

May 26, 2015

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Re: Comments to CASAC on the second draft US EPA Integrated Science Assessment for Oxides of Nitrogen – Health Criteria

Dear Mr. Yeow:

Please find attached Gradient's recent comments on the second draft Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (ISA), as well as a set of slides and handouts that I presented to EPA on May 19, 2015.

In the presentation handouts, Table 1 compares the causal determinations for various health effects between the 2008, the 2013 first draft, and the 2015 second draft ISAs. Table 1 also lists several reasons why the evidence does not support strengthened causal determinations.

We also reproduced Table 5-1 in the second draft ISA (*i.e.*, the current ISA), which outlines specified criteria US EPA used to evaluate study quality. This table is followed by Table 2, which provides a systematic perspective on overall and comparative study quality for epidemiology studies of short-term NO<sub>2</sub> exposure and hospital admissions (HAs) and emergency department (ED) visits for asthma. Epidemiology studies cited in the ISA as supporting a causal association between short-term NO<sub>2</sub> exposure and asthma exacerbation are listed in rows. Study quality criteria defined in Table 5-1 of the current ISA are listed in columns. The shaded studies were considered by EPA to be of overall higher quality than other studies. If a study meets a criterion, the cell is shaded green; otherwise, the cell is shaded pink. As shown in Table 2, the studies designated as "high quality" in the ISA do not appear to be of higher quality than others and should not have been given more weight in causal analyses. Table 2 thus demonstrates that US EPA did not apply study quality criteria in Table 5-1 in a consistent and systematic manner.

Finally, Tables 3 and 4 summarize key evidence for short-term NO<sub>2</sub> exposure and asthma exacerbation and long-term NO<sub>2</sub> exposure and the development of new-onset asthma, respectively. The left two columns are reproduced from Tables 5-45 and 6-5 of the current ISA, respectively, and provide EPA's interpretation of the key evidence and rationale for casual determinations, while the right two columns show our interpretation. Cells are shaded pink if they provide evidence for causation, green if they do not. As you can see, our systematic review of the studies indicates that none of these studies provide evidence for causation.

In our comments and our slides, we discuss issues with EPA's application of its causal framework and interpretation of study findings. Specifically, we find that the causal framework does not provide enough guidance for studies to be evaluated in a systematic manner using well-specified criteria, and therefore other investigators cannot replicate US EPA's analyses. Also, the causal framework does not require a determination of whether, as a whole, the evidence more likely indicates causation or alternative hypotheses. In addition, the framework is not applied using a true weight-of-evidence (WoE) approach. With respect to the interpretation of study findings, associations deemed to be causal (short-term NO<sub>2</sub> exposure and respiratory effects) and likely causal (long-term NO<sub>2</sub> exposure and respiratory effects) in the ISA were generally close to null and more likely the result of chance, bias, and/or confounding. In some cases, associations between NO<sub>2</sub> exposure and health effects were not consistent either within or among epidemiology studies or were not coherent with controlled-exposure studies. For several reported short-term health effects, studies reported associations at lag times that do not appear to be biologically plausible. For numerous endpoints, the modes of action (MoAs) were not established; for cases in which the ISA hypothesized a potential MoA, the MoA lacked biological plausibility.

As the current ISA acknowledges, uncertainty also exists regarding whether some observed associations are attributable to NO<sub>2</sub> *per se* or whether NO<sub>2</sub> is a surrogate for another pollutant or pollutant mixture. For example, US EPA relied primarily on longitudinal cohort studies of asthma development in children and concluded that a causal relationship likely exists between long-term exposure to NO<sub>2</sub> and respiratory effects. However, most studies evaluated multiple traffic-related air pollutants, such as PM<sub>2.5</sub> and black carbon, and often found similar positive associations with asthma for these co-pollutants in single-pollutant analyses. None of the studies conducted multi-pollutant analyses, so there is considerable uncertainty with regard to potential confounding by traffic-related co-pollutants. Also, in several instances, the ISA cites the results of multi-pollutant models as evidence that traffic-related pollutants do not appear to confound associations between health and NO<sub>2</sub>; however, in other instances, the ISA states that the results of multi-pollutant models are unreliable.

Overall, we find that the current ISA does not provide evidence that the classifications should be strengthened for any of the endpoints reviewed, because of considerable inconsistency and uncertainty in available evidence.

Thank you for your consideration.

Sincerely,

GRADIENT



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# **Comments on EPA's Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (Second External Review Draft)**

Prepared for  
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April 23, 2015



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# Abbreviations

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ACS	American Cancer Society
AHR	Airway Hyper-Responsiveness
AHSMOG	Adventist Health and Smog
CHS	Children's Health Study
CI	Confidence Interval
CO	Carbon Monoxide
CV	Cardiovascular
ECP	Eosinophil Cationic Protein
ECRHS	European Community Respiratory Health Survey
ED	Emergency Department
eNO	Exhaled Nitric Oxide
EPA	United States Environmental Protection Agency
ESCAPE	European Study of Cohorts for Air Pollution Effects
FEV <sub>1</sub>	Forced Expiratory Volume in 1 Second
FVC	Forced Vital Capacity
GEE	Generalized Estimating Equation
GIS	Geographical Information Systems
HA	Hospital Admissions
ICAM-1	Intercellular Cell Adhesion Molecule-1
IFN $\gamma$	Interferon Gamma
IgE	Immunoglobulin E
IL	Interleukin
IOM	Institute of Medicine
IQR	Interquartile Range
ISA	Integrated Science Assessment for Oxides of Nitrogen – Health Criteria
kpm	Kilopound Meter
LUR	Land Use Regression
MoA	Mode of Action
mph	Miles Per Hour
NAAQS	National Ambient Air Quality Standards
NHS	Nurses' Health Study
NO <sub>2</sub>	Nitrogen Dioxide
NO <sub>x</sub>	Oxides of Nitrogen
NTP	National Toxicology Program
O <sub>3</sub>	Ozone
OR	Odds Ratio
PC	Provocative Concentration
PD	Provocative Dose
PEF	Peak Expiratory Flow Rate
PM	Particulate Matter
PMN	Polymorphonuclear Leukocyte
ppb	Parts Per Billion
ppm	Parts Per Million
ROS	Reactive Oxygen Species

RR	Relative Risk
SE	Standard Error
SES	Socioeconomic Status
SO <sub>2</sub>	Sulfur Dioxide
Th2	T-Derived Lymphocyte Helper 2
TNF $\alpha$	Tumor Necrosis Factor Alpha
UFP	Ultrafine Particles
WoE	Weight of Evidence

# Executive Summary

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As part of its review of the National Ambient Air Quality Standards (NAAQS) for nitrogen dioxide (NO<sub>2</sub>), in February 2015, the United States Environmental Protection Agency (EPA) released the second external review draft of the *Integrated Science Assessment for Oxides of Nitrogen – Health Criteria* (ISA; US EPA, 2015). The ISA reviews short- and long-term epidemiology, controlled human exposure, animal toxicity, and mode-of-action (MoA) studies of NO<sub>2</sub> and makes causal determinations regarding several health effects. EPA will conduct risk assessments on health effects for which NO<sub>2</sub> is determined to be a causal or likely causal factor.

After reviewing EPA's causal framework, its application, and EPA's assessment of individual studies, we conclude that the current ISA does not provide evidence that any causal determination should be strengthened from those noted in the 2008 ISA.

With regard to the causal framework, we find it does not provide enough guidance for studies to be evaluated in a systematic manner using well-specified criteria, and, as such, EPA's analyses cannot be replicated by other investigators. It also does not require a determination of whether, as a whole, evidence is more likely indicative of causation or alternative hypotheses. In addition, the framework is not applied using a true weight-of-evidence (WoE) approach.

With respect to the interpretation of study findings, in general, associations deemed to be causal (short-term NO<sub>2</sub> exposure and respiratory effects) and likely causal (long-term NO<sub>2</sub> exposure and respiratory effects) in the ISA were close to null and more likely the result of chance (*e.g.*, multiple comparison), bias (*e.g.*, exposure measurement error, outcome misclassification, publication bias), and/or confounding (*e.g.*, by co-pollutants, smoking, socioeconomic status [SES]). In some cases, associations between NO<sub>2</sub> exposure and health effects were not consistent either within or among epidemiology studies or were not coherent with controlled exposure studies. For several reported short-term health effects, associations were found at lag times that do not appear to be biologically plausible. For numerous endpoints, the MoAs were not established; in cases in which the ISA hypothesized a potential MoA, the MoA lacked biological plausibility. As acknowledged in the ISA, there is also uncertainty regarding whether some observed associations are due to NO<sub>2</sub> *per se* or whether NO<sub>2</sub> is a surrogate for another pollutant or pollutant mixture. In several instances, the ISA cites the results of multi-pollutant models as evidence that traffic-related pollutants do not appear to confound associations between health and NO<sub>2</sub>; however, in other instances, the ISA states that the results of multi-pollutant models are unreliable.

The ISA concludes that the evidence for short-term NO<sub>2</sub> exposure is suggestive of causation for cardiovascular (CV) effects and total mortality, and the evidence for long-term NO<sub>2</sub> exposure is suggestive of causation for CV effects, birth outcomes, total mortality, and cancer. The evidence presented in the ISA, however, does not indicate that NO<sub>2</sub> exposure is more likely to be a causal factor for these effects than not. Even if new, high-quality studies demonstrate statistically significant associations, the results of all other relevant studies must be considered as well, to determine whether these associations are likely indicative of causation (with higher quality studies given more weight in the evaluation of evidence). If inconsistencies among the studies cannot be resolved, it is inappropriate to conclude that the evidence is suggestive of an association; rather, in this case, the WoE indicates that the evidence is inadequate for drawing conclusions.

Finally, the ISA's framework for classifying potential "at-risk" factors (*i.e.*, effect modifiers, which are factors that differentially modify the observed effect a targeted risk factor has on disease status) is not the same as the causal framework, and it too is insufficient for assessing the strength of evidence. The ISA does not conduct a systematic review of potential effect modifiers that considers study quality and relevance and weights studies based on both before drawing conclusions. We find the evidence for an increased risk among individuals with asthma who are exposed to ambient concentrations, as well as children and older adults, does not meet the ISA's criteria for "adequate evidence" of being an at-risk factor.

While we do not necessarily agree with each causal classification in the 2008 ISA, we find that the current ISA does not provide evidence that the classifications should be strengthened for any of the endpoints reviewed.

# 1 Introduction

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As part of its review of the National Ambient Air Quality Standards (NAAQS) for nitrogen dioxide (NO<sub>2</sub>), in February 2015, the United States Environmental Protection Agency (EPA) released the second external review draft of the *Integrated Science Assessment for Oxides of Nitrogen – Health Criteria* (referred to as the "ISA" throughout these comments) (US EPA, 2015). The ISA reviews short- and long-term epidemiology, controlled human exposure, toxicology, and mode-of-action (MoA) studies of NO<sub>2</sub> and makes causal determinations regarding several health effects. EPA will conduct risk assessments on health effects for which NO<sub>2</sub> is determined to be a causal or likely causal factor.

In 2008, the ISA from the previous NAAQS review cycle (US EPA, 2008) concluded that there was sufficient evidence to establish a likely causal association between short-term NO<sub>2</sub> exposure and respiratory effects, suggestive but not sufficient evidence to infer a causal association between short-term NO<sub>2</sub> exposure and total mortality, and inadequate evidence to infer the presence or absence of a causal association between short-term NO<sub>2</sub> exposure and cardiovascular (CV) effects (US EPA, 2008). For long-term NO<sub>2</sub> exposure, the 2008 ISA concluded that evidence was suggestive but not sufficient to assume a likely causal association for respiratory effects and that evidence was inadequate to infer the presence or absence of a causal association for CV effects, reproductive and developmental effects, total mortality, and cancer (US EPA, 2008; see Table 1.1).

In the current review cycle, the first external review draft (first draft) ISA, issued in November 2013, concluded that evidence was sufficient to conclude a causal association, rather than a likely causal association, between short-term NO<sub>2</sub> exposure and respiratory effects. The first draft ISA also changed the causal determination for total mortality from suggestive to likely causal, and from inadequate to likely causal for CV effects. For long-term NO<sub>2</sub> exposure, the first draft ISA strengthened the causal determination for all endpoints, concluding that there was a likely causal association with respiratory effects, and that evidence was suggestive of a causal association for CV effects, reproductive and developmental effects, total mortality, and cancer (Table 1.1).

The second draft ISA revised several of the causal determinations from the first draft. Evidence for short-term NO<sub>2</sub> exposure and both CV effects and total mortality are no longer judged to be likely causal, but rather suggestive but not sufficient to infer a causal relationship. Also, the classification of evidence for two categories of reproductive and developmental effects – fertility, reproduction, and pregnancy, and postnatal effects – has been changed from suggestive to inadequate.

In Section 2 of these comments, we discuss the framework set forth in the ISA for assessing causality (EPA's causal framework) and how it is applied in the ISA. Section 3 reviews the evidence regarding short-term NO<sub>2</sub> exposure and respiratory effects, which the ISA concludes is sufficient to establish a causal association. Section 4 reviews the evidence regarding long-term NO<sub>2</sub> exposure and respiratory effects, which the ISA concludes indicates a likely causal association. Section 5 reviews the evidence regarding associations between NO<sub>2</sub> exposure and health effects for which the ISA concludes the evidence is either suggestive or inadequate. In Section 6, we discuss the ISA's evaluation of effect modifiers (what EPA refers to as "at-risk factors," *i.e.*, variables that differentially modify the observed effect of a risk factor on disease status). Overall, we found that the current ISA does not provide evidence that the classifications should be strengthened for any of the endpoints reviewed.

**Table 1.1 Causal Determinations in the 2008, 2013 Draft, and 2015 Draft Integrated Science Assessments for Oxides of Nitrogen**

Health Effect Category	Causal Determination			Comments
	2008 ISA	First Draft ISA	Second Draft ISA	
<b>Short-term NO<sub>2</sub> Exposure</b>				
Respiratory Effects	Sufficient to determine a likely causal relationship.	Causal	Causal	Inadequate quality of epidemiology studies. Lack of coherence with controlled exposure or toxicology studies. Uncertainty regarding whether NO <sub>2</sub> is a proxy for traffic-related pollution.
Cardiovascular Effects	Inadequate to infer the presence or absence of a causal relationship.	Likely causal	Suggestive	Inadequate quality of epidemiology studies. Lack of coherence across studies. Lack of confirmed MoAs.
Total Mortality	Suggestive but not sufficient to infer a causal relationship.	Likely causal	Suggestive	Inadequate quality of epidemiology studies. Lack of confirmed MoAs.
<b>Long-term NO<sub>2</sub> Exposure</b>				
Respiratory Effects	Suggestive but not sufficient to infer a causal relationship.	Likely causal	Likely causal	Inconsistent findings among epidemiology studies. Lack of coherence with studies of short-term effects. Lack of coherence with MoAs. Uncertainty regarding whether NO <sub>2</sub> is a proxy for traffic-related pollution.
Cardiovascular Effects	Inadequate to infer the presence or absence of a causal relationship.	Suggestive	Suggestive	Inconsistent findings among epidemiology studies. Lack of confirmed MoAs.
Reproductive and Developmental Effects	Inadequate to infer the presence or absence of a causal relationship.	Fertility, Reproduction, and Pregnancy: Suggestive	Fertility, Reproduction, and Pregnancy: Inadequate	Inconsistent findings among studies. Lack of coherence across different endpoints. Lack of confirmed MoAs.
		Birth Outcomes: Suggestive	Birth Outcomes: Suggestive	Inconsistent findings among studies. Lack of coherence across different endpoints. Lack of confirmed MoAs.
		Postnatal Development: Suggestive	Postnatal Development: Inadequate	Inconsistent findings among studies. Lack of confirmed MoAs.
Total Mortality	Inadequate	Suggestive	Suggestive	Inconsistent findings among epidemiology studies. Lack of coherence with studies of morbidity endpoints. Lack of confirmed MoAs.
Cancer	Inadequate to infer the presence or absence of a causal relationship.	Suggestive	Suggestive	Inconsistent findings among epidemiology studies. Lack of confirmed MoAs.

Notes:

ISA = Integrated Science Assessment; NO<sub>2</sub> = Nitrogen Dioxide; MoA = Mode of Action. Sources: EPA (2008, 2013, 2015).

## 2 The Causal Framework

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### 2.1 Evaluation of study quality is not transparent

The ISA notes that EPA assessed study quality based on features such as the adequacy of study population selection, the representativeness of the exposure assessment, the appropriateness of the statistical analyses, the sufficient control of potential confounders, the validity and reliability of health endpoints, and the overall biological coherence, internally and externally, of the study findings. The addition of Table 5-1 in the second draft ISA (US EPA, 2015) (reproduced in this report as Appendix A), which the agency describes as a "summary and description of scientific considerations for evaluating the quality of studies on the health effects of oxides of nitrogen," is a major improvement. This table outlines specific criteria for evaluating study quality on the basis of aspects such as study design, exposure assessment, outcome assessment, potential confounding, and statistical methodology, although it is not always explicit in describing how decisions should be made regarding these factors.

While the addition of this table is a step in the right direction, it should be noted that it is not appropriate for the ISA to consider the study findings when evaluating study quality. It is also not clear how EPA applies these criteria when evaluating study quality and whether it does so in a consistent and systematic manner across studies. For example, if a particular statistical model is considered a limitation in one study, it is not clear whether the model is considered a limitation in all studies that used it, and if not, whether there is a good reason to conclude otherwise. It is also not clear whether the evaluation of study quality is based purely on methods, independent of funding source of studies; and whether studies with more robust methods received more weight in causal determinations. Currently, no explicit rationale is provided in the ISA for why certain studies are considered key evidence while others of similar quality are not.

In Table 2.1, we demonstrate that the ISA does not apply study quality criteria in a consistent and systematic manner, using short-term NO<sub>2</sub> exposure and hospital admissions (HAs) and emergency department (ED) visits for asthma as an example. The ISA indicates that 23 epidemiology studies support a causal association between short-term NO<sub>2</sub> exposure and asthma exacerbation and indicates in Table 5-45 that several of these studies are of higher quality than others (section 5.2.2.4 of the ISA and Section 3.1 of this report discuss this evidence in depth). In Table 2.1, we present each of the 23 studies according to various study quality characteristics listed in Table 5-1 in the ISA and highlight in green the characteristics that the ISA indicates are indicative of a higher quality study. Table 2.1 provides a systematic perspective on overall and comparative study quality and shows that the studies designated as "high quality" in the ISA do not appear to be of higher quality than others.

### 2.2 The weight of evidence for causal determination framework is inadequate

The EPA causal framework draws its language from sources across the federal government and scientific community, and particularly relies on an Institute of Medicine (IOM) report titled *Improving the Presumptive Disability Decision-making Process for Veterans* (IOM, 2008). Whereas IOM recommended four categories for the level of evidence for causation (Table 2.2), EPA has five categories for causal relationships (Table 2.3). Based on these categories, the ISA determines which health effects will be evaluated in quantitative risk assessments. Notably, the ISA uses a different framework (Table

2.4) for classifying effect modifiers (which it calls "at-risk factors") that is much more similar to the IOM framework, although the ISA indicates that this framework is based on EPA's causal framework (as shown in Table 2.3).

EPA's causal framework is also ostensibly based on modified Bradford Hill aspects. Both the original and modified Bradford Hill aspects (*i.e.*, strength of association, consistency and coherence, biological plausibility, biological gradient or exposure-response, specificity, temporality of effect, and adversity) are useful tools for evaluating causation; it may be difficult to ascribe observations to causation if these aspects are not met, whereas it may be difficult to ascribe observations to anything other than causation if they are met. In its current form, however, EPA's causal framework is not congruent with the judgments based on the original or modified Bradford Hill aspects. For example, the framework claims to rely heavily on the aspect of consistency across studies in its categorization scheme, but, in practice, EPA does not always fully evaluate consistency or consider other aspects such as coherence, biological plausibility, biological gradient, and strength of association. In many cases, the ISA assumes association indicates causation even when causal modeling may indicate otherwise.

The ISA states that evidence is sufficient to conclude a *causal* relationship if "chance, confounding, and other biases [can] be ruled out with reasonable confidence" (US EPA, 2015), yet there is no guidance on what constitutes "reasonable confidence." Based on the current framework, the ISA cannot reliably make that determination, because it does not fully explore chance, confounding, and other biases in a consistent manner. The ISA suggests that "controlled human exposure studies that demonstrate consistent effects" constitute evidence for a causal relationship (US EPA, 2015), but it should indicate that this is only true if the exposures are at concentrations relevant to ambient exposure and the results are coherent with other lines of evidence. The ISA also indicates that "observational studies that cannot be explained by plausible alternatives" constitute evidence for a causal relationship (US EPA, 2015). Yet, the ISA does not fully explore alternative explanations for study results. Currently, the ISA sets forth a hypothesis (*i.e.*, a criteria pollutant causes a particular health effect) and determines whether the evidence supports that hypothesis. The ISA does not, but should, fully explore whether and to what degree the evidence supports *other* hypotheses (*e.g.*, a confounder, rather than the criteria pollutant, causes a particular health effect). It is only in this manner that alternative hypotheses can truly be ruled out.

The ISA states that evidence is sufficient to conclude a *likely causal* relationship if "copollutant exposures are difficult to address and/or other lines of evidence (controlled human exposure, animal, or mode of action information) are limited or inconsistent" or if "animal toxicological evidence from multiple studies from different laboratories demonstrate effects, but limited or no human data are available" (US EPA, 2015). The ISA concludes that evidence is *suggestive of a causal relationship* if "at least one high-quality epidemiologic study shows an association with a given health outcome but the results of other studies are inconsistent" or if "a well-conducted toxicological study, such as those conducted in the National Toxicology Program (NTP), shows effects in animal species" (US EPA, 2015).

For making determinations regarding causality, it is important to evaluate all available evidence (positive, null, and negative) in what is referred to as a weight-of-evidence (WoE) evaluation. Any WoE evaluation, by definition, involves a consideration of all lines of evidence in a consistent and coherent manner. It is not about resolving all uncertainty; rather, the goal of a WoE evaluation is to determine whether the evidence as a whole supports causation more than it supports a lack of effect. If co-pollutants cannot be addressed or studies are inconsistent, the WoE may indicate a lack of causality or inadequate evidence to assess causation. If positive effects in high-dose animal studies cannot be related to humans, this does not constitute suggestive evidence; instead, these effects are essentially uninformative regarding causation in humans. Not every study evaluating criteria pollutants is informative for evaluating human health risk, and the ISA should not place undue weight on these studies.

It is notable that the EPA causal framework requires only one high-quality study for evidence of a causal relationship to be deemed *suggestive*. Under this definition, high-quality studies that are inconsistent with evidence of an association may exist, but as long as one high-quality study demonstrates an effect, there would still be enough evidence to constitute a suggestive relationship. Instead, all studies should be reviewed using the same criteria, and one should conclude a suggestive causal association only if the WoE indicates that a causal association is more likely than not, based on all the evidence combined. In situations where there are multiple, but inconsistent, high-quality studies, the appropriate conclusion is that the evidence is below equipoise (IOM, 2008).

Finally, evaluating the evidence as a whole means that one should evaluate not only how much evidence can be adduced to support (or to counter) the hypothesized causal effect, but also how separate lines of evidence support (or contradict) one another. It is critical to determine the most likely explanation for discrepancies across studies by evaluating all of the evidence and not selectively considering evidence that supports or counters a given hypothesis.

Many of the issues noted above could be resolved by updating the ISA's categories for causal determination to be more consistent with the IOM framework (on which it was based originally), outlined in Table 2.2. The ISA should evaluate all the evidence in a consistent manner, using well-specified criteria, and determine whether, as a whole, it constitutes evidence for causation or is more likely to be supportive of an alternative hypothesis. EPA should proceed with a risk assessment on a particular health effect only if the evidence is clearly supportive of causation (*i.e.*, equipoise and above in the IOM framework).

**Table 2.1 Asthma HA/ED Visit Study Quality Characteristics**

Citation	Inclusion in ISA		Study Design			Pollutant		Exposure Assessment			Outcome Assessment		Confounding by Co-pollutants			Other Confounders				Statistical Methods	
	"High-quality" Study	Main Text Only	Design	Single vs. Multi-city	Size/Duration <sup>1</sup>	NO, NO <sub>2</sub> , NO <sub>x</sub>	Comparisons Between Oxides	Central Site Monitoring	Spatial Variability Assessed	Comparison of Exposure Assessment Methods	Type of Outcome	Exclusion of Children < 2 Years Old	Traffic-related Pollutants Assessed	Correlations Reported	Relative Measurement Error in Co-pollutants Discussed	Meteorology	Day of Week	Season	Allergens	Cautious Interpretation of Multi-pollutant Models	Sensitivity Analysis: Alternate Model Specification
Strickland <i>et al.</i> (2010)	√		Case cross-over	Single	91,386 ED visits/ 12 years	NO <sub>2</sub>	No	Yes	No	No	ED visits	Yes	No <sup>2</sup>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Villeneuve <i>et al.</i> (2007)	√		Case cross-over	Single	57,912 ED visits/ 10 years	NO <sub>2</sub>	No	Yes	No	No	ED visits	Yes	CO	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Jalaludin <i>et al.</i> (2008)	√		Case cross-over	Single	1,826 ED visits/ 5 years	NO <sub>2</sub>	No	Yes	No	No	ED visits	No <sup>3</sup>	CO, PM <sub>2.5</sub>	Yes	No	Yes	Yes	Yes	No	Yes	No
Ito <i>et al.</i> (2007)	√		Time series	Single	4 years	NO <sub>2</sub>	No	Yes	Yes	No	ED visits	No	CO, PM <sub>2.5</sub>	Yes	Yes	Yes	Yes	No	Yes	Yes	
Iskandar <i>et al.</i> (2012)	√		Case cross-over	Single	8,226 HAS/ 8 years	NO <sub>2</sub> , NO <sub>x</sub>	Yes	Yes	Yes	No	HAS	No <sup>4</sup>	UFP, PM <sub>2.5</sub>	Yes	No <sup>5</sup>	Yes	Yes	Yes	No	Yes	Yes
ATSDR and NYSDOH (2006)	√		Time-series	Single	2 years	NO <sub>2</sub>	No	Yes	Yes	No	ED visits	No <sup>6</sup>	PM <sub>2.5</sub>	Yes	No	Yes	Yes	Yes	No <sup>7</sup>	Yes	Yes
Stieb <i>et al.</i> (2009)	√		Time series	Multi-city	4-10 years <sup>8</sup>	NO <sub>2</sub>	No	Yes	No	No	ED visits	No	No <sup>9</sup>	Yes	No	Yes	Yes	Yes	No	NA	Yes
Samoli <i>et al.</i> (2011)		√	Time series	Single	4 years	NO <sub>2</sub>	No	Yes	Yes	No	HAS	No	No	Yes	No	Yes	Yes	Yes	Yes <sup>10</sup>	Yes	Yes
Peel <i>et al.</i> (2005)		√	Time series	Single	8 years	NO <sub>2</sub>	No	Yes	Yes	Yes <sup>11</sup>	ED visits	No <sup>4</sup>	CO	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Son <i>et al.</i> (2013)		√	Time-series	Multi-city	6 years	NO <sub>2</sub>	No	Yes	No	No	HAS	No	No <sup>12</sup>	Yes	No	Yes	Yes	Yes	No	NA	Yes
Ko <i>et al.</i> (2007)		√	Time-series	Single	6 years	NO <sub>2</sub>	No	Yes	No	No	HAS	No	PM <sub>2.5</sub>	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Sarnat <i>et al.</i> (2013b)		√	Time series	Single	4 years	NO <sub>x</sub>	No	Yes <sup>13</sup>	Yes	Yes	ED visits	No	No <sup>9</sup>	Yes	Yes	Yes	Yes	Yes	No	NA	Yes
Orazio <i>et al.</i> (2009)		√	Case cross-over	Multi-city	53,272 ED visits/ 7 years	NO <sub>2</sub>	No	Yes	Yes	No	ED visits	No	No <sup>12</sup>	No	No	Yes	Yes	Yes	No <sup>7</sup>	NA	Yes
Strickland <i>et al.</i> (2011)		√	Time series	Single	41,741 ED visits/ 12 years	NO <sub>2</sub>	No	Yes	Yes	Yes	ED visits	Yes	No <sup>2</sup>	No	Yes	Yes	Yes	Yes	No	NA	Yes
Li <i>et al.</i> (2011)		√	Time series and case cross-over	Single	12,933 asthma events/ 3 years	NO <sub>2</sub>	No	Yes	No	No	ED visits and HAS	Yes	No <sup>9</sup>	Yes	No	Yes	Yes	Yes	No	NA	Yes
Gass <i>et al.</i> (2014)		√	Case cross-over	Single	11 years	NO <sub>2</sub>	No	Yes	No	No	ED visits	Yes	CO, PM <sub>2.5</sub>	No	No	Yes	Yes	Yes	No	Yes	No
Winqvist <i>et al.</i> (2014)		√	Time series	Single	6 years	NO <sub>2</sub>	No <sup>14</sup>	Yes	No	No	ED visits	Yes	CO, PM <sub>2.5</sub> , EC	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Burnett <i>et al.</i> (1999)		√	Time series	Single	15 years	NO <sub>2</sub>	No	Yes	No	No	HAS	No	CO, PM <sub>2.5</sub>	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Linn <i>et al.</i> (2000)		√	Time series	Single	4 years	NO <sub>2</sub>	No	Yes	Yes	No	HAS	Yes	CO	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes

Citation	Inclusion in ISA		Study Design			Pollutant		Exposure Assessment			Outcome Assessment		Confounding by Co-pollutants			Other Confounders				Statistical Methods	
	"High-quality" Study	Main Text Only	Design	Single vs. Multi-city	Size/Duration <sup>1</sup>	NO, NO <sub>2</sub> , NO <sub>x</sub>	Comparisons Between Oxides	Central Site Monitoring	Spatial Variability Assessed	Comparison of Exposure Assessment Methods	Type of Outcome	Exclusion of Children < 2 Years Old	Traffic-related Pollutants Assessed	Correlations Reported	Relative Measurement Error in Co-pollutants Discussed	Meteorology	Day of Week	Season	Allergens	Cautious Interpretation of Multi-pollutant Models	Sensitivity Analysis: Alternate Model Specification
Burra <i>et al.</i> (2009)		√	Time series	Single	10 years	NO <sub>2</sub>	No	Yes	No	No	Physician visits	No <sup>3</sup>	No <sup>15</sup>	No	No	Yes	Yes	Yes	No	NA	Yes
Sinclair <i>et al.</i> (2010)		√	Time series	Single	4 years	NO <sub>2</sub>	No	Yes	No	No	Acute out-patient visits	No	No <sup>16</sup>	Yes	No	Yes	Yes	Yes	No	NA	Yes
Tolbert <i>et al.</i> (2000)		√	Retrospective cohort	Single	5,934 ED visits for asthma/3 summers	NO <sub>x</sub>	No	Yes	No <sup>17</sup>	No	ED visits	No	No	Yes	No	Yes	Yes	Yes	No <sup>7</sup>	Yes	Yes
Jaffe <i>et al.</i> (2003)		√	Time series	Multi-city	6 summers	NO <sub>2</sub>	No	Yes	No <sup>17</sup>	No	ED visits	Yes	No	Yes	No	Yes	Yes	Yes	No	NA	No

Notes:

CO = Carbon Monoxide; EC = Elemental Carbon; ED = Emergency Department; HA = Hospital Admission; ISA = Integrated Science Assessment Oxides of Nitrogen; NO = Nitrogen Monoxide; NO<sub>2</sub> = Nitrogen Dioxide; NO<sub>x</sub> = Oxides of Nitrogen; O<sub>3</sub> = Ozone; OC = Organic Carbon; PM = Particulate Matter; UFP = Ultrafine Particles; VOC = Volatile Organic Compound.

(1) In Table 5-1, EPA did not indicate what sample size and duration are required for a study to be considered "large" and, therefore, more reliable. For the purposes of this table, we highlight time series studies of at least 10 years in duration and case cross-over studies of at least 10,000 events as higher quality.

(2) Several traffic-related co-pollutants were measured and examined in single-pollutants models, but authors did not attempt to determine whether NO<sub>2</sub> associations were confounded by traffic-related co-pollutants.

(3) < 1-year-old subjects excluded.

(4) 0- to 1-year-old subjects analyzed separately.

(5) Limited discussion of exposure measurement error in co-pollutants: only in the context of UFP and the potential that other pollutants were measured more accurately and served as proxies.

(6) Included additional diagnostic criteria for children < 1 year old to mitigate outcome misclassification.

(7) Aeroallergens measured but not included in statistical models as a confounder.

(8) Duration varied by city.

(9) CO and PM<sub>2.5</sub> measured and analyzed in separate models, but no multi-pollutant models were conducted.

(10) Desert dust, which includes bio-allergens.

(11) Compared monitoring systems.

(12) CO measured and analyzed in separate models, but no multi-pollutant models were conducted. Authors did not assess potential co-pollutant confounding in any other manner.

(13) Dispersion modeling used in addition to measurements from central site monitors.

(14) Nitrate also examined.

(15) PM<sub>2.5</sub> measured and analyzed in separate models, but no multi-pollutant models were conducted. Authors did not assess potential co-pollutant confounding in any other manner.

(16) PM<sub>2.5</sub>, CO, oxygenated VOCs, EC, OC, and metals were measured and analyzed in separate models, but no multi-pollutant models were conducted. Authors did not assess potential co-pollutant confounding in any other manner.

(17) Spatial variability of other pollutants (*i.e.*, O<sub>3</sub> and PM) only was assessed, but not variability of NO<sub>2</sub>.

**Table 2.2 Institute of Medicine's Recommended Categories for the Level of Evidence for Causation**

Causal Determination	Evidence
Sufficient	The evidence is sufficient to conclude that a causal relationship exists. For example: a) replicated and consistent evidence of an association from several high-quality epidemiologic studies that cannot be explained by plausible noncausal alternatives ( <i>e.g.</i> , chance, bias, or confounding); or b) evidence of causation from animal studies and mechanistic knowledge; or c) compelling evidence from animal studies and strong mechanistic evidence from studies in exposed humans, consistent with ( <i>i.e.</i> , not contradicted by) the epidemiologic evidence.
Equipoise and above	The evidence is sufficient to conclude that a causal relationship is at least as likely as not, but not sufficient to conclude that a causal relationship exists. For example: a) evidence of an association from the preponderance of several high-quality epidemiologic studies that cannot be explained by plausible noncausal alternatives ( <i>e.g.</i> , chance, bias, or confounding) as well as animal evidence and biological knowledge consistent with a causal relationship; or b) strong evidence from animal studies or mechanistic evidence that is not contradicted by human or other evidence.
Below Equipoise	The evidence is not sufficient to conclude that a causal relationship is at least as likely as not, or is not sufficient to make a scientifically informed judgment. For example: a) consistent human evidence of an association that is limited by the inability to rule out chance, bias, or confounding with confidence, and weak animal or mechanistic evidence; or b) animal evidence suggestive of a causal relationship, but weak or inconsistent human and mechanistic evidence; or c) mechanistic evidence suggestive of a causal relationship, but weak or inconsistent animal and human evidence; or d) the evidence base is very thin.
Against	The evidence suggests the lack of a causal relationship. For example: a) consistent human evidence of no causal association from multiple studies covering the full range of exposures encountered by humans; or b) animal or mechanistic evidence supportive of a lack of a causal relationship.

Note:

Source: IOM (2008).

**Table 2.3 EPA's Weight of Evidence for Causal Determination**

Causal Determination	Health Effects
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (e.g., doses or exposures generally within one to two orders of magnitude of recent concentrations). That is, the pollutant has been shown to result in health effects in studies in which chance, confounding, and other biases could be ruled out with reasonable confidence. For example: (1) controlled human exposure studies that demonstrate consistent effects, or (2) observational studies that cannot be explained by plausible alternatives or that are supported by other lines of evidence (e.g., animal studies or mode of action information). Generally, the determination is based on multiple high-quality studies conducted by multiple research groups.
Likely to be a causal relationship	Evidence is sufficient to conclude that a causal relationship is likely to exist with relevant pollutant exposures. That is, the pollutant has been shown to result in health effects in studies where results are not explained by chance, confounding, and other biases, but uncertainties remain in the evidence overall. For example: (1) observational studies show an association, but copollutant exposures are difficult to address and/or other lines of evidence (controlled human exposure, animal, or mode of action information) are limited or inconsistent, or (2) animal toxicological evidence from multiple studies from different laboratories demonstrate effects, but limited or no human data are available. Generally, the determination is based on multiple high-quality studies.
Suggestive of a causal relationship	Evidence is suggestive of a causal relationship with relevant pollutant exposures but is limited, and chance, confounding, and other biases cannot be ruled out. For example: (1) when the body of evidence is relatively small, at least one high-quality epidemiologic study shows an association with a given health outcome and/or at least one high-quality toxicological study shows effects relevant to humans in animal species, or (2) when the body of evidence is relatively large, evidence from studies of varying quality is generally supportive but not entirely consistent, and there may be coherence across lines of evidence (e.g., animal studies or mode of action information) to support the determination.
Inadequate to infer a causal relationship	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quantity, quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.
Not likely to be a causal relationship	Evidence indicates there is no causal relationship with relevant pollutant exposures. Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter and considering at-risk populations and lifestages, are mutually consistent in not showing an effect at any level of exposure.

Notes:

EPA = United States Environmental Protection Agency.

Source: EPA (2015, Table III).

**Table 2.4 EPA's Classification of Evidence for Potential At-risk Factors<sup>1</sup>**

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

Notes:

EPA = United States Environmental Protection Agency.

(1) An "at-risk factor" is best described as an effect modifier, which is a technical term defined in epidemiology as a variable that differentially modifies the observed effect of a risk factor on disease status.

Source: EPA (2015, Table III).

## 3 Short-term NO<sub>2</sub> Exposure and Asthma Exacerbation

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In the previous review cycle, the 2008 ISA concluded that the relationship between short-term NO<sub>2</sub> exposure and respiratory effects was likely causal, primarily based on evidence regarding asthma exacerbation. In the current review cycle, the ISA primarily relies on epidemiology studies that evaluated HAs and ED visits for asthma and concludes that the relationship between short-term NO<sub>2</sub> exposure and respiratory effects is causal. The ISA also cites evidence from controlled human exposure studies that evaluated airway responsiveness and other effects consistent with the ISA's proposed MoA for asthma exacerbation.

EPA's casual determinations and their rationales are summarized in Table 5-45 in the ISA. We have reproduced this here in Table 3.1 (at the end of this section), along with comments demonstrating why these rationales are not supported (*e.g.*, study selection may have been biased and the evaluation of the studies was not rigorous or balanced). It is clear that the few positive findings in epidemiology studies are not strong enough to support a causal determination in light of these studies' major uncertainties and limitations. Further, evidence from controlled human exposure studies does not provide clear evidence that NO<sub>2</sub> increases airway responsiveness at relevant NO<sub>2</sub> concentrations or that effects consistent with the proposed MoA for asthma exacerbation contribute to increased HAs and ED visits for asthma.

### 3.1 Asthma Hospital Admissions and Emergency Department Visits

Summarizing the evidence for a causal relationship between short-term NO<sub>2</sub> exposure and respiratory morbidity, the ISA maintains that there is "consistent epidemiologic evidence from multiple, high quality studies at relevant NO<sub>2</sub> concentrations" (Section 5.2.9.1 and Table 5-45). Increases in asthma HAs and ED visits are cited as the first type of key evidence in Table 5-45. We reviewed the studies that the ISA identifies as being "key" as well as other studies of HAs/ED visits and found that the results are inconsistent and that the ISA does not evaluate study quality or results in a thorough or objective manner.

#### 3.1.1 EPA's selection of studies informing the causal determination may be biased

In the ISA, 23 studies of NO<sub>2</sub> and HAs/ED visits are discussed in Section 5.2.2.4 and summarized in Table 5-14. At the beginning of this section, EPA indicates that these studies are only a portion of all the relevant studies identified in literatures searches. Thirty-nine studies of HAs/ED visits are tabulated in Supplemental Table S5-3, but not discussed in the main body of the ISA. EPA does not fully explain why these studies were relegated to the supplemental table, stating only that they are "not the focus of this evaluation," because they were set in small, individual cities; spanned a short study duration; had an insufficient sample size; and/or did not examine potential co-pollutant confounding. However, some of these criteria require subjective judgments (*e.g.*, what size city is considered to be "small"?), and it is difficult to determine whether EPA applied its criteria in a systematic, objective manner when selecting studies for presentation in the main text. Of the 39 studies summarized in Supplemental Table S5-3, all spanned at least a year in duration, and most were multiple-year studies (the longest was 12 years). Studies discussed in the main text of the ISA involved similar study durations overall, and one analysis included only 2 years of data (ATSDR and NYSDOH, 2006). Several studies in the supplementary table were set in large urban centers, including Sao Paulo, Shanghai, and Tokyo, and one was a multi-city study including seven cities in South Korea. The sizes of cities in the 23 studies discussed in the main text of

the ISA were similar in size. In addition, several of the 23 studies in the main text did not analyze co-pollutant models (Linn *et al.*, 2000; Burnett *et al.*, 1999; Son *et al.*, 2013; Peel *et al.*, 2005; Jaffe *et al.*, 2003; Sarnat *et al.*, 2013a; Stieb *et al.*, 2009; Orazzo *et al.*, 2009; Strickland *et al.*, 2011; Li *et al.*, 2011; Gass *et al.*, 2014; Burra *et al.*, 2009; Sinclair *et al.*, 2010).

Seven of the twenty-three studies discussed in the main text are highlighted as key references in Table 5-45 (Strickland *et al.*, 2010; Villeneuve *et al.*, 2007; Jalaludin *et al.*, 2008; Ito *et al.*, 2007; Iskandar *et al.*, 2012; ATSDR and NYSDOH, 2006; Stieb *et al.*, 2009), one of which was distinguished as a key reference that reported null associations (Stieb *et al.*, 2009). The ISA does not explain how the seven studies were chosen to be key references. Based on the wording of Table 5-45, these key references presumably represent, in the ISA's words, the "multiple high quality studies providing consistent evidence" of increased risk of HAs and ED visits following elevated NO<sub>2</sub> exposure (US EPA, 2015). As we describe in depth below, the evidence is inconsistent both within this group of key references as well as within the broader collection of studies discussed in the main text of the ISA.

As discussed in Section 2.1, EPA provides a detailed framework for assessing study quality in Table 5-1 of the ISA but does not appear to apply the framework in a systematic or transparent manner when evaluating specific studies. EPA should have evaluated study quality in a clear and thorough manner and provided full justification for relegating 39 studies to Supplemental Table S5-3 and highlighting only a small number of studies as key references in Table 5-45 in the main text of the ISA. In the absence of such transparency, it is not possible to determine how EPA screened and weighted the available evidence. When we evaluated the study quality of 23 HA and ED visit studies using the principles described in Table 5-1 of the ISA, we found that the seven key references emphasized in Table 5-45 in the ISA are not clearly or consistently of higher quality than other studies discussed in the main text of the ISA. Our evaluation indicates that EPA did not systematically assess study quality or apply its own criteria for study quality in a consistent or unbiased manner.

### **3.1.2 EPA's evaluation of evidence for HAs and ED visits is not rigorous or balanced**

Section 5.2.2.4 of the ISA discusses the results of epidemiology studies evaluating HAs/ED visits, and concludes that the evidence "consistently" supports increased risks following NO<sub>2</sub> exposure. Several studies discussed in Section 5.2.2.4 reported elevated risks, and some of these were statistically significant. However, the validity of the ISA's conclusion is undermined by several major issues with its approach to evaluating the research results.

Table 5-16 and Figure 5-7 in the ISA display results from 17 studies with effect estimates standardized to increments of 20 parts per billion (ppb) for studies of 24-h average NO<sub>2</sub> and 30 ppb for studies of 1-h maximum NO<sub>2</sub>. All-year, warm season, and cold season effect estimates from each study are included, and newer studies (*i.e.*, those published since the 2008 ISA) are distinguished from older ones. Nearly every study mentioned in this section analyzed and reported quantitative results for multiple lag relationships, but the ISA selectively presents results for the one lag relationship from each that had the most positive and statistically significant association. In this way, the ISA presents a skewed perspective on the research literature. A more balanced approach would have been to present all the lags explored by individual studies.

Similarly, the ISA fails to address substantial between-study inconsistencies in risk estimates across various lags. In fact, there are striking inconsistencies in temporal patterns among EPA's key references. For example, Villeneuve *et al.* (2007) explored associations between NO<sub>2</sub> and ED asthma visits at lag 0, lag 1, lag 2, lag 3, lag 0-2, and lag 0-4 days and found the strength of association increased with increasing lag time in all-year analysis. Specifically, the lag 0-4 relationship was the largest and the only

one to achieve statistical significance (odds ratio [OR] = 1.03, 95% confidence interval [CI]: 1.00-1.05). Results from the warm season followed the same pattern, with lag 0-4 having the largest OR. Iskandar *et al.* (2012) also found that the strength of associations appeared to increase with lag time when assessing lag days 0 through 4 in separate models. Associations at lag days 3 and 4 were statistically significant and slightly elevated for oxides of nitrogen (NO<sub>x</sub>) as well as NO<sub>2</sub>, while associations at shorter lags were consistent with null relationships. In contrast, both Jalaludin *et al.* (2008) and Strickland *et al.* (2010) reported the opposite relationship with lag time. In both studies, associations were strongest on lag days 0 and 1, and associations with asthma ED visits decreased with longer lags. EPA does not mention these inconsistencies between the results of key references in the ISA. In fact, EPA's presentation of only one lag relationship from each paper completely obscures these inconsistencies.

In Table 5-45, EPA identifies a single key reference that reported null associations between HAs/ED visits (Stieb *et al.*, 2009). Highlighting this one study in the summary table gives the false impression that this null finding was a sole exception among the studies reviewed, and implies consistent and positive associations in the remaining body of evidence. Four other studies discussed in the main text besides Stieb *et al.* (2009) reported null relationships between NO<sub>2</sub> and HAs/ED visits (Samoli *et al.*, 2011; Tolbert *et al.*, 2000; Peel *et al.*, 2005; Jaffe *et al.*, 2003). As shown in Table 2.1, several aspects of these other four null studies have characteristics of high quality studies described in the ISA's Table 5-1. In fact, Peel *et al.* (2005) appears to be one of the highest quality studies of all 23 studies discussed in the ISA, based on our qualitative assessment. Furthermore, Jaffe *et al.* (2003) and Samoli *et al.* (2011) are comparable in quality to Stieb *et al.* (2009) and the other six high quality studies in Table 5-45. It is unclear why EPA only lists Stieb *et al.* (2009) as the lone high-quality study reporting null associations between NO<sub>x</sub> and HAs/ED visits for asthma.

EPA should have conducted a systematic and transparent evaluation of study quality to identify the key references for evidence of increases in asthma HAs and ED visits in Table 5-45. If a true causal relationship exists between NO<sub>2</sub> exposure and asthma exacerbations, well-conducted epidemiology studies of HAs/ED visits should consistently demonstrate positive results. As we have described here, results of epidemiology studies discussed in the ISA are not consistent and, collectively, do not provide strong evidence of a causal association.

## 3.2 Respiratory Symptoms and Lung Function

Table 5-45 in the ISA indicates that the first rationale for its causal determination is "consistent epidemiologic evidence from multiple high-quality studies" (US EPA, 2015). Following HAs/ED visits, the second form of key evidence supporting this statement is "coherence with increases in respiratory symptoms and decrements in lung function in populations with asthma" (US EPA, 2015). As discussed below, the evidence for lung function and respiratory symptoms is decidedly mixed, both within and between studies discussed in the ISA. The ISA's presentation and discussion of study results is misleading and gives the false impression that the results are stronger and more consistent than they are.

### 3.2.1 Evidence does not indicate increased respiratory symptoms in people with asthma

In Table 5-45, seven studies are cited as key references to support EPA's assertion of "coherence with increases in respiratory symptoms and decrements in lung function in populations with asthma" (US EPA, 2015). Four of these evaluated respiratory symptoms and medication use by individuals with asthma (Schildcrout *et al.*, 2006; Gent *et al.*, 2009; Zora *et al.*, 2013; Holguin *et al.*, 2007).

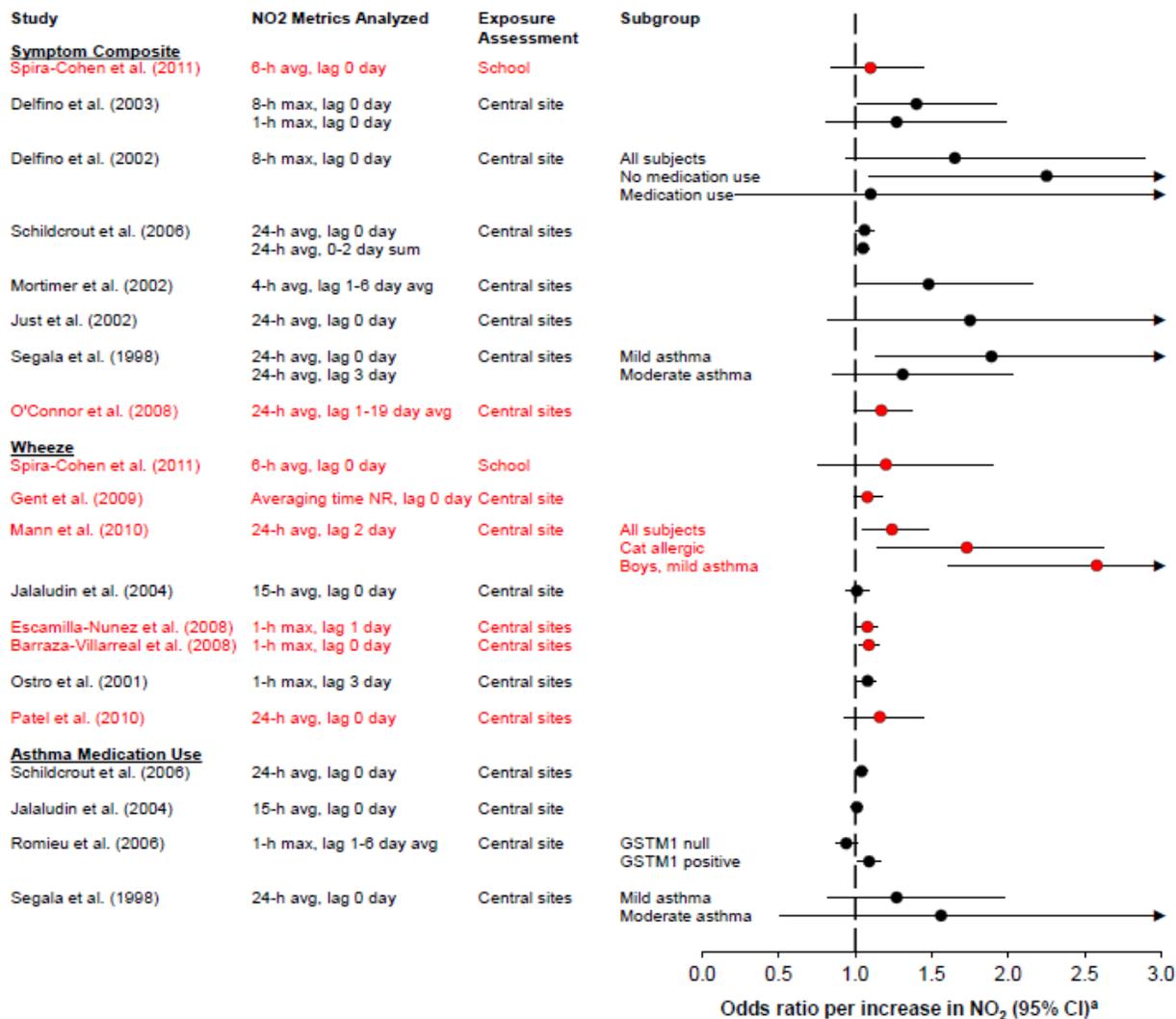
Most of the associations reported in these four key studies are null, directly contradicting the ISA's conclusion regarding coherent evidence for increases in respiratory symptoms following short-term NO<sub>2</sub> exposure. Holguin *et al.* (2007) followed 97 children with asthma in Mexico for four months with daily assessments of self-reported asthma symptoms. NO<sub>2</sub> was measured outdoors at each child's school, and the median distance between each subject's home and school was small (397 m), indicating a low potential for exposure measurement error due to geospatial variability in ambient NO<sub>2</sub>. The authors did not present quantitative results for associations between asthma symptoms and relief medication use, but simply stated that all associations were null. Zora *et al.* (2013) conducted a similar school-based panel study with 36 children. In their analysis, the outcome of interest was a composite "asthma control" index that was calculated based on symptom reports as well as the results of supervised spirometry measurements. They reported no association between school NO<sub>2</sub> concentrations and the symptom score (change in score per interquartile range [IQR] increase in NO<sub>2</sub> = 0.0096, 95% CI: -0.1345-0.1537). It is noteworthy that, in Table 5-12, the summary table of all respiratory symptom studies reviewed in the ISA, EPA does not report these main findings of Zora *et al.* (2013). Instead, the ISA presents results from analyses of two small subgroups of the overall cohort in Zora *et al.* (2013), *i.e.*, children with and without allergies. The association between NO<sub>2</sub> and symptom scores was positive and significant at a  $p < 0.10$  level for the 17 children with allergies. Presenting these findings alone is highly misleading, because the results were null for the overall cohort as well as in the nine other subgroups evaluated, besides children with allergies. None of these null findings were mentioned in the ISA.

Gent *et al.* (2009) is another key reference highlighted in the ISA as evidence for increases in asthma symptoms following NO<sub>2</sub> exposure. Investigators collected data on asthma symptoms and medication use reported by 149 children over the course of a year and estimated a very large number of associations ( $n = 240$ ) between various outcomes and components of speciated particulate matter (PM). Though these analyses of speciated PM constituted the primary investigation, Gent *et al.* (2009) also presented associations between symptoms and NO<sub>2</sub> estimated in single-pollutant models. These results included a nearly significant increase in wheezing and a null relationship with rescue medication use (OR = 1.08, 95% CI: 0.99-1.18 and OR = 1.01, 95% CI: 0.97-1.06, respectively). Yet, the ISA only presents their risk estimate for wheezing (Table 5-12 and Figure 5-4).

The final key reference highlighted as evidence for increased respiratory symptoms in Table 5-45 is a study by Schildcrout *et al.* (2006), who described results of a multi-city panel study of 990 children whose exposure was estimated using air quality measured at central site monitors. The distance between the monitor and the centroid of each child's residential ZIP code was sometimes quite large (as large as 50 miles for some subjects). Thus, the potential for exposure measurement error due to geospatial heterogeneity in ambient NO<sub>2</sub> is especially high in this study. Schildcrout *et al.* (2006) reported small and borderline significant increases in asthma symptoms and rescue medication use in single-pollutant models (OR = 1.06, 95% CI: 1.00-1.12 and OR = 1.04, 95% CI: 1.00-1.08, respectively, for same day increases of 20 parts per million [ppm] in 24-h average NO<sub>2</sub>). However, in their discussion, the authors cautioned against interpreting these effects as attributable to NO<sub>2</sub>, noting that they are likely to be surrogates for other components of traffic emissions highly correlated with NO<sub>2</sub>. The ISA does not interpret the findings of Schildcrout *et al.* (2006) with the same level of caution as the authors did.

In addition to these four key studies identified in Table 5-45, the ISA presents the results of 17 other studies of symptoms and medication use in Table 5-12 and Figure 5-4 (reproduced as Figure 3.1 below), with associations between outcome and 24-h average, 8-h maximum, or 1-h maximum NO<sub>2</sub>, standardized as described above. As with the analogous summaries of HAs/ED visits, the ISA does not give a complete perspective on all the associations explored in the subgroup analyses of individual studies. For example, Mann *et al.* (2010) explored associations between reported wheezing and NO<sub>2</sub> at lag days 0 through 14, but the ISA presents only the OR for wheezing measured at lag 2, the lag with the strongest relationship. Likewise, the ISA presents four ORs for Segala *et al.* (1998) in Figure 5-4 and Table 5-14,

but does not mention that these are only 4 of 64 associations reported by the authors in the original publication, generated through numerous variations in lag time, outcome definitions, and study population subgroup. The four ORs selected in the ISA are the few positive, statistically significant associations out of all 64. As mentioned above, the ISA also displays the results of Gent *et al.* (2009) in Figure 5-4, but only for wheezing, the outcome associated with a small, nearly significant elevation of risk. Null results for asthma medication use reported by Gent *et al.* (2009) are not included in Figure 5-4. It is not clear why the OR for wheezing is included at all, given that the authors did not specify the averaging time for NO<sub>2</sub> used in statistical models, and, as a result, the risk estimate could not be standardized in the manner applied to all the other studies presented in Figure 5-4.



**Figure 3.1 Association between Ambient NO<sub>2</sub> Concentrations and Respiratory Symptoms and Medication Use in Children with Asthma.** Red = Recent studies; Black = Previous studies. Effect estimates are standardized to a 20-ppb, 25-ppb, and 30-ppb increase for 24-h average, 8-h maximum, and 1-h maximum NO<sub>2</sub>, respectively. Source: Figure 5-4, EPA (2015).

Overall, many of the associations presented in Figure 3.1 indicate an increase in symptoms and medication use following NO<sub>2</sub> exposure, and, in several cases, these increases are statistically significant. Setting aside that this small number of associations represents a skewed selection of available results, as

described above, many of the associations are small in magnitude, and the outcomes assessed are mild in severity, but the ISA does not discuss whether the measured effects are clinically significant. It is also noteworthy that, in several analyses, a small elevation in symptoms is reported, but relationships with rescue medication use are null (Gent *et al.*, 2009; Segala *et al.*, 1998; Schildcrout *et al.*, 2006). Individuals with asthma are taught to manage bothersome symptoms with quick-relief medication, both to achieve immediate relief from discomfort and to avoid a serious asthma exacerbation (Bateman *et al.*, 2008). It follows that a positive association with symptoms such as wheezing or coughing not accompanied by associations with medication use may indicate that the perceived symptoms are too mild to be bothersome or otherwise significant to a person with asthma. The ISA states that evidence for increased medication use following NO<sub>2</sub> exposure is mixed but does not evaluate coherence between associations with asthma symptom reports and medication use.

Finally, the largest associations displayed in Figure 3.1 reflect analyses confined to small subgroups within larger cohorts, likely measured during exploratory, secondary analyses (Delfino *et al.*, 2002; Mann *et al.*, 2010; Segala *et al.*, 1998). In addition, results of Delfino *et al.* (2002) and Segala *et al.* (1998) were calculated using generalized estimating equations (GEE), a statistical modeling method vulnerable to Type I errors (*i.e.*, false positives) when there is a small number of subjects (Hubbard *et al.*, 2010). The ISA should have critically assessed whether these large, imprecise associations with asthma symptoms measured in subgroup analyses constitute reliable evidence for short-term effects of NO<sub>2</sub> exposure, especially in light of the potential biases caused by applying inappropriate statistical models.

### **3.2.2 Evidence does not consistently support lung function decrements in people with asthma**

In Section 5.2.2.2, the ISA notes that epidemiology evidence available at the time of the previous review (US EPA, 2008) provided limited support for lung function decrements associated with NO<sub>2</sub> exposure. The ISA hypothesizes that the lack of consistent evidence available in 2008 is attributable to older epidemiology studies that commonly used unsupervised lung function measurements for assessing outcome. Unsupervised lung function tests are typically conducted by children at home using portable devices and are affected by a much larger degree of measurement error than supervised spirometric tests (Self *et al.*, 2014). According to the ISA, the substantial outcome measurement errors associated with home lung function testing may have obscured true relationships between exposure and outcome in studies available during the 2008 review.

The assessment of lung function studies presented in the current ISA, however, demonstrates that the results of studies with supervised spirometry measurements are just as inconsistent as those dependent on unsupervised measurements. For example, in Table 5-45, the ISA cites four key references in support of its conclusion that lung function decrements occur in asthmatic subjects exposed to NO<sub>2</sub> (Greenwald *et al.*, 2013; McCreanor *et al.*, 2007; Holguin *et al.*, 2007; Delfino *et al.*, 2008). The results of the three that conducted supervised spirometric measurements were mixed. In Greenwald *et al.* (2013), children with asthma performed spirometry tests coached by trained research staff, and the results were evaluated by clinical pulmonologists. No associations between indoor or outdoor NO<sub>2</sub> measured at the subjects' schools and forced expiratory volume in 1 second (FEV<sub>1</sub>) were observed.<sup>1</sup> Holguin *et al.* (2007) also conducted spirometry in a clinical setting and measured NO<sub>2</sub> at school sites. They reported a small and not statistically significant decrement in FEV<sub>1</sub> associated with outdoor NO<sub>2</sub> (-2.4%, 95% CI: -5.09 to 0.24 for a 20-ppb increase in NO<sub>2</sub>). McCreanor *et al.* (2007) studied lung function in a small cohort of asthmatic adults using a natural experiment study design. Marginally significant decrements in some lung

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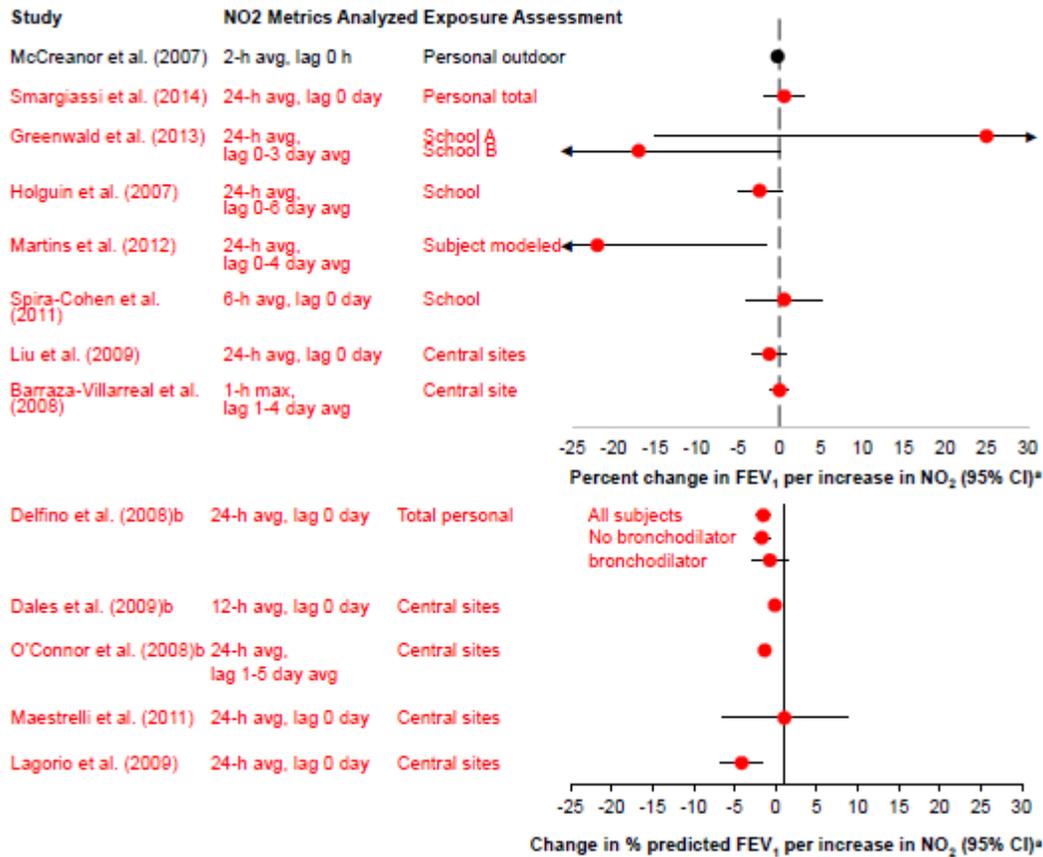
<sup>1</sup> The results presented in Greenwald *et al.* (2013) are different than those presented in Table 5-9 and Figure 5-4 of the ISA, which indicate borderline significant associations between NO<sub>2</sub> and FEV<sub>1</sub>.

function parameters, but not others, were observed in association with traffic-derived NO<sub>2</sub>, but the authors concluded that the observed lung function decrements were small and not accompanied by any clinically significant symptoms.

Notably, of the four key studies, the one in which lung function measurements were performed by children at home without supervision reported the largest lung function decrements. Delfino *et al.* (2008) reported decrements in the percentage of predicted FEV<sub>1</sub> associated with personal NO<sub>2</sub> exposure for 53 children with asthma (change in FEV<sub>1</sub>%: -1.68, 95% CI: -3.17 to -0.19 for 20-ppb increase in 24-h average NO<sub>2</sub>). However, the authors expressed caution in interpreting this as causally related to NO<sub>2</sub>. They explained that because personal NO<sub>2</sub> exposures were very low, it is more plausible that NO<sub>2</sub> is a marker for more toxic components of traffic emissions.

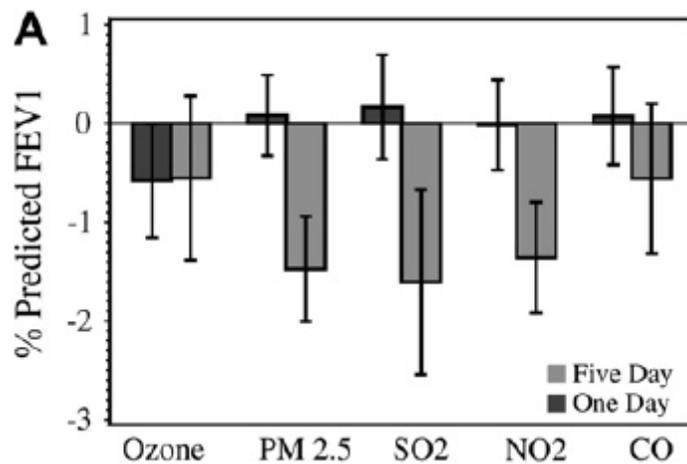
It is also informative to consider the relative quality of exposure assessment methods when evaluating the results of lung function panel studies. As shown in Table 5-1 of the ISA, studies that estimate personal NO<sub>2</sub> exposures using home- or school-based NO<sub>2</sub> measurements are much less vulnerable to the effects of exposure measurement error associated with using central site monitors to estimate NO<sub>2</sub> exposure. Figure 5-3 of the ISA, reproduced as Figure 3.2 below, shows no qualitative differences between the strength or precision of associations measured in studies of personal or school-based exposure assessment in comparison to studies involving central site monitoring.

Taken together, the presentation of results in the ISA's Table 5-9 and Figure 3.2 of this report does not provide a complete or balanced perspective on findings in the available literature. The quantitative results in Figure 3.2 are skewed towards positive findings because the figure gives no indication of the results of studies for which associations with lung function were not significant but were not presented quantitatively (Delfino *et al.*, 2003; Mortimer *et al.*, 2002; Just *et al.*, 2002; Odajima *et al.*, 2008; Canova *et al.*, 2010). Similarly, several studies that assessed peak expiratory flow rate (PEF), rather than FEV<sub>1</sub>, reported null findings (Hiltermann *et al.*, 2008, Park *et al.*, 2005; Qian *et al.*, 2009a; Wiwatanadate and Trakultivakorn, 2010), but these findings are not presented in Figure 3.5. The exclusion of a substantial amount of null findings from the main graphical display of lung function research is misleading.



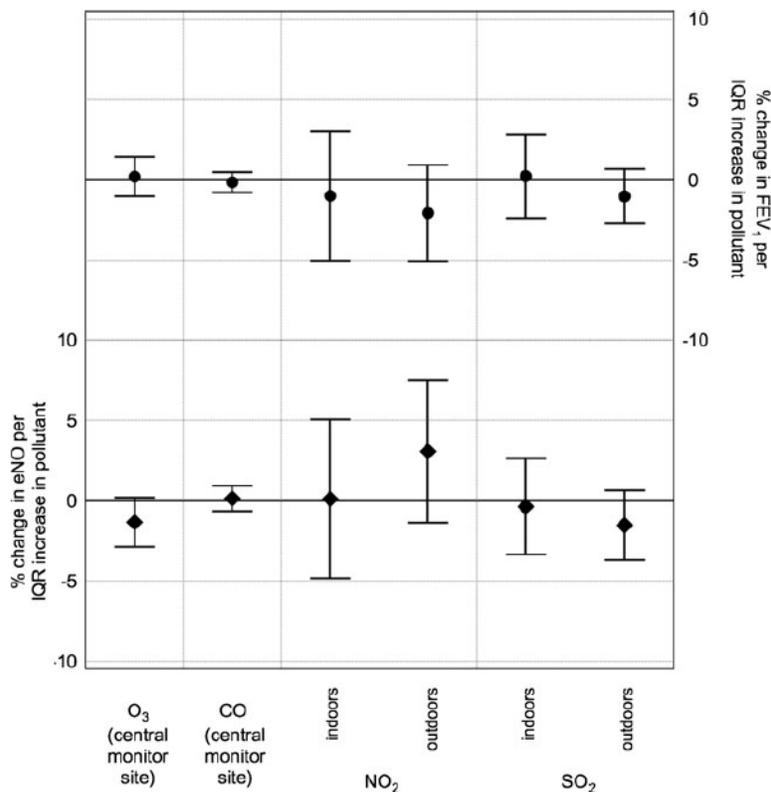
**Figure 3.2 Association between Ambient NO<sub>2</sub> Concentrations or Personal NO<sub>2</sub> Exposure with Changes in FEV<sub>1</sub> in Children and Adults with Asthma.** Red = Recent studies; Black = Previous studies. Effect estimates standardized to a 20-ppb increase in 24-h average NO<sub>2</sub> and a 30-ppb increase in 1-h maximum NO<sub>2</sub>. Source: Figure 5-3, EPA (2015).

A final issue with the ISA's presentation of lung function results in Table 5-9 (shown in Figure 3.2 of this report) is that only a single lag relationship's results from each investigation are shown. Similarly, in papers that reported a number of results based on variations in study population (*e.g.*, based on the full cohort as well as subgroups defined by subject characteristics), the ISA sometimes selects a limited number of associations to highlight, while disregarding others. In these cases, the ISA does not indicate how one lag or subgroup from a study was chosen over the others for presentation. For example, one of the largest and most precise lung function decrements displayed in Figure 3.2 comes from O'Connor *et al.* (2008). Even though, a significant FEV<sub>1</sub> decrement was observed in analyses of lag 1-5 exposures, as EPA presents in Figure 3.2, the authors also reported that associations with lag 1 were completely null (Figure 3.3).



**Figure 3.3 Changes in FEV<sub>1</sub> Associated with a 10<sup>th</sup> to 90<sup>th</sup> Percentile Pollutant Increment in Pollutant in Single-pollutant Models of 937 Children with Asthma.** Error bars represent 95% CIs. Source: Figure 2, O'Connor *et al.* (2008).

EPA's presentation of results from Greenwald *et al.* (2013) is also misleading. In Figure 3.2, EPA presents associations between school-based NO<sub>2</sub> estimates and FEV<sub>1</sub> for two schools separately, giving the appearance that decrements in FEV<sub>1</sub> for students at School B nearly achieve statistical significance. However, in the main text of the original article, these school-specific effect estimates are not presented or discussed. Instead, Greenwald *et al.* (2013) present results demonstrating null associations between FEV<sub>1</sub> and NO<sub>2</sub> measured both indoors and outdoors at subjects' schools (see the reproduced plot below, Figure 3.4). By only presenting the associations specific to each school, rather than the main findings reported by the authors in the main text of their publication, EPA gives a misleading perspective on the main findings of Greenwald *et al.* (2013).



**Figure 3.4 Percent Change in FEV<sub>1</sub> and Exhaled Nitric Oxide per Interquartile Range Increase in 96-h Concentrations of NO<sub>2</sub> and Other Pollutants among 38 Children with Asthma.** Source: Greenwald *et al.* (2013).

### 3.2.3 Evidence for exposure metrics with lower potential for exposure measurement error is not consistent

In Table 5-45, EPA states that the causal determination for short-term respiratory effects is supported by "consistent evidence for NO<sub>2</sub> metrics with lower potential for exposure measurement error" (US EPA, 2015). In support of this statement, the table cites seven panel studies (Greenwald *et al.*, 2013; Holguin *et al.*, 2007; Delfino *et al.*, 2006, 2008; McCreanor *et al.*, 2007; Sarnat *et al.*, 2012; Zora *et al.*, 2013) employing exposure assessment methodologies with better spatial alignment than the use of central site monitors, including the measurement of total and outdoor personal NO<sub>2</sub> exposure and school-based NO<sub>2</sub> exposure.

However, as discussed above, the main findings of Zora *et al.* (2013), Holguin *et al.* (2007), and Greenwald *et al.* (2013) are consistent with null associations between NO<sub>2</sub> and respiratory symptoms, medication use, and lung function. While Delfino *et al.* (2008) reported associations with lung function decrements, they found that the magnitude and precision of these associations were sensitive to whether or not subjects used asthma medication. In their overall conclusions, the authors expressed doubt that NO<sub>2</sub> was the causal agent because personal NO<sub>2</sub> concentrations were very low.

Sarnat *et al.* (2012) conducted a panel study of 58 asthmatic children in Mexico and Texas and evaluated whether short-term air pollution exposure was associated with respiratory symptoms or exhaled nitric oxide (eNO), a biomarker of airway inflammation. Compared to other panel studies discussed in the ISA, Sarnat *et al.* (2012) stands out because of its rigorous assessment of model misspecification, which included sensitivity analyses to determine whether associations were robust to pollutant averaging times, methods for calculating exposure based on multiple monitoring sites, and methods for modeling covariates. Sarnat *et al.* (2012) found that short-term NO<sub>2</sub> was associated with increases in eNO, but that these results were highly sensitive to variations in model specification. For example, when NO<sub>2</sub> exposure was modeled using different lag times prior to eNO assessment (*i.e.*, lag 1, lag 0-1, and lag 0-2 *versus* lag 0-3, as used in the core analysis), the authors found that associations between eNO and NO<sub>2</sub> changed direction. Only the 4-day lag time resulted in evidence that NO<sub>2</sub> exposure was associated with increased pulmonary inflammation; in contrast, associations with 3-day lags indicated a statistically significant decrease in pulmonary inflammation. The authors did not provide an explanation for this discrepancy. It does not seem biologically plausible that a small change in the averaging time of the air pollution metric would determine whether NO<sub>2</sub> exposure suppresses or stimulates airway inflammation.

Contrary to EPA's assertion that there is "consistent evidence" of short-term respiratory effects in studies involving personal NO<sub>2</sub> exposure, there appears to be very little support for this conclusion, even with a restricted focus on the seven studies highlighted in Table 5-45. It is notable that some panel studies of personal or school-based NO<sub>2</sub> exposure not mentioned in Table 5-45 also indicated null associations (*e.g.*, Smargiassi *et al.*, 2014; Spira-Cohen *et al.*, 2011).

### **3.2.4 Evidence from controlled human exposure studies indicates associations between NO<sub>2</sub> exposures less than 300 ppb and AHR are questionable**

The ISA indicates that observations of NO<sub>2</sub>-associated airway hyper-responsiveness (AHR) are consistent with asthma exacerbation observed in epidemiology studies, which thus "rules out chance, confounding, and other biases [in the epidemiology studies] with reasonable confidence" (US EPA, 2015). As in the first draft ISA, the second draft discusses the potential importance of AHR in terms of exposure to specific allergens; it speculates that the lack of an NO<sub>2</sub> effect on AHR in studies involving exercise is due to a refractory period<sup>2</sup> following exercise and that studies with multiple exposure concentrations provide evidence of concentration-response. The ISA further speculates that the lack of an NO<sub>2</sub> effect in studies involving exposures while subjects are exercising and studies using specific airway challenges can be attributed to a greater percentage of these studies using forced vital capacity (FVC) maneuvers<sup>3</sup> to evaluate AHR. In addition, the ISA highlights the importance of AHR based on observations of clinically relevant reductions in the provocative dose (PD) of an airway challenge.

On closer inspection, the evidence does not indicate that NO<sub>2</sub> increases AHR for specific allergens or that the paradoxical lack of an effect in studies involving exercise is due to a refractory period, nor does the evidence indicate that studies that use FVC maneuvers to evaluate AHR are less likely to observe an NO<sub>2</sub> effect. Moreover, the studies with multiple exposure concentrations do not provide clear, consistent evidence of a monotonic concentration-response relationship. Finally, the clinically relevant PD reductions reported in the ISA were 1) observed in studies involving exposure to airway challenges (*e.g.*, methacholine, carbachol, histamine, and relatively high concentrations of sulfur dioxide [SO<sub>2</sub>]) that would likely be experienced only in experimental settings, and 2) did not exhibit a concentration-response. Overall, based on the WoE, the association between NO<sub>2</sub> and AHR at NO<sub>2</sub> concentrations below 300 ppb remains questionable.

<sup>2</sup> During a refractory period, there is a diminished response to airway challenges.

<sup>3</sup> FVC and FEV<sub>1</sub> maneuvers are measured simultaneously using a spirometer, which records both total volume (FVC) and the volume exhaled in the first second of a forced exhalation (FEV<sub>1</sub>).

### 3.2.4.1 Studies using specific allergens do not report statistically significant effects

The ISA notes that, despite the lack of statistical significance in studies with specific allergens, inhalation of very low antigen concentrations may cause severe bronchoconstriction in some individuals (US EPA, 2015). The ISA further speculates that the lack of statistical significance in studies using specific allergens may be attributed to the use of FVC maneuvers in most of the studies evaluating specific allergens. To support this, the ISA cites studies that indicate that the use of repeated FVC maneuvers might cause a partial reversal of bronchospasm (*e.g.*, Jackson *et al.*, 2004; Beaupré and Orehek, 1982; Orehek *et al.*, 1981, all as cited in US EPA, 2015). As discussed in Gradient's comments on the first draft ISA (Gradient, 2014), the issue is not whether allergen exposure causes bronchoconstriction, but rather whether NO<sub>2</sub> exacerbates the effects of allergen exposure. Even if NO<sub>2</sub> exacerbates allergen-induced bronchoconstriction, the studies cited in the ISA indicate that this effect does not occur below 260 ppb NO<sub>2</sub>. Furthermore, the ISA's hypothesis that the use of FVC maneuvers accounts for the lack of statistical significance of studies evaluating specific allergens is not supported by the results of these studies. As shown in Table 3.2, studies using FVC maneuvers were more likely to observe increased bronchoconstriction with NO<sub>2</sub> *vs.* with air than studies using airway conductance or resistance.

**Table 3.2 Effect of NO<sub>2</sub> on Bronchoconstriction in Studies Evaluating Specific Allergens**

Assessment of Bronchoconstriction	Increased (p < 0.05)	Increased (n.s.)	Decreased (n.s.)
FEV <sub>1</sub>	3/7	2/7	2/7
sRAW or sGAW	0	0	2/2

Notes:

FEV<sub>1</sub> = Forced Expiratory Volume in 1 Second; n.s. = Not Significant; NO<sub>2</sub> = Nitrogen Dioxide; sGAW = Specific Airway Conductance; sRAW = Specific Airway Resistance.

Overall, the evidence regarding whether NO<sub>2</sub> exacerbates allergen-induced bronchoconstriction is, at best, inconclusive.

### 3.2.4.2 Studies involving exercise do not report statistically significant effects

The ISA indicates that the paradoxical lack of a significant effect on AHR in studies in which participants were exposed to NO<sub>2</sub> while exercising (*vs.* at rest) may be attributable to "the development of a refractory period following bouts of exercise" (US EPA, 2015), during which time the response to an airway challenge is diminished. As with studies involving exposure to specific airway challenges, the ISA further concludes that the lack of a significant AHR effect in studies involving exercise may be attributed to use of FVC maneuvers to evaluate AHR in most of these studies. However, as discussed in Gradient's prior comments, and summarized in the ISA, there is little evidence of such an effect either in the NO<sub>2</sub> AHR studies or other studies that evaluated AHR using comparable study designs. As with studies involving exposure to specific airway challenges, the ISA's conclusion that use of FVC maneuvers accounts for the lack of statistical significance for studies involving exercise is not supported by results from these studies.

**Table 3.3 Summary of Evidence Regarding NO<sub>2</sub> Effects on AHR in Studies Involving Exercise**

ISA Conclusion (Re: Lack of Statistical Significance in Studies Involving Exercise)	Response
Effect on AHR following 30-minute exposure to 250 ppb NO <sub>2</sub> at rest in Jorres and Magnussen (1990) but not following 30-minute exposure to 300 ppb NO <sub>2</sub> while at rest in Rubinstein <i>et al.</i> (1990) supports existence of a refractory period following exercise.	<ul style="list-style-type: none"> <li>• Citing these two NO<sub>2</sub> studies is circular reasoning.</li> <li>• Critical study differences other than exercise, such as use of a mouthpiece vs. exposure chamber, could account for observation of an NO<sub>2</sub> effect on AHR in Jorres and Magnussen (1990) but not Rubinstein <i>et al.</i> (1990).</li> </ul>
Studies by Inman <i>et al.</i> (1990) and Freedman <i>et al.</i> (1988) further support existence of a refractory period following exercise.	<ul style="list-style-type: none"> <li>• Study designs used by Inman <i>et al.</i> (1990) and Freedman <i>et al.</i> (1988) are not analogous to study designs used in the NO<sub>2</sub> AHR studies.</li> <li>• In the NO<sub>2</sub> AHR studies, bronchial challenge agent was administered following a period of exercise</li> <li>• Inman <i>et al.</i> administered challenge agent while participants were exercising; Freedman <i>et al.</i> administered challenge agent prior to exercise.</li> <li>• Studies in which bronchial challenge agent was administered after a period of rest vs. exercise (as in the NO<sub>2</sub> AHR studies) do not support existence of a refractory period following exercise.</li> </ul>
Lack of statistically significant effects in the NO <sub>2</sub> AHR studies involving exercise is due to a refractory period following exercise.	<ul style="list-style-type: none"> <li>• Lack of statistically significant effects in NO<sub>2</sub> AHR studies cannot be explained by refractory period.               <ul style="list-style-type: none"> <li>– Intensity and frequency of exercise in the NO<sub>2</sub> AHR studies may not have been sufficient to induce a refractory period.</li> <li>– Not all individuals experience a refractory period following exercise.</li> <li>– Even if there is a refractory period following exercise, AHR may be diminished but not necessarily abolished, and would occur following exposure to both filtered air and NO<sub>2</sub>.</li> </ul> </li> </ul>
Use of FVC maneuvers also contributes to lack of statistically significant effect in studies involving exercise.	<ul style="list-style-type: none"> <li>• Results from NO<sub>2</sub> controlled human exposure studies do not indicate that NO<sub>2</sub> enhancement of the AHR response is less likely to occur in studies using FVC maneuvers.</li> </ul>

Notes:

AHR = Airway Hyper-responsiveness; FVC = Forced Vital Capacity; ISA = Integrated Science Assessment; NO<sub>2</sub> = Nitrogen Dioxide; ppb = Parts Per Billion.

As evidence of an exercise-induced refractory period, the draft ISA notes that, while Jorres and Magnussen (1990) found statistically significant increases in AHR to SO<sub>2</sub> following 30-minute exposure to 250 ppb NO<sub>2</sub> at rest, Rubinstein *et al.* (1990) found no change in responsiveness to SO<sub>2</sub> inhalation following 30-minute exposures to 300 ppb NO<sub>2</sub>, including 20 minutes of exercise. Relying on two different NO<sub>2</sub> studies that conformed to the paradoxical pattern of showing effects when study participants were exposed at rest but not while exercising is circular reasoning and does not constitute evidence of a refractory period. Scientifically, either a single study in which the same subjects were exposed using the same protocol while at rest vs. exercising or multiple studies all using comparable exposure protocols would be required to support the existence of an exercise-induced refractory period. As in the first draft ISA, the second draft does not consider that there may be critical study differences other than exercise that could explain why Jorres and Magnussen (1990), but not Rubinstein *et al.* (1990),

observed an effect of NO<sub>2</sub> on AHR. For example, Jorres and Magnussen (1990) exposed study participants *via* mouthpiece, while Rubinstein *et al.* (1990) used an exposure chamber. As discussed by Goodman *et al.* (2009), airway effects may be enhanced by breathing *via* mouthpiece relative to normal oro-nasal breathing, due to the increased amount of pollutant delivered to the airways and alterations in pollutant distribution and deposition in the airways. Hence, the significant effect observed by Jorres and Magnussen (1990) could be related to the exposure method rather than exercise.

As further evidence of an exercise-induced refractory period, the draft ISA refers to studies by Inman *et al.* (1990) and Freedman *et al.* (1988). Inman *et al.* (1990) found that the protection afforded by exercise (in terms of reduced bronchoconstriction) increases with the level of exercise, as indicated by a concomitant increase in methacholine PC<sub>20</sub>.<sup>4</sup> Freedman *et al.* (1988) found that methacholine-induced bronchoconstriction was reversed more rapidly following periods of exercise or hyperventilation than following periods of rest. The studies by Inman *et al.* (1990) and Freedman *et al.* (1988), however, are not analogous to the NO<sub>2</sub> AHR studies, in which the bronchial challenge agent was administered following a period of exercise. In the Inman *et al.* (1990) study, methacholine was administered while study participants were exercising; in Freedman *et al.* (1988), methacholine was administered prior to exercise. Hence, results from these two studies do not necessarily apply to the NO<sub>2</sub> AHR studies.

In contrast to the studies by Inman *et al.* (1990) and Freedman *et al.* (1988), Boulet *et al.* (1987), Hahn *et al.* (1984), and Magnussen *et al.* (1986) used study designs in which the bronchial challenge agent was administered after a period of rest or exercise. Neither Boulet *et al.* (1987) or Hahn *et al.* (1984) observed an effect of exercise on subsequent AHR. As discussed in the ISA, Magnussen *et al.* (1986) found that a diminished airway response to exercise correlated with a diminished response to a methacholine challenge. However, the methacholine PD was comparable before and after exercise.

Contrary to EPA's hypothesis regarding an exercise-induced refractory period, results from several NO<sub>2</sub> AHR studies do not provide evidence of a refractory period for AHR following exercise. The ISA notes that, in studies by Jorres and Magnussen (1991) and Strand *et al.* (1996), there was a "slight tendency for the PD<sub>100</sub> to be lower following the filtered air exposures relative to control (no exposure, no exercise), with roughly 53% of the individuals having a lower PD<sub>100</sub> following filtered air (with exercise)," concluding, "these two studies do not support an effect of exercise on AHR in studies evaluating effects of NO<sub>2</sub> exposure" (US EPA, 2015). Similarly, in the study by Roger *et al.* (1990), the concentration of methacholine required to induce a doubling in specific airway resistance was greater when tested for inclusion in the study (presumably without exercise) than following exercise (mean ± standard error [SE]: 5 ± 1.0 vs. 3.3 ± 0.7).

In addition, the intensity and frequency of exercise in many of the NO<sub>2</sub> AHR studies may not have been sufficient to induce a refractory period, the occurrence of which is positively related to both the intensity and frequency of exercise (*e.g.*, Constantinou and Derman, 2004; Tan and Spector, 1998; Weiler-Ravell and Godfrey, 1981). For example, in the study by Bauer *et al.* (1986), participants exercised on a bicycle ergometer for only 10 minutes at a workload of 300 kilopound meters per minute (kpm/min), which is approximately equivalent to walking 3 miles per hour (mph) at a 5% grade (Martin, 1999). In comparison, a workload of 540 kpm/min is considered to be a low-intensity workload (Marra *et al.*, 2005). By this metric, exercise intensity in the study by Rubinstein *et al.* (1990), which was approximately 480 kpm/min, would also have been considered to be low-intensity.<sup>5</sup> Study participants in the study by Jorres and Magnussen (1991) exercised at a workload designed to achieve a ventilation rate of approximately 30 L/min. According to Martin (1999), a ventilation rate of 30 L/min would be observed in a well-trained jogger after walking on a treadmill for 5 minutes at 2.5 mph. In the study by

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<sup>4</sup> PC<sub>20</sub> is the concentration that causes a 20% decrease in FEV<sub>1</sub>. "PC" stands for provocative concentration.

<sup>5</sup> Study participants exercised at a workload of 60-80 watts, with 1 watt = 6.12 kpm/min. (ExRx.net, 2013).

Kleinman *et al.* (1983), the exercise intensity was aimed to achieve a ventilation rate twice that of resting ventilation, which is approximately 6 L/min in a well-trained jogger (Martin, 1999).

Importantly, not all individuals experience a refractory period following exercise (*e.g.*, Weiler-Ravell and Godfrey, 1981). For those individuals who do experience a refractory period, the refractory effect does not necessarily abolish the AHR effect but, rather, increases the threshold for an effect (*e.g.*, Inman *et al.*, 1990). This means that any effect of exercise would apply to both NO<sub>2</sub> and clean air exposures. If there is an allergen response following inhalation of filtered air, then one should still observe a more pronounced effect following NO<sub>2</sub> exposure if NO<sub>2</sub> were associated with AHR. In fact, AHR was observed in the five NO<sub>2</sub> studies that provided sufficient information to evaluate the extent of airway responsiveness following NO<sub>2</sub> vs. filtered air exposure (Avol *et al.*, 1988, 1989; HEI, 1989; Roger *et al.*, 1990; Witten *et al.*, 2005). Among the studies in which AHR was observed following exercise, only the study by Avol *et al.* (1989) observed a difference in AHR following exposure to NO<sub>2</sub> vs. air.

Finally, as with studies involving exposure to specific allergens, the evidence does not support EPA's conclusion that the lack of statistical significance for NO<sub>2</sub>-induced AHR in studies involving exercise can be attributed to use of FVC maneuvers in these studies. As shown in Table 3.4, below, studies that used FVC maneuvers (*i.e.*, those that used FEV<sub>1</sub> to assess bronchoconstriction) were actually more likely to observe NO<sub>2</sub>-associated AHR.

**Table 3.4 Effect of NO<sub>2</sub> on Bronchoconstriction in Studies Involving Exercise**

Assessment of Bronchoconstriction	Increased (p < 0.05)	Increased (n.s.)	Decreased (n.s.)
FEV <sub>1</sub>	4/11	7/11	0
sRAW or sGAW	0	2/6	4/6

Notes:

FEV<sub>1</sub> = Forced Expiratory Volume in 1 Second; n.s. = Not Significant; NO<sub>2</sub> = Nitrogen Dioxide; sGAW = Specific Airway Conductance; sRAW = Specific Airway Resistance.

### 3.2.4.3 The lack of a concentration-response indicates NO<sub>2</sub> does not cause AHR at concentrations less than 600 ppb

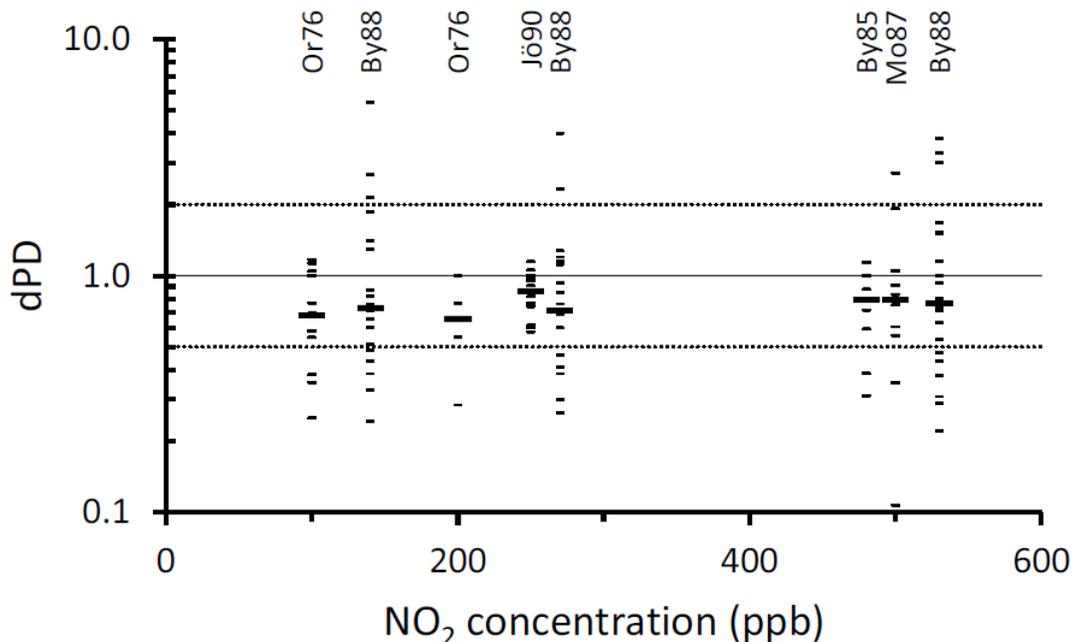
The ISA notes that studies evaluating multiple exposure concentrations, specifically those using resting exposure (*e.g.*, Bylin *et al.*, 1988; Orehek *et al.*, 1976; Tunnicliffe *et al.*, 1994), provide evidence of an NO<sub>2</sub> concentration-response for AHR. Overall, these studies provide limited, if any, evidence of a concentration-response. The ISA notes that Bylin *et al.* (1988) observed statistically significant effects on AHR at 270 ppb but not 140 ppb. However, the response at 530 ppb, as reflected by either fraction affected or the PD, was lower than the response at either 140 or 270 ppb (Bylin *et al.*, 1988). Hence, over the full range of study concentrations, Bylin *et al.* (1988) did not observe an increase in AHR with increasing NO<sub>2</sub> concentrations. As discussed in the ISA, the PD<sub>100</sub> was similar at both 100 and 200 ppb NO<sub>2</sub> for three of four individuals in the study by Orehek *et al.* (1976) (for which there was responsiveness data) and was doubled for the fourth individual. Moreover, the fraction affected at these two exposure concentrations was comparable (0.7; 95% CI: 0.46, 0.88 at 100 ppb NO<sub>2</sub> vs. 0.75; 95% CI: 0.19, 0.81).<sup>6</sup> Based on the limited data from Orehek *et al.* (1976), there does not appear to be a clear concentration-response for NO<sub>2</sub>. In the study by Tunnicliffe *et al.* (1994), although the fraction affected following NO<sub>2</sub> exposure was greater at 400 ppb (0.75; 95% 0.35-0.97) than 100 ppb (0.63; 95% CI: 0.25-0.92), the decrease in FEV<sub>1</sub> following an airway challenge was comparable at the two exposure concentrations

<sup>6</sup> 95% CIs are as reported by Goodman *et al.* (2009).

(-4.90%; 95% CI: -9.62 to -0.18 at 100 ppb vs. -5.29; 95% CI: -9.09 to -1.49). Hence, this study does not provide evidence of a concentration-response. Consistent with findings from the studies highlighted in the ISA, Goodman *et al.* (2009) concluded there was no evidence of an NO<sub>2</sub> concentration-response at concentrations less than 600 ppb in a meta-analysis evaluating AHR in controlled human exposure studies.

### 3.2.4.4 Reductions in airway provocative dose do not support a causal association between NO<sub>2</sub> and increased AHR

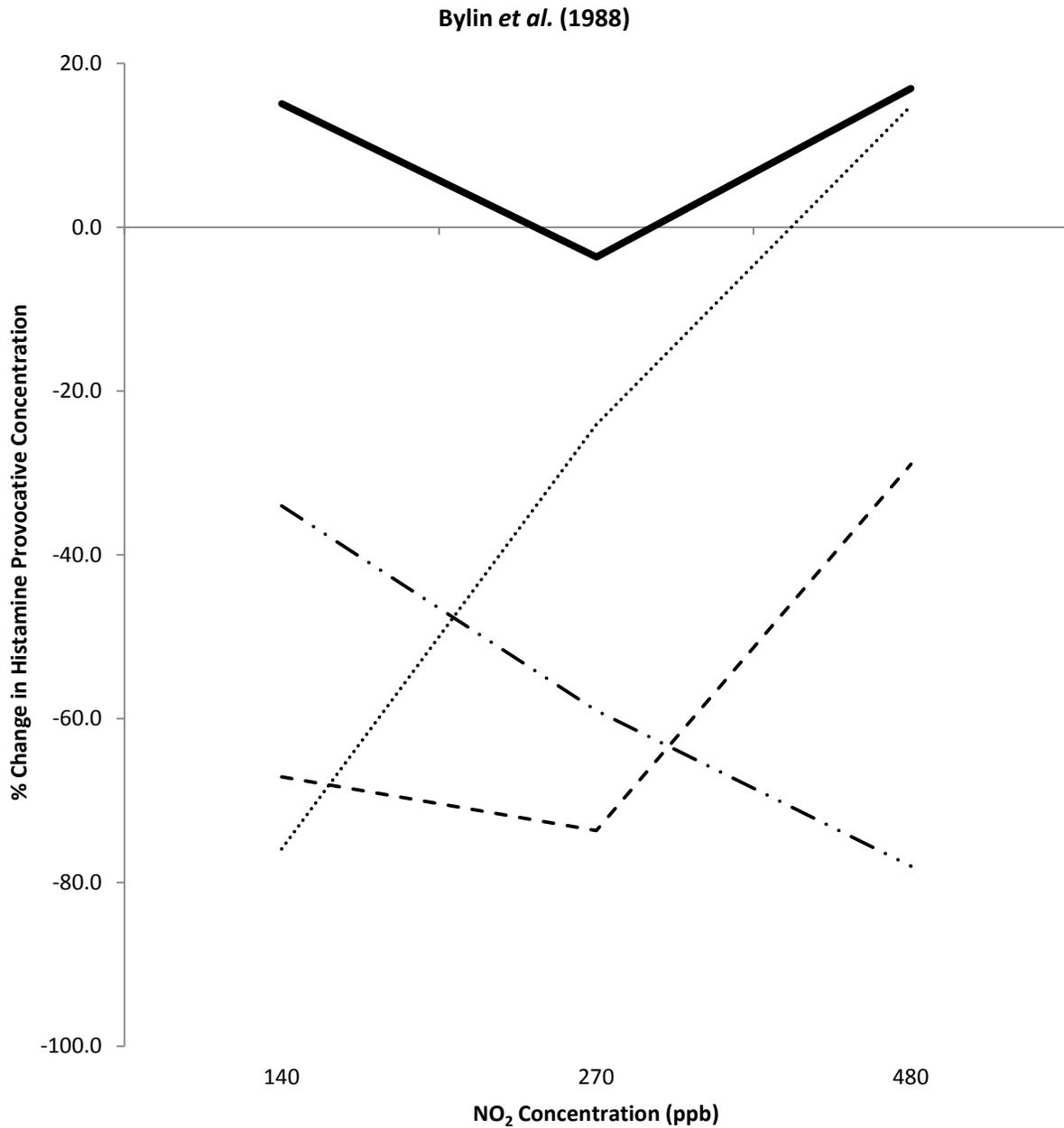
As further evidence that observations of AHR in controlled human exposure studies support a role for NO<sub>2</sub> in asthma exacerbation, the ISA highlights clinically relevant (*i.e.*, greater than 50%) reductions in the PD of airway challenges observed in several studies in Figure 5-1, reproduced below in Figure 3.5.



**Figure 3.5 Provocative Dose as a Function of NO<sub>2</sub> Exposure Concentration in Controlled Human Exposure Studies.** Small dashes are responses of 72 individuals from Orehek *et al.* (1976), Bylin *et al.* (1985, 1988), Jorres and Magnussen (1990), and Mohsenin (1987). Bars are median responses. Dotted lines indicate a doubling of dose change (*i.e.*, dPD = +2 or -0.5). Source: Figure 5-1, EPA (2015).

The studies included in Figure 3.5 used airway challenges that would likely not be encountered outside of an experimental setting, including carbachol (Orehek *et al.*, 1976), histamine (Bylin *et al.*, 1988), methacholine (Mohsenin, 1987), and 750 ppb SO<sub>2</sub> (Jorres and Magnussen, 1990). It is uncertain whether similar PD reductions would be observed outside of an experimental setting. Moreover, the PD reduction did not decrease with increasing NO<sub>2</sub> concentration, as would be expected if it were caused by NO<sub>2</sub> exposure. For example, Figure 3.6 illustrates the lack of a concentration-response for three study participants from Bylin *et al.* (1988) who experienced the greatest reduction in the histamine PD at one of the three NO<sub>2</sub> exposure concentrations. As shown in this figure, only one of the three study participants experienced a reduction in histamine PD with increasing NO<sub>2</sub> concentration. For the other two study

participants, the histamine PD increased with increasing NO<sub>2</sub> concentration, which does not support a causal association between NO<sub>2</sub> and increased AHR.



**Figure 3.6 Response to Histamine Airway Challenge after NO<sub>2</sub> Exposure in Bylin *et al.* (1988).** Solid line represents average response for all study participants. Dashed lines represent response for participants experiencing greatest reduction in provocative histamine concentration at 140, 270, and 480 ppb NO<sub>2</sub>.

### 3.3 Several lines of evidence indicate chance, confounding, and other biases cannot be ruled out

Table 5-45 of the ISA summarizes six lines of key evidence to support the claim that "epidemiologic evidence helps rule out chance, confounding, and other biases with reasonable confidence" (US EPA, 2015). A careful review of the evidence regarding asthma exacerbation indicates that, in fact, it is likely affected by chance, confounding, and other biases.

#### 3.3.1 Multi-pollutant modeling cannot rule out confounding with reasonable confidence

Of the six lines of evidence, four of them pertain to potential confounding by co-pollutants. Specifically, the ISA asserts that: 1) associations between personal NO<sub>2</sub> exposure and lung function and pulmonary inflammation persist in co-pollutant models with traffic-related co-pollutants; 2) most central site NO<sub>2</sub> associations are also robust to adjustment for traffic-related co-pollutants; 3) some associations were attenuated with adjustment for PM<sub>2.5</sub> or ultrafine particles (UFP); and 4) most associations for microenvironmental and central site NO<sub>2</sub> persist in co-pollutant models with PM<sub>10</sub>, SO<sub>2</sub>, or ozone (O<sub>3</sub>).

While it is true that many associations remain relatively unaffected after adjustment for co-pollutants, multi-pollutant models do not provide "reasonable confidence" that NO<sub>2</sub> associations are not confounded by them. Theoretically, effect estimates derived from multi-pollutant models represent independent associations between different components of air pollution and health outcomes. However, it is difficult to interpret coefficients for each pollutant when co-pollutants are correlated or when there is substantial measurement error. If two pollutants are correlated, the two covariates will "compete" for effect size in multivariate regression, and the regression coefficients of both will be unstable. In the case of differential exposure measurement error, the pollutant with the largest coefficient in regression results may be the one with the least exposure measurement error, not necessarily the one that is the most toxic (Tolbert *et al.*, 2007).

Several researchers have described the methodological issues with multi-pollutant models and have called for new approaches in analyzing complicated mixtures of pollutants, especially in consideration of environmental policymaking. Possibilities include shifting from a focus on individual pollutants to a consideration of air pollution sources or identifying chemical "fingerprints" of toxic air pollution mixtures (Vedal and Kaufman, 2011). However, these methods are still being developed and are not yet being used by most air pollution researchers.

Given the substantial limitations of multi-pollutant regression models, they cannot rule out confounding with reasonable confidence.

#### 3.3.2 Studies of indoor NO<sub>2</sub> exposure do not support a casual association

In Table 5-45, EPA states that studies of indoor NO<sub>2</sub> exposure constitute the fifth line of evidence to help rule out chance, confounding and other biases. EPA provides three key references in support of this assertion: Sarnat *et al.* (2012), Lu *et al.* (2013), and Hansel *et al.* (2008). In this summary table, EPA also cites Greenwald *et al.* (2013) as a study of indoor exposures demonstrating no associations.

It is unclear why EPA highlights Sarnat *et al.* (2012), Lu *et al.* (2013), and Hansel *et al.* (2008) as studies that support the existence of associations between indoor NO<sub>2</sub> exposure and respiratory effects, because many of the findings reported in these three studies are consistent with a null relationship. As discussed

above, Sarnat *et al.* (2012) found no associations between NO<sub>2</sub> and any respiratory associations. They reported some evidence that NO<sub>2</sub> is associated with eNO, a biomarker of pulmonary inflammation, but their results were sensitive to model specification and, therefore, should be interpreted cautiously.

Hansel *et al.* (2008) conducted a panel study with 150 asthmatic children and reported positive, statistically significant associations between NO<sub>2</sub> exposure and some respiratory symptoms, including coughing, nocturnal waking, and limited speech. However, their results indicated no association with more serious outcomes that more likely reflect clinically significant effects, including healthcare utilization, asthma medication use, unscheduled doctors' visits, and ED visits for asthma. Taken together, the results of Hansel *et al.* (2008) could be interpreted as evidence that NO<sub>2</sub> exposure is associated with relatively mild respiratory effects, but they provide no evidence that increases in symptoms lead to serious or clinically significant outcomes.

Lu *et al.* (2013) also reported some increased, statistically significant associations between reported symptoms and indoor NO<sub>2</sub> exposure in a panel study of 148 children with asthma. However, these positive associations were reported in subgroup analyses only, and the article alludes to a large number of associations explored for various outcomes that were null but not presented explicitly. It is difficult to interpret the meaning of the small number of positive findings in subgroup analyses in light of the large, but unspecified, number of null associations measured by the investigators in the same study.

Overall, EPA's summary of evidence regarding indoor NO<sub>2</sub> exposures presented in Table 5-45 is highly misleading. The table implies that null associations with indoor NO<sub>2</sub> are infrequent, citing Greenwald *et al.* (2013) as the sole source of evidence consistent with a null relationship. However, as described above, most of results from Sarnat *et al.* (2012), Lu *et al.* (2013), and Hansel *et al.* (2008) are not consistent with a causal association either.

### **3.3.3 Associations do not always persist with adjustment for potential confounders**

In Table 5-45, EPA states that the sixth line of evidence that helps rule out chance, confounding, and other biases is that "NO<sub>2</sub> associations persist with adjustment for meteorology, time trends, season, and medication use" (US EPA, 2015). The strength of this conclusion is undermined, however, by the striking between-study heterogeneity in adjustment variables included in analyses of HAs/ED visits, lung function, and asthma symptoms. While many studies adjusted for one or more of the variables listed by EPA (*i.e.*, meteorology, time trends, season, and medication use), many did not adjust for all of the variables, and there was little consistency in the precise adjustment methods.

Because of this inconsistency, it is not appropriate to make an over-arching conclusion that associations persist with adjustment for these factors. Furthermore, few investigators conducted thorough sensitivity analyses to determine whether associations were sensitive to model specification. Two studies that did present results of thorough sensitivity analyses found that associations with NO<sub>2</sub> changed substantially with variations in model specification (Son *et al.*, 2013 ; Sarnat *et al.*, 2012). This indicates that it is important not only to adjust for potential confounders but also to determine whether the precise functional form of the covariate is affecting measured associations. EPA fails to critically evaluate the methods and findings of short-term studies of asthma exacerbation in this light.

### **3.3.4 Other important biases not considered by EPA impact the interpretation of results**

There are other biases that are not discussed in Table 5-45 or elsewhere in the ISA that likely affect the interpretation of results of NO<sub>2</sub> epidemiology studies. Collectively, these indicate that the epidemiology

evidence may not "[help] rule out chance, confounding, and other biases with reasonable confidence" (US EPA, 2015).

First, the ISA does not systematically assess whether short-term studies evaluated potential confounding by upper respiratory infections and aeroallergens, such as pollen. Both these factors are strongly related to respiratory health outcomes, especially for individuals with asthma, and both follow seasonal trends that could be consistent with temporal fluctuations in air pollution. Strickland *et al.* (2010) is one of very few studies of asthma-related HAs and ED visits that thoroughly assessed potential confounding by upper respiratory infections, and, based on the results of models with and without adjustment for this factor, they concluded that associations could be confounded by URI epidemics.

Based on the study characteristics for 21 studies of respiratory symptoms and medication use summarized in Table 5-12, only 2 studies directly adjusted for cold, flu, or other respiratory infections in analysis (Sarnat *et al.*, 2012; Delfino *et al.*, 2002), and only 2 adjusted for direct measurements of pollen or other aeroallergens (Jalaludin *et al.*, 2004; Just *et al.*, 2002). Likewise, of the 27 studies of lung function summarized in Table 5-9, 2 directly accounted for occurrences of respiratory infection (Qian *et al.*, 2009b; Delfino *et al.*, 2003) and two adjusted for aeroallergen measurements (Hiltermann *et al.*, 1998; Just *et al.*, 2002). Of the other studies, only some modeled temporal trends that would capture some, but not all, of the seasonal fluctuations in these potential confounders by proxy.

In addition, the ISA did not fully evaluate potential biases caused by the joint effects of model misspecification and model selection bias. Unless researchers demonstrate the robustness of their results to variations in model specification, it is possible that measured associations between NO<sub>2</sub> and health effects reflect spurious associations created by model misspecification, rather than true causal relationships. As discussed above, some NO<sub>2</sub> studies reviewed in the ISA conducted thorough sensitivity analyses and indicated that their results were sensitive to variations in model specification. For example, Son *et al.* (2013) analyzed whether their results were robust to variations in the way weather covariates and temporal trends were modeled. They found that results change if temporal trends are modeled with fewer than 6 degrees of freedom per year. Likewise, Sarnat *et al.* (2012) found that associations between NO<sub>2</sub> and respiratory effects were highly sensitive to model specification, including the averaging time of exposure metrics.

Related to model misspecification, model selection bias is another well-described phenomenon in the air pollution research literature. This bias occurs when investigators measure many epidemiology associations generated by varying details of statistical models, such as lag relationships, averaging time for exposure metrics, and mathematical forms of temperature, temporal trends, and other influential covariates. Even though such variations are necessary, to explore the potential of model misspecification, it creates a scenario in which investigators may preferentially select the models producing findings that support their hypotheses (Anderson *et al.* 2005; Lumley and Sheppard, 2003).

Finally, publication bias, the tendency of journal editors to publish reports of positive findings over those describing null results, has an important impact on the air pollution epidemiology literature (Anderson *et al.*, 2005). The effect of this bias is likely an overrepresentation of positive associations between NO<sub>2</sub> and health effects in the published literature.

Taken together, confounding by upper respiratory infections and aeroallergens, model misspecification, and model selection bias are major issues in studies of short-term NO<sub>2</sub> exposure and respiratory effects. Because of these issues, confounding and bias cannot be ruled out with reasonable confidence, and the criteria for a casual determination cannot be met.

### **3.4 Evidence based on mode of action is not consistent with a role for NO<sub>2</sub> in asthma exacerbation**

As part of its rationale for determining that there is a causal association between NO<sub>2</sub> and asthma exacerbation, the ISA discusses evidence regarding key events related to the MoA, including allergic response, oxidative stress, and airway inflammation. The ISA concludes that the evidence for oxidative stress is inconsistent (Table 5-45 in ISA; US EPA, 2015); therefore, this subsection focuses on allergic response and airway inflammation. As discussed below, the evidence from the key studies highlighted in the ISA does not indicate that NO<sub>2</sub> exacerbates asthma at NO<sub>2</sub> concentrations in the range of the current short-term NAAQS.

#### **3.4.1 Allergic Responses**

The ISA states that increased airway inflammation associated with NO<sub>2</sub> exposure, as observed in controlled exposure studies, provides "sufficient biological plausibility for the effects of NO<sub>2</sub> exposure on asthma exacerbation" (US EPA, 2015). As support for this statement, the ISA highlights five key studies, summarized below in Table 3.5. These studies provide consistent evidence that NO<sub>2</sub> exposure increases levels of airway eosinophil cationic protein (ECP) in response to allergen exposures at NO<sub>2</sub> concentrations  $\geq 260$  ppb. An increase in ECP in and of itself, however, is not sufficient to cause asthma exacerbation. In the studies by Barck *et al.* (2002, 2005), NO<sub>2</sub> exposure did not increase allergen-associated AHR, despite the increase in airway ECP. Hence, results from the studies highlighted by ISA as providing "sufficient biological plausibility" that NO<sub>2</sub> exposure exacerbates asthma do not provide support for increased HAs and ED visits for asthma reported in epidemiology studies (discussed above, in Section 3.1) at NO<sub>2</sub> concentrations lower than those used in the controlled exposure studies.

**Table 3.5 Controlled Human Exposure Studies Evaluating Airway Allergic Responses**

Study	NO <sub>2</sub> Conc. (ppb)	Exposure Protocol	Allergen Exposure <sup>1</sup>	Effects		Allergy Status	Findings <sup>2</sup>
				Assessment (Hours Post-exposure)	Asthma Status		
Ezratty <i>et al.</i> (2014)	200	<u>Day 1</u> 1 x 30 minutes	No	6	Subjects had asthma, status not defined	HDM and/or pollen	Eosinophils and ECP: No effect
		<u>Day 2</u> 2 x 30 minutes		36 48			
Barck <i>et al.</i> (2002)	260	30 minutes	Yes	19	Mild	Pollen	Neutrophils and ECP: Increased
Barck <i>et al.</i> (2005)	260	<u>Day 1</u> 1 x 15 minutes	Yes	24	Mild	Pollen	ECP: Increased
		<u>Day 2</u> 2 x 15 minutes					
Wang <i>et al.</i> (1995)	400	6 hours	Yes	Yes	No asthma	Pollen	ECP: Increased
Ezratty <i>et al.</i> (2014)	600	<u>Day 1</u> 1 x 30 minutes	No	6	Subjects had asthma, status not defined	HDM and/or pollen	Eosinophils and ECP: Increased
		<u>Day 2</u> 2 x 30 minutes		36 48			

Notes:

ECP = Eosinophil Cationic Protein; HDM = House Dust Mites; NO<sub>2</sub> = Nitrogen Dioxide; ppb = Parts Per Billion.

(1) In studies involving allergen exposure, subjects were exposed to the allergen after exposure to filtered air or NO<sub>2</sub>.

(2) In studies involving allergen exposure, findings are for NO<sub>2</sub> + allergen exposures relative to filtered air + allergen exposures; for studies without allergen exposures, findings are for NO<sub>2</sub> relative to filtered air.

### 3.4.2 Inflammatory Effects

The ISA concludes that several controlled human exposure studies provide evidence that NO<sub>2</sub> exposure causes airway inflammation. These studies, summarized below in Table 3.6, provide consistent evidence that NO<sub>2</sub> increases airway polymorphonuclear leukocytes (PMNs) at concentrations  $\geq$  2,000 ppb, and some evidence that NO<sub>2</sub> increases various inflammatory mediators at concentrations  $\geq$  1,000 ppb. Even at these concentrations, which are an order of magnitude greater than the current short-term NO<sub>2</sub> NAAQS, the inflammation was characterized as "mild." (e.g., Azadniv *et al.*, 1998 ; Devlin *et al.*, 1999) Moreover, there was no indication that the increased PMNs and other inflammatory mediators were associated with adverse inflammatory effects. For example, there was no evidence that NO<sub>2</sub> exposure increased the permeability of the airway epithelial barrier, as indicated by total protein in airway lavage fluid, or resulted in cell damage (Blomberg *et al.*, 1999; Devlin *et al.*, 1999). Furthermore, the underlying pathophysiological mechanisms associated with pulmonary effects following short-term high-concentration NO<sub>2</sub> exposure have not been demonstrated to occur at ambient NO<sub>2</sub> levels (e.g., see Gregory *et al.*, 1983). Overall, evidence of mild inflammation, in the absence of adverse inflammatory effects, at NO<sub>2</sub> concentrations much greater than the current short-term NAAQS, do not support inflammation as a likely mode of action for increased HAs and ED visits for asthma reported in epidemiology studies (discussed above, in Section 3.1).

**Table 3.6 Controlled Human Exposure Studies Evaluating Pulmonary Inflammation**

Study	NO <sub>2</sub> (ppb)	Exposure Duration (Hours)	Exposure Frequency (Days)	Timepoint(s) (Hours)	Exercise	Asthma Status	Findings	Comments
Frampton <i>et al.</i> (2002)	600	3.5	1	3.5	Yes	No asthma	PMN: No effect	
Jorres <i>et al.</i> (1995)	1,000	3	1	1	Yes	Mild extrinsic asthma and no asthma	<p><u>Subjects with asthma</u>            PGD<sub>2</sub> and TxB<sub>2</sub>: Increased            6-keto-PGF<sub>1α</sub>: Decreased</p> <p><u>Subjects without asthma</u>            TxB<sub>2</sub>: Increased</p>	Jorres <i>et al.</i> note that cytokine alterations are indicative of a pro-inflammatory response
Azadniv <i>et al.</i> (1998)	2,000	6	1	0 18.5	Yes	No Asthma	PMNs: Increased at both timepoints	Azadniv <i>et al.</i> note that PMN increase is indicative of a "mild airway inflammatory response," "unlikely to be of clinical significance for most health subjects."
Blomberg <i>et al.</i> (1999)	2,000	4	4	1.5	Yes	No asthma	Neutrophils and myeloperoxidase: increased <sup>2,3</sup>	
Devlin <i>et al.</i> (1999)	2,000	4	1	16	Yes	No asthma	PMNs, IL-6, and IL-8: Increased	Devlin <i>et al.</i> note that results are consistent with a mild inflammatory response
Frampton <i>et al.</i> (2002)	2,000	3.5	1	3.5	Yes	No asthma	PMNs: Increased in males but not females	Concentration is very high; Different results in males vs. females
Pathmanathan <i>et al.</i> (2003)	2,000	4	4	1	Yes	No asthma	IL-5, IL-10, IL-13, ICAM-1: Increased	

Study	NO <sub>2</sub> (ppb)	Exposure Duration (Hours)	Exposure Frequency (Days)	Timepoint(s) (Hours)	Exercise	Asthma Status	Findings	Comments
Solomon <i>et al.</i> (2000)	2,000	4	4	18	Yes	No asthma	Neutrophils: Increased <sup>2</sup>	Solomon <i>et al.</i> note that findings are indicative of "mild bronchial airway inflammation" and "unlikely to produce any associated immunopathology."
Helleday <i>et al.</i> (1994)	3,500	0.33	1	24	No	No asthma	<u>Smokers</u> Alveolar macrophages and neutrophils: Increased  <u>Non-smokers</u> Neutrophils and lymphocytes: Increased	

Notes:

6-keto-PGF<sub>1α</sub> = 6-keto-prostaglandin<sub>1α</sub>; ICAM-1 = Intracellular Cell Adhesion Molecule-1; IL = Interleukin; NO<sub>2</sub> = Nitrogen Dioxide; PGD<sub>2</sub> = Prostaglandin D<sub>2</sub>; PMN = Polymorphonuclear Leukocyte; ppb = Parts Per Billion; TxB<sub>2</sub> = Thromboxane B<sub>2</sub>.

(1) Findings reported here are only for endpoints related to airway inflammation, as discussed by study authors; all treatment-related findings reported in table were statistically significant;

(2) Neutrophils are the most abundant type of PMNs.

(3) Myeloperoxidase is released by activated neutrophils.

### **3.5 The weight of evidence does not support a causal association between short-term NO<sub>2</sub> exposure and asthma exacerbation.**

Table 5-45 in the ISA summarizes key evidence, along with the ISA's rationale for concluding that there is a causal association between short-term NO<sub>2</sub> exposure and asthma exacerbation. We reproduced this table and included our evaluation of this evidence in Table 3.1, below. We found that the ISA's selection of epidemiology studies may have been biased and that the studies reviewed in the ISA had important uncertainties and limitations. In addition, the controlled human exposure studies do not provide clear evidence that NO<sub>2</sub> plays a role in asthma exacerbation. Taken together, the evidence does not meet EPA's criteria for establishing a causal association.

**Table 3.1 Summary of Evidence Regarding the Relationship between Short-term NO<sub>2</sub> Exposure and Respiratory Effects**

ISA		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Consistent epidemiologic evidence from multiple, high-quality studies at relevant NO <sub>2</sub> concentrations.	Increases in asthma HAs, ED visits in diverse populations in association with 24-h avg. and 1-h max. NO <sub>2</sub> , lags 0 and 3- to 5-day avg. among all ages and children.	Inconsistent evidence from multiple epidemiology studies at relevant NO <sub>2</sub> concentrations.	Positive, statistically significant associations observed in some studies, but many associations appeared only in subgroup analyses. Associations for varying lags were inconsistent within and between studies. Study quality was not systematically assessed by EPA.
	No association in recent Canadian multicity study.		Null findings reported in other studies, in addition to the recent Canadian multicity study.
	Coherence with increases in respiratory symptoms and decrements in lung function in populations with asthma in association with 24-h avg., 2-4 h avg. NO <sub>2</sub> , 1-h max., lags 0, 3 to 6-day avg.  Panel studies of children examined representative populations recruited from schools.  No reports of selective participation by particular groups.		Evidence related to respiratory symptoms and lung function is mixed. Null associations from individual studies are not presented in ISA figures. Results from studies of supervised lung function measurements are not more consistent or stronger than those based on home lung function tests.
Consistent evidence for NO <sub>2</sub> metrics with lower potential for exposure measurement error.	Asthma-related effects associated with NO <sub>2</sub> measured in subjects' locations: total and outdoor personal, school outdoor.  Better spatial alignment with subjects compared to central site NO <sub>2</sub> .	Inconsistent evidence for NO <sub>2</sub> metrics with lower potential for exposure measurement error.	References cited in ISA as key evidence mainly reported null associations.
Consistent evidence from multiple, high-quality controlled human exposure studies. Rules out chance, confounding, and other biases with reasonable confidence.	NO <sub>2</sub> increases airway responsiveness in adults with asthma exposed at rest following nonspecific or allergen challenge in several individual studies and meta-analyses. Clinical relevance supported by findings of a doubling reduction in PD in response to NO <sub>2</sub> .	Findings from controlled human exposure studies do not provide clear evidence that NO <sub>2</sub> increases airway responsiveness at concentrations less than 600 ppb.	Studies that evaluated airway responsiveness to specific allergen challenge, which are most relevant for understanding potential effects of ambient NO <sub>2</sub> , do not provide evidence that NO <sub>2</sub> increases airway responsiveness. Paradoxical effect for studies involving exposure while at rest but not while exercising. Lack of a concentration-response both within and across studies, including for studies that observed a clinically relevant doubling reduction in PD.

ISA		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Epidemiologic evidence helps rule out chance, confounding, and other biases with reasonable confidence.	NO <sub>2</sub> associations with lung function and pulmonary inflammation persist in co-pollutant models with a traffic-related co-pollutant: PM <sub>2.5</sub> , EC/BC, OC, UFP, or VOCs in studies with exposure assessment in subjects' locations.	Insufficient epidemiology evidence to rule out chance confounding and other biases. Model selection bias and publication bias are key issues.	Potential confounding by co-pollutants assessed by inspecting results of multi-pollutant models, which are highly unreliable.
	Ambient and total personal NO <sub>2</sub> weakly-moderately correlated with other traffic-related pollutants in some studies (r = -0.43 to 0.49).		Differential exposure measurement error is not rigorously or systematically assessed in multi-pollutant analyses discussed in EPA.
	Most central site NO <sub>2</sub> associations persist with adjustment for PM <sub>2.5</sub> , EC/metals factor, UFP, or CO.		Potential confounding by co-pollutants assessed by inspecting results of multi-pollutant models, which are highly unreliable.
	Differential exposure measurement error limits inference from co-pollutant models based on central site NO <sub>2</sub> and co-pollutants.		Studies of indoor NO <sub>2</sub> mainly report null findings.
	Some associations were attenuated with adjustment for PM <sub>2.5</sub> or UFP.		Several studies show that results are sensitive to changes in model specification.
	Most associations for microenvironmental and central site NO <sub>2</sub> persist in co-pollutant models with PM <sub>10</sub> , SO <sub>2</sub> , or O <sub>3</sub> .		
	Indoor NO <sub>2</sub> associated with increases in respiratory effects in children with asthma.		
NO <sub>2</sub> associations persist with adjustment for meteorology, time trends, season, medication use.			
<b>Evidence for Key Events in Mode of Action</b>			
Allergic responses	Increases in eosinophil activation, IgE, Th2 cytokines in adults with asthma.	Studies do not provide robust evidence that allergic responses contribute to increased HAs or ED visits for asthma.	Increases in markers of allergic responses were not accompanied by increased airway responsiveness or sufficient to exacerbate asthma.
Inflammation	Increases in PMNs and prostaglandins in healthy adults.	Studies do not provide robust evidence that pulmonary inflammation contributes to increased HAs or ED visits for asthma.	Increases in PMNs were not observed at NO <sub>2</sub> concentrations less than 2,000 ppb. No evidence that increases in PMNs were accompanied by relevant physiological changes, such as increased permeability of the airway epithelial barrier, or cell damage.

Notes:

BC = Black Carbon; CO = Carbon Monoxide; EC = Elemental Carbon; ED = Emergency Department; EPA = United States Environmental Protection Agency; HA = Hospital Admission; IgE = Immunoglobulin E; ISA = Integrated Science Assessment; NO<sub>2</sub> = Nitrogen Dioxide; O<sub>3</sub> = Ozone; OC = Organic Carbon; PD = Provocative Dose; PM = Particulate Matter; PMN = Polymorphonuclear Leukocyte; ppb = Parts Per Billion; SO<sub>2</sub> = Sulfur Dioxide; Th2 = T-Derived Lymphocyte Helper 2; UFP = Ultrafine Particles; VOC = Volatile Organic Compound.

## 4 Long-term NO<sub>2</sub> Exposure and Asthma Development

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In the previous review cycle, the 2008 ISA concluded that the evidence was suggestive, but not sufficient, to infer a causal relationship between long-term NO<sub>2</sub> exposure and respiratory effects, primarily based on evidence regarding the development of new-onset asthma. In the current review cycle, the ISA primarily relies on recent epidemiology studies of asthma development in children and adults and concludes that the relationship between long-term NO<sub>2</sub> exposure and respiratory effects is likely causal. The ISA also cites evidence from studies using laboratory animals to evaluate asthma development and evidence regarding effects consistent with the ISA's proposed MoA for development of asthma.

EPA's causal determinations for long-term NO<sub>2</sub> exposure and their rationales are summarized in Table 6-5 in the ISA. We have reproduced this here in Table 4.1 (at the end of this section), along with comments demonstrating how the validity of the ISA's causal determination is undermined by major limitations in its evaluation of the evidence. The ISA does not evaluate available epidemiology studies in a systematic, balanced, and rigorous manner; instead, it emphasizes studies with positive findings while overlooking studies with null results. Further, the evidence of new-onset asthma associated with long-term NO<sub>2</sub> exposure in animal studies is not robust, and the evidence regarding effects associated with the MoA for asthma development is not compelling. Considering the significant limitations of and uncertainties in the epidemiology studies, the inconsistency and lack of coherence across these studies, and the lack of robust, compelling evidence from animal toxicity and MoA studies, it is clear that the evidence is not sufficient to support a likely causal relationship, and the body of new evidence does not support changing the causal determination from the 2008 ISA.

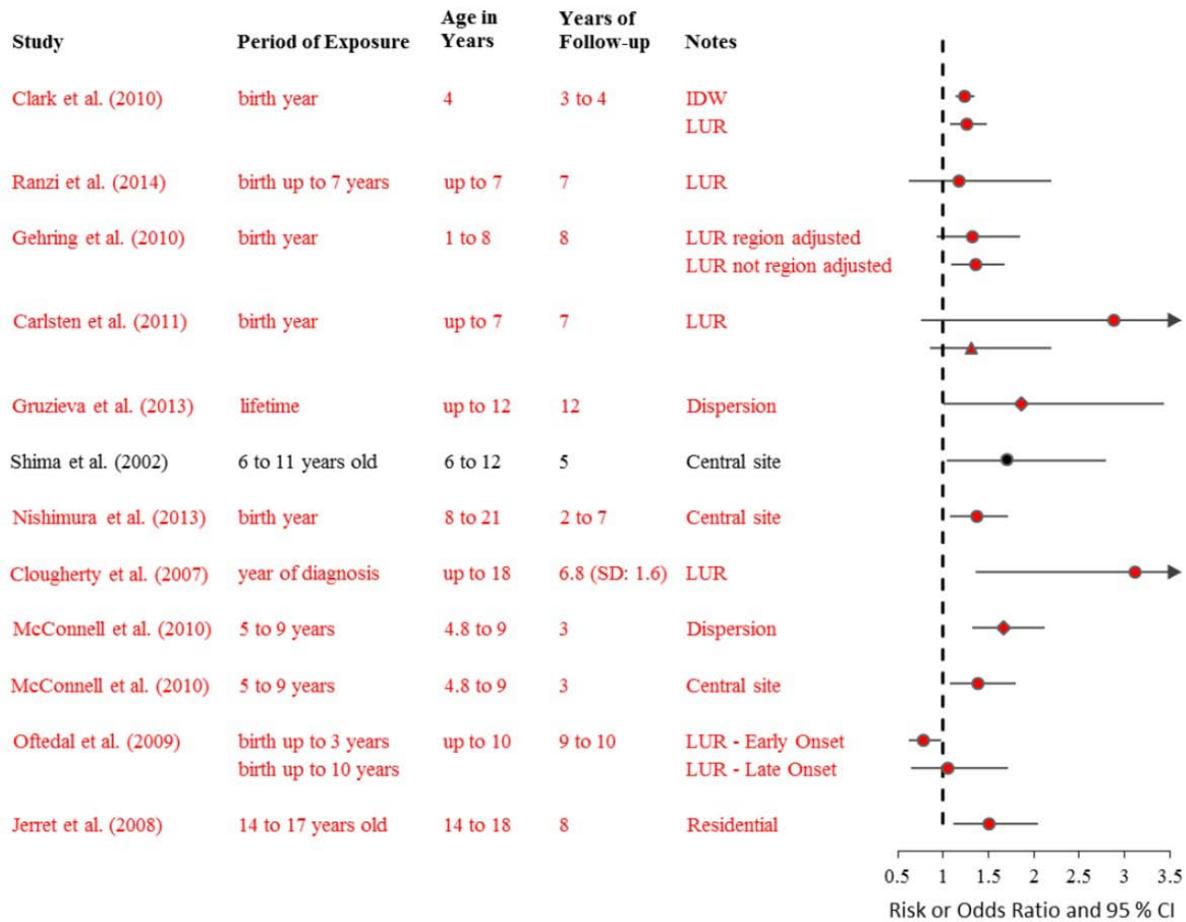
### 4.1 Evidence is inconsistent regarding asthma development in children

The ISA states that recent key longitudinal cohort studies generally demonstrate a positive relationship between asthma incidence in children and long-term NO<sub>2</sub> exposure, and these studies support a likely causal relationship between long-term NO<sub>2</sub> exposure and respiratory effects (US EPA, 2015, Section 6.2.2.1 and Table 6-5).

#### 4.1.1 EPA's selection of studies informing the causal determination appear to be biased

The ISA only considers longitudinal cohort studies of long-term NO<sub>2</sub> exposure and asthma development in its analysis. Because this is one of the most robust epidemiology study designs, this is an appropriate approach. Twelve of these studies are discussed in Section 6.2.2.1 and summarized in Table 6-1 and Figure 6-1 (shown below as Figure 4.1). Five of the twelve studies discussed in the main text are highlighted as key references in Table 6-5 (Carlsten *et al.*, 2011; Clougherty *et al.*, 2007; Gehring *et al.*, 2010; Jerrett *et al.*, 2008; Shima *et al.*, 2002), and one study is marked as "weak evidence" (Ranzi *et al.*, 2015). The ISA does not explain how the six studies were selected to be key references. Based on Table 6-5, these studies presumably represent "multiple high quality studies with relevant NO<sub>2</sub> concentrations" and with "asthma ascertainment by parental report of doctor diagnosis" (US EPA, 2015). However, three studies had small sample sizes (Carlsten *et al.*, 2011; Clougherty *et al.*, 2007; Jerrett *et al.*, 2008), and two did not ascertain incident asthma cases by parental report or doctor diagnosis (Jerrett *et al.*, 2008; Shima *et al.*, 2002). Also, diagnosing asthma in young children is difficult, and it is hard to be completely sure of the diagnosis (Mayo Clinic, 2013), but the ISA does not address this uncertainty or acknowledge the

potential for outcome misclassification in every study of asthma development in children. These six key references do not appear to be of higher quality than the other studies discussed in Section 6.2.2.1 and presented in Table 6-5 of the ISA.



**Figure 4.1 Association between Long-term Exposure to NO<sub>2</sub>, NO, and NO<sub>x</sub> and New-onset Asthma in Prospective Cohort Studies of Children.** All risk estimates are standardized to 10 ppb, except Gruzieva *et al.* (2013) and Oftedal *et al.* (2009), which are standardized to percentile increases in NO<sub>2</sub> of 5 to 95 and 25 to 75, respectively. Studies in red are reviewed in the current ISA; studies in black were reviewed in the 2008 ISA. Circles = NO<sub>2</sub>; Triangle = NO; Diamonds = NO<sub>x</sub>. Source: Figure 6-1, US EPA (2015).

#### 4.1.2 The ISA's evaluation of evidence for asthma development in children is not rigorous or balanced

Section 6.2.2.1 of the ISA discusses epidemiology studies of asthma development in children and concludes that there is consistent evidence of a positive relationship. The validity of the ISA's conclusion is undermined by several limitations in its evaluation of the evidence.

Table 6-1 and Figure 6-1 in the ISA (Figure 4-1 below) summarize the characteristics of 12 cohort studies and present the risk estimates that are standardized to a 10-ppb increment in long-term ambient NO<sub>2</sub> concentrations. These estimates provide a skewed perspective on the published literature. For example, the ISA presents the overall risk estimates for the entire study period for the majority of studies, but it

does not show the inconsistent results generated from subgroup or stratified analyses (Carlsten *et al.*, 2011; Gehring *et al.*, 2010). For one study, the ISA presents the significantly positive risk estimate for a specific subgroup but does not present the null results for the other subgroup (Clougherty *et al.*, 2007). EPA's presentation of the results makes them appear to be more consistent than they actually are.

The ISA states that epidemiology evidence from multiple high-quality studies is consistent with regard to an elevated risk for asthma development in children who were exposed to higher NO<sub>2</sub> concentrations (US EPA, 2015, Table 6-5). However, the ISA does not address the inconsistency among findings within certain studies. For example, exposure to violence, a chronic social stressor, was found to modify the observed effects of NO<sub>2</sub> exposure on asthma incidence in children (Clougherty *et al.*, 2007); an NO<sub>2</sub>-associated effect on asthma was observed among children with high exposure to violence (OR = 1.63, 95% CI: 1.14-2.33) but not among children with low exposure to violence (OR = 0.99, 95% CI: 0.73-1.34). Another key reference evaluated overall and age-specific associations between NO<sub>2</sub> and asthma during the first 8 years of life (Gehring *et al.*, 2010). Despite a positive overall association, some age-specific associations were null. A study in Japan evaluated both incident and prevalent asthma and reported a positive and null association with NO<sub>2</sub> exposure for these two endpoints, respectively (Shima *et al.*, 2002). The inconsistent findings of prevalent and incident asthma were not fully addressed by the study authors, nor are they acknowledged in the ISA.

In addition, the ISA fails to address the considerable between-study inconsistencies in effect estimates for NO<sub>2</sub> exposures. The majority of studies that assessed NO<sub>2</sub> exposure in birth year reported moderately increased incidence of asthma (risk estimates < 1.5) in children with higher NO<sub>2</sub> exposure (Clark *et al.*, 2010; Gehring *et al.*, 2010; Nishimura *et al.*, 2013), while one study with a smaller sample size reported a non-statistically significant OR of 3.12 (Carlsten *et al.*, 2011). Four studies evaluated NO<sub>2</sub> exposure that was closer in time to asthma diagnosis (Shima *et al.*, 2002; Clougherty *et al.*, 2007; McConnell *et al.*, 2010; Jerret *et al.*, 2008). Although a positive association was consistently observed in all four studies, the magnitude of the risk estimates varied considerably. In addition, three studies assessed lifetime NO<sub>2</sub> exposure in children and reported mixed results. Two studies using land use regression (LUR) models or dispersion models to assess exposure reported null or negative associations between long-term NO<sub>2</sub> exposure and asthma incidence (Ranzi *et al.*, 2015; Oftedal *et al.*, 2009), while one study using dispersion models for exposure assessment reported a positive association (Gruzieva *et al.*, 2013).

The ISA considers four key references to have a lower potential for exposure measurement error because they estimated residential NO<sub>2</sub> exposure using well-validated LUR models or by monitoring (Carlsten *et al.*, 2011; Clougherty *et al.*, 2007; Gehring *et al.*, 2010; Jerrett *et al.*, 2008). The ISA concludes that these studies consistently showed a positive association between asthma and NO<sub>2</sub> exposure but fails to consider the numbers of actual measurements taken for ambient NO<sub>2</sub> concentrations in these studies. With the exception of Clougherty *et al.* (2007), the studies took very limited numbers of measurements, which may not have been sufficient to fully account for temporal variation in NO<sub>2</sub> levels (Carlsten *et al.*, 2011; Gehring *et al.*, 2010; Jerrett *et al.*, 2008). In addition, as discussed above, two studies that estimated NO<sub>2</sub> exposures using well-validated LUR or dispersion models reported null associations between NO<sub>2</sub> and asthma (Ranzi *et al.*, 2015; Oftedal *et al.*, 2009). It is unclear why the ISA does not consider these two studies to have a lower potential for exposure measurement error or take their null findings into account.

#### **4.1.3 We agree with EPA that there is uncertainty regarding confounding by traffic-related co-pollutants**

Table 6-5 of the ISA states, and we concur, that there is uncertainty with regard to potential confounding by traffic-related co-pollutants. The majority of the studies evaluated multiple traffic-related air pollutants, such as PM<sub>2.5</sub> and black carbon, and often found similar positive associations with asthma for

these co-pollutants. None of the studies conducted co-pollutant analyses, so the ISA is correct in suggesting that significant uncertainty remains with regard to whether the observed association between NO<sub>2</sub> and asthma suggests a causal relationship or that NO<sub>2</sub> is a marker of traffic-related exposures.

## **4.2 Evidence regarding asthma development in children is not consistent with other respiratory effects**

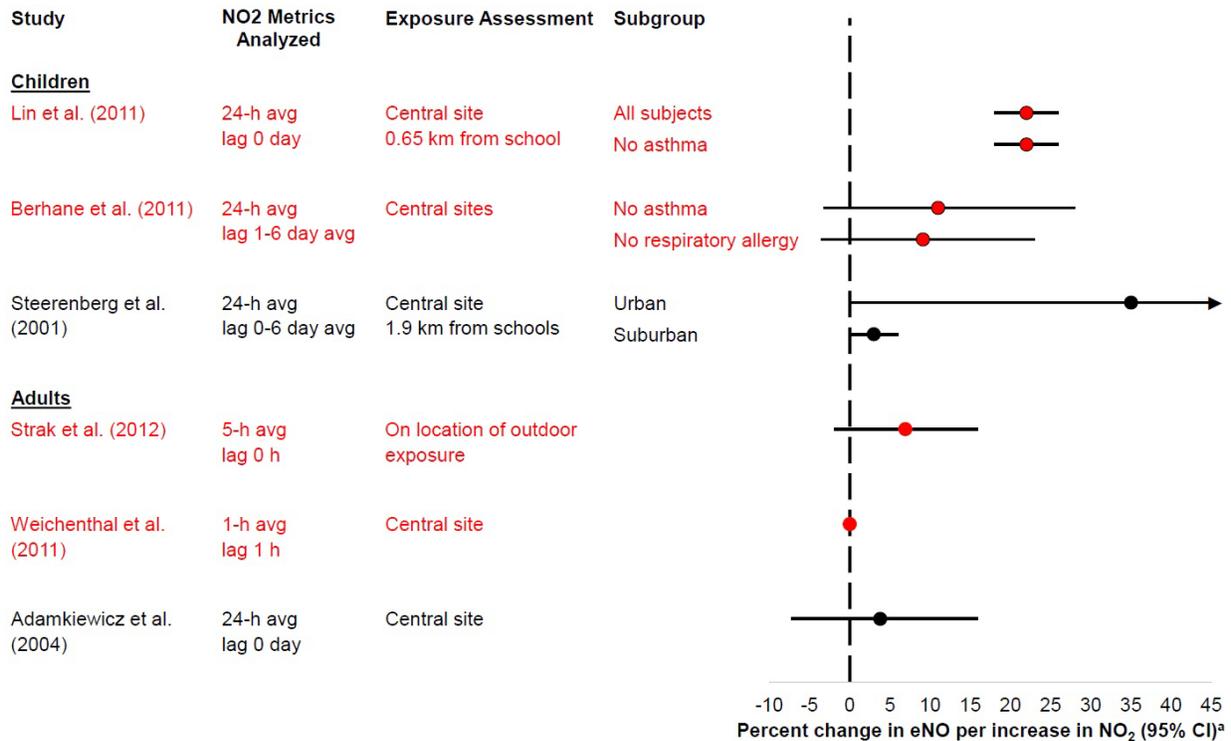
### **4.2.1 Evidence is inconsistent regarding asthma incidence and chronic bronchitis in adults**

In Section 6.2.2.2, the ISA discusses three epidemiology studies that examined asthma/chronic bronchitis in adults in relation to long-term exposure to NO<sub>2</sub> (Sunyer *et al.*, 2006; Jacquemin *et al.*, 2009; Modig *et al.*, 2009). All of these studies were based on the same study population – the European Community Respiratory Health Survey (ECRHS) cohort. In Table 6-5, the ISA cites these three studies as supporting evidence for a causal determination for asthma development. However, one study evaluated chronic bronchitis in adults instead of asthma (Sunyer *et al.*, 2006), and the ISA does not address the relevance of this study. The other two studies relied on self-reported asthma and reported a positive overall association between incident asthma and long-term NO<sub>2</sub> exposure (Jacquemin *et al.*, 2009; Modig *et al.*, 2009), but there was considerable heterogeneity among sex-specific and city-specific risk estimates for NO<sub>2</sub> exposure, which was not fully explained by the study investigators, nor is it acknowledged in the ISA. In addition, similar to epidemiology studies of asthma in children, these studies did not conduct co-pollutant analyses. Therefore, uncertainty remains with regard to potential confounding by traffic-related co-pollutants. The ISA should have acknowledged this uncertainty and its impact on its causal determination.

### **4.2.2 Epidemiology evidence is not coherent with short-term respiratory effects**

The ISA cites epidemiology studies that evaluated eNO concentrations as a marker of pulmonary inflammation following short-term NO<sub>2</sub> exposure in healthy children and adults as evidence for the coherence between respiratory effects of long-term and short-term NO<sub>2</sub> exposures. Figure 5-14 in the ISA (shown here as Figure 4.2) presents the study results and clearly shows between-study inconsistencies.

Although eNO is considered a valid indicator of airway inflammation (Dweik *et al.*, 2011), there is uncertainty when using this marker in epidemiology studies of short-term NO<sub>2</sub> exposures. As the ISA acknowledges in Section 4.2.3, eNO is affected by a variety of factors, including disease state, diet, sex (or height), species, smoking history, and environmental exposure, and that "endogenous NO production, even during inflammatory states, is at best modest compared to dietary intake" (US EPA, 2015). Yet, these factors have not been consistently or comprehensively evaluated in NO<sub>2</sub> epidemiology studies that evaluate eNO. The ISA should have acknowledged that such limitations undermine any observed effects on eNO following short-term NO<sub>2</sub> exposure and do not support coherence between long-term and short-term respiratory effects of NO<sub>2</sub>.



**Figure 4.2 Association between Short-term Exposure to NO<sub>2</sub> and Exhaled Nitric Oxide among Healthy Children and Adults.** All risk estimates are standardized to a 20-ppb increase in 24-h average ambient NO<sub>2</sub> concentrations. Studies in red are reviewed in the current ISA; studies in black were reviewed in the 2008 ISA. Source: Figure 5-14, US EPA (2015).

#### 4.2.3 Animal studies do not provide evidence that ambient NO<sub>2</sub> leads to new-onset asthma

The ISA concludes that, although the epidemiology evidence is uncertain, the experimental animal evidence demonstrates the biological plausibility (*i.e.*, the "pathophysiological basis") of long-term exposure to NO<sub>2</sub> contributing to the development of new-onset asthma (US EPA, 2015). Yet, as noted in the ISA, this evidence is limited. Evidence of AHR induced by acute or long-term NO<sub>2</sub> exposure is variable, and responses have been observed only in guinea pigs exposed continuously to high (> 1,000 ppb) NO<sub>2</sub> concentrations. It is unknown whether the biological response to such high NO<sub>2</sub> concentrations would occur at ambient concentrations or whether AHR observed in guinea pigs predicts asthma development in humans. Thus, this evidence may not be informative regarding biological plausibility.

The ISA highlights two key studies in guinea pigs, by the same research group, in which increased AHR was reported. Kobayashi and Shinozaki (1990) demonstrated that subacute (3-day) exposure to 4,000 ppb NO<sub>2</sub> induced AHR in guinea pigs, but the increase in AHR was transient, and neither 1 nor 7 days of exposure caused a permanent change in AHR. In a subsequent study by Kobayashi and Miura (1995), subchronic (6- to 12-week) continuous exposure of guinea pigs to 60, 500, 1,000, 2,000, or 4,000 ppb NO<sub>2</sub> induced an increase in AHR in a dose- and time-dependent manner. Statistically significant increases in airway resistance were observed at 2,000 and 4,000 ppb NO<sub>2</sub> following 6 weeks of exposure and at 1,000 and 2,000 ppb NO<sub>2</sub> following 12 weeks of exposure (the results for 12 weeks exposure to 4,000 ppb were not reported).

Overall, these results suggest that continuous high-level NO<sub>2</sub> may induce an initial transient effect on AHR, followed by a progressive increase in responsiveness after several months of exposure. The ISA acknowledges that the NO<sub>2</sub> concentrations used in these studies were exceptionally high. Continuous exposure to 1,000 ppb NO<sub>2</sub> can damage rat lung epithelial tissue (Gregory *et al.*, 1983); 1,000 ppb is also the lowest concentration at which Kobayashi and Miura (1995) observed increased AHR. Thus, the limited experimental animal evidence does not provide evidence regarding whether long-term exposure to ambient NO<sub>2</sub> levels leads to lung function decrements that may precede new-onset asthma.

### **4.3 Mode-of-action evidence does not indicate that ambient NO<sub>2</sub> induces pathophysiological responses that would lead to new-onset asthma**

There is no direct experimental evidence regarding the development of new-onset asthma from either controlled human exposure or laboratory animal studies. In the absence of such evidence, the ISA considers experimental evidence regarding pathophysiological responses as potential predictors of asthma development – specifically, evidence of an association between NO<sub>2</sub> exposure and pulmonary allergic response, airway remodeling, lung inflammation, and oxidative stress. The ISA concludes that the experimental evidence is consistent, but limited, regarding associations between NO<sub>2</sub> exposure and these endpoints. Below, we show that the key studies cited in the ISA only observed effects at levels of NO<sub>2</sub> well above ambient exposures; at such high levels, NO<sub>2</sub> may cause overt damage to the lung tissue. Effects observed at such high exposure levels are unlikely to predict potential effects associated with ambient exposures.

#### **4.3.1 Allergic Responses**

The ISA addresses the potential for NO<sub>2</sub> to enhance the development of immune responses to inhaled allergens, which could contribute to the development of asthma in sensitive individuals (Holgate *et al.*, 2010). EPA cites experimental evidence that NO<sub>2</sub> exposure can lead to a T-derived lymphocyte helper 2 (Th2) bias in the lymphocyte response, the recruitment and activation of mast cells, and development of an immunoglobulin E (IgE) antibody response (US EPA, 2015). Activation of CD4+ lymphocytes in the lungs, skin, and gut by local dendritic cell populations often results in the formation of Th2 polarized lymphocytes. These antigen-specific Th2 lymphocytes secrete cytokines, such as interleukin (IL)-4 and IL-13, that are associated with, and likely support, B cell maturation and mast cell recruitment (Maier *et al.*, 2012). Accordingly, a bias towards a Th2 lymphocyte response is considered an important immunological hallmark of allergy development, although it is unlikely that Th2 CD4+ T cells alone can cause asthma (Umetsu and DeKruyff, 2010). EPA proposes that NO<sub>2</sub>-induced recruitment of Th2 CD4+ T cells and mast cells and the maturation of B cells to produce allergen-specific IgE contribute to the development of new-onset asthma (US EPA, 2015).

The ISA highlights three studies as providing evidence that NO<sub>2</sub> may enhance the development of immune responses to inhaled allergens. In one study, slightly enhanced IgE-mediated mast cell activity was observed in guinea pigs continuously exposed to 4,000 ppb NO<sub>2</sub> (but not 1,000 or 2,000 ppb) for 12 weeks (Fujimaki and Nohara, 1994). Mast cell activity in NO<sub>2</sub>-exposed guinea pigs was modestly (but not consistently) reduced compared to controls. In another study, in which guinea pigs were exposed to NO<sub>2</sub> for 6 hours/day, 6 days/week for 2 weeks, Ohashi *et al.* (1994) observed a modest eosinophilic response at 3,000 ppb NO<sub>2</sub>, and both nasal pathology and eosinophilia at 9,000 ppb NO<sub>2</sub>. Overall, these studies provide some evidence of enhanced allergic response in guinea pigs, but no evidence of a Th2 bias. Finally, Pathmanathan *et al.* (2003) observed increased levels of IL-5, IL-10, IL-13, and intercellular cell adhesion molecule-1 (ICAM-1) in lung tissue biopsies from people exposed to 2,000 ppb NO<sub>2</sub> for 4 hours/day for 4 consecutive days. Pathmanathan *et al.* (2003) presented data for 17 matched

biopsies that were isolated from 12 subjects. The potential for sample bias in this study is high, and no representative immunohistological data was shown. Although this study provides evidence that high NO<sub>2</sub> exposures can induce certain facets of a Th2-type response in humans, the lack of representative immunohistological data and the potential bias in data presentation and analysis reduces the reliability of the results from this study.

In the studies highlighted in the ISA as providing evidence of pulmonary allergic responses, no such effects were observed with repeated or continuous exposure for up to 12 weeks at concentrations below 2,000 ppb NO<sub>2</sub>. The experimental animal data show that high concentrations of NO<sub>2</sub> can elicit components of an allergic response, but these effects were only observed at concentrations associated with overt respiratory damage in rodents (*e.g.*, see Gregory *et al.*, 1983), and no study specifically evaluated allergic responses. Overall, the studies highlighted in the ISA do not provide evidence that ambient NO<sub>2</sub> exposure leads to new onset-asthma *via* enhanced allergic responses.

### **4.3.2 Airway Remodeling**

EPA cites the study by Kobayashi and Miura (1995) as providing evidence of airway remodeling based on increased airway resistance in guinea pigs exposed to 2,000 or 4,000 ppb NO<sub>2</sub> for 12 weeks (US EPA, 2015, p. 4-40). However, Kobayashi and Miura (1995) did not examine the airways for histopathological changes, and increased airway resistance alone is not direct evidence of airway remodeling (Cockcroft and Davis, 2006). At present, experimental evidence regarding whether NO<sub>2</sub> exposure can lead to airway remodeling is lacking.

### **4.3.3 Inflammation**

As further support for evidence consistent with the MoA for development of new-onset asthma, the ISA cites studies that evaluated inflammatory responses in animal, controlled human exposure, and epidemiology studies. Overall, the studies highlighted in the ISA do not provide compelling evidence that NO<sub>2</sub>-associated pulmonary inflammation underlies the development of new-onset asthma.

#### **4.3.3.1 Increased Lymphocytes and PMNs in Rats**

Depending on the chronicity and severity, airway inflammation can contribute to increased AHR, airway remodeling, and the development of allergies, thus increasing the likelihood of new-onset asthma (Cockcroft and Davis, 2006; Holgate *et al.*, 2010). The ISA cites several experimental animal studies that measured cytokines (*e.g.*, tumor necrosis factor alpha [TNF $\alpha$ ] and interferon gamma [IFN $\gamma$ ]) and cells (*e.g.*, neutrophils) associated with inflammation following long-term NO<sub>2</sub> exposure. The results of these studies are inconsistent and generally insufficient to conclude that NO<sub>2</sub> causes lung inflammation.

As evidence that long-term NO<sub>2</sub> exposure causes lymphocyte recruitment in association with lung inflammation, the ISA highlights a study by Kumae and Arakawa (2006), in which rats were exposed to 200, 500, or 2,000 ppb NO<sub>2</sub> for 12 weeks beginning *in utero* or at weaning. The authors observed a statistically significant increased percentage of lung lymphocytes in weanling rats after 12 weeks at 500 and 2,000 ppb NO<sub>2</sub>. This effect, however, was only observed at 200 ppb NO<sub>2</sub> in the rats exposed during gestation and did not have a monotonic dose-response relationship in either the weanling or gestational exposure groups. The authors also assessed the production of inflammatory cytokines from cultured alveolar macrophages isolated from the exposed animals. Increased levels of inflammatory cytokines, TNF $\alpha$ , and IFN $\gamma$  were observed in the gestational exposure group at 8 weeks (but not at 12 weeks) and in the weanling exposure groups at 12 weeks (but not at 8 weeks) at 500 and 2,000 ppb NO<sub>2</sub> (but not at 200

ppb NO<sub>2</sub>). Unexpectedly, this response was associated with a reduced percentage of lung macrophages and suppressed reactive oxygen species (ROS) production by lung macrophages in the weanling groups following 12 weeks of exposure to 500 and 2,000 ppb NO<sub>2</sub>.

Overall, the observations by Kumae and Arakawa (2006) provide limited evidence that NO<sub>2</sub> exposure leads to lymphocyte recruitment or lung inflammation. The proportional changes in lung macrophages and lymphocytes are suggestive of an immune response, but, because the authors did not report the number of cells isolated from the lungs, the data are inadequate to conclude there was a change in that number. Furthermore, in the absence of direct measures of inflammatory cytokines in lung tissue or fluid, the observed production of TNF $\alpha$  and IFN $\gamma$  by lung macrophages in culture for 24 hours is suggestive of, but not direct evidence of, lung inflammation. Importantly, the responses observed in rats exposed to NO<sub>2</sub> starting from gestation and those exposed from weaning were often discordant. This raises questions regarding whether the response was treatment-related.

#### **4.3.3.2 Increased PMNs in Controlled Human Exposure Studies**

As discussed in Section 3.3.2 of this report, several controlled human exposure studies provide evidence of increased airway PMNs following short-term NO<sub>2</sub> exposure. However, the increase in PMNs, which has been observed only at NO<sub>2</sub> concentrations greater than 2,000 ppb, did not appear to be associated with overt inflammatory effects, such as increased permeability of the airway epithelial barrier or cell damage. In the absence of overt inflammatory effects at more relevant ambient NO<sub>2</sub> levels, the findings regarding increased PMNs do not provide sufficient evidence that inflammation contributes to the development of new-onset asthma.

#### **4.3.3.3 eNO in Longitudinal Epidemiology Study**

Berhane *et al.* (2014) conducted a longitudinal study of school children with asthma in southern California as part of the Children's Health Study (CHS). They analyzed the effects of long-term changes in NO<sub>2</sub> and other pollutants on eNO, with adjustment for short-term effects of each pollutant, to distinguish potential long-term effects. Annual averages of NO<sub>2</sub> and PM<sub>2.5</sub> were associated with changes in eNO levels, adjusted for short-term effects of the same air pollutant as well as several subject characteristics, such as asthma medication use, history of allergy, age, and season. Although this study had a relatively large study size and other methodological strengths, such as the use of a prospective, longitudinal study design, the study authors acknowledged the potential for residual spatio-temporal confounding that could not be fully addressed (due to budgetary limitations). It is also noteworthy that the study authors did not adjust associations for co-pollutants, so confounding could be an issue in this study. Perhaps most importantly, even if there is a causal association, there is substantial uncertainty regarding whether eNO is an accurate marker of pulmonary inflammation. As a whole, this study does not provide sufficient evidence that long-term NO<sub>2</sub> exposure contributes to pulmonary inflammation.

#### **4.3.4 Oxidative Stress**

The ISA concludes that evidence regarding long-term NO<sub>2</sub> and lung oxidative stress is inconsistent. In mice exposed to 1,000 ppb NO<sub>2</sub> for 17 months, Ayaz and Csallany (1978) observed reduced glutathione peroxidase activity compared to mice exposed to air. Because antioxidant defenses are expected to increase during continuous exposure to oxidative stressors, the reduction in glutathione peroxidase activity suggests that exposure to air results in greater oxidative stress than exposure to 1,000 ppb NO<sub>2</sub>. A reduction in glutathione peroxidase activity was also observed by Sagai *et al.* (1984) in rats exposed to 400 or 4,000 ppb NO<sub>2</sub> for 18 months. These findings are inconsistent with those in the Gregory *et al.*

(1983) study, in which rats exposed to 1,000 ppb NO<sub>2</sub> with 5,000 ppb spikes<sup>7</sup> or 5,000 ppb NO<sub>2</sub> for 7 hours/day, 5 days/week for 3 weeks showed lung damage with concomitant increases in glutathione peroxidase activity. One possible explanation for the differences between these studies may be the duration of exposure; adaptation to long-term NO<sub>2</sub> exposure may lead to the reversal of markers of oxidative stress. Overall, these studies show that high-level NO<sub>2</sub> may transiently alter the activity of antioxidant enzymes, but this effect was only observed in the presence of overt lung damage, which has not been demonstrated to occur at ambient NO<sub>2</sub> concentrations.

#### **4.4 The weight of evidence does not support a likely causal association between long-term NO<sub>2</sub> exposure and development of new-onset asthma**

Table 6-5 in the ISA summarizes key evidence, along with the ISA's rationale for concluding that there is a likely causal association between long-term NO<sub>2</sub> exposure and the development of new-onset asthma. We reproduced this table and included our evaluation of this evidence in Table 4.1 below. We found that evidence from the epidemiology studies was inconsistent both within and across studies and that there was a high potential for confounding from co-pollutant exposures and uncertainties in exposure estimates in this body of literature. In addition, the evidence from animal toxicology studies is limited, both in number of relevant studies and with respect to the relevance of their findings. Taken together, the evidence does not meet EPA's criteria for establishing a likely causal association.

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<sup>7</sup> Gregory *et al.* (1983) spiked NO<sub>2</sub> levels from 1,000 ppb to 5,000 ppb for 1.5 hours twice each exposure day.

**Table 4.1 Summary of Key Evidence Regarding the Relationship between Long-term NO<sub>2</sub> Exposure and Development of New-onset Asthma**

ISA		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Consistent epidemiology evidence from multiple, high-quality studies with relevant NO <sub>2</sub> concentrations.	Consistent evidence for increases in asthma incidence in diverse cohorts of children in the US, Europe, Canada, and Asia.	Epidemiology studies have limitations and uncertainties, and evidence is inconsistent.	Inconsistent evidence for asthma incidence within and across cohorts of children in the US, Europe, Canada, and Asia.
	Asthma ascertainment by parental report of doctor diagnosis.		Asthma diagnosis is uncertain in young children and doctor diagnosis was not always reported by parents.
	Supporting evidence for asthma incidence or chronic bronchitis in the ECRHS cohort of adults.		Inconsistent evidence for asthma incidence in the ECRHS cohort of adults. Findings on chronic bronchitis not relevant.
Consistent evidence for NO <sub>2</sub> metrics with lower potential for exposure measurement error.	In children, asthma associated with residential NO <sub>2</sub> estimated using well-validated LUR models or by monitoring.	Studies have considerable error and uncertainty in exposure estimation, and evidence is inconsistent.	The majority of the studies had a limited number of measurements and could not fully account for temporal variation in NO <sub>2</sub> levels.
Uncertainty regarding potential confounding by traffic-related co-pollutants.	When reported, correlations with PM <sub>2.5</sub> and EC often were high (r = 0.7-0.96). No co-pollutant models analyzed.	Uncertainty regarding potential confounding by traffic-related co-pollutants.	Similar effects on asthma incidence in children often found for traffic-related co-pollutants in the same studies.
	Associations found with adjustment for SES, family history of asthma, smoking exposure, housing characteristics, and presence of gas stove.		No co-pollutant analyses conducted.

ISA		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Coherence with respiratory effects of short-term NO <sub>2</sub> exposure.	Limited epidemiologic evidence for increases in pulmonary inflammation in healthy children and adults with exposures assessed in subjects' locations and associations adjusted for BC/EC, OC, PNC, or PM <sub>2.5</sub> .	Lack of coherence with respiratory effects of short-term NO <sub>2</sub> exposure.	Limited epidemiology evidence for pulmonary inflammation (eNO) associated with short-term NO <sub>2</sub> exposure in healthy children and adults.  Uncertainty in findings due to influence of diet and other factors on individual eNO levels.
	Evidence from controlled human exposure studies for increased airway responsiveness in healthy adults.		Studies not discussed in ISA with respect to association between long-term NO <sub>2</sub> exposure and development of new-onset asthma.
Limited and supporting toxicological evidence at relevant NO <sub>2</sub> exposures.	Increased AHR in guinea pigs with long-term or short-term NO <sub>2</sub> exposure.	No evidence of increased AHR at ambient NO <sub>2</sub> levels.	Effects may be secondary to tissue damage due to high exposure level.
<b>Some Evidence for Key Events in Mode of Action</b>			
Allergic responses	Increased IgE-mediated histamine release in mast cells from rodents.	No evidence of enhanced mast cell activation at ambient NO <sub>2</sub> exposure levels.	Inconsistent evidence of IgE-mediated histamine release in mast cells among rodent species (increased in guinea pigs, no effect in rats). Effects may be secondary to tissue damage due to high exposure level.
	Experimental findings for development of Th2 phenotype with short-term NO <sub>2</sub> exposure.	No evidence of Th2 phenotype at ambient NO <sub>2</sub> exposure levels.	Controlled human exposure data may be confounded by reporting bias. No direct evidence of Th2 phenotype in experimental animal data. Effects may be secondary to tissue damage due to high exposure level.
Airway remodeling	Increased airway resistance with AHR in guinea pigs.	No direct evidence of airway remodeling.	Increased airway resistance appears to be transient. Effects may be secondary to tissue damage due to high exposure level.

ISA		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Inflammation	Increases in lymphocytes, PMNs, in rats with long-term exposure.	No evidence of inflammation at ambient NO <sub>2</sub> exposure levels.	No evidence of increased lymphocyte or PMN cells in rats; change in number cannot be determined from proportions. Discordant effects observed between gestational and juvenile exposure group in rats.
	Increases in PMNs in healthy adults with repeated short-term exposure.	Insufficient evidence that inflammation contributes to development of new-onset asthma.	Increase in PMNs observed only at NO <sub>2</sub> concentrations of at least 2,000 ppb. Increase in PMNs not associated with overt inflammatory effects.
	Longitudinal changes in eNO in children independent of asthma status.	Evidence is not sufficient to support long-term NO <sub>2</sub> exposure contributing to pulmonary inflammation.	Potential for residual spatio-temporal confounding not fully addressed. No adjustment for potential confounding by co-pollutants. Substantial uncertainty regarding whether eNO is an accurate marker of pulmonary inflammation.
Oxidative stress	Varying and transient effects on antioxidant levels and enzyme activity.	No evidence of pulmonary oxidative stress at ambient NO <sub>2</sub> levels.	Effects were variable and transient. Effects may be secondary to tissue damage due to high exposure level.

Notes:

AHR = Airway Hyper-responsiveness; BC = Black Carbon; EC = Elemental Carbon; ECRHS = European Community Respiratory Health Study; eNO = Exhaled Nitric Oxide; IgE = Immunoglobulin E; ISA = Integrated Science Assessment; LUR = Land Use Regression; NO<sub>2</sub> = Nitrogen Dioxide; OC = Organic Carbon; PM = Particulate Matter; PMN = Polymorphonuclear Leukocyte; PNC = Particle Number Concentration; ppb = Parts Per Billion; SES = Socioeconomic Status; Th2 = T-Derived Lymphocyte Helper 2.

## 5 Evidence for Other Health Effects Is Inadequate

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EPA evaluates associations between short- and long-term NO<sub>2</sub> exposure and several other endpoints, in addition to the respiratory health effects discussed here in Sections 3 and 4 (see Table 1.1). The second draft ISA concludes that the evidence for short-term NO<sub>2</sub> exposure and both CV effects and total mortality is suggestive, but not sufficient to infer a causal relationship, whereas the first draft ISA concluded that evidence for both indicated a likely causal relationship. With respect to long-term exposure, EPA evaluates CV effects, reproductive and developmental effects, total mortality, and cancer. The second draft ISA concludes that the evidence for an association between NO<sub>2</sub> and fertility, reproduction, and pregnancy, and for postnatal developmental effects, is inadequate, and that the evidence for CV effects, total mortality, cancer, and birth outcomes is suggestive. (In the first draft ISA, evidence for fertility, reproduction, and pregnancy, and postnatal development was considered suggestive.) As discussed below, the evidence for all of these endpoints is inadequate to infer a causal relationship.

### 5.1 Short-term Exposure

#### 5.1.1 Evidence for cardiovascular effects is inadequate

The causal determination regarding short-term NO<sub>2</sub> exposure and CV effects was changed from likely causal in the first draft ISA to suggestive in the second draft ISA. This change is based, in part, on EPA's greater acknowledgement of the potential for confounding by traffic-related pollutants and the difficulty of determining the independent effects of NO<sub>2</sub>, as well as potential exposure measurement error.

The majority of CV effect estimates are small and very close to the null. Given the various methodological limitations in the epidemiology studies, these estimates are more likely the result of bias, confounding, or chance than evidence of a causal relationship between NO<sub>2</sub> and CV health outcomes.

Although the majority of the observed effects were for 0- or 1-day lags, which are biologically plausible, there appeared to be inconsistency among the studies reviewed in reported seasonal patterns of NO<sub>2</sub> effects. Setting aside the inconsistencies among studies, the reported associations between NO<sub>2</sub> and CV hospitalization were stronger in colder conditions, while the reported associations with CV mortality were more pronounced in summer. It is not clear why this would be the case.

It is notable that none of the CV effects examined in the ISA are specific to NO<sub>2</sub>. Each outcome has several other risk factors that are much more likely to contribute to CV effects and that were not fully accounted for in most analyses (Petito Boyce *et al.*, 2015). Given these limitations, evidence regarding the relationship between short-term NO<sub>2</sub> exposure and CV effects is inadequate.

#### 5.1.2 Evidence for total mortality is inadequate

In contrast to the first draft ISA, which concluded that there is likely to be a causal relationship between short-term NO<sub>2</sub> exposure and total mortality, the second draft ISA concludes that the evidence for such a relationship is only suggestive. This classification is consistent with the conclusion of the 2008 ISA (US EPA, 2008) and acknowledges key uncertainties and data gaps. Specifically, as indicated in the second draft ISA, the body of evidence does not include co-pollutant model analyses that focus on traffic-related

pollutants, and the independent association between NO<sub>2</sub> exposure and total mortality is unknown (US EPA, 2015). Given this critical data gap and the uncertainties in the body of literature (discussed below), the evidence for an association between short-term NO<sub>2</sub> exposure and total mortality should be classified as inadequate rather than suggestive.

All the studies that evaluated total mortality estimated city-level NO<sub>2</sub> concentrations based on measurements at a single central monitor or a small network air monitors (US EPA, 2015). This method likely resulted in substantial measurement error because air monitors generally measure background ambient NO<sub>2</sub> levels and do not account for emissions from traffic or industrial sources.

The recent multi-city studies evaluated in the ISA conducted time-series analyses, except for Chiusolo *et al.* (2011). Time-series analyses are ecological in nature, and, in these analyses, causal inference at the individual level is often subject to ecological bias. In contrast, Chiusolo *et al.* (2011) employed a case-crossover study design, which allows causal inference at the individual level, but the authors used a time-stratified approach, in which the study period was divided into monthly strata, and the controls days (days on which a person is at risk) were chosen in the stratum of the case day (the day of death) and matched on day of the week. Such an approach can lead to biased results because a case cannot be exposed after he or she is deceased. Chiusolo *et al.* (2011) also relied on city-level NO<sub>2</sub> concentrations, and the study results were likely impacted by measurement error of covariates (*e.g.*, meteorological factors). For example, temperature was often measured at a single location that did not accurately reflect the area-level temperature or account for individual exposure to any heat islands (Bukowski, 2008).

Epidemiology studies have generally reported significant overall excess risks of total mortality, but there are inconsistencies both within and across studies. Considerable heterogeneity was present in reported city-specific effect estimates in all the multi-city studies reviewed in the ISA (Bellini *et al.*, 2007; Berglind *et al.*, 2009; Wong *et al.*, 2008; Wong, 2010), except for one (Chen *et al.*, 2012). Bellini *et al.* (2007) reported that the association between NO<sub>2</sub> and total mortality was null or negative in 10 out of 15 Italian cities examined; the overall excess risk was driven by five positive city-specific estimates. Berglind *et al.* (2009) found that the association between NO<sub>2</sub> and mortality was null in three out of five European cities examined; the overall excess risk was driven by the estimates for Augsburg, Germany and Barcelona, Spain. Wong *et al.* (2008) and Wong (2010) reported consistent positive associations in four Asian cities using single-pollutant models. The city-specific associations remained robust against co-pollutants, except for Bangkok, Thailand, for which the association became null when adjusted for PM<sub>10</sub>.

The majority of the observed effects occurred within a day, which is biologically plausible. In addition, the ISA proposes that the CV and respiratory effects of NO<sub>2</sub> may lead to CV and respiratory mortality, which accounts for over 40% of total mortality, also providing support for biological plausibility. However, as noted in the ISA, uncertainties remain about the "biological plausibility for NO<sub>2</sub>-related cardiovascular mortality," and "the biological mechanism that explains the continuum of effects that could lead to respiratory-related mortality also remains unclear" (US EPA, 2015). These uncertainties indicate that the evidence for total mortality may not support causality.

It is difficult to assess the biological gradient of any effects of NO<sub>2</sub> on mortality because most of the studies reviewed in the ISA assumed response linearity and reported effect estimates associated with increments in NO<sub>2</sub> concentrations. Four studies presented exposure-response curves (Moolgavkar *et al.*, 2013; Chen *et al.*, 2012; Wong *et al.*, 2008; Wong, 2010). For studies that were conducted in Asia (Chen *et al.*, 2012; Wong *et al.*, 2008; Wong, 2010), linear or J-shaped exposure-response curves were presented with mortality outcomes at concentrations exceeding the current NAAQS. Moolgavkar *et al.* (2013), who studied 72 cities in the US, found an association between NO<sub>2</sub> and total mortality based on a single-pollutant model. They found that the exposure-response curve may have been exaggerated because the point estimate for NO<sub>2</sub> decreased by 40% when adjusted for co-pollutants. Finally, the association

between NO<sub>2</sub> and total mortality in Berglind *et al.* (2009) was null in the two cities with the lowest ambient NO<sub>2</sub> levels (Helsinki, Finland and Stockholm, Sweden), and the one city with the highest ambient NO<sub>2</sub> levels (Rome, Italy).

Overall, the ISA's conclusion that the relationship between short-term NO<sub>2</sub> exposure and total mortality is suggestive is not supported by the evidence.

## **5.2 Long-term Exposure**

### **5.2.1 Evidence for cardiovascular effects is inadequate**

The ISA states that the WoE regarding associations between long-term NO<sub>2</sub> exposure and CV effects is suggestive, but not sufficient to infer a causal relationship. The ISA highlights several studies that reported statistically significant associations with various CV endpoints but indicates that "there is uncertainty regarding the extent to which findings can be explained by noise or copollutant exposure" (US EPA, 2015).

As the ISA discusses, several studies (*e.g.*, Weitzberg and Lungberg, 2013; Yadav *et al.*, 2011) suggest that nitrate, the primary metabolite of NO<sub>2</sub> formed in the respiratory tract, may have a protective effect against CV outcomes. The ISA also concludes that there is limited toxicology evidence and limited evidence for key events to inform an MoA. The ISA should discuss whether studies indicating no effect are of higher quality than those that reported associations (particularly in light of the limited toxicology evidence) to determine whether the WoE regarding causation qualifies as suggestive or if the evidence is inadequate to infer a causal relationship.

### **5.2.2 Evidence for reproductive and developmental effects is inadequate**

The ISA distinguishes between three subcategories of reproductive and developmental health effects – fertility, reproduction, and pregnancy; postnatal development; and birth outcomes – and makes separate causal determinations for each. Unlike the first draft (which concluded evidence was suggestive for all three categories), the second draft ISA concludes that new data since the 2008 ISA (US EPA, 2008) does not change the balance of the WoE regarding causation for two of these subcategories. It also no longer draws conclusions about broad reproductive and developmental categories based on one endpoint each (*e.g.*, fertility, low birth weight, gestational age). Rather, the ISA concludes that the evidence for an association between NO<sub>2</sub> exposure and fertility, reproduction, and pregnancy, as well as for postnatal development effects, is inadequate.

The evidence for birth outcomes has the same limitations and inconsistencies that the ISA notes for the other reproductive and developmental effects evidence evaluated, and, therefore, it should also be classified as inadequate. For example, the ISA states that "the biological mechanisms by which air pollutants may influence the developing fetus remain largely unknown" (US EPA, 2015). In addition to the lack of a confirmed MoA, the ISA discusses the inconsistent epidemiology evidence for the birth outcomes assessed, including birth weight and pre-term birth. As with the ISA's revised classification for the other subcategories of reproductive outcomes, the lack of consistent evidence and the lack of evidence of biological plausibility indicate that the evidence is inadequate.

### 5.2.3 Evidence for total mortality is inadequate

The ISA indicates that there are several epidemiology studies that reported positive associations between long-term NO<sub>2</sub> exposure and total mortality, but there are no such associations in several large prospective cohorts, such as the California Seventh-day Adventists (Adventist Health and Smog [AHSMOG]) cohort, the American Cancer Society (ACS) cohort, the California Teachers cohort, the Nurses' Health Study (NHS) cohort, and the multicentre European Study of Cohorts for Air Pollution Effects (ESCAPE) cohort. There is also limited coherence between respiratory and CV morbidity endpoints in these studies, indicating that increased mortality may not be biologically plausible. The majority of mortality studies included in the ISA suffered from several common methodological limitations. Control for key confounders (*e.g.*, smoking, physical activity, and socioeconomic status [SES]) was generally inadequate or incomplete. Geographic Information System (GIS)-facilitated modeling was often employed to interpolate individual exposure to NO<sub>2</sub>, but the accuracy and validity of the models were seldom addressed. Confounding effects of co-pollutants also contributed to the uncertainty of the results, as some observed associations between NO<sub>2</sub> and total mortality became null when adjusted for co-pollutants. Exposure estimates based on stationary air monitor measurements and residential addresses usually did not account for indoor exposure or exposure while commuting. Loss to follow-up was also substantial in some cohort studies. These methodological issues have considerable impact on the validity of the findings but are not fully considered in the ISA.

The ISA should have evaluated the evidence across studies, particularly those evaluating the ACS and ESCAPE cohorts, to determine whether the reported associations are more likely to be indicative of causation or are attributable to other factors (*e.g.*, confounding, bias). This evaluation should have been based on a review of all the relevant data, including other epidemiology, toxicology, and MoA studies. Because the ISA does not show that it is more likely than not that the positive associations between long-term NO<sub>2</sub> exposure and total mortality are indicative of causation, the ISA should conclude that the evidence is inadequate to infer causation.

### 5.2.4 Evidence for cancer is inadequate

The ISA indicates that the available evidence is suggestive of a causal relationship between long-term NO<sub>2</sub> exposure and cancer, based primarily on associations between ambient NO<sub>x</sub> and NO<sub>2</sub> concentrations and lung cancer incidence and mortality in some previous and recent high-quality epidemiology studies, but not in other studies of comparable quality. However, the epidemiology studies considered to be of high quality in the ISA were not without limitations. For example, smoking and SES were not (or were not adequately) adjusted for in statistical analyses in these studies.

The ISA also indicates that the evidence regarding the biological plausibility of a causal relationship with cancer is limited and that "toxicological data provide no clear evidence of NO<sub>2</sub> acting as a complete carcinogen" (US EPA, 2015). Although lung tumor promotion and hyperplasia of lung epithelial cells with NO<sub>2</sub> exposure has been reported, often at high concentrations, there is no evidence for direct carcinogenicity. Because the evidence is not consistent and there is uncertainty due to limited biological plausibility, the ISA should conclude that the evidence is inadequate to infer causation with regard to long-term NO<sub>2</sub> exposure and cancer.

## 6 EPA's Conclusions Regarding "At-risk" Factors Are Not Generally Supported by the Evidence

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Chapter 7 of the ISA evaluates what it calls "at-risk" factors. The ISA defines these factors as characteristics that may increase a person's risk of adverse health effects from exposure to NO<sub>2</sub> (US EPA, 2015). These characteristics include both intrinsic (*e.g.*, genetic or developmental factors, race, sex, lifestage, pre-existing diseases) and extrinsic, non-biological factors (*e.g.*, SES, occupation). The ISA used the framework outlined above in Table 2.4 to evaluate the WoE for potential "at risk" factors.

It is unclear why the ISA does not use the same framework it uses for causal determination to evaluate at-risk factors. Although the former is an assessment of direct causation and the latter an assessment of factors that can contribute to (or prevent) causation from NO<sub>2</sub>, in both cases, the goal of the ISA is to critically, systematically, and transparently review the weight of scientific evidence. The same rules should be applied for both types of analysis; if not, there needs to be justification for using different sets of rules. The ISA should adopt the IOM-recommended (2008) categories for the level of evidence for causation (*i.e.*, sufficient, equipoise and above, below equipoise, against) and use the criteria outlined in Table 5-1 of the ISA to consider whether the WoE indicates that an association is more likely than not.

Despite the two different frameworks, the issues we identified with respect to EPA's WoE evaluation for causal determination (discussed in Section 2 of this report) also apply to the risk factor classifications. Specifically, there is no indication that EPA's evaluation was systematic and was conducted so as to ensure that all studies were evaluated in the same manner. Study quality and relevance are not discussed, despite the condition that adequate evidence includes "multiple high-quality studies." Rather, Chapter 7 of the ISA briefly summarizes study results and makes classifications regardless of study quality and the WoE from which the classification of at-risk factors were derived. Even if a study is discussed in another part of the ISA, a critical review that includes an evaluation of study quality and its impact on the interpretation of results should be considered in EPA's evaluation of at-risk factors.

The ISA concludes that there is suggestive evidence of an increased risk for NO<sub>2</sub>-related health effects among individuals with a low SES, women, and people with reduced antioxidant intake (US EPA, 2015). EPA's requirement for evidence to be suggestive is not based on a robust, systematic review of the evidence; in fact, inconsistency or a lack of coherence is deemed acceptable for evidence to be classified as such. Without high-quality studies with consistent and coherent results, the evidence that these populations have an increased risk for NO<sub>2</sub>-related health effects should be classified as inadequate.

Consistent with the 2008 ISA, the current ISA concludes that there is adequate evidence that people with asthma, children, and older adults are at an increased risk for NO<sub>2</sub>-related health effects (US EPA, 2015). The evidence for an increased risk among these three sub-populations does not meet the criteria for adequate evidence, as outlined in Table 2.4 above. The comments that follow address the uncertainties and inconsistencies in the body of evidence that evaluates NO<sub>2</sub>-related risk among people with asthma, children, and older adults.

## 6.1 Evidence does not indicate that individuals with asthma are at increased risk at ambient NO<sub>2</sub> concentrations

In contrast to the first draft ISA, which concluded that the evidence for individuals with asthma having an increased risk of experiencing NO<sub>2</sub>-related respiratory effects was suggestive, the second draft concludes that the evidence is adequate. It does not appear that the ISA's evaluation differs between the two drafts, only the conclusion has changed. The ISA states that the findings from epidemiology studies are variable, and it relies on what it says is compelling evidence from human controlled exposure studies to conclude that individuals with asthma are at an increased risk for NO<sub>2</sub>-related respiratory health effects.

Although the evidence from controlled exposure studies demonstrates that individuals with asthma may be more sensitive to NO<sub>2</sub>-associated AHR than people without asthma (*e.g.*, Folinsbee, 1992), these effects were observed only at NO<sub>2</sub> concentrations of 300 ppb and greater. There is no evidence regarding whether NO<sub>2</sub> would exacerbate asthma at relevant ambient concentrations.

## 6.2 Evidence does not indicate that children are at increased risk

The ISA suggests that children may have an increased risk of NO<sub>2</sub>-related health effects because the human respiratory system is not fully developed until 18-20 years of age, and because both higher ventilation rates and child-specific time-activity patterns can result in a higher inhaled dose of NO<sub>2</sub> in children *versus* adults (US EPA, 2015). As discussed below, the evidence for an increased risk during this lifestage is not supported by the available evidence and is not coherent across disciplines, which is required to meet the EPA classification of adequate evidence.

According to the ISA, there is consistent evidence from epidemiology studies demonstrating that increases in short-term NO<sub>2</sub> exposure have a greater effect on asthma-related HAs, ED visits, and outpatient visits in children *versus* adults (US EPA, 2015). As discussed in Section 3 of this report, the ISA's evaluation of evidence for HA and ED visits associated with short-term NO<sub>2</sub> exposure is not rigorous or balanced and does not fully address the limitations of the cited studies. In Table 7-15, the ISA cites four recent studies that evaluated these outcomes and provide evidence of effect modification in children (Ko *et al.*, 2007; Son *et al.*, 2013; Villeneuve *et al.*, 2007; Samoli *et al.*, 2011). Only Villeneuve *et al.* (2007) and Samoli *et al.* (2011) stratified by cases age in a multi-pollutant model. Carbon monoxide (CO) and NO<sub>2</sub> (as 5-day averages) were the only pollutants included in a two-pollutant model by Villeneuve *et al.* (2007); other traffic-related pollutants, such as PM<sub>2.5</sub> or elemental or black carbon, were not considered. Samoli *et al.* (2011) found no association between a 5-ppb<sup>8</sup> increase in NO<sub>2</sub> and asthma-related HAs in children 14 years and younger (lag 0) in co-pollutant models with PM<sub>10</sub>, SO<sub>2</sub>, and O<sub>3</sub>. Further, when children were stratified into the two age groups the ISA cited in Table 7-13 (0-4 years old and 5-14 years old), the percentage increase in asthma-related HAs was not significantly associated with increasing NO<sub>2</sub> concentrations in either age group in single-pollutant models (Samoli *et al.*, 2011).

The ISA acknowledges that the toxicology evidence suggests that adult animals may actually have a greater response to NO<sub>2</sub> exposure compared to juvenile animals. As shown in Table 7-14 of the ISA, a smaller effect on both mortality and lung injury and inflammation was observed in juvenile *versus* adult animals following exposure to 2,000 ppb NO<sub>2</sub>. The ISA dismisses this incompatibility and concludes that the overall body of evidence indicates increased sensitivity for children, because the specific endpoints examined "are not directly related to asthma and are not considered to contradict epidemiologic evidence" (US EPA, 2015). The ISA's conclusion that these endpoints are not relevant is inconsistent with the its

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<sup>8</sup> The authors presented results as 10 µg/m<sup>3</sup> NO<sub>2</sub>; 5 ppb is based on the conversion factor 1 ppb NO<sub>2</sub> = 1.88 µg/m<sup>3</sup> NO<sub>2</sub>.

review of the toxicology evidence for respiratory health effects. As discussed in Sections 3 and 4 in this report, the ISA suggests that inflammation is part of the MoA for both asthma exacerbation and development and cites evidence of NO<sub>2</sub>-induced inflammation to support its causal and likely causal determinations, respectively, for these endpoints. It is not clear why the ISA is not consistent in how it considers this evidence.

According to the criteria established by EPA (see Table 2.4 in this report), adequate evidence that specific sub-populations are at a greater risk for NO<sub>2</sub>-related respiratory health effects requires substantial and consistent evidence from within a discipline and multiple high-quality studies. The ISA provides no discussion of how it reviewed and weighted the epidemiology studies for children included in Table 7-13 (US EPA, 2015). In light of the epidemiology studies' limitations and the lack of supporting evidence from the available toxicity studies, the evidence for a potential increased risk among children does not meet EPA's criteria for adequate evidence.

### 6.3 Evidence does not indicate that older adults are at increased risk

The ISA concludes that there is adequate evidence for an increased risk of NO<sub>2</sub>-related respiratory health effects in older (*versus* younger) adults based on epidemiology studies that evaluated asthma and respiratory-related HAs and ED visits, and that uncertainties about the independent effects of NO<sub>2</sub> prevent inferences about increased risks of total mortality and CV effects in older adults. In fact, the evidence that older adults are at a greater risk for NO<sub>2</sub>-related respiratory effects is not consistent across epidemiology and controlled exposure studies. In addition, as with the other at-risk factors evaluated, the ISA does not provide any description of which studies it considers high-quality, despite determining that adequate evidence for a risk factor "includes multiple high-quality studies" (US EPA, 2015). Given the limitations in the body of evidence and the framework used to evaluate it, the ISA should not have concluded that there is adequate evidence that older adults are at an increased risk for NO<sub>2</sub>-related health effects.

Although there is some evidence from short-term epidemiology studies that compared respiratory-related HAs and ED visits in older adults (*e.g.*, over 65 years of age) to those events in younger adults that suggests an increased risk for older adults (Table 7-15 of the ISA; US EPA, 2015), many of the observed effects were small and were reported in studies with methodological limitations (discussed in Section 3 of this report). In Ko *et al.* (2007), for example, the relative risk (RR) for hospitalization due to acute exacerbation per 5-ppb<sup>8</sup> increase in NO<sub>2</sub> concentration for participants over 65 years of age was 1.023 (95% CI: 1.014-1.033), while the RR for younger adults (between 15 and 65 years of age) was 1.018 (95% CI: 1.007-1.029). The CIs around these RRs overlap, indicating that they are not statistically different. In addition, the authors found significant correlations between NO<sub>2</sub> and SO<sub>2</sub>, O<sub>3</sub>, PM<sub>10</sub>, and PM<sub>2.5</sub>, but they did not stratify by age in the multi-pollutant model, making it impossible to assess whether NO<sub>2</sub> had different effects in different age groups.

The conclusion that there is adequate evidence of an increased risk for older adults is also not supported by evidence from controlled exposure studies. As outlined in Table 7-16 of the ISA, neither of the controlled exposure studies reviewed found statistically significant respiratory effects in healthy older adults following NO<sub>2</sub> exposure *versus* exposure to clean air (US EPA, 2015).

The lack of coherence between controlled exposure and epidemiology studies that assessed the potential sensitivity of older adults to NO<sub>2</sub>-related health effects, particularly considering the methodological limitations of the epidemiology studies, indicates that the evidence does not meet EPA's criteria for classifying it as adequate (see Table 2.4 in this report). Until study limitations and the lack of coherence

are addressed, the body of evidence that evaluates the effects of NO<sub>2</sub> exposure for older adults cannot be classified as adequate.

## **6.4 Conclusion**

The ISA's determination that there is adequate evidence that individuals with asthma, children, and older adults are at a greater risk of NO<sub>2</sub>-related health effects compared to the general population is not based on a systematic, comprehensive review that considered study quality and relevance. The epidemiology studies of these populations have major methodological limitations, and there is no coherence across disciplines to meet EPA's criteria for adequate evidence for an increased risk of NO<sub>2</sub>-related effects in these populations. For these reasons, the evidence for NO<sub>2</sub>-related health effects in children and older adults should be classified as inadequate. Although there is sufficient evidence to suggest that asthma is an at-risk factor at higher concentrations of NO<sub>2</sub>, the evidence for an increased risk of asthma at lower, ambient concentrations is inadequate.

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# Appendix A

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## **EPA Summary and Description of Scientific Considerations for Evaluating the Quality of Studies on the Health Effects of Oxides of Nitrogen**

**Table 5-1 Summary and description of scientific considerations for evaluating the quality of studies on the health effects of oxides of nitrogen.**

NOTE: Study aspects of interest are reported in gray boxes (e.g., Study Design). Summary bullets are provided in the top sections and are described in more detail in the text immediately following.

Study Design		
Controlled Human Exposure	Animal Toxicology	Epidemiology
<ul style="list-style-type: none"> <li>Clearly defined hypotheses/aims</li> <li>Appropriately matched control exposures</li> <li>Randomization and allocation concealment</li> <li>Balanced crossover (repeated measures) or parallel design studies</li> </ul>	<ul style="list-style-type: none"> <li>Clearly defined hypotheses/aims</li> <li>Appropriately matched control exposures</li> <li>Randomization and allocation concealment</li> <li>All groups handled and cared for equally</li> </ul>	<ul style="list-style-type: none"> <li>Clearly defined hypotheses/aims</li> <li>Key designs for short-term exposure: time series, case crossover, panel</li> <li>Key designs for long-term exposure: prospective cohort, nested case-control</li> <li>High power studies key: large sample sizes, multiple years, multicity studies</li> </ul>

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

<b>Controlled Human Exposure:</b>		
<p>Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Study subjects should be randomly exposed without knowledge of the exposure condition. Preference is given to balanced crossover (repeated measures) or parallel design studies which include control exposures (e.g., to clean filtered air). In crossover studies, a sufficient and specified time between exposure days should be provided to avoid carry over effects from prior exposure days. In parallel design studies, all arms should be matched for individual characteristics such as age, sex, race, anthropometric properties, and health status. Similarly, in studies evaluating effects of disease, appropriately matched healthy controls are desired for interpretative purposes.</p>		
<b>Animal Toxicology:</b>		
<p>Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Studies should include appropriately matched control exposures (e.g., to clean filtered air, time matched). Studies should use methods to limit differences in baseline characteristics of control and exposure groups. Studies should randomize assignment to exposure groups and where possible conceal allocation to research personnel. Groups should be subjected to identical experimental procedures and conditions and care of animals, including housing, husbandry, etc. Blinding of research personnel to study group may not be possible due to animal welfare and experimental considerations; however, differences in the monitoring or handling of animals in all groups by research personnel should be minimized.</p>		
<b>Epidemiology:</b>		
<p>Studies should clearly describe the primary and any secondary aims of the study, or specific hypotheses being tested.</p> <p>For short-term exposure, time-series, case crossover, and panel studies are emphasized over cross-sectional studies because they examine temporal correlations and are less prone to confounding by factors that differ between individuals (e.g., SES, age). Studies with large sample sizes and conducted over multiple years are considered to produce more reliable results. If other quality parameters are equal, multicity studies carry more weight than single-city studies because they tend to have larger sample sizes and lower potential for publication bias.</p> <p>For long-term exposure, inference is considered to be stronger for prospective cohort studies and case-control studies nested within a cohort (e.g., for rare diseases) than cross-sectional, other case-control, or ecologic studies. Cohort studies can better inform the temporality of exposure and effect. Other designs can have uncertainty also related to the appropriateness of the control group or validity of inference about individuals from group-level data. Study design limitations can bias health effect associations in either direction.</p>		
<b>Study Population/Test Model</b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>• Similarly matched control and exposed subjects</li> <li>• Subject characteristics reported</li> <li>• Clearly indicated inclusion and exclusion criteria</li> <li>• Independent, clinical assessment of the health condition</li> <li>• Loss or withdrawal of subjects should be reported with rationale</li> </ul>	<ul style="list-style-type: none"> <li>• Animal characteristics reported</li> <li>• Studies testing and reporting both sexes and multiple life stages preferred</li> <li>• Loss or exclusion of animals should be reported with rationale</li> </ul>	<ul style="list-style-type: none"> <li>• Representative of population of interest</li> <li>• High participation and low drop-out over time that is not dependent on exposure or health status</li> <li>• Clearly indicated inclusion and exclusion criteria</li> <li>• Independent, clinical assessment of health condition</li> <li>• Groups are compared if from same source population</li> </ul>

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

<b>Controlled Human Exposure:</b>		
<p>In general, the subjects recruited into study groups should be similarly matched for age, sex, race, anthropometric properties, and health status. In studies evaluating effects of specific subject characteristics (e.g., disease, genetic polymorphism, etc.), appropriately matched healthy controls are preferred. Relevant characteristics and health status should be reported for each experimental group. Criteria for including and excluding subjects should be clearly indicated. For the examination of populations with an underlying health condition (e.g., asthma), independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory diseases and history of cardiovascular events.<sup>a</sup> The loss or withdrawal of recruited subjects during the course of a study should be reported. Specific rationale for excluding subject(s) from any portion of a protocol should be explained.</p>		
<b>Animal Toxicology:</b>		
<p>Ideally, studies should report species, strain, sub-strain, genetic background, age, sex, and weight. However, differences in these parameters across studies do not make the studies incomparable. Unless data indicate otherwise, all animal species and strains are considered appropriate for evaluating effects of NO<sub>2</sub> or NO exposure. It is preferred that the authors test for effects in both sexes and multiple life stages, and report the result for each group separately. All animals used in a study should be accounted for, and rationale for exclusion of animals or data should be specified.</p>		
<b>Epidemiology:</b>		
<p>The ideal study population is recruited from and is representative of the target population. Studies with high participation and low drop-out over time that is not dependent on exposure or health status are considered to have low potential for selection bias. Criteria for including and excluding subjects should be clearly indicated. For populations with an underlying health condition, independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory diseases and history of cardiovascular events.<sup>a</sup> Groups with and without an underlying health condition should be compared if they are from the same source population. Selection bias can influence results in either direction or may not affect the validity of results but rather reduce the generalizability of findings to the target population.</p>		
<b>Pollutant</b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>• Studies of NO<sub>2</sub> are emphasized</li> </ul>	<ul style="list-style-type: none"> <li>• Studies of NO<sub>2</sub> are emphasized</li> </ul>	<ul style="list-style-type: none"> <li>• NO<sub>2</sub> emphasized over NO, NO<sub>x</sub></li> <li>• Comparisons of health effect associations among gaseous oxides of nitrogen species ideal</li> </ul>
<b>Controlled Human Exposure:</b>		
<p>The focus is on studies testing NO<sub>2</sub> exposure.</p>		
<b>Animal Toxicology:</b>		
<p>The focus is on studies testing NO<sub>2</sub> exposure.</p>		
<b>Epidemiology:</b>		
<p>Health effects are evaluated mostly for NO<sub>2</sub>, and less so for NO or NO<sub>x</sub>. Studies that compare health effect associations among these species are informative. Typically, one species is examined, and studies of NO<sub>2</sub> are emphasized. It is not clear that ambient-relevant NO exposures induce negative health effects (<a href="#">Section 4.2.3</a>). The relationship of NO<sub>x</sub> to NO<sub>2</sub> varies with distance from roads, and thus, may vary among subjects. Hence, there is uncertainty about the extent to which associations with NO<sub>x</sub> reflect those for NO<sub>2</sub> vs. other pollutants from traffic.</p>		

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

Exposure Assessment or Assignment		
Controlled Human Exposure	Animal Toxicology	Epidemiology
<ul style="list-style-type: none"> <li>Well characterized and reported exposure conditions</li> <li>Limited to studies that utilize NO<sub>2</sub> and/or NO concentrations less than or equal to 5,000 ppb</li> <li>Preference is given to studies that include exposure control groups</li> <li>Randomized exposure groups</li> </ul>	<ul style="list-style-type: none"> <li>Well characterized and reported exposure conditions</li> <li>Inhalation exposure</li> <li>Limited to studies that utilize NO<sub>2</sub> and/or NO concentrations less than or equal to 5,000 ppb</li> <li>All studies should include exposure control groups</li> <li>Randomized exposure groups</li> </ul>	<ul style="list-style-type: none"> <li>Exposure metrics that accurately represent temporal or spatial variability for study area</li> <li>Comparisons of exposure measurement methods</li> <li>Indoor and total personal exposures can inform independent effects of NO<sub>2</sub></li> <li>Lag/duration of exposure metric correspond with time course for health effect</li> </ul>
<b>Controlled Human Exposure:</b>		
<p>Studies should well characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions for subject safety. For this assessment, the focus is on studies that utilize NO<sub>2</sub> and/or NO concentrations less than or equal to 5,000 ppb (<a href="#">Section 1.2</a>). Studies that utilize higher exposure concentrations may provide information relevant to MOA, dosimetry, or at-risk human populations. Preference is given to balanced crossover or parallel design studies which include control exposures (e.g., to clean filtered air). Study subjects should be randomly exposed without knowledge of the exposure condition. Method of exposure (e.g., chamber, facemask, etc.) should be specified and activity level of subjects during exposures should be well characterized.</p>		
<b>Animal Toxicology:</b>		
<p>Studies should characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. The focus is on inhalation exposure. Non-inhalation exposure experiments may provide information relevant to MOA. In vitro studies may be included if they provide mechanistic insight or examine similar effects as in vivo, but are generally not included. All studies should include exposure control groups (e.g., clean filtered air). For this assessment, the focus will be on studies that utilize NO<sub>2</sub> and/or NO concentrations less than or equal to 5,000 ppb (<a href="#">Section 1.2</a>). Studies that utilize higher exposure concentrations may provide information relevant to MOA, dosimetry, interspecies variation, or at-risk human populations.</p>		

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

Epidemiology:		
<p>Of primary relevance are relationships of health effects with the ambient component of exposure to oxides of nitrogen. However, information about ambient exposure rarely is available for individual subjects; most often, inference is based on ambient concentrations. Studies that compare exposure assessment methods are considered to be particularly informative. The duration or lag of the exposure metric should correspond with the time course for physiological changes in the outcome (e.g., up to a few days for symptoms) or latency of disease (e.g., several years for cancer).</p> <p>Given the spatial heterogeneity in ambient oxides of nitrogen and variable relationships between personal exposures and ambient concentrations (<a href="#">Section 3.4.3</a>), validated methods that capture the extent of variability for the particular study design (temporal vs. spatial contrasts) and location carry greater weight. Central site measurements, whether averaged across multiple monitors or assigned from the nearest or single available monitor, have well-recognized limitations in capturing spatial variation in oxides of nitrogen. Inference from central site measurements can be adequate if correlated with personal exposures, closely located to study subjects, highly correlated across monitors within a location, used in locations with well-distributed sources, or combined with time-activity information.</p> <p>In studies of short-term exposure, metrics that may capture variation in ambient oxides of nitrogen and strengthen inference include concentrations in subjects' microenvironments (e.g., outdoor home, school, in-vehicle) and individual-level outdoor concentrations combined with time-activity data. Results for total personal and indoor NO<sub>2</sub> exposure are other lines of evidence that inform judgments about causality of NO<sub>2</sub> because inference is based on an individual's microenvironmental exposures and potential for copollutant confounding may be lower or different than that for ambient concentrations. Results for total personal exposure can inform the effects of ambient exposure when well correlated with ambient concentrations. For long-term exposures, LUR models that well represent spatial variation in ambient NO<sub>2</sub> can provide estimates of individual exposure. Less weight is placed on NO<sub>x</sub> from dispersion models because of limitations in accurate estimation of within-community conditions (<a href="#">Section 3.2.1.2</a>). And because NO<sub>x</sub> from dispersion models often shows near perfect correlations (<math>r = 0.94-0.99</math>) with EC, PM<sub>2.5</sub>, and CO, the effects of NO<sub>x</sub> cannot be distinguished from traffic-related copollutants.</p> <p>Exposure measurement error often attenuates health effect estimates or increases the precision of the association (i.e., width of 95% CIs), particularly associations based on temporal variation in short-term exposure (<a href="#">Section 3.4.5.1</a>). However, exposure measurement error can bias estimates away from the null, particularly for long-term exposures.</p>		
Outcome Assessment/Evaluation		
Controlled Human Exposure	Animal Toxicology	Epidemiology
<ul style="list-style-type: none"> <li>• Same manner of outcome assessment for all groups</li> <li>• Validated, reliable methods</li> <li>• Reporting of outcome assessment details</li> <li>• Blinding of endpoint evaluators</li> <li>• Appropriate timing of endpoint evaluation</li> </ul>	<ul style="list-style-type: none"> <li>• Same manner of outcome assessment for all groups</li> <li>• Validated, reliable methods</li> <li>• Reporting of outcome assessment details</li> <li>• Blinding of endpoint evaluators</li> <li>• Appropriate timing of endpoint evaluation</li> </ul>	<ul style="list-style-type: none"> <li>• Same manner of outcome assessment for all groups</li> <li>• Validated, reliable methods</li> <li>• Assessment is blind to exposure status</li> <li>• Appropriate timing of endpoint evaluation</li> </ul>
Controlled Human Exposure:		
<p>Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on the endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.</p>		
Animal Toxicology:		
<p>Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on the endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.</p>		

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

<b>Epidemiology:</b>		
<p>Outcomes should be assessed or reported without knowledge of exposure status. Such bias could produce artifactual associations. Outcomes assessed by interview, self-report, clinical examination, or analysis of biological indicators should be defined by consistent criteria and collected by validated, reliable methods. Independent, clinical assessment is ideal for outcomes such as lung function or incidence of disease, but report of physician diagnosis has shown good reliability.<sup>a</sup> Outcomes should be assessed at time intervals that correspond with the time course for physiological changes (e.g., up to a few days for symptoms). When health effects of long-term exposure are assessed by acute events such as symptoms or hospital admissions, inference is strengthened when results are adjusted for short-term exposure. Validated questionnaires for subjective outcomes such as symptoms are regarded to be reliable,<sup>b</sup> particularly when collected frequently and not subject to long recall. For biological samples, the stability of the compound of interest and the sensitivity and precision of the analytical method is considered. If not based on knowledge of exposure status, errors in outcome assessment tend to bias results toward the null.</p>		
<b>Potential Copollutant Confounding</b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>Well-characterized exposure</li> </ul>	<ul style="list-style-type: none"> <li>Well-characterized exposure</li> </ul>	<ul style="list-style-type: none"> <li>Traffic-related copollutants are key: CO, PM<sub>2.5</sub>, BC/EC, OC, UFP, metal PM components, VOCs</li> <li>Also considered: PM<sub>10</sub>, SO<sub>2</sub>, O<sub>3</sub></li> </ul>
<b>Controlled Human Exposure:</b>		
Exposure should be well characterized to evaluate independent effects of NO <sub>2</sub> .		
<b>Animal Toxicology:</b>		
Exposure should be well characterized to evaluate independent effects of NO <sub>2</sub> .		
<b>Epidemiology:</b>		
<p>Not accounting for copollutant confounding can produce artifactual associations; thus, studies that examine copollutant confounding carry greater weight. The predominant method is copollutant modeling, which is especially informative when measurement error is comparable for copollutants and correlations are not high. Interaction and joint effect models are examined to a lesser extent. Copollutant confounding also can be informed by evaluating correlations between oxides of nitrogen and copollutants and comparing health associations between gaseous oxides of nitrogen and copollutants in single-pollutant models if exposure measurement error is comparable among pollutants. Studies that examine only gaseous oxides of nitrogen are considered poorly to inform the potential for copollutant confounding. Copollutant confounding is evaluated based on the extent of their correlations typically observed with oxides of nitrogen and relationships observed with health effects.</p> <p>Among copollutants, of primary concern are traffic-related pollutants, which include CO, PM<sub>2.5</sub>, BC/EC, OC, UFP, metal PM components such as copper, zinc, and iron, as well as VOCs such as benzene, acetaldehyde, toluene, ethylbenzene, and xylene. Short-term and long-term metrics for these pollutants consistently show moderate to high correlations with oxides of nitrogen (Figure 3-6). Many traffic-related pollutants also are characterized to have common modes of action.<sup>c</sup> Common key events include formation of secondary oxidation products, inflammation, and for respiratory effects, increases in airway responsiveness. They also show relationships with many of the health effects evaluated in this ISA<sup>d</sup> except as follows. For long-term exposure, there is uncertainty regarding confounding by UFP because of their short atmospheric lifetime. Also for long-term exposure, CO is not considered to be an important confounding copollutant for mortality or lung cancer.<sup>d</sup></p> <p>Of less concern is confounding by PM<sub>10</sub>, SO<sub>2</sub>, and O<sub>3</sub> because they show varying and often lower correlations with NO<sub>2</sub> (Figure 3-6). O<sub>3</sub> generally is negatively or weakly positively correlated with NO<sub>2</sub> but may be a confounding copollutant where moderate positive correlations are observed. O<sub>3</sub> and SO<sub>2</sub> in particular show similarities with NO<sub>2</sub> in mode of action. PM<sub>10</sub>, SO<sub>2</sub>, and O<sub>3</sub> show relationships with the health effect evaluated in this ISA<sup>d</sup> except as follows. For short-term exposure, SO<sub>2</sub> is not considered to be a strong confounding copollutant for cardiovascular effects. For long-term exposure, neither O<sub>3</sub> nor SO<sub>2</sub> is considered to be a strong confounding copollutant.</p>		

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

<b>Other Potential Confounding Factors<sup>e</sup></b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>• Preference given to studies with adequate control of factors influencing health response</li> </ul>	<ul style="list-style-type: none"> <li>• Preference given to studies with adequate control of factors influencing health response</li> </ul>	<ul style="list-style-type: none"> <li>• Potential confounders related to health effect and correlated with oxides of nitrogen should be examined</li> <li>• Potential confounders vary by study design (temporally vs. spatially correlated) and by health effects</li> </ul>
<b>Controlled Human Exposure:</b>		
Preference is given to studies utilizing experimental and control groups that are matched for individual level characteristics (e.g., body weight, smoking history, age) and time varying factors (e.g., seasonal and diurnal patterns).		
<b>Animal Toxicology:</b>		
Preference is given to studies utilizing experimental and control groups that are matched for individual level characteristics (e.g., body weight, litter size, food and water consumption) and time varying factors (e.g., seasonal and diurnal patterns).		
<b>Epidemiology:</b>		
Factors are considered to be potential confounders if demonstrated in the scientific literature to be related to health effects and correlated with oxides of nitrogen and/or traffic indicators. Not accounting for confounders can produce artifactual associations; thus, studies that statistically adjust for multiple factors or control for them in the study design are emphasized. Less weight is placed on studies that adjust for factors that mediate the relationship between oxides of nitrogen and health effects, which can bias results toward the null. In the absence of information linking health risk factors to oxides of nitrogen or traffic indicators, a factor may be evaluated as a potential effect measure modifier, but uncertainty is noted as to its role as a confounder. Confounders vary according to study design, exposure duration, and health effect and include the following: For time-series and panel studies of short-term exposure:		
<ul style="list-style-type: none"> <li>• Respiratory Effects—meteorology, day of week, season, medication use, allergen exposure (potential effect modifier)</li> <li>• Cardiovascular Effects—meteorology, day of week, season, medication use</li> <li>• Total Mortality—meteorology, day of week, season, long-term temporal trends</li> </ul>		
For studies of long-term exposure:		
<ul style="list-style-type: none"> <li>• Respiratory Effects—socioeconomic status, race, age, medication use, smoking, stress</li> <li>• Cardiovascular, Reproductive, and Development Effects—socioeconomic status, race, age, medication use, smoking, stress, noise</li> <li>• Total Mortality—socioeconomic status, race, age, medication use, smoking, comorbid health conditions</li> <li>• Cancer—socioeconomic status, race, age, occupational exposure</li> </ul>		
<b>Statistical Methodology</b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>• Clearly described and appropriate statistical methods for the study design and research question</li> <li>• Preference given to adequately powered studies</li> <li>• Consideration given to trends in data and reproducibility</li> </ul>	<ul style="list-style-type: none"> <li>• Clearly described and appropriate statistical methods for the study design and research question</li> <li>• Preference given to adequately powered studies</li> <li>• Consideration given to trends in data and reproducibility</li> </ul>	<ul style="list-style-type: none"> <li>• Multivariable regression adjusting for potential confounders ideal</li> <li>• Exception is multipollutant models. Multicollinearity can produce unreliable results</li> <li>• Results based on small sample sizes can be unreliable</li> </ul>

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

**Controlled Human Exposure:**

Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of controlled human exposure studies. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.

**Animal Toxicology:**

Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of animal toxicology studies. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.

**Epidemiology:**

Multivariable regression models that include potential confounding factors are emphasized. However, multipollutant models (more than two pollutants) are considered to produce too much uncertainty because of copollutant collinearity to be informative. Models with interaction terms aid in the evaluation of potential confounding as well as effect modification. Sensitivity analyses with alternate specifications for potential confounding inform the stability of findings and aid in judgments of the strength of inference of results. In the case of multiple comparisons, consistency in the pattern of association can increase confidence that associations were not found by chance alone. Statistical methods should be appropriate for the power of the study. For example, categorical analyses with small sample sizes can be prone to bias results toward or away from the null. Statistical tests such as t-tests and Chi-squared tests are not considered sensitive enough for adequate inferences regarding pollutant-health effect associations. For all methods, the effect estimate and precision of the estimate (i.e., width of 95% CI) are important considerations rather than statistical significance.

BC = black carbon, CI = confidence interval, CO = carbon monoxide, EC = elemental carbon, ISA = Integrated Science Assessment, LUR = land use regression, MOA = mode of action, NO = nitric oxide, NO<sub>2</sub> = nitrogen dioxide, NO<sub>x</sub> = sum of NO and NO<sub>2</sub>, O<sub>3</sub> = ozone, OC = organic carbon, PM = particulate matter, SES = socioeconomic status, SO<sub>2</sub> = sulfur dioxide, UFP = ultrafine particles, VOC = volatile organic compound.

<sup>a</sup>Toren et al. (1993); (Murgia et al. (2014); Weakley et al. (2013); Yang et al. (2011); Heckbert et al. (2004); Barr et al. (2002); Muhajjanine et al. (1997))

<sup>b</sup>Burney et al. (1989)

<sup>c</sup>Information on modes of action for NO<sub>2</sub> is described in [Section 4.3](#). The characterization of similar modes of action for many traffic-related pollutants is based on information described in the most recently completed ISAs ([U.S. EPA, 2013a, 2010, 2009, 2008b](#)) and the Health Effects Institute's 2010 review of Traffic-related air pollution ([HEI, 2010](#)).

<sup>d</sup>Judgments regarding potential confounding by other criteria pollutants are based on studies evaluated in this ISA, causal determinations made in the most recently completed ISAs ([U.S. EPA, 2013a, 2010, 2009, 2008b](#)), as well as recent reviews published by the Health Effects Institute. Judgments regarding potential confounding by the PM components EC/BC, OC, metals, and UFP as well as VOCs should not be inferred as conclusions regarding causality. Their consideration is based on associations with oxides of nitrogen and health effects observed in the studies examined in this ISA and reviews conducted by the Health Effects Institute ([HEI Review Panel on Ultrafine Particles, 2013; HEI, 2010](#)). Judgments regarding potential confounding by PM<sub>10</sub> should not be inferred as conclusions regarding causality specifically for that size fraction. The 2009 ISA for PM evaluated PM<sub>10</sub> studies but did not form individual causal determinations for that size fraction because PM<sub>10</sub> comprises both fine and thoracic coarse particles.

<sup>e</sup>Many factors evaluated as potential confounders can be effect measure modifiers (e.g., season, comorbid health condition) or mediators of health effects related to oxides of nitrogen (comorbid health condition).

# Comments on US EPA's Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (Second External Review Draft)

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Julie E. Goodman, PhD, DABT, ACE, ATS

Research Triangle Park, North Carolina  
May 19, 2015

# Conclusions

The current ISA does not provide evidence that the classifications in the 2008 ISA should be strengthened for any of the endpoints reviewed.

- Issues with framework
- Study quality not adequately addressed
- Evidence not consistent or coherent
- At-risk factors evidence not evaluated systematically

# Short-term Exposure

# Short-term NO<sub>2</sub> Epidemiology Studies

- Evaluation of evidence for HAs and ED visits is not rigorous or balanced
- Evidence does not indicate increased respiratory symptoms in people with asthma
- Evidence of lung function decrements in people with asthma is not consistent
- Chance, confounding, and other biases cannot be ruled out

# Evaluation of Evidence for HAs and ED Visits is Not Rigorous or Balanced

- Results generally presented for only the most positive and statistically significant lag
- Substantial between-study inconsistencies not addressed
- Null studies not given equal weight

# Evidence Does Not Indicate Increased Respiratory Symptoms in People With Asthma

- Small number of positive associations presented, many small in magnitude
- Outcomes mild, but clinical significance not discussed
- In several analyses, small elevation in symptoms but not rescue medication use
- Rescue medication use results mixed
- Largest associations in sub-group analyses

# Epidemiology Evidence of Lung Function Decrements in People With Asthma Not Consistent

- Several null studies not included in main presentation of results
- Results of studies with either supervised or unsupervised spirometry measurements are inconsistent
- No qualitative differences between strength or precision of associations in studies of personal or school-based exposure vs. central site monitoring
- Choice of lag or subgroup presented is not transparent

# Chance, Confounding, and Other Biases in Epidemiology Studies Cannot be Ruled Out

- Multi-pollutant modeling has not ruled out confounding with reasonable confidence
- Effects of NO<sub>2</sub> independent from those of other traffic-derived pollutants have not been resolved
- Studies of indoor NO<sub>2</sub> exposure do not support a casual association
- Associations do not always persist after adjustment for potential confounders
- Confounding by upper respiratory infections and aeroallergens, model misspecification, and model selection bias are major issues not fully addressed

## Weight of Evidence from Controlled Human Exposure Studies Indicates Increased AHR Would Not Occur at < 300 ppb NO<sub>2</sub>

- NO<sub>2</sub> does not increase AHR for specific allergens
- Lack of effect with exercise is not due to refractory period
- Clinically relevant reductions in the provocative dose (PD) of an airway challenge were observed in studies involving experimental conditions that do not represent plausible exposure scenarios
- Studies with multiple concentrations do not provide consistent evidence of a concentration-response relationship

# Increased Bronchoconstriction More Likely for Studies Using FVC Maneuvers

## Effect of NO<sub>2</sub> on Bronchoconstriction with Specific Allergens

Assessment of Bronchoconstriction	Increased (p < 0.05)	Increased (n.s.)	Decreased (n.s.)
FEV <sub>1</sub>	3/7	2/7	2/7
sRAW or sGAW	0	0	2/2

## Effect of NO<sub>2</sub> on Bronchoconstriction with Exercise

Assessment of Bronchoconstriction	Increased (p < 0.05)	Increased (n.s.)	Decreased (n.s.)
FEV <sub>1</sub>	4/11	7/11	0
sRAW or sGAW	0	2/6	4/6

Note: FEV<sub>1</sub>=Forced expiratory volume in 1 second; sGAW =specific airway conductance; sRAW =specific airway resistance.

# Lack of Stat Sig Effects in Controlled Exposure Studies w/Exercise Can Not be Explained by a Refractory Period

- Studies cited as support are dissimilar to NO<sub>2</sub> studies
- Intensity and frequency of exercise may not have been sufficient to induce a refractory period
- Not all individuals experience a refractory period following exercise
- Even if there is a refractory period, AHR may be diminished but not necessarily abolished

# Clinically Relevant Reductions in Airway PD in Controlled Exposure Studies Do Not Indicate NO<sub>2</sub> Increases AHR under Plausible Exposure Scenarios

- Pharmacological airway challenges or high concentrations of sulfur dioxide used in many studies do not represent plausible exposure scenarios
- Lack of exposure-response in studies with multiple exposures (as discussed in REA planning document)

ISA, Figure 5-1

# Lack of Conc-Response in Controlled Exposure Studies Indicates NO<sub>2</sub> Does Not Cause AHR at Concentrations of At Least 300 ppb

Results are highly variable across these studies. The available information does not demonstrate an exposure-dependent response and, therefore, this information is not sufficient to support the derivation of an exposure-response function for use in quantitative estimates of NO<sub>2</sub> health risks. Goodman et al. (2009) reached a similar conclusion, based on meta-analyses and meta-regressions of information from studies of NO<sub>2</sub>-induced specific and non-specific airway responsiveness. In addition, there is not strong evidence of an exposure-response relationship in individual studies that evaluated exposures to multiple NO<sub>2</sub> concentrations (Bylin et al., 1988; Orehek et al., 1976). Therefore, while the available information is sufficient to support the identification of health effect benchmarks for NO<sub>2</sub>, as described above (section 2.2.2), we reach the preliminary conclusion that a quantitative risk assessment based on information from controlled human exposure studies is not supported by the evidence available in the current review.

Risk and Exposure Planning Document, pp. 4-2 - 4-3.

# Mode of Action Evidence Does Not Support a Role for NO<sub>2</sub> in Asthma Exacerbation

## Allergic response

- Increased eosinophil cationic protein (ECP) is not sufficient to cause asthma exacerbation
- Increase only at exposures  $\geq 260$  ppb

## Inflammation

- Evidence of mild inflammation, but not adverse inflammatory effects
- Increased airway polymorphonuclear leukocytes (PMNs) at NO<sub>2</sub> exposures  $\geq 2,000$  ppb
- Some evidence of increased inflammatory mediators at NO<sub>2</sub> exposures  $\geq 1,000$  ppb

# Long-term Exposure

# Evaluation of Evidence for Asthma Development in Children is Not Rigorous or Balanced

- Positive epidemiology associations preferentially presented
- Uncertainty of asthma diagnosis in small children (*i.e.* potential for outcome misclassification) not fully acknowledged
- Inconsistent findings within and across studies not fully considered
- Several null studies not included in main presentation of results

# Evidence Does Not Indicate Long-term NO<sub>2</sub> Exposure Causes Asthma Development

- Considerable inconsistencies in epidemiology evidence of asthma development in children and adults
- Significant uncertainty with regard to the independent effects of NO<sub>2</sub> on asthma development
- Lack of coherence with short-term respiratory effects
- Lack of coherence with animal studies
- Lack of established mode of action

# Other Health Endpoints

# Inadequate Evidence for Short-Term Exposures

Health Effect	ISA Causal Determination	Comments
<b>Cardiovascular effects</b>	Suggestive	<p>Majority of effects <u>small or close to null</u>; more likely the result of bias or confounding.</p> <p>Outcomes have other risk factors that contribute to CV effects.</p> <p>Lack of confirmed MoAs.</p>
<b>Total mortality</b>	Suggestive	<p><u>Exposure measurement error</u> from city-level or small networks of monitors.</p> <p><u>Heterogeneity of effects</u> in multi-city studies.</p>

# Inadequate Evidence for Long-Term Exposures

Health Effect	ISA Causal Determination	Comments
<b>Cardiovascular effects</b>	Suggestive	Confounding by noise or copollutant exposure Limited toxicology and MoA evidence
<b>Reproductive and Developmental effects</b>	Fertility, Reproduction and Pregnancy: Inadequate	Inconsistent epidemiology evidence Lack of confirmed MoAs
	Birth outcomes: Suggestive	
	Postnatal development: Inadequate	
<b>Total mortality</b>	Suggestive	Inconsistent and uncertain epidemiology evidence
<b>Cancer</b>	Suggestive	Lack of confirmed MoA

# Conclusions Regarding "At-risk" Factors Are Not Supported by the Evidence

*ISA does not systematically review evidence for at-risk factors.*

Factor	Comments
People with asthma	No evidence at ambient concentrations
Children	Inconsistent epidemiology evidence Lack of support from toxicity studies
Older adults	Lack of coherence between controlled exposure and epidemiology studies

# Questions?

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Gradient

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# **Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (Second External Review Draft)**

## **Summary Tables**

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May 19, 2015



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**Table 1 Causal Determinations in the 2008, 2013 Draft, and 2015 Draft ISAs for Oxides of Nitrogen**

Health Effect Category	Causal Determination			Comments
	2008 ISA	First Draft ISA	Second Draft ISA	
<b>Short-term NO<sub>2</sub> Exposure</b>				
Respiratory Effects	Sufficient to determine a likely causal relationship.	Causal	Causal	Inadequate quality of epidemiology studies. Lack of coherence with controlled exposure or toxicology studies. Uncertainty regarding whether NO <sub>2</sub> is a proxy for traffic-related pollution.
Cardiovascular Effects	Inadequate to infer the presence or absence of a causal relationship.	Likely causal	Suggestive	Inadequate quality of epidemiology studies. Lack of coherence across studies. Lack of confirmed MoAs.
Total Mortality	Suggestive but not sufficient to infer a causal relationship.	Likely causal	Suggestive	Inadequate quality of epidemiology studies. Lack of confirmed MoAs.
<b>Long-term NO<sub>2</sub> Exposure</b>				
Respiratory Effects	Suggestive but not sufficient to infer a causal relationship.	Likely causal	Likely causal	Inconsistent findings among epidemiology studies. Lack of coherence with studies of short-term effects. Lack of coherence with MoAs. Uncertainty regarding whether NO <sub>2</sub> is a proxy for traffic-related pollution.
Cardiovascular Effects	Inadequate to infer the presence or absence of a causal relationship.	Suggestive	Suggestive	Inconsistent findings among epidemiology studies. Lack of confirmed MoAs.
Reproductive and Developmental Effects	Inadequate to infer the presence or absence of a causal relationship.	Fertility, Reproduction, and Pregnancy: Suggestive	Fertility, Reproduction, and Pregnancy: Inadequate	Inconsistent findings among studies. Lack of coherence across different endpoints. Lack of confirmed MoAs.
		Birth Outcomes: Suggestive	Birth Outcomes: Suggestive	Inconsistent findings among studies. Lack of coherence across different endpoints. Lack of confirmed MoAs.
		Postnatal Development: Suggestive	Postnatal Development: Inadequate	Inconsistent findings among studies. Lack of confirmed MoAs.
Total Mortality	Inadequate	Suggestive	Suggestive	Inconsistent findings among epidemiology studies. Lack of coherence with studies of morbidity endpoints. Lack of confirmed MoAs.
Cancer	Inadequate to infer the presence or absence of a causal relationship.	Suggestive	Suggestive	Inconsistent findings among epidemiology studies. Lack of confirmed MoAs.

Notes:

ISA = Integrated Science Assessment; NO<sub>2</sub> = Nitrogen Dioxide; MoA = Mode of Action. Sources: EPA (2008, 2013, 2015).

**Table 5-1 Summary and description of scientific considerations for evaluating the quality of studies on the health effects of oxides of nitrogen.**

NOTE: Study aspects of interest are reported in gray boxes (e.g., Study Design). Summary bullets are provided in the top sections and are described in more detail in the text immediately following.

Study Design		
Controlled Human Exposure	Animal Toxicology	Epidemiology
<ul style="list-style-type: none"> <li>Clearly defined hypotheses/aims</li> <li>Appropriately matched control exposures</li> <li>Randomization and allocation concealment</li> <li>Balanced crossover (repeated measures) or parallel design studies</li> </ul>	<ul style="list-style-type: none"> <li>Clearly defined hypotheses/aims</li> <li>Appropriately matched control exposures</li> <li>Randomization and allocation concealment</li> <li>All groups handled and cared for equally</li> </ul>	<ul style="list-style-type: none"> <li>Clearly defined hypotheses/aims</li> <li>Key designs for short-term exposure: time series, case crossover, panel</li> <li>Key designs for long-term exposure: prospective cohort, nested case-control</li> <li>High power studies key: large sample sizes, multiple years, multicity studies</li> </ul>

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

<b>Controlled Human Exposure:</b>		
<p>Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Study subjects should be randomly exposed without knowledge of the exposure condition. Preference is given to balanced crossover (repeated measures) or parallel design studies which include control exposures (e.g., to clean filtered air). In crossover studies, a sufficient and specified time between exposure days should be provided to avoid carry over effects from prior exposure days. In parallel design studies, all arms should be matched for individual characteristics such as age, sex, race, anthropometric properties, and health status. Similarly, in studies evaluating effects of disease, appropriately matched healthy controls are desired for interpretative purposes.</p>		
<b>Animal Toxicology:</b>		
<p>Studies should clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Studies should include appropriately matched control exposures (e.g., to clean filtered air, time matched). Studies should use methods to limit differences in baseline characteristics of control and exposure groups. Studies should randomize assignment to exposure groups and where possible conceal allocation to research personnel. Groups should be subjected to identical experimental procedures and conditions and care of animals, including housing, husbandry, etc. Blinding of research personnel to study group may not be possible due to animal welfare and experimental considerations; however, differences in the monitoring or handling of animals in all groups by research personnel should be minimized.</p>		
<b>Epidemiology:</b>		
<p>Studies should clearly describe the primary and any secondary aims of the study, or specific hypotheses being tested.</p> <p>For short-term exposure, time-series, case crossover, and panel studies are emphasized over cross-sectional studies because they examine temporal correlations and are less prone to confounding by factors that differ between individuals (e.g., SES, age). Studies with large sample sizes and conducted over multiple years are considered to produce more reliable results. If other quality parameters are equal, multicity studies carry more weight than single-city studies because they tend to have larger sample sizes and lower potential for publication bias.</p> <p>For long-term exposure, inference is considered to be stronger for prospective cohort studies and case-control studies nested within a cohort (e.g., for rare diseases) than cross-sectional, other case-control, or ecologic studies. Cohort studies can better inform the temporality of exposure and effect. Other designs can have uncertainty also related to the appropriateness of the control group or validity of inference about individuals from group-level data. Study design limitations can bias health effect associations in either direction.</p>		
<b>Study Population/Test Model</b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>• Similarly matched control and exposed subjects</li> <li>• Subject characteristics reported</li> <li>• Clearly indicated inclusion and exclusion criteria</li> <li>• Independent, clinical assessment of the health condition</li> <li>• Loss or withdrawal of subjects should be reported with rationale</li> </ul>	<ul style="list-style-type: none"> <li>• Animal characteristics reported</li> <li>• Studies testing and reporting both sexes and multiple life stages preferred</li> <li>• Loss or exclusion of animals should be reported with rationale</li> </ul>	<ul style="list-style-type: none"> <li>• Representative of population of interest</li> <li>• High participation and low drop-out over time that is not dependent on exposure or health status</li> <li>• Clearly indicated inclusion and exclusion criteria</li> <li>• Independent, clinical assessment of health condition</li> <li>• Groups are compared if from same source population</li> </ul>

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

<b>Controlled Human Exposure:</b>		
<p>In general, the subjects recruited into study groups should be similarly matched for age, sex, race, anthropometric properties, and health status. In studies evaluating effects of specific subject characteristics (e.g., disease, genetic polymorphism, etc.), appropriately matched healthy controls are preferred. Relevant characteristics and health status should be reported for each experimental group. Criteria for including and excluding subjects should be clearly indicated. For the examination of populations with an underlying health condition (e.g., asthma), independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory diseases and history of cardiovascular events.<sup>a</sup> The loss or withdrawal of recruited subjects during the course of a study should be reported. Specific rationale for excluding subject(s) from any portion of a protocol should be explained.</p>		
<b>Animal Toxicology:</b>		
<p>Ideally, studies should report species, strain, sub-strain, genetic background, age, sex, and weight. However, differences in these parameters across studies do not make the studies incomparable. Unless data indicate otherwise, all animal species and strains are considered appropriate for evaluating effects of NO<sub>2</sub> or NO exposure. It is preferred that the authors test for effects in both sexes and multiple life stages, and report the result for each group separately. All animals used in a study should be accounted for, and rationale for exclusion of animals or data should be specified.</p>		
<b>Epidemiology:</b>		
<p>The ideal study population is recruited from and is representative of the target population. Studies with high participation and low drop-out over time that is not dependent on exposure or health status are considered to have low potential for selection bias. Criteria for including and excluding subjects should be clearly indicated. For populations with an underlying health condition, independent, clinical assessment of the health condition is ideal, but self-report of physician diagnosis generally is considered to be reliable for respiratory diseases and history of cardiovascular events.<sup>a</sup> Groups with and without an underlying health condition should be compared if they are from the same source population. Selection bias can influence results in either direction or may not affect the validity of results but rather reduce the generalizability of findings to the target population.</p>		
<b>Pollutant</b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>• Studies of NO<sub>2</sub> are emphasized</li> </ul>	<ul style="list-style-type: none"> <li>• Studies of NO<sub>2</sub> are emphasized</li> </ul>	<ul style="list-style-type: none"> <li>• NO<sub>2</sub> emphasized over NO, NO<sub>x</sub></li> <li>• Comparisons of health effect associations among gaseous oxides of nitrogen species ideal</li> </ul>
<b>Controlled Human Exposure:</b>		
<p>The focus is on studies testing NO<sub>2</sub> exposure.</p>		
<b>Animal Toxicology:</b>		
<p>The focus is on studies testing NO<sub>2</sub> exposure.</p>		
<b>Epidemiology:</b>		
<p>Health effects are evaluated mostly for NO<sub>2</sub>, and less so for NO or NO<sub>x</sub>. Studies that compare health effect associations among these species are informative. Typically, one species is examined, and studies of NO<sub>2</sub> are emphasized. It is not clear that ambient-relevant NO exposures induce negative health effects (<a href="#">Section 4.2.3</a>). The relationship of NO<sub>x</sub> to NO<sub>2</sub> varies with distance from roads, and thus, may vary among subjects. Hence, there is uncertainty about the extent to which associations with NO<sub>x</sub> reflect those for NO<sub>2</sub> vs. other pollutants from traffic.</p>		

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

Exposure Assessment or Assignment		
Controlled Human Exposure	Animal Toxicology	Epidemiology
<ul style="list-style-type: none"> <li>Well characterized and reported exposure conditions</li> <li>Limited to studies that utilize NO<sub>2</sub> and/or NO concentrations less than or equal to 5,000 ppb</li> <li>Preference is given to studies that include exposure control groups</li> <li>Randomized exposure groups</li> </ul>	<ul style="list-style-type: none"> <li>Well characterized and reported exposure conditions</li> <li>Inhalation exposure</li> <li>Limited to studies that utilize NO<sub>2</sub> and/or NO concentrations less than or equal to 5,000 ppb</li> <li>All studies should include exposure control groups</li> <li>Randomized exposure groups</li> </ul>	<ul style="list-style-type: none"> <li>Exposure metrics that accurately represent temporal or spatial variability for study area</li> <li>Comparisons of exposure measurement methods</li> <li>Indoor and total personal exposures can inform independent effects of NO<sub>2</sub></li> <li>Lag/duration of exposure metric correspond with time course for health effect</li> </ul>
<b>Controlled Human Exposure:</b>		
<p>Studies should well characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions for subject safety. For this assessment, the focus is on studies that utilize NO<sub>2</sub> and/or NO concentrations less than or equal to 5,000 ppb (<a href="#">Section 1.2</a>). Studies that utilize higher exposure concentrations may provide information relevant to MOA, dosimetry, or at-risk human populations. Preference is given to balanced crossover or parallel design studies which include control exposures (e.g., to clean filtered air). Study subjects should be randomly exposed without knowledge of the exposure condition. Method of exposure (e.g., chamber, facemask, etc.) should be specified and activity level of subjects during exposures should be well characterized.</p>		
<b>Animal Toxicology:</b>		
<p>Studies should characterize pollutant concentration, temperature, and relative humidity and/or have measures in place to adequately control the exposure conditions. The focus is on inhalation exposure. Non-inhalation exposure experiments may provide information relevant to MOA. In vitro studies may be included if they provide mechanistic insight or examine similar effects as in vivo, but are generally not included. All studies should include exposure control groups (e.g., clean filtered air). For this assessment, the focus will be on studies that utilize NO<sub>2</sub> and/or NO concentrations less than or equal to 5,000 ppb (<a href="#">Section 1.2</a>). Studies that utilize higher exposure concentrations may provide information relevant to MOA, dosimetry, interspecies variation, or at-risk human populations.</p>		

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

<b>Epidemiology:</b>		
<p>Of primary relevance are relationships of health effects with the ambient component of exposure to oxides of nitrogen. However, information about ambient exposure rarely is available for individual subjects; most often, inference is based on ambient concentrations. Studies that compare exposure assessment methods are considered to be particularly informative. The duration or lag of the exposure metric should correspond with the time course for physiological changes in the outcome (e.g., up to a few days for symptoms) or latency of disease (e.g., several years for cancer).</p> <p>Given the spatial heterogeneity in ambient oxides of nitrogen and variable relationships between personal exposures and ambient concentrations (<a href="#">Section 3.4.3</a>), validated methods that capture the extent of variability for the particular study design (temporal vs. spatial contrasts) and location carry greater weight. Central site measurements, whether averaged across multiple monitors or assigned from the nearest or single available monitor, have well-recognized limitations in capturing spatial variation in oxides of nitrogen. Inference from central site measurements can be adequate if correlated with personal exposures, closely located to study subjects, highly correlated across monitors within a location, used in locations with well-distributed sources, or combined with time-activity information.</p> <p>In studies of short-term exposure, metrics that may capture variation in ambient oxides of nitrogen and strengthen inference include concentrations in subjects' microenvironments (e.g., outdoor home, school, in-vehicle) and individual-level outdoor concentrations combined with time-activity data. Results for total personal and indoor NO<sub>2</sub> exposure are other lines of evidence that inform judgments about causality of NO<sub>2</sub> because inference is based on an individual's microenvironmental exposures and potential for copollutant confounding may be lower or different than that for ambient concentrations. Results for total personal exposure can inform the effects of ambient exposure when well correlated with ambient concentrations. For long-term exposures, LUR models that well represent spatial variation in ambient NO<sub>2</sub> can provide estimates of individual exposure. Less weight is placed on NO<sub>x</sub> from dispersion models because of limitations in accurate estimation of within-community conditions (<a href="#">Section 3.2.1.2</a>). And because NO<sub>x</sub> from dispersion models often shows near perfect correlations (<math>r = 0.94-0.99</math>) with EC, PM<sub>2.5</sub>, and CO, the effects of NO<sub>x</sub> cannot be distinguished from traffic-related copollutants.</p> <p>Exposure measurement error often attenuates health effect estimates or increases the precision of the association (i.e., width of 95% CIs), particularly associations based on temporal variation in short-term exposure (<a href="#">Section 3.4.5.1</a>). However, exposure measurement error can bias estimates away from the null, particularly for long-term exposures.</p>		
<b>Outcome Assessment/Evaluation</b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>• Same manner of outcome assessment for all groups</li> <li>• Validated, reliable methods</li> <li>• Reporting of outcome assessment details</li> <li>• Blinding of endpoint evaluators</li> <li>• Appropriate timing of endpoint evaluation</li> </ul>	<ul style="list-style-type: none"> <li>• Same manner of outcome assessment for all groups</li> <li>• Validated, reliable methods</li> <li>• Reporting of outcome assessment details</li> <li>• Blinding of endpoint evaluators</li> <li>• Appropriate timing of endpoint evaluation</li> </ul>	<ul style="list-style-type: none"> <li>• Same manner of outcome assessment for all groups</li> <li>• Validated, reliable methods</li> <li>• Assessment is blind to exposure status</li> <li>• Appropriate timing of endpoint evaluation</li> </ul>
<b>Controlled Human Exposure:</b>		
<p>Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on the endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.</p>		
<b>Animal Toxicology:</b>		
<p>Endpoints should be assessed in the same manner for control and exposure groups (e.g., time after exposure, methods, endpoint evaluator) using valid, reliable methods. Blinding of endpoint evaluators is ideal, especially for qualitative endpoints (e.g., histopathology). For each experiment and each experimental group, including controls, precise details of all procedures carried out should be provided including how, when, and where. Time of the endpoint evaluations is a key consideration that will vary depending on the endpoint evaluated. Endpoints should be assessed at time points that are appropriate for the research questions.</p>		

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

<b>Epidemiology:</b>		
<p>Outcomes should be assessed or reported without knowledge of exposure status. Such bias could produce artifactual associations. Outcomes assessed by interview, self-report, clinical examination, or analysis of biological indicators should be defined by consistent criteria and collected by validated, reliable methods. Independent, clinical assessment is ideal for outcomes such as lung function or incidence of disease, but report of physician diagnosis has shown good reliability.<sup>a</sup> Outcomes should be assessed at time intervals that correspond with the time course for physiological changes (e.g., up to a few days for symptoms). When health effects of long-term exposure are assessed by acute events such as symptoms or hospital admissions, inference is strengthened when results are adjusted for short-term exposure. Validated questionnaires for subjective outcomes such as symptoms are regarded to be reliable,<sup>b</sup> particularly when collected frequently and not subject to long recall. For biological samples, the stability of the compound of interest and the sensitivity and precision of the analytical method is considered. If not based on knowledge of exposure status, errors in outcome assessment tend to bias results toward the null.</p>		
<b>Potential Copollutant Confounding</b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>Well-characterized exposure</li> </ul>	<ul style="list-style-type: none"> <li>Well-characterized exposure</li> </ul>	<ul style="list-style-type: none"> <li>Traffic-related copollutants are key: CO, PM<sub>2.5</sub>, BC/EC, OC, UFP, metal PM components, VOCs</li> <li>Also considered: PM<sub>10</sub>, SO<sub>2</sub>, O<sub>3</sub></li> </ul>
<b>Controlled Human Exposure:</b>		
<p>Exposure should be well characterized to evaluate independent effects of NO<sub>2</sub>.</p>		
<b>Animal Toxicology:</b>		
<p>Exposure should be well characterized to evaluate independent effects of NO<sub>2</sub>.</p>		
<b>Epidemiology:</b>		
<p>Not accounting for copollutant confounding can produce artifactual associations; thus, studies that examine copollutant confounding carry greater weight. The predominant method is copollutant modeling, which is especially informative when measurement error is comparable for copollutants and correlations are not high. Interaction and joint effect models are examined to a lesser extent. Copollutant confounding also can be informed by evaluating correlations between oxides of nitrogen and copollutants and comparing health associations between gaseous oxides of nitrogen and copollutants in single-pollutant models if exposure measurement error is comparable among pollutants. Studies that examine only gaseous oxides of nitrogen are considered poorly to inform the potential for copollutant confounding. Copollutant confounding is evaluated based on the extent of their correlations typically observed with oxides of nitrogen and relationships observed with health effects. Among copollutants, of primary concern are traffic-related pollutants, which include CO, PM<sub>2.5</sub>, BC/EC, OC, UFP, metal PM components such as copper, zinc, and iron, as well as VOCs such as benzene, acetaldehyde, toluene, ethylbenzene, and xylene. Short-term and long-term metrics for these pollutants consistently show moderate to high correlations with oxides of nitrogen (Figure 3-6). Many traffic-related pollutants also are characterized to have common modes of action.<sup>c</sup> Common key events include formation of secondary oxidation products, inflammation, and for respiratory effects, increases in airway responsiveness. They also show relationships with many of the health effects evaluated in this ISA<sup>d</sup> except as follows. For long-term exposure, there is uncertainty regarding confounding by UFP because of their short atmospheric lifetime. Also for long-term exposure, CO is not considered to be an important confounding copollutant for mortality or lung cancer.<sup>d</sup> Of less concern is confounding by PM<sub>10</sub>, SO<sub>2</sub>, and O<sub>3</sub> because they show varying and often lower correlations with NO<sub>2</sub> (Figure 3-6). O<sub>3</sub> generally is negatively or weakly positively correlated with NO<sub>2</sub> but may be a confounding copollutant where moderate positive correlations are observed. O<sub>3</sub> and SO<sub>2</sub> in particular show similarities with NO<sub>2</sub> in mode of action. PM<sub>10</sub>, SO<sub>2</sub>, and O<sub>3</sub> show relationships with the health effect evaluated in this ISA<sup>d</sup> except as follows. For short-term exposure, SO<sub>2</sub> is not considered to be a strong confounding copollutant for cardiovascular effects. For long-term exposure, neither O<sub>3</sub> nor SO<sub>2</sub> is considered to be a strong confounding copollutant.</p>		

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

<b>Other Potential Confounding Factors<sup>a</sup></b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>• Preference given to studies with adequate control of factors influencing health response</li> </ul>	<ul style="list-style-type: none"> <li>• Preference given to studies with adequate control of factors influencing health response</li> </ul>	<ul style="list-style-type: none"> <li>• Potential confounders related to health effect and correlated with oxides of nitrogen should be examined</li> <li>• Potential confounders vary by study design (temporally vs. spatially correlated) and by health effects</li> </ul>
<b>Controlled Human Exposure:</b>		
Preference is given to studies utilizing experimental and control groups that are matched for individual level characteristics (e.g., body weight, smoking history, age) and time varying factors (e.g., seasonal and diurnal patterns).		
<b>Animal Toxicology:</b>		
Preference is given to studies utilizing experimental and control groups that are matched for individual level characteristics (e.g., body weight, litter size, food and water consumption) and time varying factors (e.g., seasonal and diurnal patterns).		
<b>Epidemiology:</b>		
Factors are considered to be potential confounders if demonstrated in the scientific literature to be related to health effects and correlated with oxides of nitrogen and/or traffic indicators. Not accounting for confounders can produce artifactual associations; thus, studies that statistically adjust for multiple factors or control for them in the study design are emphasized. Less weight is placed on studies that adjust for factors that mediate the relationship between oxides of nitrogen and health effects, which can bias results toward the null. In the absence of information linking health risk factors to oxides of nitrogen or traffic indicators, a factor may be evaluated as a potential effect measure modifier, but uncertainty is noted as to its role as a confounder. Confounders vary according to study design, exposure duration, and health effect and include the following: For time-series and panel studies of short-term exposure:		
<ul style="list-style-type: none"> <li>• Respiratory Effects—meteorology, day of week, season, medication use, allergen exposure (potential effect modifier)</li> <li>• Cardiovascular Effects—meteorology, day of week, season, medication use</li> <li>• Total Mortality—meteorology, day of week, season, long-term temporal trends</li> </ul>		
For studies of long-term exposure:		
<ul style="list-style-type: none"> <li>• Respiratory Effects—socioeconomic status, race, age, medication use, smoking, stress</li> <li>• Cardiovascular, Reproductive, and Development Effects—socioeconomic status, race, age, medication use, smoking, stress, noise</li> <li>• Total Mortality—socioeconomic status, race, age, medication use, smoking, comorbid health conditions</li> <li>• Cancer—socioeconomic status, race, age, occupational exposure</li> </ul>		
<b>Statistical Methodology</b>		
<b>Controlled Human Exposure</b>	<b>Animal Toxicology</b>	<b>Epidemiology</b>
<ul style="list-style-type: none"> <li>• Clearly described and appropriate statistical methods for the study design and research question</li> <li>• Preference given to adequately powered studies</li> <li>• Consideration given to trends in data and reproducibility</li> </ul>	<ul style="list-style-type: none"> <li>• Clearly described and appropriate statistical methods for the study design and research question</li> <li>• Preference given to adequately powered studies</li> <li>• Consideration given to trends in data and reproducibility</li> </ul>	<ul style="list-style-type: none"> <li>• Multivariable regression adjusting for potential confounders ideal</li> <li>• Exception is multipollutant models. Multicollinearity can produce unreliable results</li> <li>• Results based on small sample sizes can be unreliable</li> </ul>

**Table 5-1 (Continued): Summary and description of scientific considerations for evaluating the quality of studies on the health effects from oxides of nitrogen.**

**Controlled Human Exposure:**

Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of controlled human exposure studies. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.

**Animal Toxicology:**

Statistical methods should be clearly described and appropriate for the study design and research question (e.g., correction for multiple comparisons). Generally, statistical significance is used to evaluate the findings of animal toxicology studies. Detection of statistical significance is influenced by a variety of factors including, but not limited to, the size of the study, exposure and outcome measurement error, and statistical model specifications. Sample size is not a criterion for exclusion; ideally, the sample size should provide adequate power to detect hypothesized effects (e.g., sample sizes less than three are considered less informative). Because statistical tests have limitations, consideration is given to both trends in data and reproducibility of results.

**Epidemiology:**

Multivariable regression models that include potential confounding factors are emphasized. However, multipollutant models (more than two pollutants) are considered to produce too much uncertainty because of copollutant collinearity to be informative. Models with interaction terms aid in the evaluation of potential confounding as well as effect modification. Sensitivity analyses with alternate specifications for potential confounding inform the stability of findings and aid in judgments of the strength of inference of results. In the case of multiple comparisons, consistency in the pattern of association can increase confidence that associations were not found by chance alone. Statistical methods should be appropriate for the power of the study. For example, categorical analyses with small sample sizes can be prone to bias results toward or away from the null. Statistical tests such as t-tests and Chi-squared tests are not considered sensitive enough for adequate inferences regarding pollutant-health effect associations. For all methods, the effect estimate and precision of the estimate (i.e., width of 95% CI) are important considerations rather than statistical significance.

BC = black carbon, CI = confidence interval, CO = carbon monoxide, EC = elemental carbon, ISA = Integrated Science Assessment, LUR = land use regression, MOA = mode of action, NO = nitric oxide, NO<sub>2</sub> = nitrogen dioxide, NO<sub>x</sub> = sum of NO and NO<sub>2</sub>, O<sub>3</sub> = ozone, OC = organic carbon, PM = particulate matter, SES = socioeconomic status, SO<sub>2</sub> = sulfur dioxide, UFP = ultrafine particles, VOC = volatile organic compound.

<sup>a</sup>Toren et al. (1993); (Murgia et al. (2014); Weakley et al. (2013); Yang et al. (2011); Heckbert et al. (2004); Barr et al. (2002); Muhajarine et al. (1997))

<sup>b</sup>Bumey et al. (1989)

<sup>c</sup>Information on modes of action for NO<sub>2</sub> is described in Section 4.3. The characterization of similar modes of action for many traffic-related pollutants is based on information described in the most recently completed ISAs (U.S. EPA, 2013a, 2010, 2009, 2008b) and the Health Effects Institute's 2010 review of Traffic-related air pollution (HEI, 2010).

<sup>d</sup>Judgments regarding potential confounding by other criteria pollutants are based on studies evaluated in this ISA, causal determinations made in the most recently completed ISAs (U.S. EPA, 2013a, 2010, 2009, 2008b), as well as recent reviews published by the Health Effects Institute. Judgments regarding potential confounding by the PM components EC/BC, OC, metals, and UFP as well as VOCs should not be inferred as conclusions regarding causality. Their consideration is based on associations with oxides of nitrogen and health effects observed in the studies examined in this ISA and reviews conducted by the Health Effects Institute (HEI Review Panel on Ultrafine Particles, 2013; HEI, 2010). Judgments regarding potential confounding by PM<sub>10</sub> should not be inferred as conclusions regarding causality specifically for that size fraction. The 2009 ISA for PM evaluated PM<sub>10</sub> studies but did not form individual causal determinations for that size fraction because PM<sub>10</sub> comprises both fine and thoracic coarse particles.

<sup>e</sup>Many factors evaluated as potential confounders can be effect measure modifiers (e.g., season, comorbid health condition) or mediators of health effects related to oxides of nitrogen (comorbid health condition).

**Table 2 Asthma HA/ED Visit Study Quality Characteristics Based on ISA Table 5-1**

Citation	Inclusion in ISA		Study Design			Pollutant		Exposure Assessment			Outcome Assessment		Confounding by Co-pollutants			Other Confounders				Statistical Methods	
	"High-quality" Study	Main Text Only	Design	Single vs. Multi-city	Size/Duration <sup>1</sup>	NO, NO <sub>2</sub> , NO <sub>x</sub>	Comparisons Between Oxides	Central Site Monitoring	Spatial Variability Assessed	Comparison of Exposure Assessment Methods	Type of Outcome	Exclusion of Children < 2 Years Old	Traffic-related Pollutants Assessed	Correlations Reported	Relative Measurement Error in Co-pollutants Discussed	Meteorology	Day of Week	Season	Allergens	Cautious Interpretation of Multi-pollutant Models	Sensitivity Analysis: Alternate Model Specification
Strickland <i>et al.</i> (2010)	√		Case cross-over	Single	91,386 ED visits/ 12 years	NO <sub>2</sub>	No	Yes	No	No	ED visits	Yes	No <sup>2</sup>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Villeneuve <i>et al.</i> (2007)	√		Case cross-over	Single	57,912 ED visits/ 10 years	NO <sub>2</sub>	No	Yes	No	No	ED visits	Yes	CO	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Jalaludin <i>et al.</i> (2008)	√		Case cross-over	Single	1,826 ED visits/ 5 years	NO <sub>2</sub>	No	Yes	No	No	ED visits	No <sup>3</sup>	CO, PM <sub>2.5</sub>	Yes	No	Yes	Yes	Yes	No	Yes	No
Ito <i>et al.</i> (2007)	√		Time series	Single	4 years	NO <sub>2</sub>	No	Yes	Yes	No	ED visits	No	CO, PM <sub>2.5</sub>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Iskandar <i>et al.</i> (2012)	√		Case cross-over	Single	8,226 HAs/ 8 years	NO <sub>2</sub> , NO <sub>x</sub>	Yes	Yes	Yes	No	HAs	No <sup>4</sup>	UFP, PM <sub>2.5</sub>	Yes	No <sup>5</sup>	Yes	Yes	Yes	Yes	Yes	Yes
ATSDR and NYSDOH (2006)	√		Time-series	Single	2 years	NO <sub>2</sub>	No	Yes	Yes	No	ED visits	No <sup>6</sup>	PM <sub>2.5</sub>	Yes	No	Yes	Yes	Yes	No <sup>7</sup>	Yes	Yes
Stieb <i>et al.</i> (2009)	√		Time series	Multi-city	4-10 years <sup>8</sup>	NO <sub>2</sub>	No	Yes	No	No	ED visits	No	No <sup>9</sup>	Yes	No	Yes	Yes	Yes	No	NA	Yes
Samoli <i>et al.</i> (2011)		√	Time series	Single	4 years	NO <sub>2</sub>	No	Yes	Yes	No	HAs	No	No	Yes	No	Yes	Yes	Yes	Yes <sup>10</sup>	Yes	Yes
Peel <i>et al.</i> (2005)		√	Time series	Single	8 years	NO <sub>2</sub>	No	Yes	Yes	Yes <sup>11</sup>	ED visits	No <sup>4</sup>	CO	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Son <i>et al.</i> (2013)		√	Time-series	Multi-city	6 years	NO <sub>2</sub>	No	Yes	No	No	HAs	No	No <sup>12</sup>	Yes	No	Yes	Yes	Yes	No	NA	Yes
Ko <i>et al.</i> (2007)		√	Time-series	Single	6 years	NO <sub>2</sub>	No	Yes	No	No	HAs	No	PM <sub>2.5</sub>	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Sarnat <i>et al.</i> (2013b)		√	Time series	Single	4 years	NO <sub>x</sub>	No	Yes <sup>13</sup>	Yes	Yes	ED visits	No	No <sup>9</sup>	Yes	Yes	Yes	Yes	Yes	No	NA	Yes
Orazzo <i>et al.</i> (2009)		√	Case cross-over	Multi-city	53,272 ED visits/ 7 years	NO <sub>2</sub>	No	Yes	Yes	No	ED visits	No	No <sup>12</sup>	No	No	Yes	Yes	Yes	No <sup>7</sup>	NA	Yes
Strickland <i>et al.</i> (2011)		√	Time series	Single	41,741 ED visits/ 12 years	NO <sub>2</sub>	No	Yes	Yes	Yes	ED visits	Yes	No <sup>2</sup>	No	Yes	Yes	Yes	Yes	No	NA	Yes
Li <i>et al.</i> (2011)		√	Time series and case cross-over	Single	12,933 asthma events/ 3 years	NO <sub>2</sub>	No	Yes	No	No	ED visits and HAs	Yes	No <sup>9</sup>	Yes	No	Yes	Yes	Yes	No	NA	Yes
Gass <i>et al.</i> (2014)		√	Case cross-over	Single	11 years	NO <sub>2</sub>	No	Yes	No	No	ED visits	Yes	CO, PM <sub>2.5</sub>	No	No	Yes	Yes	Yes	No	Yes	No
Winqvist <i>et al.</i> (2014)		√	Time series	Single	6 years	NO <sub>2</sub>	No <sup>14</sup>	Yes	No	No	ED visits	Yes	CO, PM <sub>2.5</sub> , EC	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Burnett <i>et al.</i> (1999)		√	Time series	Single	15 years	NO <sub>2</sub>	No	Yes	No	No	HAs	No	CO, PM <sub>2.5</sub>	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Linn <i>et al.</i> (2000)		√	Time series	Single	4 years	NO <sub>2</sub>	No	Yes	Yes	No	HAs	Yes	CO	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes

**Table 2 Asthma HA/ED Visit Study Quality Characteristics (Continued)**

Citation	Inclusion in ISA		Study Design			Pollutant		Exposure Assessment			Outcome Assessment		Confounding by Co-pollutants			Other Confounders				Statistical Methods	
	"High-quality" Study	Main Text Only	Design	Single vs. Multi-city	Size/Duration <sup>1</sup>	NO, NO <sub>2</sub> , NO <sub>x</sub>	Comparisons Between Oxides	Central Site Monitoring	Spatial Variability Assessed	Comparison of Exposure Assessment Methods	Type of Outcome	Exclusion of Children < 2 Years Old	Traffic-related Pollutants Assessed	Correlations Reported	Relative Measurement Error in Co-pollutants Discussed	Meteorology	Day of Week	Season	Allergens	Cautious Interpretation of Multi-pollutant Models	Sensitivity Analysis: Alternate Model Specification
Burra <i>et al.</i> (2009)	√		Time series	Single	10 years	NO <sub>2</sub>	No	Yes	No	No	Physician visits	No <sup>3</sup>	No <sup>15</sup>	No	No	Yes	Yes	Yes	No	NA	Yes
Sinclair <i>et al.</i> (2010)	√		Time series	Single	4 years	NO <sub>2</sub>	No	Yes	No	No	Acute out-patient visits	No	No <sup>16</sup>	Yes	No	Yes	Yes	Yes	No	NA	Yes
Tolbert <i>et al.</i> (2000)	√		Retrospective cohort	Single	5,934 ED visits for asthma/3 summers	NO <sub>x</sub>	No	Yes	No <sup>17</sup>	No	ED visits	No	No	Yes	No	Yes	Yes	Yes	No <sup>7</sup>	Yes	Yes
Jaffe <i>et al.</i> (2003)	√		Time series	Multi-city	6 summers	NO <sub>2</sub>	No	Yes	No <sup>17</sup>	No	ED visits	Yes	No	Yes	No	Yes	Yes	Yes	No	NA	No

Notes:

CO = Carbon Monoxide; EC = Elemental Carbon; ED = Emergency Department; HA = Hospital Admission; ISA = Integrated Science Assessment Oxides of Nitrogen; NO = Nitrogen Monoxide; NO<sub>2</sub> = Nitrogen Dioxide; NO<sub>x</sub> = Oxides of Nitrogen; O<sub>3</sub> = Ozone; OC = Organic Carbon; PM = Particulate Matter; UFP = Ultrafine Particles; VOC = Volatile Organic Compound.

(1) In Table 5-1, EPA did not indicate what sample size and duration are required for a study to be considered "large" and, therefore, more reliable. For the purposes of this table, we highlight time series studies of at least 10 years in duration and case cross-over studies of at least 10,000 events as higher quality.

(2) Several traffic-related co-pollutants were measured and examined in single-pollutants models, but authors did not attempt to determine whether NO<sub>2</sub> associations were confounded by traffic-related co-pollutants.

(3) < 1-year-old subjects excluded.

(4) 0- to 1-year-old subjects analyzed separately.

(5) Limited discussion of exposure measurement error in co-pollutants: only in the context of UFP and the potential that other pollutants were measured more accurately and served as proxies.

(6) Included additional diagnostic criteria for children < 1 year old to mitigate outcome misclassification.

(7) Aeroallergens measured but not included in statistical models as a confounder.

(8) Duration varied by city.

(9) CO and PM<sub>2.5</sub> measured and analyzed in separate models, but no multi-pollutant models were conducted.

(10) Desert dust, which includes bio-allergens.

(11) Compared monitoring systems.

(12) CO measured and analyzed in separate models, but no multi-pollutant models were conducted. Authors did not assess potential co-pollutant confounding in any other manner.

(13) Dispersion modeling used in addition to measurements from central site monitors.

(14) Nitrate also examined.

(15) PM<sub>2.5</sub> measured and analyzed in separate models, but no multi-pollutant models were conducted. Authors did not assess potential co-pollutant confounding in any other manner.

(16) PM<sub>2.5</sub>, CO, oxygenated VOCs, EC, OC, and metals were measured and analyzed in separate models, but no multi-pollutant models were conducted. Authors did not assess potential co-pollutant confounding in any other manner.

(17) Spatial variability of other pollutants (*i.e.*, O<sub>3</sub> and PM) only was assessed, but not variability of NO<sub>2</sub>.

**Table 3 Summary of Evidence Regarding the Relationship between Short-term NO<sub>2</sub> Exposure and Respiratory Effects**

ISA Table 5-45		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Consistent epidemiologic evidence from multiple, high-quality studies at relevant NO <sub>2</sub> concentrations.	Increases in asthma HAs, ED visits in diverse populations in association with 24-h avg. and 1-h max. NO <sub>2</sub> , lags 0 and 3- to 5-day avg. among all ages and children.	Inconsistent evidence from multiple epidemiology studies at relevant NO <sub>2</sub> concentrations.	Positive, statistically significant associations observed in some studies, but <b>many associations appeared only in subgroup analyses</b> . Associations for varying lags were inconsistent within and between studies. <b>Study quality was not systematically assessed by EPA.</b>
	No association in recent Canadian multicity study.		<b>Null findings reported in other studies</b> , in addition to the recent Canadian multicity study.
	Coherence with increases in respiratory symptoms and decrements in lung function in populations with asthma in association with 24-h avg., 2-4 h avg. NO <sub>2</sub> , 1-h max., lags 0, 3 to 6-day avg.  Panel studies of children examined representative populations recruited from schools.  No reports of selective participation by particular groups.		<b>Evidence related to respiratory symptoms and lung function is mixed. Null associations from individual studies are not presented in ISA figures.</b> Results from studies of supervised lung function measurements are not more consistent or stronger than those based on home lung function tests.
Consistent evidence for NO <sub>2</sub> metrics with lower potential for exposure measurement error.	Asthma-related effects associated with NO <sub>2</sub> measured in subjects' locations: total and outdoor personal, school outdoor.  Better spatial alignment with subjects compared to central site NO <sub>2</sub> .	Inconsistent evidence for NO <sub>2</sub> metrics with lower potential for exposure measurement error.	<b>References cited in ISA as key evidence mainly reported null associations.</b>
Consistent evidence from multiple, high-quality controlled human exposure studies. Rules out chance, confounding, and other biases with reasonable confidence.	NO <sub>2</sub> increases airway responsiveness in adults with asthma exposed at rest following nonspecific or allergen challenge in several individual studies and meta-analyses. Clinical relevance supported by findings of a doubling reduction in PD in response to NO <sub>2</sub> .	Findings from controlled human exposure studies do not provide clear evidence that NO <sub>2</sub> increases airway responsiveness at concentrations less than 600 ppb.	<b>Studies that evaluated airway responsiveness to specific allergen challenge, which are most relevant for understanding potential effects of ambient NO<sub>2</sub>, do not provide evidence that NO<sub>2</sub> increases airway responsiveness.</b> Paradoxical effect for studies involving exposure while at rest but not while exercising. <b>Lack of a concentration-response both within and across studies,</b> including for studies that observed a clinically relevant doubling reduction in PD.

**Table 3 Summary of Evidence Regarding Short-term NO<sub>2</sub> Exposure and Respiratory Effects (Continued)**

ISA		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Epidemiologic evidence helps rule out chance, confounding, and other biases with reasonable confidence.	NO <sub>2</sub> associations with lung function and pulmonary inflammation persist in co-pollutant models with a traffic-related co-pollutant: PM <sub>2.5</sub> , EC/BC, OC, UFP, or VOCs in studies with exposure assessment in subjects' locations.	Insufficient epidemiology evidence to rule out chance confounding and other biases. Model selection bias and publication bias are key issues.	<b>Potential confounding by co-pollutants assessed by inspecting results of multi-pollutant models, which are highly unreliable.</b>
	Ambient and total personal NO <sub>2</sub> weakly-moderately correlated with other traffic-related pollutants in some studies (r = -0.43 to 0.49).		<b>Differential exposure measurement error is not rigorously or systematically assessed in multi-pollutant analyses discussed in EPA.</b>
	Most central site NO <sub>2</sub> associations persist with adjustment for PM <sub>2.5</sub> , EC/metals factor, UFP, or CO.		<b>Potential confounding by co-pollutants assessed by inspecting results of multi-pollutant models, which are highly unreliable.</b>
	Differential exposure measurement error limits inference from co-pollutant models based on central site NO <sub>2</sub> and co-pollutants.		<b>Studies of indoor NO<sub>2</sub> mainly report null findings.</b>
	Some associations were attenuated with adjustment for PM <sub>2.5</sub> or UFP.		Several studies show that <b>results are sensitive to changes in model specification.</b>
	Most associations for microenvironmental and central site NO <sub>2</sub> persist in co-pollutant models with PM <sub>10</sub> , SO <sub>2</sub> , or O <sub>3</sub> .		
	Indoor NO <sub>2</sub> associated with increases in respiratory effects in children with asthma.		
NO <sub>2</sub> associations persist with adjustment for meteorology, time trends, season, medication use.			
<b>Evidence for Key Events in Mode of Action</b>			
Allergic responses	Increases in eosinophil activation, IgE, Th2 cytokines in adults with asthma.	Studies do not provide robust evidence that allergic responses contribute to increased HAs or ED visits for asthma.	<b>Increases in markers of allergic responses were not accompanied by increased airway responsiveness or sufficient to exacerbate asthma.</b>
Inflammation	Increases in PMNs and prostaglandins in healthy adults.	Studies do not provide robust evidence that pulmonary inflammation contributes to increased HAs or ED visits for asthma.	<b>Increases in PMNs were not observed at NO<sub>2</sub> concentrations less than 2,000 ppb. No evidence that increases in PMNs were accompanied by relevant physiological changes, such as increased permeability of the airway epithelial barrier, or cell damage.</b>

Notes:

BC = Black Carbon; CO = Carbon Monoxide; EC = Elemental Carbon; ED = Emergency Department; EPA = United States Environmental Protection Agency; HA = Hospital Admission; IgE = Immunoglobulin E; ISA = Integrated Science Assessment; NO<sub>2</sub> = Nitrogen Dioxide; O<sub>3</sub> = Ozone; OC = Organic Carbon; PD = Provocative Dose; PM = Particulate Matter; PMN = Polymorphonuclear Leukocyte; ppb = Parts Per Billion; SO<sub>2</sub> = Sulfur Dioxide; Th2 = T-Derived Lymphocyte Helper 2; UFP = Ultrafine Particles; VOC = Volatile Organic Compound.

Key Evidence Shading	Definition
	Evidence supports a causal association
	Evidence does not support a causal association

**Table 4 Summary of Key Evidence Regarding the Relationship between Long-term NO<sub>2</sub> Exposure and Development of New-onset Asthma**

ISA Table 6-5		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Consistent epidemiology evidence from multiple, high-quality studies with relevant NO <sub>2</sub> concentrations.	Consistent evidence for increases in asthma incidence in diverse cohorts of children in the US, Europe, Canada, and Asia.	Epidemiology studies have limitations and uncertainties, and evidence is inconsistent.	<b><u>Inconsistent evidence for asthma incidence within and across cohorts of children in the US, Europe, Canada, and Asia.</u></b>
	Asthma ascertainment by parental report of doctor diagnosis.		<b><u>Asthma diagnosis is uncertain in young children</u></b> and doctor diagnosis was not always reported by parents.
	Supporting evidence for asthma incidence or chronic bronchitis in the ECRHS cohort of adults.		<b><u>Inconsistent evidence for asthma incidence in the ECRHS cohort of adults.</u></b> Findings on chronic bronchitis not relevant.
Consistent evidence for NO <sub>2</sub> metrics with lower potential for exposure measurement error.	In children, asthma associated with residential NO <sub>2</sub> estimated using well-validated LUR models or by monitoring.	Studies have considerable error and uncertainty in exposure estimation, and evidence is inconsistent.	<b><u>The majority of the studies had a limited number of measurements and could not fully account for temporal variation in NO<sub>2</sub> levels.</u></b>
Uncertainty regarding potential confounding by traffic-related co-pollutants.	When reported, correlations with PM <sub>2.5</sub> and EC often were high (r = 0.7-0.96). No co-pollutant models analyzed.  Associations found with adjustment for SES, family history of asthma, smoking exposure, housing characteristics, and presence of gas stove.	Uncertainty regarding potential confounding by traffic-related co-pollutants.	<b><u>Similar effects on asthma incidence in children often found for traffic-related co-pollutants in the same studies.</u></b>  No co-pollutant analyses conducted.

**Table 4 Summary of Key Evidence Regarding Long-term NO<sub>2</sub> Exposure and New-onset Asthma (Continued)**

ISA Table 6-5		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Coherence with respiratory effects of short-term NO <sub>2</sub> exposure.	Limited epidemiologic evidence for increases in pulmonary inflammation in healthy children and adults with exposures assessed in subjects' locations and associations adjusted for BC/EC, OC, PNC, or PM <sub>2.5</sub> .	Lack of coherence with respiratory effects of short-term NO <sub>2</sub> exposure.	Limited epidemiology evidence for pulmonary inflammation (eNO) associated with short-term NO <sub>2</sub> exposure in healthy children and adults.
	Evidence from controlled human exposure studies for increased airway responsiveness in healthy adults.		Uncertainty in findings due to influence of diet and other factors on individual eNO levels.
Limited and supporting toxicological evidence at relevant NO <sub>2</sub> exposures.	Increased AHR in guinea pigs with long-term or short-term NO <sub>2</sub> exposure.	No evidence of increased AHR at ambient NO <sub>2</sub> levels.	<b>Effects may be secondary to tissue damage due to high exposure level.</b>
<b>Some Evidence for Key Events in Mode of Action</b>			
Allergic responses	Increased IgE-mediated histamine release in mast cells from rodents.	No evidence of enhanced mast cell activation at ambient NO <sub>2</sub> exposure levels.	<b>Inconsistent evidence of IgE-mediated histamine release in mast cells among rodent species</b> (increased in guinea pigs, no effect in rats). Effects may be secondary to tissue damage due to high exposure level.
	Experimental findings for development of Th2 phenotype with short-term NO <sub>2</sub> exposure.	No evidence of Th2 phenotype at ambient NO <sub>2</sub> exposure levels.	Controlled human exposure data may be confounded by reporting bias. No direct evidence of Th2 phenotype in experimental animal data. <b>Effects may be secondary to tissue damage due to high exposure level.</b>
Airway remodeling	Increased airway resistance with AHR in guinea pigs.	No direct evidence of airway remodeling.	Increased airway resistance appears to be transient. Effects may be secondary to tissue damage due to high exposure level.

**Table 4 Summary of Key Evidence Regarding Long-term NO<sub>2</sub> Exposure and New-onset Asthma (Continued)**

ISA Table 6-5		GRADIENT	
Rationale for Causal Determination	Key Evidence	Rationale for Causal Determination	Key Evidence
Inflammation	Increases in lymphocytes, PMNs, in rats with long-term exposure.	No evidence of inflammation at ambient NO <sub>2</sub> exposure levels.	<b>No evidence of increased lymphocyte or PMN cells in rats;</b> change in number cannot be determined from proportions. Discordant effects observed between gestational and juvenile exposure group in rats.
	Increases in PMNs in healthy adults with repeated short-term exposure.	Insufficient evidence that inflammation contributes to development of new-onset asthma.	<b>Increase in PMNs observed only at NO<sub>2</sub> concentrations of at least 2,000 ppb.</b> Increase in PMNs not associated with overt inflammatory effects.
	Longitudinal changes in eNO in children independent of asthma status.	Evidence is not sufficient to support long-term NO <sub>2</sub> exposure contributing to pulmonary inflammation.	Potential for residual spatio-temporal confounding not fully addressed. No adjustment for potential confounding by co-pollutants. <b>Substantial uncertainty regarding whether eNO is an accurate marker of pulmonary inflammation.</b>
Oxidative stress	Varying and transient effects on antioxidant levels and enzyme activity.	No evidence of pulmonary oxidative stress at ambient NO <sub>2</sub> levels.	Effects were variable and transient. Effects may be secondary to tissue damage due to high exposure level.

Notes:

AHR = Airway Hyper-responsiveness; BC = Black Carbon; EC = Elemental Carbon; ECRHS = European Community Respiratory Health Study; eNO = Exhaled Nitric Oxide; IgE = Immunoglobulin E; ISA = Integrated Science Assessment; LUR = Land Use Regression; NO<sub>2</sub> = Nitrogen Dioxide; OC = Organic Carbon; PM = Particulate Matter; PMN = Polymorphonuclear Leukocyte; PNC = Particle Number Concentration; ppb = Parts Per Billion; SES = Socioeconomic Status; Th2 = T-Derived Lymphocyte Helper 2.

Key Evidence Shading	Definition
	Evidence supports a causal association
	Evidence does not support a causal association