**Summary**

* In general, USEPA made significant improvements in the second draft NO2 ISA.
* In the second draft NO2 ISA, USEPA continued to use ecological epidemiology studies to support causal associations between NO2 exposure and certain health endpoints. Key uncertainties remain regarding this procedure.
* Respiratory effects (short-term exposure): TCEQ agrees with the causal determination between short-term NO2 exposure and increased airway responsiveness in asthmatics for concentrations at or above the current 1-hour NAAQS of 100 ppb, based on evidence from controlled human and animal studies and to a limited extent, epidemiological studies. Evidence for causal associations between other respiratory effects and short-term exposure to NO2 concentrations is inconsistent, weak, or limited to high exposure concentrations.
* Respiratory effects (long-term exposure): In the second draft ISA, USEPA strengthened the causal determination to “likely to be a causal relationship” based on new evidence from epidemiological studies for asthma incidence and respiratory symptoms in children, and respiratory effects in adults. In the absence of more conclusive evidence from controlled exposure studies in humans or animals, TCEQ does not agree with strengthening this causal determination based on the information presented in the second draft ISA.
* Cardiovascular effects (short-term and long-term exposure): In the current NO2 ISA, the USEPA concluded that available evidence is suggestive but not sufficient to infer causal relationships for cardiovascular disease with short- and long-term NO2 exposure. These conclusions represent a change from the 2008 ISA based upon epidemiologic evidence linking myocardial infarction to short-term exposure and new evidence linking heart disease to long-term exposure. The majority of available epidemiological studies fail to demonstrate that the observed outcomes are independently associated with NO2 exposure (i.e., inclusive to). Some experimental studies indicate that short-term NO2 exposure increases inflammation and oxidative stress in the blood or heart tissue. While increases in inflammation and oxidative stress reveal a possible mechanism for NO2 exposure to induce cardiovascular and related metabolic disease, the available data does not link these observations to the manifestation of disease (in epidemiological studies) or demonstrate that these effects occur at environmentally-relevant concentrations (in animal studies). Thus, the TCEQ agrees that available evidence is suggestive of a causal relationship for cardiovascular disease and NO2 exposure.
* Metabolic effects (short-term and long-term exposure): The TCEQ agrees that available evidence is suggestive, but not sufficient to infer, a causal relationship for short- and long-term NO2 exposure and metabolic effects. Importantly, available data is limited by uncertainties produced by confounding factors and lack of definitive data from well-controlled exposure studies in humans and animals.
* Mortality (short-term exposure): In the second draft ISA, USEPA concluded that the evidence for short-term NO2 exposure and total mortality is suggestive, but not sufficient to infer a causal relationship. This conclusion is the same as the conclusion reached in the 2008 ISA for Oxides of Nitrogen. The TCEQ agrees that the causal association should not be strengthened based on the available information. Additionally, the TCEQ thinks that a strong case can be made for reducing the causal determination to “inadequate to infer a causal relationship” because of major uncertainties in the available evidence for short-term NO2 exposure and total mortality.
* Mortality (long-term exposure): Based on the available evidence presented in the draft ISA, the TCEQ thinks that it is not appropriate to conclude the overall evidence is “suggestive of a causal relationship” between long-term exposure to NO2 and mortality among adults.
* Reproductive and Developmental Effects (long-term exposure): Based on the evidence presented in the second draft ISA, TCEQ agrees with the causal determination for long-term NO2 exposure and fertility, reproduction, and pregnancy as well as postnatal development of “inadequate to infer a causal relationship.” TCEQ agrees with the causal determination for long-term NO2 exposure and birth outcomes of “suggestive, but not sufficient, to infer a causal relationship,” but *only at high concentrations*. The experimental evidence available suggests that effects such as maternal toxicity and reduced litter size only occur at concentrations high enough to cause overt toxicity.
* Cancer (long-term exposure): TCEQ does not agree with strengthening the causal determination to “suggestive, but not sufficient, to infer a causal relationship” based on the existing body of evidence. Not only is there a lack of clear experimental evidence and mechanistic data to inform a potential mode of action for NO2 acting as a direct carcinogen, but the epidemiological studies used as supporting evidence show weak, inconsistent, or no association between cancer and long-term exposure to NO2. Based on the existing body of evidence, the TCEQ thinks that the causal determination for this endpoint should be “inadequate to infer a causal relationship.”

**General Comments**

In general, USEPA made significant improvements in the second draft NO2 ISA.

One improvement is that USEPA clarified how the ISA evaluates the aspects listed in the Preamble (consistency, coherence, biological plausibility, exposure-response, strength of association, experimental evidence, temporal relationship, specificity of the observed association, and analogy) and how they were integrated into causal determinations.

Another significant improvement is that, with some exceptions, USEPA standardized the results of epidemiology studies to increase comparability among studies and they clearly defined the methodology used for standardization. Comprehensive tables of available studies are presented at the end of the various chapters; however, it is not clear why summary figures only show a subset of studies presented in the tables. USEPA should provide an explanation for why only a subset of studies was presented in summary tables in some of the sections.

The TCEQ agrees with the statement in Section 5.1.2.4 that “Controlled human exposure and animal toxicological studies can provide direct evidence for health effects related to NO2 or NO exposure. Coherence between experimental and epidemiological studies can address uncertainties within the collective body of evidence.” However, USEPA repeatedly uses epidemiology study results as evidence of a causal association between certain health endpoints and NO2 exposure, even in the absence of experimental evidence. With a notable lack of experimental evidence and mechanistic data, there is significant uncertainty in the interpretation of certain realms of evidence, especially epidemiologic studies. Throughout the document, the evidence for specific endpoints is often inconsistent or weak and effect estimates are small and/or not statistically significant. Nevertheless, the document then proceeds to combine these and draw causal determinations for the overall endpoint. USEPA should include a discussion addressing the rationale for this practice since it contradicts what is stated in Section 5.1.2.4.

**Use of Ecological Epidemiology Studies**

1. Ecological epidemiology studies are not designed to determine if oxides of nitrogen caused the health effects observed. Instead, these studies simply report statistical associations.

For example, epidemiology studies evaluating cardiovascular diseases are often very broad in their scope and include diseases of the circulatory system such as heart disease and cerebrovascular disease. The assumption that oxides of nitrogen caused all evaluated cardiovascular health effects (i.e., cardiac causes such as MI and heart failure, hypertension, heart rate and heart rate variability, venous thromboembolism, cerebrovascular diseases and stroke, and other cardiovascular causes of hospital admission or ED visit) is not supported by the ecological epidemiology studies.

Ecological epidemiology studies do not collect data on when, how long, and how much exposure occurred; if exposure occurred before the health effects; or if it makes biological sense that the chemical could cause the effect. In other words, the study designs are limited. There is general agreement that this study design does not provide enough information to determine the actual cause of studied effects.[[1]](#footnote-1) Ecological epidemiology studies are not supposed to be used quantitatively and they certainly are not rigorous enough to set environmental policy.

2. Lack of personal exposure data severely limits the utility of ecological epidemiology studies.

The issue of limited or entirely absent personal exposure data is significant. Personal exposure is a measurement of the amount of an air pollutant that a person actually breathes. Ecological epidemiology studies generally rely on ambient air monitoring data as a surrogate for personal exposure. Central site monitors are limited in their capacity to resolve individual variations in NO2 exposure. It is very unlikely that people would ever be exposed to those pollutants at concentrations measured at outdoor monitors for long durations of time. This is due, at least partly, to the fact that the average American spends the majority of their time indoors, as indicated by USEPA in the 2008 Oxides of Nitrogen ISA Annexes (Section AX3.4.1) and Klepeis et al. (2001). In fact, a study conducted by Leaderer et al. (1986) found that concentrations of NO2 inside the home (which are not measured in the vast majority of ecological epidemiology studies) accounted for 80% of the variance in total personal exposure. The second draft NO2 ISA acknowledges that many uncertainties exist regarding the extent to which ambient, personal, and indoor exposure is correlated (USEPA 2015).

4. Ecological epidemiology studies have considerable uncertainty in their identification of health effects.

To determine the prevalence of a health issue, epidemiologists frequently use readily-available information, including hospital admissions records and death certificates or participant surveys. Use of this type of information can be problematic when paired with the lack of personal exposure data, making it impossible to know if decedents were actually well enough to be outdoors in the days preceding their deaths. The data is further confounded by the frequent use of a single monitor to represent exposures throughout the city – as if a single monitor can accurately reflect personal exposure with measurements sometimes miles away.

**Health Effect Category**

**Respiratory Effects**

***Section 5.2 Respiratory Effects (Short-term Exposure)***

In the 2008 ISA for Oxides of Nitrogen, USEPA determined that the evidence was “sufficient to infer a likely causal relationship” between short-term NO2 exposure and respiratory effects. Similarly, in the draft ISA, USEPA determined that recent evidence gave additional support to the association between short-term NO2 exposure and respiratory effects and concluded there was a “causal relationship.” TCEQ agrees with the causal determination between short-term NO2 exposure and increased airway responsiveness in asthmatics for concentrations at or above the current 1-hour NAAQs of 100 ppb, based on evidence from controlled human and animal studies and, to a limited extent, epidemiological studies. Evidence for causal associations between other respiratory effects (i.e., allergy exacerbation, reductions in lung function, increases in respiratory infection and chronic obstructive pulmonary disease, and respiratory effects in healthy populations) and short-term exposure to NO2 concentrations is inconsistent, weak, or limited to high exposure concentrations.

TCEQ does not agree that USEPA presented enough evidence to demonstrate a causal link between NO2 exposure and increases in respiratory hospital admissions, ED visits, and respiratory mortality. Major uncertainties remain regarding the causal factor(s) of respiratory effects associated with short-term ambient NO2 exposure because of the high correlation of NO2 with other traffic-related pollutants (i.e., ozone, carbon monoxide, PM10, PM2.5) and the potential for NO2 to serve primarily as an indicator for another pollutant or mixture of combustion-related pollutants.

**Section 5.2.2.1 Airway Hyperresponsiveness (AHR)/Airway Responsiveness (AR)**

In the 2008 ISA for Oxides of Nitrogen, the USEPA identified multiple lines of evidence as support for a causal association between short-term NOx exposure and respiratory effects. As stated in Section of the second draft NO2 ISA:

“Controlled human exposure studies demonstrated NO2-induced increases in airway responsiveness in adults with asthma. These findings for increased airway responsiveness, a characteristic feature of asthma, provided biological plausibility for epidemiologic evidence for asthma exacerbation. Further, airway responsiveness was increased following <1 to 6-hour exposures to NO2 at concentrations in the range of 100 to 300 ppb, which are not much higher than peak ambient concentrations.”

The 2008 ISA for Oxides of Nitrogen also noted some support for pulmonary inflammation and impaired host defenses in controlled human exposure and animal toxicological studies, albeit at higher concentrations of 1,500 to 5,000 ppb NO2. Increases in airway hyperresponsiveness (AHR)/airway responsiveness (AR) occur after exposure to lower NO2 concentrations than other respiratory system effects observed in controlled human exposure and animal toxicological studies (including pulmonary inflammation and impaired host defenses); therefore, increased airway responsiveness is considered the most sensitive endpoint for respiratory effects.

In the 2008 ISA, USEPA conducted a meta-analysis of controlled human studies evaluating the effects of short-term NO2 exposure on AHR. The USEPA meta-analysis was based on a meta-analysis conducted by Follinsbee 1992. USEPA concluded that a 1-hour exposure to 100 ppb NO2 caused increased airway responsiveness in 66% of mild asthmatics. In addition, 67% of asthmatics experienced an increase in airway responsiveness following exposure to NO2 concentrations from 100 to 150 ppb, 75% of asthmatics experienced an increase in airway responsiveness following exposure to NO2 concentrations from 200 to 300 ppb, and 73% of asthmatics experienced an increase in airway responsiveness following exposure to NO2 concentrations above 300 ppb. The fraction of resting asthmatics experiencing an increase in airway responsiveness was statistically significant at all of these NO2 concentrations. Major uncertainties remained for the USEPA (2008) meta-analysis. The magnitude of response could not be determined from the meta-analysis conducted by USEPA (2008) and it was not clear if the observed effects were clinically significant. The primary short-term NAAQs of 100 ppb was based, in part, on studies showing an increase in AHR in asthmatics after a 1-hour exposure to NO2; therefore, the results and interpretation of this meta-analysis are of particular importance.

In an attempt to address some of the limitations of the USEPA (2008) meta-analysis and one of the few more recent studies of NO2 effects on AHR, Goodman et al. (2009) conducted meta-regression and meta-analyses studies evaluating the effects of NO2 exposure (100 to 600 ppb) on AHR in asthmatics. Details about this study were discussed in the first draft ISA. Several effect estimates from the meta-analysis were statistically significant; however, they were so small that the clinical relevance of these effect estimates was questionable. They found no clear exposure-response associations for any effect estimates based on linear meta-regressions or analyses of effect estimates for exposure groups, and in general for analyses stratified by airway challenge, exposure method, and activity during exposure. Goodman et al. (2009) concluded that “to the extent that the effects observed are associated with NO2 exposure, they are sufficiently small such that they do not provide evidence that NO2 has a significant adverse effect on AHR at concentrations up to 600 ppb.” Another conclusion was that exposure duration was not significantly associated with AHR for any of the effect metrics.

In the first draft NO2 ISA, USEPA discussed the results of the Goodman et al. (2009) study but supported their decision to use 100 ppb as a concentration at which adverse effects on AR have been observed in asthmatics after a 1 hour exposure. USEPA also pointed out differences in methodology used by Goodman et al. (2009) which may give rise to different conclusions (USEPA 2013).

In the second draft ISA, USEPA conducted a meta-analysis to address the uncertainties remaining for the USEPA (2008) meta-analysis of AR in asthmatics. The meta-analysis in the second draft ISA provided a comprehensive assessment of the clinical relevance of changes in airway responsiveness, the potential for methodological biases in the original papers, and the distribution of responses. USEPA stated in Section 5.2.2.1:

“This section provides analyses showing that a statistically significant fraction (i.e., 70% of individuals with asthma exposed to NO2 at rest) experience increases in airway responsiveness following 30-minute exposures to NO2 in the range of 200 to 300 ppb and following 60-minute exposures to 100 ppb. The distribution of changes in airway responsiveness is log-normally distributed with a median change of 0.75 and [provocative dose following NO2, divided by PD following filtered air exposure] and geometric standard deviation of 1.88. About a quarter of the exposed individuals experience a clinically relevant reduction in their provocative dose due to NO2 relative to air exposure. The fraction experiencing an increase in responsiveness was statistically significant and robust to exclusion of individual studies. The results of the meta-analysis showed minimal change in airway responsiveness for individuals exposed to NO2 during exercise. A variety of factors that may affect the assessment of airway responsiveness and how those factors may directionally bias the results of individual studies and the analyses in this current assessment are considered.”

The TCEQ believes USEPA adequately addressed the uncertainties remaining in the meta-analysis conducted in the first draft ISA and strengthened the conclusion that AR is indeed an adverse effect observed in asthmatics after short-term exposure to *NO2* concentrations of 100 ppb for 1 hour and 200 to 300 ppb for 30 minutes.

***Section 5.2 Respiratory Effects (Long-term Exposure)***

In the 2008 ISA for Oxides of Nitrogen, USEPA determined that the evidence was “suggestive but not sufficient to infer a causal relationship” between long-term NO2 exposure and respiratory effects. In the second draft ISA, USEPA strengthened the causal determination to “likely to be a causal relationship” based on new evidence from epidemiological studies for asthma incidence and respiratory symptoms in children, and respiratory effects in adults. Epidemiological studies are designed to evaluate possible associations, not determine causation, as discussed in TCEQ general comments above. In the absence of more conclusive evidence from controlled exposure studies in humans or animals, TCEQ does not agree with strengthening the causal determination based on the information presented in the second draft ISA.

**Cardiovascular Effects**

***Section 5.3 Cardiovascular Effects (Short-term Exposure)***

In the 2008 ISA for Oxides of Nitrogen USEPA determined that the evidence was "inadequate to infer the presence or absence of a causal relationship for oxides of nitrogen to contribute to cardiovascular-related morbidity and associated mortality". In the first draft ISA from the current review (USEPA 2013) the USEPA strengthened the association to “likely to be causal”. In the current second draft ISA (USEPA 2015), the USEPA down-graded the causal determination to “suggestive but not sufficient to infer causal relationships for cardiovascular disease.” The TCEQ agrees with this latest determination.

***Epidemiological Studies***

The current draft of the NOx ISA evaluated many endpoints to determine whether NO2 exposure was associated with cardiovascular disease, including myocardial infarction, angina pectoris, arrhythmia, hypertension, cardiac arrest, cerebrovascular disease, stroke, decompensation of heart failure, venous thromboembolism, cardio-metabolic effects, and subclinical cardiovascular effects. Endpoints were often evaluated in the context of epidemiological studies where associations were inconsistent and confounders (e.g., traffic copollutants) were not accounted for.

For example, the ISA indicates that the Ito et al. (2011) study reported a positive association with NO2 and CVD hospitalization at lag 0 and that the study did not include results from copollutant models. Other epidemiology studies reported associations to be less precise when adjustments were made for copollutants such as sulfur dioxide [Guo et al. (2009); Chen et al. (2010b)]. Additionally, several other studies from Denmark, Spain, and Taiwan reported null or negative associations between NO2 concentrations and risk of hospital admissions for CVD (Andersen et al., 2008b, Linares and Diaz, 2010; Chen et al., 2008).

Another general concern with the vast majority of available epidemiological studies is that NO2 exposure is often represented as an estimate using central site monitors or city-wide averages. These types of estimates do not accurately describe variance in individual NO2 exposure and likely represent a source of error (exposure measurement error). This is of particular concern given that the studies where personal monitors were used showed no association between NO2 and cardiovascular health (Williams et al., 2012a; Liu et al., 2014b). Due to lack of consistency and potential confounding, the USEPA reasonably assigned a designation of the evidence being suggestive of a causal association between NO2 exposure and cardiovascular disease. The TCEQ agrees that the data supports this designation, according to the USEPA’s own criteria, regarding the epidemiological data for short-term cardiovascular effects.

***Controlled Human Exposure Studies***

The results from the controlled human exposure studies do not support the hypothesis that *typical ambient concentrations* of NO2 affects cardiovascular function. Many of the controlled human exposure studies included NO2 concentrations (400 ppb - 4000 ppb) that are more than an order of magnitude higher than the average ambient concentrations reported in the epidemiological studies (20-60 ppb). Many controlled exposure studies reported no changes in in heart rate (HR), cardiac output, and/or blood pressure (BP) even with high exposure concentrations of NO2 (i.e., 400 ppb – 4000 ppb) in healthy and/or asthmatic volunteers. For example Linn et al. (1985) reported no change in BP, either in healthy or asthmatic volunteers after exposure to 4000 ppb of NO2. Likewise,Langrish et al. (2010) did not report any effects of NO2 on vascular endothelial tone or fibrinolytic function after exposure to 4000 ppb NO2 for 1-h with intermittent exercise in healthy adults.

Clinical studies of both healthy volunteers (Huang et al. 2012) or individuals with coronary heart disease (Scaife et al. 2012) exposed to high concentrations of NO2 (i.e., 400 ppb) reported no statistically significant changes in heart rate variability (HRV) or heart rate (HR). Results from controlled exposures in healthy older adults to 600 ppb NO2 (Folinsbee et al. 1978 and Drechsler-Parks 1995) or asthmatics (Gong et al. 2005) also reported no changes in HR.

A number of new studies have been recently published investigating the impact of NO2 inhalation on the induction of biomarkers associated with inflammation and oxidative stress. For example, Huang and colleagues (2012b) observed that exposure to 500 ppb NO2 for 2 hours with intermittent exercise induced a statistically non-significant increase in IL-6. Similarly, plasma was isolated from healthy volunteers exposed to 500 ppb NO2 while intermittently exercising and was used to treat primary human coronary artery endothelial cells (hCAECs). Plasma collected immediately after NO2 exposure and exercise induced IL-8 in the hCAEC cells, indicating the possibility for induction of inflammatory mediators in NO2-exposed volunteers.

The limitation that exists in drawing conclusions from the induction of inflammatory biomarkers such as C-Reactive Protein (CRP), interleukin-8 (IL-8), or IL-6 to indicate physiological changes that could precipitate cardiovascular disease is that all inflammatory conditions can increase the circulating levels of such molecules. Many of the biomarkers that are used as predictors of cardiovascular disease are also elevated in many conditions including central nervous system disorders, autoimmunity, fibromyalgia, bacterial infections, tumors, cardiac diseases, viral infections, allergies, asthma, and diabetes, among others.

Given these human clinical experimental data, there is little evidence that NO2 can affect cardiovascular health at ambient concentrations (ie. < 150 ppb).

***Animal Toxicological Studies***

Like the aforementioned human exposure studies, several publications have investigated the impact of NO2 exposure on biomarkers of inflammation, oxidative stress, and cellular adhesion in rodents exposed to NO2. These studies have similar limitations as the human studies in that the NO2 exposure concentrations are significantly higher than those observed in ambient air. Li and colleagues (2011a) reported that rats exposed to 2660 or 5320 ppb NO2 for 7 days had increased biomarkers of oxidative stress and mild pathological changes in heart tissue. Campen et al (2010) observed an NO2 concentration-dependent increase in the antioxidant enzyme HO-1 in aortic tissue of apolipoprotein E knockout mice at 200 to 2000 ppb. While many of these studies demonstrate that NO2 inhalation can induce systemic inflammation and changes in cardiovascular tissue, their applicability to human health is limited by the concentrations required to induce such pathological changes.

Taken together, data from controlled human exposure and animal toxicological studies support USEPA’s conclusion that evidence is suggestive of a causal relationship between NO2 exposure and cardiovascular disease. TCEQ agrees with this conclusion, but questions their applicability to human health due to a *lack of consistent concentration-response data at environmentally-relevant concentrations* *of NO2*.

***Section 5.3 Cardiovascular and Metabolic Effects (Long-term Exposure)***

The USEPA strengthened the causal determination for long-term NO2 exposure and cardiovascular effects to “suggestive of a causal relationship” and added diabetes and insulin deficiency effects to the second draft ISA. The TCEQ agrees with the draft ISA’s conclusion. However, the TCEQ notes that the strongest data include controlled human exposure studies and toxicological studies conducted at NO2 concentrations significantly higher than ambient concentrations.

***Epidemiological Studies***

The evidence for cardiovascular and metabolic diseases was not consistent across the different disease categories and in some cases the associations seem to weaken when adjustments for other confounders were included. The second draft ISA includes studies that reported both positive associations and/or null or negative associations. The long-term exposure to NO2 and cardiac heart disease (CHD) hospital admission was not supported by the Gan et al. (2011) study. Miller et al. (2007) also reported a null association between NO2 and cardiovascular events (MI, revascularization, angina, CHF, CHD death). However, the inclusion of recent, large epidemiological studies in weight of evidence (WOE) analysis generally supports the hypothesis that long-term NO2 exposure is associated with *some* *forms of heart disease and diabetes* (Cesaroni et al., 2013; Beckerman et al., 2012). It is important to note that the association with stroke and hypertension is weak and inconsistent. Likewise, conclusions are limited by the fact that exposure estimates were derived using monitoring or modeling data (which generally does not take into account personal exposure) and outcomes were probably confounded by copollutant exposure.

Despite the weak WOE for cardiovascular morbidity, there seems to be a suggestion of positive association between long-term NO2 exposure and cardiovascular disease mortality, supporting USEPA’s designation of evidence being suggestive of a causal relationship.

***Animal Toxicological Studies***

There was limited data regarding the long-term effects of NO2 inhalation in animal models for the previous ISA. More data has become available for this draft, which supports that NO2 exposure may be associated with some forms of cardiovascular disease and diabetes. The most significant weakness of these studies is that they were generally conducted at higher concentrations of NO2 than are observed in ambient air, or with mixtures of air copollutants. For example, Seilkop and colleagues (2012) exposed mice to a mix of air pollutants, including various concentrations of NO2 (0-3670 ppb), for 50 days. Using a data mining program, the authors found that NO2 strongly associated with markers of oxidative stress and cardiovascular disease progression. While the *in silico* data *suggest* exposure related induction of these effects, it is not possible to exclusively attribute them to NO2.

In summary, epidemiological and animal toxicological evidence is suggestive but not sufficient to infer a causal relationship between long-term exposure to NO2 and some cardiovascular and metabolic effects. It is important to note that uncertainty remains regarding whether or not NO2 causes these effects at environmentally relevant concentrations, as available data is strongest at high concentrations. The lack of conclusive evidence in long-term animal exposure data weakens the proposed mechanisms responsible for these associations.

## Mortality

***Section* *5.4 Total Mortality (Short-term Exposure)***

Epidemiology studies provide the only evidence showing an association between total mortality and short-term exposure to NO2. The majority of studies were conducted in Asia and only a limited number of studies have been conducted in more relevant populations (i.e., in the US, Canada, and Europe). Additionally, most of the recent studies published since the 2008 ISA did not focus specifically on the NO2 -mortality relationship, but rather on other pollutants. Given the limited number of studies in relevant populations and the lack of NO2-specific data, significant uncertainties remain regarding whether there is a relationship between short-term exposure to NO2 and total mortality. Other major uncertainties are caused by relying solely on epidemiological evidence to show a causal association. Uncertainties associated with epidemiological studies were addressed in our General Comments section.

Figure 5-22 of the second draft ISA (USEPA 2015) summarized available total mortality studies and standardized results to show percent increase in mortality per 20-ppb increase in 24-h average NO2 concentration or 30-ppb increase in 1-h max NO2 concentrations. The figure does not specify how individual study results were standardized (ie. which studies were standardized to show percent increase in mortality per 20-ppb increase in 24-h average NO2 concentration and which were standardized to show 30-ppb increase in 1-h max NO2 concentrations). USEPA should clearly indicate how each individual study was standardized.

A key uncertainty of the NO2-mortality relationship identified in the 2008 ISA was whether NO2 or another unmeasured pollutant was responsible for the mortality. Measuring potential confounding of results has been complicated because many available studies do not focus on traffic-related pollutants only. Effect modifiers like age, smoking status, socioeconomic status, and income have only been evaluated in a small percentage of available epidemiological studies. Studies that have looked at effect modifiers have found that certain effect modifiers can alter the NO2-mortality relationship, which further complicates the interpretation of results across all studies. Seasonal variability and lag structure have also been found to affect the NO2-mortality relationship, further increasing the uncertainty associated with making causal determinations using the available epidemiological studies.

**Section 5.4.8 Summary and Causal Determination (Mortality)**

USEPA concludes that the evidence for short-term NO2 exposure and total mortality is suggestive, but not sufficient to infer a causal relationship. This conclusion is the same as the conclusion reached in the 2008 ISA for Oxides of Nitrogen. Table 5-63 is a summary of evidence used to support the causal determination of “suggestive, but not sufficient to infer, a causal relationship” between short-term NO2 exposure and total mortality. The TCEQ agrees that the causal association should not be strengthened based on the available information.

Additionally, the TCEQ thinks that a strong case can be made for reducing the causal determination to “inadequate to infer a causal relationship” because of major uncertainties in the available evidence for short-term NO2 exposure and total mortality. Until key uncertainties can be addressed, either with experimental studies or additional epidemiological studies, the association between short-term NO2 exposure and mortality is tenuous.

***Section* *5.5 Total Mortality (Long-term Exposure)***

Studies appear to be missing from this section of the second draft NO2 ISA. These include, but are not necessarily limited to:

*Raaschou-Nielsen et al. 2012. Traffic air pollution and mortality from cardiovascular disease and all causes: a Danish cohort study. Environmental Health. 11:60.*

*Zhang et al. 2011. Long-term exposure to ambient air pollution and mortality due to cardiovascular disease and cerebrovascular disease in Shenyang, China. PLoS One. 6:e20827.*

Table 6-15 of the second draft NO2 ISA does not include information on risk estimates as did the corresponding table in the first draft ISA, and instead of risk estimates, hazard ratios are presented for each study. The USEPA should explain this change and describe why the methodology is appropriate. The first draft ISA listed risk estimates for each study and indicated that approximately half of the included studies report statically significant risk estimates for long-term exposure to NO2 while the other half do not. In addition, some estimates were positive and some were negative. In the second draft ISA the hazard ratios showed a similar pattern, with some being less than one, some equal to one, and some greater than 1. Similar observations can be made for cause-specific mortality in Tables 6-16 and 6-17. The second draft ISA should include an extended discussion on the interpretation of such a dataset. Does the USEPA believe small, inconsistent hazard ratios indicate true increased risk due to long-term exposure to NO2?

In the summary for this section, the draft ISA states that “…there were several well-designed, well conducted studies that did not observe an association between long-term exposure to NO2 and mortality…” However it is not clear how this information was integrated into the causal determination. Similarly, the second draft ISA indicates limited coherence with morbidity endpoints and no information on potential mode of action for this section. Based on the available evidence presented in the second draft ISA, the TCEQ thinks that it is not appropriate to conclude the overall evidence is “suggestive of a causal relationship” between long-term exposure to NO2 and mortality among adults.

**Reproductive and Developmental Effects**

***Section 6.4 Reproductive and Developmental Effects (Long-term Exposure)***

Animal toxicological studies are available that evaluate the reproductive and developmental effects of NO2 exposure. Table 6-13 summarizes available animal studies. Effects occurred at high concentrations and developmental effects were secondary to maternal toxicity. Maternal toxicity or reductions in maternal weight gain were reported in pregnant rats inhaling 5,300 ppb NO2 for 6 hours/day, 7 days/week, throughout gestation (Tabacova et al. 1984). DiGiovanni et al. (1994) found no differences in maternal weight gain in pregnant rats exposed to concentrations up to 3,000 ppb NO2 over the duration of the pregnancy. Decreased litter sizes were reported after dams were exposed to 1,300 ppb NO2 for 12 hours/day for three months during pregnancy (Shalamberidze and Tsereteli 1971a and b). Litter size was not affected in dams exposed to 1,500 or 3,000 ppb NO2 exposure over the duration of the pregnancy (DiGiovanni et al. 1994).

Based on the evidence presented in the second draft ISA, TCEQ agrees with the causal determination for long-term NO2 exposure and fertility, reproduction, and pregnancy as well as postnatal development of “inadequate to infer a causal relationship.”

TCEQ agrees with the causal determination for long-term NO2 exposure and birth outcomes of “suggestive, but not sufficient, to infer a causal relationship,” but *only at high concentrations* (lowest adverse effect level of 1,300 ppb for 12 hours/day for 30 days in rodents). The experimental evidence available suggests that effects such as maternal toxicity and reduced litter size only occur at concentrations high enough to cause overt toxicity. Epidemiological studies have also been used as evidence to support this causal determination. As mentioned previously, many key uncertainties exist when using epidemiological studies to infer causal relationships in the absence of supporting experimental studies and mechanistic data (i.e., uncertainties about personal exposure versus ambient exposure since exposure concentrations are obtained from ambient monitoring data; co-pollutant confounding issues; effect modifiers such as age, smoking, and socioeconomic factors; and selection of relevant exposure period).

**Cancer**

***Section 6.6 Cancer (Long-term Exposure)***

The draft ISA presented results of both animal experimental studies and several epidemiological studies to support strengthening the causal determination of long-term exposure to NO2 and cancer to “suggestive, but not sufficient, to infer a causal relationship.”

Available animal studies were described in Section 6.6.1.2 and summarized in Table 6-19. USEPA should clearly indicate which concentration(s) of NO2 caused a statistically significant effect in each study. *In vivo* animal studies have demonstrated that NO2 can act as a tumor promoter at the site of contact (respiratory tract) when administered with a known carcinogen. Other animal studies have shown NO2 inhalation exposure can significantly facilitate lung cancer metastases. *Ex vivo* studies of human cells showed mixed genotoxicity results.

Section 6.6 of the draft ISA includes a number of epidemiological studies that evaluate the association between long-term NO2 exposure and cancer. The ISA includes studies that reported both positive associations and/or null or negative associations. However, it appears that results from the studies that reported negative or null associations were not included and/or were given less weight, while the studies that reported positive associations seem to have been given a greater weight when the causal determination was made.

TCEQ does not agree with strengthening the causal determination based on the existing body of evidence. Not only is there a lack of clear experimental evidence and mechanistic data to inform a potential mode of action for NO2 acting as a direct carcinogen, but the epidemiological studies used as supportive evidence show weak, inconsistent, or no association between cancer and long-term exposure to NO2. Based on the existing body of evidence, the TCEQ thinks the causal determination for this endpoint should be “inadequate to infer a causal relationship.”

**References[[2]](#footnote-2)**

Andersen, ZJ; Wahlin, P; Raaschou-Nielsen, O; Ketzel, M; Scheike, T; Loft, S. 2008b. Size distribution and total number concentration of ultrafine and accumulation mode particles and hospital admissions in children and the elderly in Copenhagen, Denmark. Occup Environ Med 65: 458-466. <http://dx.doi.org/10.1136/oem.2007.033290>

Beckerman, BS; Jerrett, M; Finkelstein, M; Kanaroglou, P; Brook, JR; Arain, MA; Sears, MR; Stieb, D; Balmes, J; Chapman, K. (2012). The association between chronic exposure to traffic-related air pollution and ischemic heart disease. J Toxicol Environ Health A 75: 402-411. <http://dx.doi.org/10.1080/15287394.2012.670899>

Bhaskaran, K; Hajat, S; Armstrong, B; Haines, A; Herrett, E; Wilkinson, P; Smeeth, L. 2011. The effects of hourly differences in air pollution on the risk of myocardial infarction: case crossover analysis of the MINAP database. B M J 343: d5531. <http://dx.doi.org/10.1136/bmj.d5531>

Cesaroni, G; Badaloni, C; Gariazzo, C; Stafoggia, M; Sozzi, R; Davoli, M; Forastiere, F. 2013. Long-term exposure to urban air pollution and mortality in a cohort of more than a million adults in rome. Environ Health Perspect 121: 324-331. <http://dx.doi.org/10.1289/ehp.1205862>

Chen, R; Chu, C; Tan, J; Cao, J; Song, W; Xu, X; Jiang, C; Ma, W; Yang, C; Chen, B; Gui, Y; Kan, H. 2010b. Ambient air pollution and hospital admission in Shanghai, China. J Hazard Mater 181: 234-240. <http://dx.doi.org/10.1016/j.jhazmat.2010.05.002>

Chen, G; Song, G; Jiang, L; Zhang, Y; Zhao, N; Chen, B; Kan, H. 2008. Short-term effects of ambient gaseous pollutants and particulate matter on daily mortality in Shanghai, China. J Occup Health 50: 41-47. <http://dx.doi.org/10.1539/joh.50.41>

Di Giovanni, V; Cagiano, R; Carratù, MR; De Salvia, MA; Giustino, A; Cuomo, V. 1994. Alterations in the ontogeny of rat pup ultrasonic vocalization produced by prenatal exposure to nitrogen dioxide. Psychopharmacology 116: 423-427.

Drechsler-Parks, DM. 1995. Cardiac output effects of O3 and NO2 exposure in healthy older adults. Toxicol Ind Health 11: 99-109. <http://dx.doi.org/10.1177/074823379501100109>

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

Folinsbee, LJ; Horvath, SM; Bedi, JF; Delehunt, JC. 1978. Effect of 0.62 ppm NO2 on cardiopulmonary function in young male nonsmokers. Environ Res 15: 199-205. <http://dx.doi.org/10.1016/0013-9351(78)90096->

Gan, W; Koehoorn, M; Davies, H; Demers, P; Tamburic, L; Brauer, M. 2011. Long-term exposure to traffic-related air pollution and the risk of coronary heart disease hospitalization and mortality. Environ Health Perspect 119: 501-507. <http://dx.doi.org/10.1289/ehp.1002511>

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

Guo, Y; Jia, Y; Pan, X; Liu, L; Wichmann, HE. 2009. The association between fine particulate air pollution and hospital emergency room visits for cardiovascular diseases in Beijing, China. Sci Total Environ 407: 4826-4830. <http://dx.doi.org/10.1016/j.scitotenv.2009.05.022>

Huang, W; Zhu, T; Pan, X; Hu, M; Lu, SE; Lin, Y; Wang, T; Zhang, Y; Tang, X. 2012a. Air pollution and autonomic and vascular dysfunction in patients with cardiovascular disease: Interactions of systemic inflammation, overweight, and gender. Am J Epidemiol 176: 117-126. <http://dx.doi.org/10.1093/aje/kwr511>

Huang, YC; Rappold, AG; Graff, DW; Ghio, AJ; Devlin, RB. 2012b. Synergistic effects of exposure to concentrated ambient fine pollution particles and nitrogen dioxide in humans. Inhal Toxicol 24: 790-797. <http://dx.doi.org/10.3109/08958378.2012.718809>

Ito, K; Mathes, R; Ross, Z; Nádas, A; Thurston, G; Matte, T. 2011. Fine particulate matter constituents associated with cardiovascular hospitalizations and mortality in New York City. Environ Health Perspect 119: 467-473. <http://dx.doi.org/10.1289/ehp.1002667>

Klepeis, N. E.; Nelson, W. C.; Ott. W. R.; Robinson, J. P. Tsang, A. M.; Switzer, P.; Behar, J. V.; Hern, S. C.; Engelmann, W. H. 2001. The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants. J. Exposure Anal. Environ. Epidemiol. 11: 231-252.

Langrish, JP; Lundbäck, M; Barath, S; Söderberg, S; Mills, NL; Newby, DE; Sandström, T; Blomberg, A. 2010. Exposure to nitrogen dioxide is not associated with vascular dysfunction in man. Inhal Toxicol 22: 192-198. <http://dx.doi.org/10.3109/08958370903144105>

Leaderer, B. P.; Zagraniski, R. T.; Berwick, M.; Stolwijk, J. A. J. 1986. Assessment of exposure to indoor air contaminants from combustion sources: methodology and application. Am. J. Epidemiol. 124: 275-289.

Linares, C; Diaz, J. 2010. Short-term effect of concentrations of fine particulate matter on hospital admissions due to cardiovascular and respiratory causes among the over-75 age group in Madrid, Spain. Public Health 124: 28-36. <http://dx.doi.org/10.1016/j.puhe.2009.11.007>

Linn, WS; Shamoo, DA; Spier, CE; Valencia, LM; Anzar, UT; Venet, TG; Avol, EL; Hackney, JD. 1985a. Controlled exposure of volunteers with chronic obstructive pulmonary disease to nitrogen dioxide. Arch Environ Health 40: 313-317. <http://dx.doi.org/10.1080/00039896.1985.10545939>

Liu, C; Flexeder, C; Fuertes, E; Cyrys, J; Bauer, CP; Koletzko, S; Hoffmann, B; von Berg, A; Heinrich, J; Groups, ftGaLS. 2014a. Effects of air pollution on exhaled nitric oxide in children: Results from the GINIplus and LISAplus studies. Int J Hyg Environ Health 217: 483-491. <http://dx.doi.org/10.1016/j.ijheh.2013.09.006>

Miller, KA; Siscovick, DS; Sheppard, L; Shepherd, K; Sullivan, JH; Anderson, GL; Kaufman, JD. 2007. Long-term exposure to air pollution and incidence of cardiovascular events in women. N Engl J Med 356: 447-458.

Raaschou-Nielsen et al. 2012. Traffic air pollution and mortality from cardiovascular disease and all causes: a Danish cohort study. Environmental Health. 11:60.

Scaife, A; Barclay, J; Hillis, GS; Srinivasan, J; Macdonald, DW; Ross, JA; Ayres, JG. 2012. Lack of effect of nitrogen dioxide exposure on heart rate variability in patients with stable coronary heart disease and impaired left ventricular systolic function. Occup Environ Med 69: 587-591. <http://dx.doi.org/10.1136/oemed-2011-100126>

Seilkop, SK; Campen, MJ; Lund, AK; McDonald, JD; Mauderly, JL. 2012. Identification of chemical components of combustion emissions that affect pro-atherosclerotic vascular responses in mice. Inhal Toxicol 24: 270-287. <http://dx.doi.org/10.3109/08958378.2012.667455>

Shalamberidze, OP; Tsereteli, NT. 1971a. Effect of low concentrations of sulfur and nitrogen dioxides on the estrual cycle and reproductive functions of experimental animals. Gig Sanit 36: 178-182.

Shalamberidze, OP; Tsereteli, NT. 1971b. [Effect of small concentrations of sulfur dioxide and nitrogen peroxide on the estrus cycle and reproductive function in experiments on animals]. Gig Sanit 36: 13-17.

Tabacova, S; Balabaeva, L; Vardev, F. 1984. Nitrogen dioxide: maternal and fetal effects. 25th Congress of the European Society of Toxicology, June 11-14, 1984, Budapest, Hungary.

USEPA. 2015. Integrated Science Assessment for Oxides of Nitrogen — Health Criteria. Second External Review Draft. U.S. Environmental Protection Agency, Washington, DC. EPA/600/R-14/006, 2015. Accessible here: <http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=288043>

USEPA. 2013. Integrated Science Assessment for Oxides of Nitrogen — Health Criteria. First External Review Draft. U.S. Environmental Protection Agency, Washington, DC . EPA/600/R-13/202, 2013. Accessible here: <http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=259167>

USEPA. 2008. Integrated Science Assessment for Oxides of Nitrogen — Health Criteria Annexes. U.S. Environmental Protection Agency, Washington, DC . EPA/600/R-08/07. Accessible here: <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=194645>

USEPA. 2008. Integrated Science Assessment for Oxides of Nitrogen — Health Criteria. U.S. Environmental Protection Agency, Washington, DC . EPA/600/R-08/07. Accessible here: <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=194645>

Williams, R; Brook, R; Bard, R; Conner, T; Shin, H; Burnett, R. 2012a. Impact of personal and ambient-level exposures to nitrogen dioxide and particulate matter on cardiovascular function. Int J Environ Health Res 22: 71-91. <http://dx.doi.org/10.1080/09603123.2011.588437>

Zhang et al. 2011. Long-term exposure to ambient air pollution and mortality due to cardiovascular disease and cerebrovascular disease in Shenyang, China. PLoS One. 6:e20827.

1. EPA has recently stated: “[E]pidemiological studies do not generally provide direct evidence of causation; instead they indicate the existence or absence of a statistical relationship.” ATIELC v. USEPA case 1:12-cv-01066-ATJ-TCB. October 4, 2012. [↑](#footnote-ref-1)
2. References cited from USEPA 2015 Integrated Science Assessment for Oxides of Nitrogen - Health Criteria (Second Draft) were cited exactly as they were in the USEPA document. [↑](#footnote-ref-2)