Comments on the Integrated Science Assessment for Ozone and Related Photochemical Oxidants (External Review Draft)
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Executive Summary

The "Integrated Science Assessment for Ozone and Related Photochemical Oxidants External Review Draft" (hereafter, the draft Ozone ISA) is a comprehensive and critical evaluation of the body of scientific knowledge relevant to the review of the National Ambient Air Quality Standards (NAAQS) for ozone for the purpose of making key evidence-based judgments to inform policy and risk assessment (US EPA, 2019a). The draft Ozone ISA aims to assess whether new information further informs the relationship between exposure to ozone and specific health and welfare effects and provides new information as to whether the primary and secondary NAAQS for ozone are appropriate. As discussed below, new scientific evidence does not support ozone health effects below the current primary standard.

For short-term ozone exposure, the 2013 Ozone ISA concluded that there was a causal association between short-term ozone exposure and respiratory effects, and that there were likely to be causal relationships for total mortality and cardiovascular effects (US EPA, 2013). For long-term ozone exposure, the 2013 Ozone ISA concluded that evidence indicated that there was likely to be a causal relationship for respiratory effects and that evidence was suggestive of causal relationships for total mortality and cardiovascular, reproductive, and central nervous system effects. Only a few causal determinations changed from the 2013 Ozone ISA. Metabolic effects were evaluated in the context of a mode of action for cardiovascular effects in 2013, but the 2019 draft Ozone ISA concludes a likely causal relationship for both short- and long-term ozone exposure. Furthermore, the draft Ozone ISA downgraded the causal conclusions between short-term ozone exposure and cardiovascular effects and total mortality from likely to be causal to suggestive of, but not sufficient to infer, a causal relationship.

The draft Ozone ISA includes new details on the literature search and study selection, including links to view a database of the studies included in the draft Ozone ISA and some brief information on study quality. In addition, biological plausibility assessments play a larger role than they have in the past. However, several issues still remain. Study quality information is limited and presented in an unclear manner on the online database, and in the draft Ozone ISA, study quality is not fully or consistently considered. Furthermore, while the draft Ozone ISA emphasizes biological plausibility for each health outcome with regard to ozone exposure, the evidence presented does not demonstrate a complete pathway from exposure to downstream health endpoints. Overall, the NAAQS systematic review and causal determination framework should be updated to allow for conclusions that are reflective of the weight of scientific evidence, and this framework should be followed and described in a transparent manner in the ISA.

The evidence for respiratory effects does not support the conclusion of the United States Environmental Protection Agency (EPA) that there is a causal relationship between short- or long-term ozone exposure and respiratory morbidity and mortality at relevant concentrations. The controlled human exposure studies indicate that there are no statistically significant adverse respiratory effects associated with ozone exposures below 70 ppb. Effects reported at 60 ppb are also not adverse. In addition, the 2013 Ozone ISA did not properly consider key limitations in the epidemiology evidence, and new studies have the same critical issues of that impact the validity of the results. Furthermore, key toxicity studies on which EPA relied to support the epidemiology data were conducted at very high exposure levels that are not relevant for assessing health effects of ambient ozone.

The evidence for metabolic effects does not support EPA's conclusion that there is a likely causal relationship between for short- and long-term ozone exposure at relevant concentrations. The draft Ozone ISA acknowledges that there is limited evidence from epidemiology and controlled human exposure studies

but indicates that animal toxicity studies provide robust evidence of the effects of short-term ozone on metabolic effects. While key animal toxicity studies support the effects of short-term ozone on glucose impairment at 500-1,000 ppb, the evidence for other metabolic endpoints is not consistent, and many studies only evaluate ozone exposure concentrations that are higher than the ambient levels. Also, animal toxicity and human epidemiology studies are limited regarding long-term effects of ozone on metabolic endpoints. Overall, the evidence presented is inadequate to assess causation between ozone and metabolic effects in humans.

As indicated in the draft Ozone ISA, evidence for short-term ozone exposure and cardiovascular effects and total mortality certainly does not support a likely causal relationship. However, it also is not suggestive of a causal relationship, but rather it is inadequate to address causality, if not suggestive of a lack of association. Finally, we concur with the draft Ozone ISA that evidence for other endpoints does not support causal or likely causal associations; however, like the evidence for short-term ozone exposure and cardiovascular effects and total mortality, this evidence falls short of suggestive.

In conclusion, the draft Ozone ISA has adopted several important aspects of systematic review that have been absent in other ISAs. However, it is still not fully transparent, and it does not adequately take study quality or relevance into consideration. Taken together, the currently available science does not provide evidence that supports health effects at ozone concentrations below the current primary standard.

1   Introduction

The United States Environmental Protection Agency (EPA) released the "Integrated Science Assessment for Ozone and Related Photochemical Oxidants (External Review Draft)" (draft Ozone ISA) on September 26, 2019 (US EPA, 2019a). Most causal determinations did not change from the 2013 Ozone ISA. Exceptions include metabolic effects; these were evaluated in the context of a mode of action for cardiovascular effects in 2013, but the 2019 draft Ozone ISA concludes a likely causal relationship for both short- and long-term exposure and metabolic effects. The 2013 Ozone ISA concluded there was a likely causal relationship between short-term exposure and cardiovascular effects and total mortality, but these associations have been downgraded to suggestive.

There have been several improvements in the ISA process, but several issues still remain, particularly with respect to the literature search and study selection, study quality evaluation, biological plausibility evaluation, evidence integration, and causal conclusions. We briefly discuss these issues below. This is followed by a discussion of the evidence for respiratory effects and metabolic effects; we show that evidence for short- and long-term ozone exposure, at ambient concentrations, fall short of causal and likely causal conclusions, respectively. We then discuss evidence for short-term ozone exposure and cardiovascular effects and total mortality; while the evidence certainly does not support a likely causal relationship, we discuss how it is not suggestive, but rather inadequate. Finally, we concur with the draft Ozone ISA that evidence for other endpoints does not support causal or likely causal associations; however, like the evidence for short-term ozone exposure and cardiovascular effects and total mortality, we also conclude this evidence falls short of suggestive.

2 There have been some improvements in the ISA process, but many issues remain.

There have been several improvements in the ISA process, but several issues remain, particularly with respect to the procedures for the literature search and study selection, study quality evaluation, biological plausibility evaluation, evidence integration, and causal conclusions. More specifically, there are inconsistencies in the selection and review of evidence, and the reliance on toxicity studies that evaluate high ozone concentrations. The ISA process could be improved by adding transparent criteria for assessing study quality in the systematic review and causal framework, as well as detailed methods for integrating evidence in a way that fully and systematically considers individual study quality and relevance and considers the coherence of results across studies within and across scientific disciplines (see example in Appendix A).

2.1 Literature search and study selection is improved (IS1.2.1, 10.2, 10.3).

For the first time, this draft Ozone ISA includes details on the literature search and study selection. The draft Ozone ISA has a literature flow diagram (Figure 10-2) that describes the literature search process and the number of studies included in each section of the draft Ozone ISA. The final list of studies can be downloaded from the Health Assessment Workplace Collaborative (HAWC) database (US EPA, 2019b), and it is clear how to determine which studies are in which section of the draft Ozone ISA. In addition, the full set of literature search results (49,561 records) can be found on the Health & Environmental Research Online (HERO) database (US EPA, 2019c). However, there does not appear to be an option to only select excluded studies or to ascertain reasons for exclusion. This feature is important for transparency.

The draft Ozone ISA also describes how EPA used the Population, Exposure, Comparison, Outcome, and Study design (PECOS) tool to help identify literature relevant to the ISA. Essentially, PECOS is used to explicitly define parameters for every realm of evidence and health outcome to help ensure the review includes all relevant studies and excludes ones that are not relevant. This is an improvement over past ISAs.

2.2 Study quality evaluation is not consistent or transparent (10.3.2).

Similar to the most recent particulate matter (PM), nitrogen oxide (NO_x), and sulfur oxide (SO_x) ISAs, the draft Ozone ISA has very detailed tables regarding aspects of study quality that should be considered for various study designs that are specific to ozone (US EPA, 2018, 2016, 2017). There are two paragraphs of text and a set of tables in Annexes to Appendices 3 to 7 for respiratory, cardiovascular, metabolic effects, mortality, and other outcomes, respectively. It appears that the same text and tables are cut and pasted in each Annex. There is no discussion of *in vitro* studies. The text also seems to be the same as that found in the draft PM ISA (US EPA, 2018).

The quality of 150 epidemiology and toxicity studies is documented on the HAWC database (US EPA, 2019b) for only a small subset of studies deemed as policy relevant by the draft Ozone ISA. Policy-relevant studies are defined as health studies for which there was a causal or likely to be causal relationship, or for which the causality determination changed from that made in the 2013 Ozone ISA. The quality of every

study in the draft Ozone ISA should have been evaluated. Also, while detailed study quality information is provided, including short descriptions of strengths and weaknesses, it is done in a narrative form, so it is not clear whether it is done in a consistent manner across studies. There is also no place where evidence quality across studies was evaluated. More importantly, much of this study quality information is overlooked in the draft Ozone ISA, when instead it should play a key role when evaluating individual studies and when integrating evidence across studies and across disciplines.

2.3 Biological plausibility evaluations overstate certainty (IS.4.2, 3.1.3, 3.2.3, 5.1.2, 5.2.2).

It is encouraging that biological plausibility assessments play a major role in the draft Ozone ISA. For each health endpoint, a schematic of potential biological pathways is presented in a figure and discussed in text. However, a major issue is that a good portion of these biological pathways are hypothesized; the evidence is not sufficient to determine whether they are plausible in humans exposed to ambient ozone. In other cases, ozone has been shown to cause upstream effects within a pathway, and downstream effects are assumed. There also is no systematic discussion of the quality of studies that support pathways and, importantly, although the draft Ozone ISA notes in several places that animal studies use very high doses, that does not seem to have a modifying impact on conclusions regarding biological plausibility. Different mechanisms may be involved at higher ozone doses, and these should not be reflected in the pathways outlined in the biological plausibility figures in the draft Ozone ISA. Overall, the draft Ozone ISA's evaluations are inadequate to assess biological plausibility at ambient ozone concentrations.

2.4 Evidence integration could be improved (IS.1.2.4).

The draft Ozone ISA focuses on key studies when integrating evidence, but does not consistently discuss the quality of these studies or whether they are consistent or coherent with other evidence. It also does not fully consider the exposure concentration used in several of these studies and how that impacts the extrapolation of results to humans exposed at ambient levels of ozone. A strong evaluation of study quality, subsequent consistent reliance on high quality work, and increased transparency are necessary improvements that would create confidence in the draft Ozone ISA's assessment of the health evidence. A good example of a critical evaluation of study quality can be seen in Zu *et al*. (2018).

2.5 Causal determinations should be based on a four-level framework (IS.1.2.4).

As discussed extensively in Gradient's comments on the "Integrated Review Plan for the Review of the Ozone National Ambient Air Quality Standards, External Review Draft" (Gradient, 2018), the causal determination framework should be updated to only include four categories for the levels of evidence for causation (causal, suggestive, inadequate, not causal) instead of five categories currently used (causal, likely, suggestive, inadequate, not causal). EPA uses a four-level framework (adequate, suggestive, and inadequate evidence or evidence of no effect) to evaluate "at-risk populations," but provides no justification for not using a similar four-level framework for causation.

2.6 Recommendations for Systematically Evaluating and Integrating Evidence

The causal framework could be improved by adding transparent criteria for assessing study quality, as well as detailed methods for integrating evidence in a way that fully and systematically considers individual

study quality and relevance, and considers the coherence of results across studies within and across scientific disciplines. For example, the framework should include not just a list of study quality aspects for evaluating human and animal studies, but also aspects for evaluating *in vitro* studies. In addition, for all realms of evidence, the framework should specify the criteria for each study quality aspect that must be met to demonstrate that a study is of high quality. An example of how these frameworks could be applied is shown in Appendix A. These aspects should be considered in a transparent and systematic fashion for each individual study, with the quality evaluations forming the basis for weighing evidence as it is integrated within and across disciplines, and ultimately for reaching conclusions regarding causality. The human relevance of experimental evidence should also be considered, particularly with respect to studies that evaluate upstream events vs. apical effects, as well as how informative these studies are for interpreting the results of epidemiology studies. These additions to the NAAQS systematic review and causal determination framework will make NAAQS causality assessments more transparent and reflective of the weight of scientific evidence and will allow for scientifically defensible decision-making.

3   The evidence does not support a causal classification for respiratory effects.

3.1   Short-term exposure evidence does not support a causal determination.

The 2013 Ozone ISA concluded there was a causal relationship between short-term ozone exposure and respiratory health effects. The 2013 Ozone ISA claimed studies reported statistically significant decreases in group mean pulmonary function in healthy young adults after 6.6 hours of 60 ppb ozone exposure with moderate exertion and that controlled human exposure and animal studies reported increases in respiratory symptoms, lung inflammation, airway permeability, and airway responsiveness. It also cited epidemiology studies conducted in the US, Europe, and Canada that evaluated respiratory hospital admissions and emergency department (ED) visits, panel studies of respiratory symptoms in children with asthma, epidemiology studies of airway inflammation and oxidative stress in children with asthma, and epidemiology studies of respiratory mortality.

The 2019 draft Ozone ISA (US EPA, 2019a) states:

> Evidence from recent controlled human exposure studies augment previously available studies. There are, however, no new 6.6-hour ozone exposure studies since the 2013 Ozone ISA. Evidence in the 2013 Ozone ISA demonstrated increases in FEV_1 decrements, respiratory symptoms, and inflammation following ozone exposures of 6.6 hours, with exercise, as low as 60 to 70 ppb (Section 3.1.4). Evidence from recent epidemiologic studies of short-term ozone exposure and hospital admission or emergency department visits observed associations at concentrations as low as 31 ppb. Controlled human exposure studies also provide consistent evidence of ozone-induced increases in airway responsiveness (Section 3.1.4.3 and Section 3.1.5.5) and inflammation in the respiratory tract (Section 3.1.4.4 and Section 3.1.5.6). Recent animal toxicological studies are consistent with evidence summarized in the 2013 Ozone ISA (U.S. EPA, 2013b); these studies support the evidence observed in healthy humans.

Below, we describe how new studies do not strengthen the evidence reviewed in the 2013 Ozone ISA and discuss how EPA fails to consider two important concepts of the exposure-response relationship. First, EPA does not adequately consider thresholds in its evaluation of the scientific evidence. Thresholds are observed in controlled human exposure studies and are supported by current understanding of ozone's mode of action.

Second, it is unclear whether the subjects' physical state (*i.e.*, exercise *vs*. rest) is considered in the draft Ozone ISA review. Physical activity increases both the ventilation rate and the distribution of ozone in the lung, which in turn increases the dose and the depth in the lung of inhaled ozone relative to an individual at rest (McCant *et al.*, 2017). Thus, as discussed in McCant *et al.* (2017), there was a misconception amongst researchers as a result of findings from Hatch *et al.* (1994). Many researchers incorrectly believe that, due to interspecies differences, rats must be exposed to ozone concentrations that are 3-5 times greater than human doses. In fact, the physical state (*i.e.*, resting *vs.* exercising) matters for ozone toxicity. As a result, animal studies do not reflect relevant exposure scenarios for humans at ambient ozone concentrations.

This concept plays an important role when interpreting results from human studies investigating ozone toxicity. In several key controlled human exposure studies discussed throughout the draft Ozone ISA, human volunteers are often performing some level of exercise, and this may limit the generalizability of the results. In two controlled human exposure studies, the researchers noted that the commonly employed exercise regimen in these studies simulates heavy manual labor performed by outdoor workers (Goodman *et al.*, 2015a). As a result, this exposure scenario does not apply to the general population or people who spend a majority of their days indoors, where ozone levels are lower than that in controlled human exposure studies (McClellan *et al.*, 2009). Furthermore, sensitive populations such as asthmatics will likely be unable to achieve the same level of ventilation rate that is required, so it is unclear how changes in respiratory health as a result of ozone exposure in these studies apply to sensitive populations.

3.1.1 There are no statistically significant adverse lung function effects associated with ozone below 70 ppb.

The draft Ozone ISA states (US EPA, 2019a):

> Controlled human exposure studies of young, healthy adults demonstrate ozone-induced decreases in FEV1 at concentrations as low as **60 ppb** and the combination of FEV1 decrements and respiratory symptoms at ozone concentrations **70 ppb** or greater following 6.6-h exposures while exercising. Studies show interindividual variability with some individuals being intrinsically more responsive. Results from recent epidemiologic studies are consistent with evidence from the 2013 Ozone ISA of an association with lung function decrements as low as **33 ppb** (mean 8-h avg ozone concentrations (7:50 a.m.−5:50 p.m.).

In the 2013 Ozone ISA, EPA reviewed controlled exposure studies of ozone in healthy adults, focusing on four studies that assessed the association between ozone and lung function at exposures below 80 ppb (Adams, 2002, 2006; Schelegle *et al.*, 2009; Kim *et al.*, 2011). Together, these studies indicate there is nonlinear relationship between ozone and lung function; this is consistent with biological data that support a threshold mechanism of action. Effects at 60 ppb are also not adverse, nor do they occur statistically more often than do those associated with filtered air (FA) exposures. These issues are summarized below.

3.1.1.1 There are no lung function effects at 60 ppb.

EPA presented a cross-study analysis of controlled ozone exposures between 40 and 120 ppb and lung function in the 2013 Ozone ISA (Adams, 2002, 2003 2006; Folinsbee *et al.*, 1988; Horstman *et al.*, 1990 ; McDonnell *et al.*, 1991, 2007). In this figure, EPA incorporated a smooth curve that represented a linear relationship between ozone and forced expiratory volume in one second (FEV_1), but it did not include the 95% confidence intervals (CIs) around each point.

We compiled the same dataset that EPA used in its evaluations and calculated the group mean decrease in ΔFEV_1 from available data for a given ozone concentration and corresponding FA controls. We also estimated, where possible, the standard deviation of the group mean decrease in ΔFEV_1. We fit two different models (linear and sigmoid) to the group mean decrease in ΔFEV_1 from across the studies. Table 3.1, below, shows the predictions of group mean decrease in ΔFEV_1 (%) at various ozone concentrations, based on the two models.

Table 3.1 Predictions of Group Mean Decreases in ΔFEV_1

O_3 Concentration (ppb)	Predicted Group Mean Decrease in ΔFEV_1 (%)	
	Linear Model	**Sigmoid Model**
0	-8.05	0.07
20	-4.25	0.24
40	-0.45	0.84
60	**3.35**	**2.71**
80	7.15	6.84
120	14.75	14.55
140	18.55	15.58

Notes:

ΔFEV_1 = Forced Expiratory Volume in 1 second; O_3 = Ozone.

The linear model suggests there is a protective effect of ozone at concentrations below ~50 ppb, which is biologically implausible. The sigmoid model fits the data and indicates there is likely a threshold. This model predicts a group mean decrement in ΔFEV_1 at 60 ppb ozone of 2.71%, with the lower 95% confidence band even lower. Such a small decrement is within the intraday variability of FEV_1 in normal subjects and does not meet established criteria for a clinically adverse effect on lung function.

Regarding EPA's conclusion that there is a smooth dose-response curve at exposures between 40 and 120 ppb, the fact that a statistical curve can be fit to the data does not itself provide evidence that another model is not more appropriate. Information regarding the mode of action of an agent should inform the statistical curves that fit the data. One should not choose one curve when the mode of action clearly indicates another. There is evidence suggesting that antioxidant defenses against ozone indicate a threshold mode of action for effects on lung function (Schelegle *et al*., 2007).

We also note that EPA's conclusion that 60 ppb ozone can cause lung function decrements is based on the studies by Kim *et al*. (2011), Schelegle *et al*. (2009), and Adams (2006), as well as a re-analysis of Adams (2006) by Brown (2008). The group mean change in FEV_1 at 60 ppb, however, was only statistically significant in the study by Kim *et al*. (2011).

In his re-analysis of Adams (2006), Brown (2008) reported a statistically significant decrement in FEV_1 at a 60 ppb square-wave mean ozone concentration using a t-test applied to the 6.6 hour data. This analysis excluded all other time points (*i.e.*, 1, 2, 3, 4.6, and 5.6 hours) and did not account for other responses from different exposure scenarios (*i.e.*, triangular mean 40, 60, and 80 ppb and square-wave 80 ppb ozone). Thus, this statistically significant finding can be attributed to the majority of the data being selectively omitted from the analysis.

Discarding data is inappropriate, especially in light of more powerful and complex statistical models (*e.g.*, mixed effect models) that can be employed (Gradient, 2011). Such post hoc selection of a data subset when valid and otherwise non-problematic observations exist calls into question the rationale for such action. The primary rationale for Brown (2008) to remove data from other experimental conditions was apparently to avoid stringent reductions in the critical *p*-value for statistical significance due to multiple comparisons procedures. These other data still exist, so leaving them out of the analysis does not eliminate the issue.

Nicolich conducted an analysis of the full dataset from Adams (2006) using a mixed model analysis of variance and Dunnett's *post hoc* test instead of the Scheffe test (Nicolich, 2007). This reanalysis, using a technique that is less likely to produce false negatives, was consistent with the original finding by Adams (2006), confirming that there was no statistically significant decrement in group mean FEV_1 after exposure to 60 ppb ozone *versus* FA after 6.6 hours of exercise. Lefohn *et al*. (2010) reanalyzed five controlled ozone exposure studies, including those by Adams (2006) and Schelegle *et al*. (2009), and did not find any

statistically significant changes in FEV_1 at any measurement time associated with 40 ppb and 60 ppb exposures.

The 2013 Ozone ISA gave no scientifically acceptable justification for relying on the Brown (2008) statistical analyses over the original analyses conducted by the authors, or those of Nicolich (2007), or Lefohn *et al.* (2010). While each statistical method has strengths and limitations, several scientifically accepted statistical methods indicate that there is no association between exposure to 60 ppb ozone and lung function decrements. EPA should give greater weight to analyses using methods and approaches that incorporate all of the exposure concentrations and time points.

3.1.1.2 Effects at 60 ppb are not adverse.

Section 109 of the Clean Air Act directs the EPA Administrator to set and revise a primary National Ambient Air Quality Standard (NAAQS) "to protect against adverse health effects" of criteria pollutants. EPA did not, however, fully consider the criteria for determining the adversity of health effects associated with controlled ozone exposures.

There is no indication that the reported FEV_1 decrements at 60 ppb in the controlled human exposure studies are adverse. Regarding what constitutes an adverse effect on pulmonary function, the American Thoracic Society (ATS) stated:

> The committee recommends that a small, transient loss of lung function, by itself should not automatically be designated as adverse. In drawing the distinction between adverse and nonadverse reversible effects, this committee recommended that reversible loss of lung function in combination with the presence of symptoms should be considered adverse. (ATS, 2000)

Average FEV_1 decrements reported at 60 ppb ranged from 1.7 to 3.5% and were not accompanied by an increase in respiratory symptoms. The 2013 Ozone ISA noted that changes in FEV_1 measurement should exceed 5% to overcome the intraday variability of FEV_1 in normal subjects (Pellegrino *et al.*, 2005,), and yet it considers the 1.7 to 3.5% decrements as an indication of an adverse effect on lung function from ozone. It should also be noted that the decrements observed in the controlled exposure studies were transient, reversible, and of low severity, did not interfere with normal activity, and would not result in permanent respiratory injury or progressive respiratory dysfunction (Goodman *et al.*, 2010). Although some individuals had larger decrements, these cannot be attributed to ozone because the lung function effects at 60 ppb ozone in controlled exposure studies are within the range of intraindividual variability in normal subjects and are not considered adverse with respect to broadly recognized clinical guidelines (*e.g.*, ATS and the European Respiratory Society). The lowest ozone concentration associated with both an FEV_1 decrement >10% and increased respiratory symptoms, which is considered an adverse effect based on clinical guidelines, is 88 ppb (as reported in the study by Schelegle *et al.*, 2009). This is supported by the more recent study by Arjomandi *et al.* (2018).

3.1.1.3 Other analyses of controlled human exposure studies support a threshold.

Two analyses incorporated an extended database of controlled human exposure studies to derive concentration-response functions (CRFs) for lung function effects from ozone exposure (Schelegle *et al.*, 2012; McDonnell *et al.*, 2012). Schelegle *et al.* (2012) developed a two-compartment exposure-response model with three coefficients for the kinetics of ozone-induced FEV_1 impairment based on data from 220

subjects who participated in 14 controlled human exposure studies, including those by Schelegle *et al.* (2009). The first compartment represented the dose of onset, and the second compartment modeled a fixed volume with a constant elimination rate of the bioactive substance. The third parameter in the model was a proportionality or responsiveness coefficient. The model's ability to predict group mean responses was validated in two ways: each study/protocol was systematically eliminated from the model fit, and observed and predicted FEV_1 decrements were compared; predicted model values from the original 220 subjects were compared with data from eight additional studies.

Schelegle *et al.* (2012) reported that the model described an increasing variability in FEV_1 decrements as a skewed response with increasing exposure, which is generally consistent with observed data. This skewed response likely occurred because of the variability of the cumulative dose needed to cause a response, which the authors determined was an individual characteristic independent of the magnitude of ozone-induced response and changing ozone concentrations. Once an individual's minimum cumulative threshold dose is reached, individual elimination rates and responsiveness coefficients determine the FEV_1 decrement. This exposure-response model, which incorporates age and baseline FEV_1 decrements, not only predicted observed group mean FEV_1 decrements reliably but also reproduced the frequency distribution of responses observed in the controlled human exposure literature.

McDonnell *et al.* (2012) presented a refinement to a previous model the authors developed (McDonnell, *et al.*, 2010), incorporating an expanded dataset (including both Kim *et al.* [2011] and Schelegle *et al.* [2009]) and providing further validation of the model. The authors also included a nonlinear model that incorporated a threshold ozone concentration below which no effects have been observed. In their threshold model, the authors defined the threshold as 59 parts per million (ppm)-liters of inhaled air (accumulated ozone dose), thus accounting for both the level of exercise and the ozone concentration. The authors predicted that exposures to the following would not reach the threshold: 0.06 and 0.08 ppm (60 to 80 ppb) during near-continuous exercise for one hour, 0.04 ppm (40 ppb) for two hours of near-continuous exercise, 0.18 and 0.24 ppm (180 to 240 ppb) for one hour at rest, and 0.12 ppm (120 ppb) ozone for two hours. The authors found that the threshold model fit the observed data better than the original (*i.e.*, no-threshold) model, especially at earlier time points and at the lowest exposure levels. McDonnell *et al.* (2012) concluded that the threshold model would likely provide better estimates of risk for populations exposed to low ozone levels. They also reported a better fit for models that incorporated body mass index, a potential confounder.

3.1.1.4   Arjomandi *et al.* (2018) does not support effects at 70 ppb.

As part of the Multicenter Ozone Study in Older Subjects (MOSES), Arjomandi *et al.* (2018) conducted a randomized crossover controlled exposure study of 87 healthy older adults (age 59.9 ± 4.5 years) to 0, 70, and 120 ppb ozone for three hours with intermittent exercise. Spirometry, sputum markers of airway inflammation, and plasma club cell protein-16 (CC16) were measured. The authors reported:

> The mean (95% confidence interval) FEV_1 and FVC increased from preexposure values by 2.7% (2.0–3.4) and 2.1% (1.3–2.9), respectively, 15 minutes after exposure to filtered air (0 ppb). Exposure to ozone reduced these increases in a concentration-dependent manner. After 120-ppb exposure, FEV_1 and FVC decreased by 1.7% (1.1–2.3) and 0.8% (0.3–1.3), respectively. A similar concentration dependent pattern was still discernible 22 hours after exposure. At 4 hours after exposure, plasma CC16 increased from preexposure levels in an ozone concentration–dependent manner. Sputum neutrophils obtained 22 hours after exposure showed a marginally significant increase in a concentration dependent manner ($P = 0.012$), but proinflammatory cytokines (IL-6, IL-8, and tumor necrosis factor-α) were not significantly affected. (Arjomandi *et al.* 2018)

In fact, this study shows a difference between lung function after exposure to filtered air *vs.* 120 ppb ozone, but no difference between lung function after exposure to filtered air *vs.* 70 ppb ozone. The statistical test used to compare all three groups was a Type III sum of squares p value. This does not test dose-response; rather, it tests whether there is any significant difference among the three dose groups (0, 70, 120 ppb). A statistically significant result only indicates that results for all three exposure doses are not the same. This is clearly driven by the 120 ppb dose. Furthermore, even if there were a statistical difference, as discussed in Section 3.1.1.4 below, there is no indication that this same difference in lung function is clinically relevant. As demonstrated in Figure 3.1 below (Figure 2 from Arjomandi *et al.*, 2018), there is an improvement in FEV_1 from pre-test baseline except for the 120 ppb 22-hour measurement.
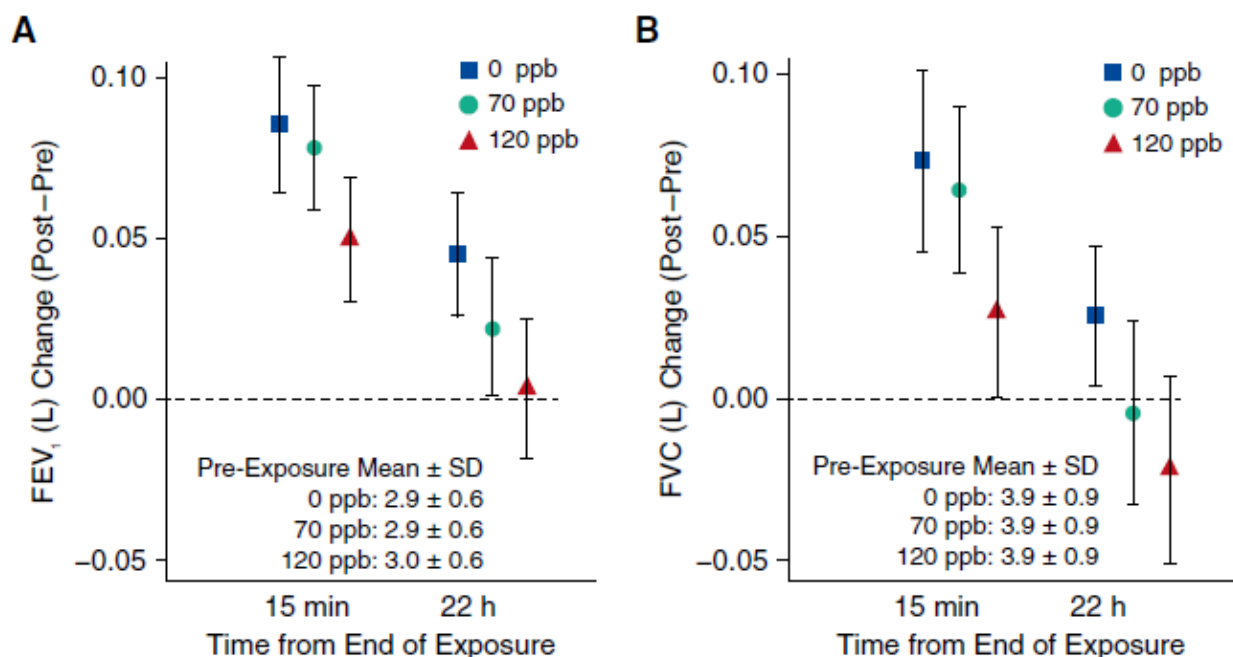
Figure 3.1 Ozone-induced Changes in Forced Expiratory Volume in One Second (FEV_1) and Forced Vital Capacity (FVC). Source: Figure 2 from Arjomandi *et al.* (2018).

3.1.1.5   Conclusion

The controlled exposure studies indicate that there are no statistically significant adverse effects associated with ozone below 70 ppb, and this is consistent with biological data that support a threshold mechanism of action. Effects at 60 ppb are not adverse, nor do they occur statistically more often than do those associated with FA exposures.

3.1.2   Airway responsiveness is not impacted below 80 ppb.

3.1.2.1   Controlled human exposure studies do not show airway responsiveness below 80 ppb.

As stated in the draft Ozone ISA, no controlled human exposure studies provide evidence for effects on airway responsiveness below 80 ppb.

3.1.2.2   Animal toxicity studies are not informative regarding ambient exposures.

In studies that investigated the effects of ozone in animals with asthma or airway hyperresponsiveness (AHR), the asthmatic phenotype is modeled by allergic sensitization of the respiratory tract. The majority of animal studies used elevated ozone concentrations that do not reflect human exposures to ambient ozone. There are only a limited number of studies that have observed airway hyperresponsiveness in rodents and guinea pigs at less than 300 ppb. Depuydt *et al*. (1999) reported that after exposure to 50 ppb ozone for four hours, two (BDII and Long-Evans) of the nine strains of rats tested experienced airway hyperresponsiveness as measured by inflammatory cells and markers in bronchioalveolar lavage fluid (BALF). This concentration is lower than that in any other studies that reported AHR and more relevant to ozone standards; however, there is uncertainty regarding the validity and applicability of the findings to humans. The study lacked a proper control group; more recent studies use a control group exposed to filtered air instead of "room air," as used in Depuydt *et al*. (1999). Furthermore, EPA concluded that the concentration used "warrants verification in other species," and the authors acknowledged that "the biological effects that are observed in these different rat strains may not be easily extrapolated to humans" (Depuydt *et al*., 1999).

More recent studies comparing ovalbumin-sensitized rodents to nonsensitized rodents showed that responses occurred in sensitized animals at levels of 120 ppb (Chhabra *et al*., 2010) and 100 to 250 ppb (Larsen *et al*., 2010). The endpoints indicating AHR included lipid peroxidation, superoxide anion generation in the bronchial lavage cells, red cell superoxide dismutase and glutathione peroxidase, and goblet-cell metaplasia. It is unclear from these studies whether these biomarkers were clinically significant or whether they were transient and reversible effects. Other studies discussed in the 2013 Ozone ISA included Funabashi *et al*. (2004; US EPA, 2013), who demonstrated changes in pulmonary function (increased respiratory resistance and decreased dynamic compliance) in mice exposed to 1,000 ppb ozone, and Wagner *et al*. (2007) , who reported enhanced inflammatory responses (such as intraepithelial mucosubstances, subepithelial eosinophils, and IL-6 production in BALF) in rats exposed to 1,000 ppb ozone in the mice sensitized to allergen. Again, these concentrations were extremely high and not relevant to ambient exposures, and it was unclear if these effects were transient or clinically relevant.

New animal toxicity studies discussed in the draft Ozone ISA also do not provide evidence of effects on lung function in humans at ambient exposures. Many of the cited studies in the draft Ozone ISA report increased airway responsiveness at exposure concentrations as high as 2,000 ppb (*e.g*., Cho *et al*., 2018 ; Stober *et al*., 2017; Kasahara *et al*., 2015; US EPA, 2019a). As noted by the draft Ozone ISA, the lowest ozone dose that increased airway responsiveness was 800 ppb. Groves *et al*. (2012) analyzed chronic macrophage inflammation in wildtype C57Bl/6J mice and mice lacking surfactant protein-D (Stfpd) following exposure to 800 ppb ozone for three hours. Acute ozone exposure resulted in airway responsiveness in mice lacking native *Stfpd*. The draft Ozone ISA states that no studies reported increases in airway responsiveness following exposures to 250 and 500 ppb ozone (US EPA, 2019a, Section 3.1.4.3.1, p. 3-27). This new evidence does not address potential effects at ambient concentrations of ozone.

The species differences in airway morphology in rodents compared with humans also leads to uncertainty regarding the relevance of these rodent studies to humans. In addition, although three other studies in more biologically relevant species (non-human primates; Schelegle *et al*., 2003; Joad *et al*., 2006; US EPA, 2013; Fanucchi *et al*., 2006) found that cyclic episodes of ozone exposure (at 500 ppb) produced alterations in airways that could lead to chronic airway disease and decreased lung function. However, these results are not informative as to whether long-term, environmentally relevant exposures could cause similar changes.

3.1.3 There is no evidence that ambient ozone concentrations lead to pulmonary inflammation, injury, or oxidative stress.

3.1.3.1 Controlled human exposure studies do not provide evidence for effects at ambient concentrations.

The draft Ozone ISA states controlled human exposure studies reviewed in the 1996 and 2006 ozone air quality criteria documents establish a relationship between short-term ozone exposure and respiratory tract inflammation, injury, and oxidative stress. Many studies focus on polymorphonuclear neutrophils (PMN) or BALF markers as evidence of increased inflammation and impaired lung function. In the 2013 Ozone ISA, studies reported increased inflammation (*i.e.*, increased sputum PMN) in young healthy adults following exposures to 60 ppb ozone.

Three studies cited in the draft Ozone ISA used ozone concentrations ranging from 40 to 70 ppb (Table 3-9, p. 3-126); however, it is unclear from the draft Ozone ISA whether ozone induced statistically significant and clinically relevant effects on inflammation at these exposure concentrations. In addition, as discussed previously in Section 3.1.1.2 and 3.1.1.4 with regard to lung function, results from controlled human exposure studies do not indicate statistically significant adverse effects on lung function associated with ozone below 70 ppb, and the lung function effects reported at 60 ppb are not adverse or statistically significant.

3.1.3.2 Animal toxicity studies do not provide evidence for effects at ambient concentrations.

The draft Ozone ISA states that ozone-induced changes to pulmonary inflammation, injury, and oxidative stress occurred at concentrations as low as 300 ppb. EPA highlighted several studies that reported increased inflammation at 300 ppb, and the evidence was fairly consistent across studies using different rodent strains (Mathews *et al*., 2015; Verhein *et al*., 2015; Cho *et al*., 2013; Kasahara *et al*., 2013,2012). However, when considering all the animal toxicity evidence, there is a lack of consistency. While several studies reported increased BALF markers following exposure to 300 ppb ozone for 72 hours (Mathews *et al*., 2015; Verhein *et al*., 2015; Cho *et al*., 2013; Kasahara *et al*., 2013, 2012), several other studies cited in the draft Ozone ISA report no changes in BALF at 250-500 ppb of ozone (Michaudel *et al*., 2018; Kodavanti *et al*., 2015; Kumarathasan *et al.*, 2015; Kurhanewicz *et al.*, 2014; McIntosh-Kastrinsky *et al.*, 2013; Thomson *et al.*, 2013). It is unclear how the draft Ozone ISA considered the entire of body of evidence in light of several studies reporting contradictory results. Furthermore, several other key animal toxicity studies used high ozone concentrations (*e.g*., 800-2,000 ppb) that are not relevant to ambient concentrations.

3.1.4 There are limited data on respiratory symptoms and medication use.

The draft Ozone ISA relies on controlled human exposure studies from the 2013 Ozone ISA as evidence of ozone-induced increases in respiratory symptoms such as pain on deep inspiration, shortness of breath, and cough. According to the draft Ozone ISA, there are no new studies that contradict the results of previous studies or provide stronger evidence. However, as discussed previously in Section 3.1.1.2 and 3.1.1.4, controlled human exposure studies do not indicate statistically significant adverse effects associated with ozone below 70 ppb, and effects at 60 ppb are not adverse or statistically significant. In addition, in general, these symptoms are subjective and not associated with lung function. As the draft Ozone ISA indicates, there are only limited data regarding whether lung function responses depend on baseline lung function and medication use.

3.1.5   New evidence from experimental studies on lung host defenses is limited.

EPA evaluated controlled human exposure studies investigating ozone effects on multiple components of lung host defenses. These effects included the mucociliary escalator; the phagocytic, bactericidal, and regulatory role of alveolar macrophages; the adaptive immune system; and host responses to experimental pulmonary infections. Since the 2013 Ozone ISA, there have been no new controlled human exposure studies investigating ozone-induced effects on lung host defenses.

In the 2013 Ozone ISA, EPA reported increased susceptibility to challenge with infectious agents at 80-500 ppb ozone from animal toxicity studies. The draft Ozone ISA cites two recent studies reporting increased ozone-induced susceptibility for infections in mice. In the Durrani *et al.* (2012) and Mikerov *et al.* (2011) studies, mice were exposed to 2,000 ppb of ozone for three hours. Durrani *et al.* (2012) used gonadectomized mice to investigate the influence of sex hormones on ozone-induced oxidative stress and lung function. Treatment with steroid hormones and ozone significantly decreases survival in both male and female mice. This exposure is an order of magnitude higher than ambient ozone concentrations and is not informative regarding risks at these lower exposure concentrations.

3.1.6   New evidence does not support allergy- and asthma-related responses to ozone.

3.1.6.1   Animal toxicity studies do not provide evidence for respiratory effects at ambient concentrations.

In the draft Ozone ISA, EPA evaluated evidence from studies investigating the effects of ozone on respiratory effects using animal models of allergic airway disease. Three studies cited by the draft Ozone ISA reported statistically significant respiratory responses to ozone exposure in allergic rodents compared to naïve rodents (Bao *et al.*, 2013; Hansen *et al.*, 2016; Schelegle and Walby, 2012). However, these findings do not provide evidence of ozone-induced health effects in people with asthma at relevant ambient concentrations of ozone, as rodents were dosed with 1,000 or 2,000 ppb of ozone. These concentrations are orders of magnitude higher than ambient ozone concentrations.

3.1.6.2   Recent controlled human exposure studies provide limited evidence of increased responses to ozone due to allergy or asthma.

The 2013 Ozone ISA reviewed two studies that indicated that the severity of asthma increased the response to ozone (albeit at high concentrations) in patients using bronchodilators, medications that open the airways and allow patients to breath (Section 3.1.5.4.1, p. 3-45). In a study by Horstman *et al.* (1995), volunteers were exposed to 160 ppb for 7.6 hours during light quasi-continuous exercise. In a study conducted by Kreit *et al.* (1989), asthmatic volunteers experienced significantly greater reductions in FEV_1 than nonasthmatics following exposure to 400 ppb ozone for two hours during heavy intermittent exercise.

However, as acknowledged in the draft Ozone ISA, newer studies do not support increased responses to ozone with more severe asthma. Two studies, Arjomandi *et al.* (2015) and Fry *et al.* (2012), found no difference in reported FEV_1 based on the presence of asthma. Another study lacked an appropriate control group of healthy, nonasthmatics and was thus uninformative (Bartoli *et al.*, 2013). In the final study cited in the draft Ozone ISA, Leroy *et al.* (2015) reported no significant association between ozone-induced reductions in lung function and people with asthma. Overall, recent studies provide no evidence to validate the results presented from studies from the 2013 Ozone ISA.

3.1.7 Epidemiology studies are insufficient to provide evidence on short-term ozone exposure and respiratory effects.

The draft Ozone ISA discusses epidemiology studies investigating the effects of short-term ozone exposure on multiple respiratory endpoints such as lung function, markers of pulmonary inflammation, injury, oxidative stress, hospital admissions, emergency department and physician visits, respiratory symptoms, and medication use. Throughout the draft Ozone ISA, EPA references epidemiology evidence from the 2013 Ozone ISA as evidence of ozone-induced effects. In fact, the draft Ozone ISA states that "[e]vidence from epidemiologic studies of healthy populations is generally coherent with experimental evidence, although the majority of the evidence comes from panel studies that were previously evaluated in the 2013 Ozone ISA (U.S. EPA, 2013a)" (US EPA, 2019a). As discussed in previous Gradient comments, there are several key limitations of these previous studies, and newer studies presented in the draft Ozone ISA have similar issues (Goodman and Sax, 2012). In fact, in the 2014 proposed rule, the EPA Administrator acknowledged the critical uncertainties and limitations of these studies that hinder the use of epidemiology data in the risk assessment (US EPA, 2014; Gradient, 2015).

Below, we discuss some of the critical limitations of the human epidemiology studies investigating the effects of short-term ozone exposure on respiratory effects.

3.1.7.1 Exposure measurement error due to central site monitoring is not adequately considered.

Most human epidemiology studies use air pollution data collected from a central ambient air monitoring site; this is the case for the epidemiology studies cited as key evidence regarding short-term ozone exposure (Spektor *et al.*, 1988; Salam *et al.*, 2012; Winquist *et al.*, 2012; Malig *et al.*, 2016 219-11187 ; Lewis *et al.*, 2013). Yet, the exposures collected from central monitoring sites are assumed to reflect personal ozone exposures, and this can be a source of exposure measurement error. Prior Clean Air Science Advisory Committee (CASAC) reviews have highlighted the uncertainty due to the use of central monitors as a surrogate for personal exposures (CASAC, 2006). CASAC (2006) reported that personal ozone exposures are typically much lower than ambient ozone levels and, more importantly, often show little or no correlation with concentrations measured at the central ambient sites. For example, in a study conducted by Sarnat *et al.* (2001), researchers found no correlation between personal and ambient ozone concentrations in a Baltimore-based cohort for both winter and summertime ozone concentrations (resulting correlation slopes of 0.00 and 0.01 respectively).

Potential issues with exposure measurement error also influence the shape of concentration-response functions derived from statistical models. Gradient has assessed how the various kinds of exposure measurement error can contribute to bias in concentration-response functions (Rhomberg *et al.*, 2011). For example, as discussed in Rhomberg *et al.* (2011), Meng *et al.* (2005) hypothesized that potential biases can arise in $PM_{2.5}$ associations because of seasonal variations in infiltration behavior. Their data showed that seasonal differences in infiltration behavior not only coincide with fluctuations in ambient particle concentrations, but they also vary with location. While this hypothetical scenario uses $PM_{2.5}$ as an example, this issue is directly applicable to ozone. As previously discussed in Gradient's comments on the 2013 Ozone ISA, "it is well established that relatively weak personal-ambient ozone correlations and low personal-ambient attenuation factors are a function of the interplay of a number of individual-, season-, and city-specific factors, including time activity patterns, building characteristics, and ventilation practices" (Goodman and Sax, 2012).

3.1.7.2 Confounding by copollutants is poorly considered in weight of evidence.

Confounding in respiratory morbidity studies is a key limitation in studies cited as key evidence in the draft Ozone ISA. Many studies fail to consider the role of copollutants in their statistical models, and as a result, it is unclear whether the adverse health effects are attributed to exposure to ozone or coexposure to other correlated air pollutants such as fine particulate matter ($PM_{2.5}$), sulfur dioxide (SO_2), nitrogen dioxide (NO_2) or carbon monoxide (CO). The draft Ozone ISA states that there were not many studies evaluating copollutants in the 2013 Ozone ISA and acknowledges the complexity of determining the effects of ozone alone due to its high correlation with other copollutants. Only 12 studies cited as key evidence in the draft Ozone ISA evaluated the role of copollutants in the draft Ozone ISA. It is unclear whether the findings from these studies were given more weight than others that did not evaluate copollutants. For example, with regard to short-term ozone exposure and mortality, the draft Ozone ISA states that there is "[g]enerally consistent epidemiologic evidence from multiple, high-quality studies" (Section 3.1.11, p. 3-84). However, only one of the key studies considered potential confounding by copollutants in their statistical models. Overall, the draft Ozone ISA appears to downplay confounding by copollutants, and it does not appear to have been considered in causality determinations.

3.1.7.3 The draft Ozone ISA does not appropriately consider and weight all of the evidence.

The draft Ozone ISA frequently highlights studies with "positive" findings and fails to properly acknowledge null results (Goodman and Sax, 2012). In many instances, null results throughout the draft Ozone ISA are presented with qualifiers in order to discount them. The implication is that null results would have been positive so long as certain limitations or biases had been addressed. Yet, conversely, positive results are not subject to the same level of scrutiny. In addition, it is unclear whether, or to what degree, null studies are considered in the causal determinations.

3.1.7.4 Conclusion

Overall, there are critical limitations and uncertainties associated with the epidemiology studies cited in the 2013 Ozone ISA and more recent studies included in the 2019 draft Ozone ISA. It is unclear how the draft Ozone ISA evaluates the evidence in light of the issues that can bias study results, but it does not appear that study quality was considered in causality determinations.

3.2 Long-term exposure evidence does not support a likely causal determination.

The 2013 Ozone ISA concluded that there was likely a causal relationship between long-term exposure to ozone and respiratory health effects based primarily on epidemiology studies that evaluated the annual average of daily ozone concentrations and new onset asthma, respiratory symptoms in children with asthma, and respiratory mortality (primarily in studies that looked at ozone interactions with exercise or genetic variants). The 2013 Ozone ISA stated this conclusion was supported by studies in which infant monkeys were exposed to biweekly cycles of alternating filtered air and ozone.

According to the 2019 draft Ozone ISA:

> Recent studies continue to examine the relationship between long-term exposure to ozone
> and respiratory effects. Key evidence supporting the causality determination is presented

in Table IS-5. A limited number of recent epidemiologic studies provide generally consistent evidence that long-term ozone exposure is associated with the development of asthma in children (Section 3.2.4.1.1). In addition to investigating the development of asthma, epidemiologic studies have evaluated the relationship between ozone exposure and asthma severity (Section 3.2.4.5). Like the studies described in the 2013 Ozone ISA (U.S. EPA, 2013b), recent studies provide evidence of consistent positive associations between long-term exposure to ozone and hospital admissions and ED visits for asthma and prevalence of bronchitic symptoms in children with asthma. Notably, some uncertainty remains about the validity of the results from studies examining long-term ozone exposure and hospital admissions and ED visits for asthma, because most of these studies do not adjust for short-term ozone concentrations, despite the causal relationship between short-term exposure and asthma exacerbation. (US EPA, 2019a)

Below, we describe how new studies do not strengthen the evidence reviewed in the 2013 Ozone ISA. Overall, recent epidemiology evidence is limited, both in quantity or quality, and epidemiology study limitations create uncertainty in the study findings. Furthermore, the evidence from animal toxicity studies is not relevant due to the use of high ozone concentrations.

3.2.1 Epidemiology and toxicological evidence does not support asthma development in children at ambient ozone concentrations.

The draft Ozone ISA states that "in general, the epidemiologic and toxicological evidence provided evidence of a likely to be causal relationship between long-term exposure to ozone and respiratory effects" (US EPA, 2019a). Epidemiology studies evaluated in the 2013 Ozone ISA did not provide evidence of the effect of ozone-induced asthma development in children. However, according to the draft Ozone ISA, recent studies conducted in the US or Canada suggest an association, and evidence from animal toxicity studies in infant monkeys suggest that ozone can cause alterations to the airway and immune system. The draft Ozone ISA also states that rodent studies provide evidence of the biological plausibility of long-term ozone and asthma development.

However, the epidemiology evidence for new onset asthma in children is inconsistent, and studies reporting positive associations suffer from critical uncertainties and limitations that impact the interpretation of results and their application to causal determinations. In addition, concentrations in animal studies are orders of magnitude higher than ambient ozone concentrations and are thus not applicable to human exposures as mechanisms of biological effects may differ at high *vs.* low ozone concentrations.

3.2.1.1 New evidence does not support asthma development in children.

The draft Ozone ISA cites a limited number of epidemiology studies as evidence of ozone-induced asthma development in children. Yet, the evidence from these studies is conflicting, so it is unclear how the draft Ozone ISA considered the entire body of evidence, in light of null findings.

Tétreault *et al*. (2016a) investigated the association between new onset asthma in children using the Québec Integrated Chronic Disease Surveillance System (QICDSS) (Tétreault *et al*., 2016a). QICDSS used data from several databases: health insurance, medical services, hospital discharge, and deaths. Asthma cases were identified using data from hospital discharge records or physician visits. Ozone levels were estimated using a Bayesian maximum entropy model and assigned yearly based on residential postal codes. The authors reported positive and statistically significant associations between estimated ozone levels and children's onset asthma. Notably, the authors used time-varying exposure estimates and accounted for

residential mobility. Yet, all associations were estimated in relation to a child's residential address and failed to account for exposures that happen elsewhere. The authors acknowledged that this is a source of bias since children spent a considerable amount of time in places other than the home. Furthermore, the authors did not adjust for multiple pollutants; as a result, it is unclear whether the adverse health effects are solely attributed to ozone exposure.

The others studies cited by the draft Ozone ISA do not provide consistent evidence of ozone-induced asthma development in children. Garcia *et al*. (2019) investigated whether decreasing regional air pollutants were associated with reduced incidence of asthma in children enrolled in the Southern California Children's Health Study (Garcia *et al*., 2019). The draft Ozone ISA states that decreases in ozone concentrations were associated with deceased asthma in this study; yet, these findings were not statistically significant and not consistent throughout the study. Furthermore, there are study limitations that create uncertainty in the findings. The authors did not account for confounding by copollutants or residential mobility and did not use time-varying exposure estimates to account for temporal variability, the latter of which likely resulted in considerable exposure measurement error. In addition, the questionnaires used to collect information on asthma incidence in children did not record specific dates of diagnosis. As result, the authors imputed all dates of the asthma diagnosis.

A study by Nishimura *et al*. (2013) investigated the association between early-life air pollution exposure and childhood asthma in Latino and African-American children living in US cities (Nishimura *et al*., 2013). Early-life ozone exposure was not associated with increased odds of childhood asthma across the study regions. The draft Ozone ISA acknowledges the null results but attributes them to the smaller study population size. This is not the only possible explanation for null results. The draft Ozone ISA does not consider other study limitations such potential exposure measurement error associated with using residential ozone measures as a surrogate for personal ozone measures.

Overall, the epidemiology evidence is inconsistent. Only Tétreault *et al*. (2016a) reported a positive association between long-term ozone and new onset asthma, and this study has several methodological limitations. Furthermore, one study is not sufficient evidence for causality. In general, the evidence is limited and inconsistent, and thus not sufficient to draw causal conclusions.

3.2.1.2   Evidence from animal toxicity studies is not relevant for health effects in humans.

Several studies cited in the draft Ozone ISA evaluated the effects of long-term ozone exposure on respiratory health in both rodents and infant rhesus monkeys. Infant rhesus monkeys are ideal animal models because their lung branching pattern and airway distribution are more closely related to humans than other animals'. The draft Ozone ISA summarizes findings from several studies in infant monkeys that showed postnatal ozone exposure compromised airway growth and development, caused the development of an allergic phenotype, and caused persistent alterations to the immune system. However, this all occurred following exposure to 500 ppb ozone (Clay *et al*., 2014; Murphy *et al*., 2012, 2013; Crowley *et al*., 2017; Chou *et al*., 2011; Moore *et al*., 2012). This concentration is orders of magnitude higher than ambient ozone concentrations. Thus, the evidence from animal toxicity does not inform asthma development in humans.

3.2.2 Evidence from asthma hospital admissions studies is insufficient.

The draft Ozone ISA states that "[r]ecent studies support a relationship between long-term ozone and the severity of respiratory disease" (US EPA, 2019a). The increase of asthma-related respiratory symptoms

has been associated with more hospital admissions and emergency department (ED) visits. However, the draft Ozone ISA also acknowledges that there are some uncertainties regarding these findings but downplays how these uncertainties call into question the validity of the study results. For example, in Tétreault *et al*. (2016b), the authors reported summertime ozone levels were associated with increased hospital admissions and ED visits related to asthma. However, the authors did not adjust for short-term asthma, so the increased medical services for asthma-related health problems could be a result of acute *vs*. chronic (*i.e.*, long-term) ozone exposure. Gilliland *et al*. (2017) reported decreases in respiratory symptoms in children with asthma associated with reductions in ambient ozone concentrations (Gilliland *et al*., 2017). However, in two-pollutant models with ozone and NO_2, PM_{10}, and $PM_{2.5}$, the effects of ozone were attenuated and became non-significant, which suggests confounding. Berhane *et al*. (2016) also reported decreased bronchitic symptoms in asthmatic children with reductions in ambient ozone. The authors found similar associations in copollutant models with NO_2. Although the authors used longitudinal outcome and covariate data, the ecological study design cannot be used to establish causality. In addition, all studies relied on ambient ozone concentrations as a surrogate for personal exposure, which can be a source of exposure measurement error.

3.2.3 Recent evidence for the effects of long-term ozone exposure on lung function development is limited.

3.2.3.1 Epidemiology evidence investigating long-term ozone and lung development is inconsistent.

The draft Ozone ISA states that the evidence from epidemiology studies investigating the association between long-term ozone exposure and lung development and lung function is inconsistent. The draft Ozone ISA cites several studies; however, the majority of recent evidence for respiratory effects in children comes from cross-sectional studies. While cross-sectional studies often rely on nationally collected survey data, which increases generalizability, a key limitation of these studies is the inability to infer temporality between exposure and outcome. In addition, the majority of studies focus on children and only one study focuses on elderly adults. Two studies in children reported no changes in lung function growth or lung function measurements with decreasing ozone concentrations (Gilliland *et al*., 2017; Gauderman *et al*., 2015). In addition, the draft Ozone ISA cites two other studies that reported modest decreases in lung function (Urman *et al*., 2014; Neophytou *et al*., 2016). Urman *et al*. (2014) investigated ozone exposure and lung function changes in children. The authors estimated ozone exposures from a central monitoring location within each community, which is a source of exposure measurement error. Neophytou *et al*. (2016) investigated the effects of ozone on lung function measured by spirometry in African American and Latino children throughout the US and Puerto Rico. They also reported "suggestive associations" for ozone exposure; however, ozone was not consistently associated with the three measures from the spirometry testing. Furthermore, the cross-sectional nature of these studies limits the ability to infer causality. As a result, as stated in the draft Ozone ISA, the evidence is limited and inconsistent and is not sufficient to make conclusions about causality.

As evidence of effects in elderly adults, the draft Ozone ISA cites one prospective longitudinal study by Eckel *et al*. (2012) that investigated long-term ozone exposure and FEV_1 and forced vital capacity (FVC) in adults aged 65 years or older. Increased ozone was associated with FEV_1 and FVC. However, the authors reported moderate correlation coefficients between PM_{10} and ozone in some study communities and did not perform analyses including copollutants. Furthermore, the authors relied on ambient exposure as surrogates from personal exposures. Yet, it is very likely that elderly and frail participants included in this study spend more time indoors and less outdoors. As result, there may be a greater potential for exposure measurement error.

3.2.3.2   Animal toxicity studies on pulmonary inflammation, injury, and oxidative stress do not provide evidence for human health effects at ambient concentrations.

The draft Ozone ISA states that postnatal exposure to ozone "resulted in altered lung development in the infant monkeys and increased oxidative stress, inflammation, and injury in neonatal rodents" (US EPA, 2019a). As discussed in Section 3.2.1.2, these studies in infant monkeys use ozone concentrations that are orders of magnitude higher than ambient ozone concentrations (*i.e*., 500 ppb). In addition, rodent studies also only report adverse effects at elevated ozone concentrations (*e.g*., 500, 800, 1,000 ppb) that are not informative of effects in humans (Miller *et al*., 2016a; Gordon *et al*., 2016).

3.2.4   Epidemiology and animal toxicity data provide insufficient evidence of long-term ozone effects on allergic responses.

According to the draft Ozone ISA, epidemiology evidence reviewed in the 2013 Ozone ISA reported generally positive associations between long-term ozone exposure and various indicators of allergies. The draft Ozone ISA also indicates that one additional recent study provides support for this association.

All the studies cited as evidence are cross-sectional in design. In the most recent study, Weir *et al*. (2013) used data from the National Health and Nutrition Examination Survey (NHANES) to investigate the association between NO_2, PM_{10}, $PM_{2.5}$, and summer O_3 and allergen-specific immunoglobulin E (IgE) in adults and children aged 6 years and older. Demographic and lifestyle information was collected *via* questionnaires, and survey participants were tested for allergen-specific antibodies as a part of NHANES collection of health data. The authors estimated air pollutant concentrations using monitors and Community Multiscale Air Quality (CMAQ) modeling. Exposures were assigned to participants using their addresses and the year they were tested for allergen antibodies. The authors reported positive associations between long-term ozone exposure and different allergens that were not statistically significant. Confounding by copollutants cannot be ruled out in this study. In addition, there is a temporality issue. Ozone was measured in May-September of each year, and it is unclear whether the exposure assigned to participants occurred before they were tested for each outcome. When determining causality, it is essential that the exposure precedes the health outcome.

The draft Ozone ISA states that previous animal studies presented in the 2013 Ozone ISA demonstrate that repeated exposure to 500 ppb ozone can cause "increased injury, inflammation, and allergic responses in a rodent model of allergic airway disease" (US EPA, 2019a). Furthermore, the draft Ozone ISA presents more recent evidence that lower concentrations can induce similar effects. In a study by Hansen *et al*. (2013), female BALB/cJ mice were exposed to 100 ppb ozone for 20 minutes/day for 5 days/week for 12 weeks and a low dose of ovalbumin to induce sensitization. Mice were challenged with a high dose of ovalbumin after 14 weeks. The authors reported that ozone exposure promoted eosinophilic airway inflammation. While this study suggests ozone induces inflammation in rodent models, there is uncertainty regarding the relevance of the evidence because of the differences in airway morphology in rodents compared with humans. Furthermore, this is only one study; additional studies are needed to confirm this finding.

3.2.5 New evidence for Chronic Obstructive Pulmonary Disease (COPD) is limited.

3.2.5.1 One epidemiology study is not sufficient evidence of long-term ozone effects on COPD.

The draft Ozone ISA appears to make causal conclusions regarding the effects of long-term ozone on specific respiratory endpoints from limited epidemiology evidence. For example, according to the draft Ozone ISA, there were no studies in the 2013 Ozone ISA that examined the association between ozone and chronic obstructive pulmonary disease (COPD); one new study is discussed in the 2019 draft Ozone ISA (To *et al*., 2016). To *et al*. (2016) investigated the association between long-term ozone exposure and COPD incidence in adults with incident asthma. Notably, the authors included multiple individual and ecological level covariates and information on other comorbidities in both single- and two-pollutant models (*i.e*., ozone and $PM_{2.5}$). The authors reported statistically significant association between ozone and COPD incidence in people with asthma; however, the results were positive yet attenuated in the two-pollutant model, which suggests confounding by $PM_{2.5}$. There is also potential for exposure measurement error since air pollution data was collected from fixed monitoring sites. In addition, a majority of the health risk factor data (*e.g*., smoking, body mass index) were collected at baseline from surveys. Even setting aside these issues, one study is not sufficient evidence to suggest an association.

3.2.5.2 Animal toxicity studies do not provide evidence of respiratory effects at relevant concentrations.

The draft Ozone ISA states that several recent animal studies demonstrate the effects of repeated subchronic ozone exposure on airway inflammation and injury. Yet, the draft Ozone ISA acknowledges that these effects occur at elevated ozone concentrations (*e.g*., 500, 800, 1,000 ppb), and, furthermore, studies using lower doses did not report any statistically significant effects (Gordon *et al*., 2016; Miller *et al*., 2016a). For example, Miller *et al*. (2016a) reported increased markers of lung injury and inflammation by analysis of the BALF from male Wistar Kyoto rats exposed to 1,000 ppb ozone for 5 hours/day for 3 days/week for 13 weeks. The authors included a lower dose group (*i.e*., 250 ppb); the effects in animals exposed to 250 ppb were almost indistinguishable from the effects in animals exposed to filtered air.

3.2.6 Evidence for respiratory mortality is inconsistent.

The draft Ozone ISA cites that evidence of the effect long-term ozone on respiratory morality is limited due to inconsistencies. It states that the strongest evidence comes from a previously reviewed study from the 2013 Ozone ISA and a more recent study reviewed in the draft Ozone ISA.

In Jerrett *et al*. (2009), the authors used data from the American Cancer Society Cancer Prevention Study II to investigate the association between long-term ozone and cardiopulmonary and respiratory causes of death. The authors reported statistically significant and positive associations between long-term ozone and respiratory-specific mortality, which includes a weakly positive risk estimate in a multipollutant model with $PM_{2.5}$. However, this study does not provide clear evidence of an association. The authors did not properly control for the potential confounding effects of copollutants because they utilized ozone and $PM_{2.5}$ data from two different periods due to a lack of available $PM_{2.5}$ data. In addition, Jerrett *et al*. (2009) found significant differences in effects by region and reported potential for confounding by temperature. Overall, due to the critical study limitations, this study is insufficient evidence of the effects of long-term ozone on respiratory mortality.

The draft Ozone ISA also cites a more recent prospective cohort study conducted by Turner *et al.* (2016) as additional recent evidence of ozone-induced effects on respiratory mortality. Turner *et al.* (2016) used data from the Cancer Prevention Study II and reported positive associations between ozone and respiratory mortality in single and multipollutant models adjusted for $PM_{2.5}$ and NO_2. However, ozone exposures were estimated based on residential postal codes and did not account for ozone exposure that could occur elsewhere, which could have introduced exposure measurement error. There is potential for unmeasured confounding as a result of a lack of information on physical activity included in the statistical models.

In addition to these studies conducted in the US, the draft Ozone ISA cites another US study by Jerrett *et al.* (2013) and a study conducted in Canada by Crouse *et al.* (2015) that reported null associations. Overall, as stated in the draft Ozone ISA, the evidence for long-term ozone exposure and respiratory mortality is inconsistent, and thus does not support causation.

3.3   Conclusions

The draft Ozone ISA indicates that recent evidence from epidemiology, controlled human exposure, and animal toxicity studies provide robust evidence for respiratory effects from short- and long-term ozone exposure. In fact, the evidence for respiratory effects does not support EPA's conclusion that there is a causal relationship between short- or long-term ozone exposure and respiratory morbidity and mortality at relevant concentrations. The controlled human exposure studies indicate that there are no statistically significant adverse respiratory effects associated with ozone exposures below 70 ppb. Effects reported at 60 ppb are also not adverse. In addition, the 2013 Ozone ISA did not properly consider key limitations in the epidemiology evidence, and new studies have the same critical issues that impact the validity of the results. Furthermore, key toxicity studies on which EPA relied to support the epidemiology data were conducted at very high exposure levels that are not relevant for assessing health effects of ambient ozone. Overall, the evidence presented in the draft Ozone ISA does not indicate that short- or long-term ozone exposure below the current ozone standard likely causes adverse respiratory effects at ambient concentrations.

4 Evidence for metabolic disease should be classified as inadequate.

Metabolic syndrome is a cluster of conditions including high blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels (National Heart, Lung, and Blood Institute, 2019). Metabolic effects were evaluated in the context of a mode of action for cardiovascular effects in the 2013 Ozone ISA. The 2019 draft Ozone ISA concludes a likely causal relationship for both short- and long-term ozone exposure giving rise to metabolic syndrome endpoints.

The draft Ozone ISA states in their Integrated Synthesis (IS-1):

> Emerging evidence indicates that short- and long-term ozone exposure contributes to metabolic disease, including diabetes. Specifically, animal toxicological studies demonstrate impaired glucose tolerance, increased triglycerides, fasting hyperglycemia, and increased hepatic gluconeogenesis in laboratory animals. A limited number of epidemiology studies observed associations between ozone and increased incidence of type 2 diabetes and mortality from diabetes. (US EPA, 2019a)

However, a more careful review of the evidence indicates that it is not consistent or sufficient for every metabolic endpoint, and that it does not support any effects at ambient ozone concentrations.

4.1 Evidence for short-term exposure does not support a likely causal determination.

The draft Ozone ISA indicates that there is limited evidence from epidemiology and controlled human exposure studies but that animal toxicity studies provide robust evidence of the effects of short-term ozone on metabolic effects. While the evidence presented in the draft Ozone ISA supports the effects of short-term ozone on glucose impairment at 800 and 1,000 ppb, the evidence for ozone-induced effects on other metabolic endpoints is not consistent and does not support the likely causal determination. Overall, the evidence presented in the draft Ozone ISA does not suggest that short-term ozone exposure at levels below the current ozone standard causes adverse effects on metabolic endpoints.

4.1.1 Animal toxicity evidence is not consistent for all metabolic endpoints.

EPA evaluated animal studies that were conducted to assess the effects of short-term ozone exposure on various markers related to metabolic health, including indicators of impaired glucose and insulin homeostasis, triglyceride levels, hepatic gluconeogenesis, and markers of inflammation. The draft Ozone ISA states that the strongest evidence comes from "animal toxicological studies that show impaired glucose tolerance, increased triglycerides, fasting hyperglycemia, decreased insulin, and increased hepatic gluconeogenesis" (Section 5.1.8, p. 5-23). The draft Ozone ISA also indicates that these studies use relevant ozone concentrations.

Animal toxicity studies support the conclusions regarding impaired glucose tolerance and fasting hyperglycemia at high ozone exposure concentrations. Evidence supporting ozone-induced hyperglycemia

and glucose intolerance was fairly consistent across studies using different rodent strains (Miller *et al*., 2016a; Miller *et al*., 2015; Gordon *et al*., 2017; Bass *et al*., 2013; Miller *et al*., 2016b). In studies investigating the effects of short-term ozone exposure on glucose, authors often used multiple doses of ozone to establish a concentration-response curve. However, significant impairments occurred primarily in the highest exposure groups (*i.e*., 800 or 1,000 ppb). The draft Ozone ISA states glucose impairments occur at concentrations as low as 250 ppb; however, there is a lack of consistent evidence at these lower concentrations from other studies. Only one study showed effects at exposures as low as 500 ppb ozone (Miller *et al*., 2015); a separate study cited in the draft Ozone ISA found no significant changes in glucose at 500 ppb (Zhong *et al*., 2016). Significant glucose intolerance was reported by Gordon *et al*. (2017) in rats exposed to 250, 500, or 1,000 ppb for five hours/day for two days; however, the effect of exercise confounded the effect of ozone on glucose tolerance in the 250 and 500 ppb exposure groups. Furthermore, other results with animals exposed to lower doses of ozone (*i.e*., 250 ppb) were indistinguishable from the results with control animals exposed to filtered air, suggesting a threshold for these metabolic endpoints that is considerably higher than ambient concentrations. Since all of these doses are much higher than ambient ozone concentrations, these studies do not provide evidence for effects in humans at lower ozone levels.

The draft Ozone ISA also evaluated evidence regarding ozone-induced alterations to serum lipids, including triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL) cholesterol. Animal studies were inconsistent with regard to changes in serum lipids as a result of short-term ozone exposure both within and across rodent strains. Miller *et al*. (2015) reported a statistically significant increase in LDL cholesterol in male Wistar Kyoto rats following exposure to 1,000 ppb ozone for six hours/day for two days, but Farraj *et al*. (2016) reported no changes in serum triglycerides, HDL, or LDL cholesterol in male Long-Evans rats following exposures up to 1,000 ppb for five hours/day for two days. Gordon *et al*. (2016) found no effect of ozone exposure (800 ppb for four days/week for three weeks) on cholesterol in male and female Brown Norway rats but found increased serum triglycerides in male rats only. The findings from these studies demonstrate a lack of coherence of effects across animal toxicity studies.

Other metabolic endpoints assessed in animal toxicity studies included inflammation, insulin impairments, and hepatic gluconeogenesis. The draft Ozone ISA cites a few studies reporting positive associations between ozone and inflammation in adipose tissue, albeit at high ozone doses (500 ppb). Furthermore, the draft Ozone ISA states that the effects of ozone on systemic inflammation varies based on the rodent strain (Section 5.1.5.1, p. 5-13) but provides no explanation for why this is the case or how inconsistent results across strains should be extrapolated to humans. In fact, other studies cited as key evidence in other parts of the draft Ozone ISA (Table 5-1, p. 5-24) also tested for inflammatory markers but did not report significant changes at 1,000 ppb (Miller *et al*., 2016a; Bass *et al*., 2013). There is no indication that these other studies reporting null effects were considered. This calls into question EPA's process of assessing the collective body of evidence for causal determinations.

In addition, while a few studies assessed the effects of ozone on insulin homeostasis, the results were not consistent across studies. Only one study reported significant results from a pyruvate tolerance test, a measurement of liver gluconeogenesis, in Wistar Kyoto rats exposed to 1,000 ppb of ozone for five hours/day for one week (Miller *et al*., 2016a).

Finally, it is notable that a majority of the animal toxicity studies cited in the draft Ozone ISA come from the same research group (*e.g*., Gordon *et al*., Bass *et al*., and Miller *et al*.). These results should be confirmed by other research groups before they are considered probative for causal determinations.

Overall, aside from glucose impairments, there is no consistent evidence for short-term effects of ozone on other indicators of metabolic effects. A majority of the toxicity studies only reported adverse effects at

high exposure levels of ozone. These doses are much higher than ambient ozone concentrations and are not informative regarding human health risks below the current standard.

4.1.2 Epidemiology evidence for diabetes and metabolic syndrome is limited in both quality and quantity.

The draft Ozone ISA states that there is "[c]onsistent epidemiologic evidence of increased risk of diabetes or metabolic syndrome" and "positive associations between short-term ozone exposure and increased indicators of impaired glucose and insulin homeostasis, including HOMA-IR, dyslipidemia, elevated HbA1c, and increased fasting glucose" (Table 5-1, p. 5-32). However, the draft Ozone ISA only presents one study that reported associations between long-term ozone exposure and metabolic endpoints. This study was conducted in Taiwan (see Chuang *et al.*, 2010) using cross-sectional health survey data and air pollutant data from monitors across Taiwan. Ozone exposure was assigned to participants based on their residential addresses and matched with the date blood was collected for testing of metabolic biomarkers. The authors reported positive associations between measured ozone, apolipoprotein B (a component of LDL cholesterol), and diastolic blood pressure. Increased ozone concentrations were also associated with very small, statistically significant increases (0.05-0.07%) in levels of hemoglobin A1c (HbA1c) at all lag times examined. Because the authors relied on self-reported questionnaire data for information on individual level confounders and did not account for other ecological covariates, the possibility for unmeasured confounding cannot be ruled out. There is also the potential for exposure measurement error from using ambient ozone concentrations as a surrogate for personal exposure levels. In addition, the authors only applied single-pollutant models, so confounding by copollutants cannot be ruled out.

It is unclear how this one study provides consistent evidence for effects on diabetes and metabolic syndrome, given that neither diabetes nor metabolic syndrome incidence or prevalence were directly assessed in the study. Importantly, the findings from Chuang *et al.* (2010) are not consistent with findings from other human epidemiology studies with more robust study designs. The draft Ozone ISA acknowledges that findings from other studies of metabolic effects, including case-crossover and panel studies, although limited in number, are generally null. EPA did not evaluate study quality or properly weigh the evidence from all the relevant studies for its causality determination. The lack of associations in these more robust human epidemiology studies calls into question the positive associations reported from Chuang *et al.* (2010). It is also worth noting that Chuang *et al.* (2010) and some of the other more robust studies relied on data from populations outside of the US, which calls into the question whether their results are generalizable to the US.

4.1.3 Few epidemiology studies evaluated copollutant models.

The draft Ozone ISA states, "[t]he magnitude of ozone associations remains relatively unchanged in a limited number of studies evaluating copollutant models, including $PM_{2.5}$ and other gaseous pollutants." As discussed above in Section 4.1.2, only one study was presented as key evidence. As a result, there is not enough evidence to definitively rule out copollutants, and, furthermore, this study has other methodological limitations that were not fully considered, such as unmeasured confounding and exposure measurement error. Furthermore, a few studies listed in the draft Ozone ISA had generally null findings (see Table 5-1, p. 5-24); this does not support positive associations in other studies. Overall, the evidence does not collectively suggest that reported associations with short-term ozone exposure are not confounded by the presence of copollutants.

4.1.4 Controlled human exposure studies do not demonstrate metabolic changes with ozone exposure at ambient concentrations.

The draft Ozone ISA states that there is "[c]ontrolled human exposure evidence of increased metabolic changes with ozone exposure at relevant concentrations," although only one key study is cited. In this study, Miller *et al*. (2016c), exposed healthy adult volunteers to either ozone (300 ppb) or fresh air for two hours in a controlled chamber while performing 15 minutes on/off exercise (Miller *et al*., 2016c). Following a two-week wash-out period, volunteers received the alternate exposure; serum samples were collected for metabolomic assessment after each exposure. Ozone exposure was only positively associated with increased concentrations of circulating metabolites (carnitine conjugates of long-chain free fatty acid and acetyl carnitine) related to ketone body formation. The authors found no significant changes in homeostatic model assessment of insulin resistance (HOMA-IR) or insulin levels. In addition, the authors did not find significant changes in the relevant cytokines and adipokines (indicators of inflammation often associated with obesity and metabolic syndrome). The levels of these biomarkers of inflammation should have increased in response to ozone exposure if it truly induces systemic inflammation (Goodman *et al*., 2015b).

The positive associations with ketone body formation from this one study are not sufficient to conclude that ozone induces metabolic changes in humans at 300 ppb, particularly in light of the null effects for other related endpoints. It is also notable that, although the 300 ppb exposure concentration is lower than some of the ozone exposure doses in animal toxicity studies, it is still much higher than ambient ozone levels.

4.1.5 Evidence does not support pathways for biological plausibility.

The draft Ozone ISA states (Table 5-1, p. 5-24), "Experimental studies provide evidence of metabolic syndrome mediated by pulmonary irritant receptor stimulation and activation of the neuroendocrine system with short-term ozone exposure provides biological plausibility to the effects of ozone on metabolic syndrome and diabetes." In addition to the text in the draft Ozone ISA outlining the evidence from experimental studies, a figure (Figure 5-1, p.5-4, reproduced below) describes hypothesized biological pathways for metabolic outcomes following short-term ozone exposure.

The solid lines in Figure 4.1 indicate essentiality, meaning direct evidence of ozone's impact on the upstream or downstream effects. The dotted lines denote "possible pathways," with presumably less evidence of the direct impacts of ozone. However, a review of the evidence discussed in the draft Ozone ISA indicates that the direct evidence is not necessarily robust or consistent and is often at exposure concentrations much higher than ambient ozone concentrations.
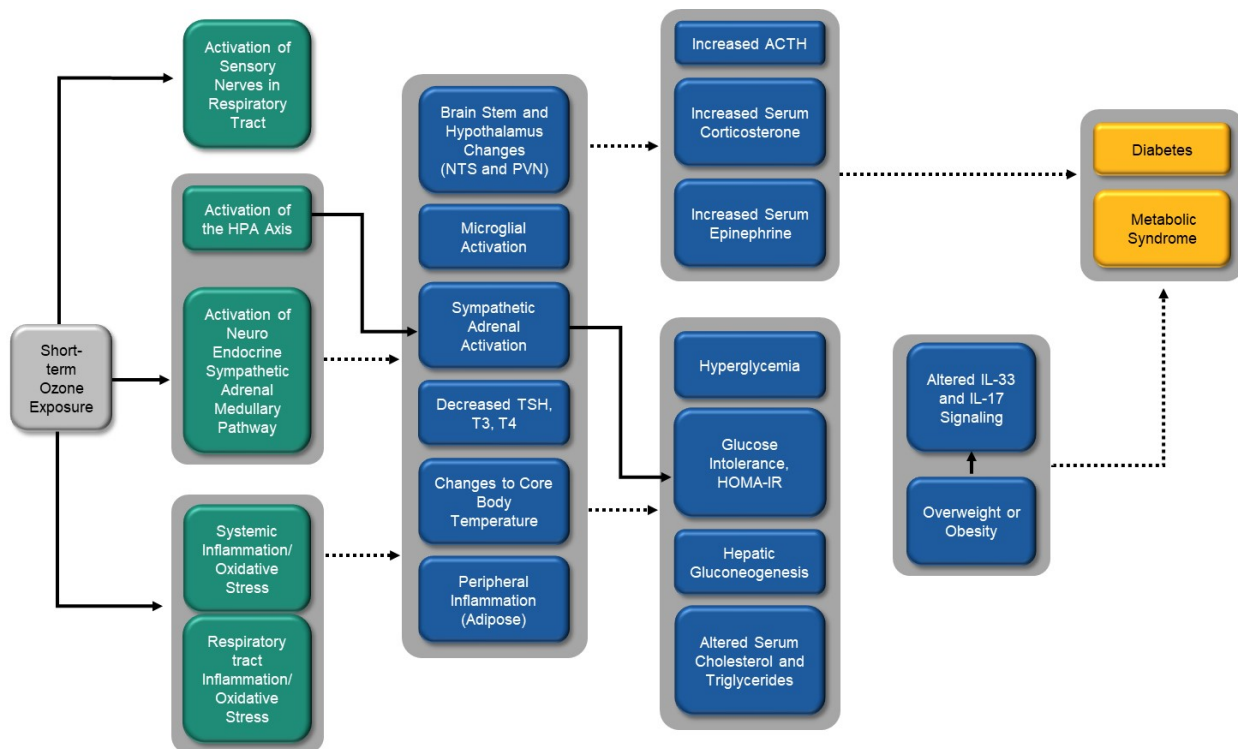
Figure 4.1   Potential Biological Pathways for Metabolic Outcomes Following Short-term Ozone Exposure. Reproduction of Figure 5-1 from EPA (2019a).

For example, the draft Ozone ISA cites several studies as evidence that pulmonary irritants, such as ozone, can activate sensory nerves in the respiratory tract (Zellner *et al*., 2011; Gackiere *et al*., 2011; Dorado-Martinez *et al*., 2001; Mumaw *et al*., 2016). The draft Ozone ISA indicates that this action can have subsequent downstream effects on the hypothalamic pituitary adrenal (HPA) axis and eventually impact metabolic health overall. While these studies provide plausible theories that connect sensory nerve activity to downstream metabolic effects, the evidence appears to be inconsistent in that the molecular endpoints studied vary across studies, thus making it difficult to discern the consistency of effects.

The draft Ozone ISA also states that there is direct evidence of the effects of ozone on activation of the HPA axis or the neuroendocrine sympathetic adrenal medullary pathway. These pathways are responsible for controlling and mediating the body's stress responses, and the draft Ozone ISA suggests that the resulting multiorgan response to stress related to ozone exposure, communicated throughout the body *via* changing levels of stress hormones, can lead to downstream metabolic effects such as glucose intolerance, hyperglycemia, and hepatic gluconeogenesis. Yet, the evidence cited by the draft Ozone ISA includes several of the key studies described above in Section 4.1.1, 4.1.2, and 4.1.3. As discussed in these Sections, aside from glucose impairment at 800 and 1,000 ppb, overall, the animal toxicity studies do not provide consistent evidence of ozone-induced effects on metabolic endpoints at ozone concentrations relevant to the standards.

Although the evidence from human epidemiology and controlled human exposure studies are limited, among these few studies, there appears to be a lack of coherence in that human studies do not consistently report associations between ozone and these downstream effects. In fact, as described in Section 4.1.2, the draft Ozone ISA cites several human epidemiology studies with largely null findings. As a result, the evidence presented by the draft Ozone ISA does not provide sufficient evidence to connect short-term ozone exposure through initial upstream effects to downstream effects.

4.2 Evidence for long-term exposure does not support a likely causal determination.

The draft Ozone ISA states:

> Experimental animal studies address some of the uncertainty in the epidemiologic evidence related to the independent effect of ozone exposure by providing evidence of direct effects on metabolic function. The animal toxicological studies provided evidence that long-term ozone exposure resulted in impaired insulin signaling, glucose intolerance, hyperglycemia, and insulin resistance (Section 5.2.3.1). In addition, these pathophysiological changes were often accompanied by increased inflammatory markers in peripheral tissues, and activation of the neuroendocrine system (Section 7.2.1.5). A limited number of epidemiologic studies have evaluated potential copollutant cofounding for PM or NO_X [Jerrett et al. (2017); Renzi et al. (2017); Section 5.2.3]. Importantly, short-term ozone exposure studies also provided evidence that ozone exposure could contribute to the development of metabolic syndrome and show consistency with the evidence that long-term ozone exposure could lead to development or worsening of metabolic syndrome or its risk factors. **Overall, the collective evidence is sufficient to conclude that a likely to be causal relationship exists between long-term ozone exposure and metabolic effects.** (US EPA, 2019a)

Similar to the evidence for short-term ozone effects on metabolic endpoints, evidence regarding long-term effects is limited overall. The draft Ozone ISA also considers findings from short-term studies as evidence of long-term ozone induced metabolic effects. As discussed above in Section 4.1, the short-term evidence is limited and inconsistent, with the exception of glucose impairment at exposures well above the ozone standards (*e.g.*, 500-1,000 ppb). Overall, the evidence presented in the draft Ozone ISA does not suggest that long-term ozone exposure below the current ozone standard causes adverse effects on metabolic endpoints.

4.2.1 Animal toxicity studies are limited.

The draft Ozone ISA states there is "consistent animal toxicology evidence from multiple, high-quality studies at relevant ozone concentrations" from "studies of impaired glucose tolerance, fasting hyperglycemia, dyslipidemia, insulin resistance, and activation of the neuroendocrine pathway with ozone exposure." The draft Ozone ISA cites three key studies as evidence of the effects of long-term ozone on metabolic endpoints; two of these studies were also considered to provide evidence for short-term ozone-induced effects.

The draft Ozone ISA stated long-term ozone exposure animal studies show adverse effects on glucose and insulin homeostasis. Similar to the evidence from short-term ozone exposure, adverse metabolic effects were primarily consistent at the highest dose groups (800 and/or 1,000 ppb) among the few animal toxicity studies investigating the effects of long-term ozone exposure. Bass *et al*. (2013) exposed Brown Norway rats, aged 1, 4, 12, and 24 months, to 250 and 1,000 ppb ozone for six hours/day for two days/week for 13 weeks and conducted glucose tolerance tests. Based on their results, the draft Ozone ISA states that all ozone-exposed animals had glucose impairment. However, ozone-reductions in glucose tolerance are only consistent across all rodent age groups in the highest ozone exposure group of 1,000 ppb (Bass *et al*., 2013). In addition, the subchronic effects on glucose were less severe than they were in rats after acute ozone exposure. Miller *et al*. reported similar effects in adult male Wistar Kyoto rats following 1,000 ppb ozone exposure for five hours/day for three consecutive days for 12 weeks. Following 12 weeks of exposure, there was also a statistically significant decrease in serum insulin (Miller *et al*., 2016a).

The third study by Gordon *et al*. (2013) only exposed animals to 800 ppb ozone, so there is no low dose evidence to compare with other key evidence. Importantly, these high doses are not relevant to ambient ozone concentrations. In addition, contrary to results from Miller *et al*., Gordon *et al*. reported an *increase* in serum insulin following episodic exposure to 800 ppb of ozone over 17 weeks in elderly (but not adult) rats (Gordon *et al*., 2013).

4.2.2 Epidemiology evidence regarding morbidity is not sufficient.

The draft Ozone ISA states there is consistent evidence for associations between long-term ozone exposure and an increased risk of diabetes and metabolic syndrome, citing four key studies. The evidence from these studies is not sufficient to conclude that long-term exposure to ozone is associated with either metabolic syndrome or diabetes. Three studies present conflicting evidence regarding the effects of long-term ozone exposure on diabetes, and only one study investigates metabolic syndrome. Issues with potential confounding and exposure measurement error are also major sources of uncertainty in these studies.

EPA noted two studies investigating the effect of ozone on diabetes in adults. Jerrett *et al*. (2017) reported positive associations between ambient ozone exposure and type 2 diabetes incidence in the Black Women's Health Study, a national US-based cohort of African-American women. However, the addition of NO_2 to the model weakened the results, suggesting confounding by NO_2 (Jerrett *et al*., 2017).

Renzi *et al*. (2018) reported positive associations between ozone and the incidence of type 2 diabetes in a cohort of men and women in Rome, and the associations remained significant in copollutant models with NO_2. However, the study did not have robust information on physical activity or diet.

Yang *et al*. (2018) conducted a secondary analysis of 18- to 74-year-old adults using data from 33 communities in China to investigate the effects of long-term ozone exposure on metabolic syndrome. Notably, for their main analyses, Yang *et al*. used one clear definition of metabolic syndrome and included categorizations for waist circumference that were specific to their study population. In addition, they conducted sensitivity analyses using other definitions of metabolic syndrome from organizations such as the American Heart Association, Chinese Diabetes Society, and International Diabetes Federation. The authors reported positive associations between ozone and metabolic syndrome in their main analyses and significant, albeit slightly weakened, associations in their sensitivity analyses. However, we are in agreement with the draft Ozone ISA regarding key limitations of this study and the resulting uncertainties of its findings. Confounding by copollutants cannot be ruled out; the authors reported high correlations between ozone and PM_{10} and SO_2. Furthermore, there is likely unmeasured confounding because baseline questionnaires were used to collect information on individual confounders. Importantly, the cross-sectional study design impedes the ability to demonstrate causality.

The final study evaluated in the draft Ozone ISA focused on maternal ozone exposure and the incidence of type 1 diabetes in children (Malmqvist *et al*., 2015). While Malmqvist *et al*. (2015) reported elevated odds ratios for type 1 diabetes in the highest quartile of ozone exposure in the first and second trimester, these findings were not statistically significant and had wide confidence intervals. In addition to the potential exposure measurement error from using ambient ozone concentrations as a surrogate for personal exposure levels, the authors acknowledge that the methods for assessing ozone exposure were crude.

Overall, the results from the human epidemiology studies are inconsistent and not suggestive of an association between long-term exposure to ozone and metabolic changes. The few studies focused on different metabolic endpoints, so there was limited evidence to review for consistency. Two studies present conflicting results regarding the association between long-term ozone and the incidence of type 2 diabetes.

As discussed in Section 3.1.7.1, there is potential for exposure measurement error since all studies use ambient ozone levels as a surrogate for personal ozone exposure. It is also worth noting that two of the key studies evaluated in the draft ISA relied on data from international cohorts (*e.g.*, China and Italy). Regardless of their results and study limitations, it is unclear whether these study results are generalizable to the US population.

4.2.3 Epidemiology evidence regarding diabetes-associated mortality is limited.

The draft Ozone ISA states that "[a] limited number of studies observed positive associations between long-term ozone exposure and mortality from diabetes and cardiometabolic diseases." The draft Ozone ISA cites two key studies. Notably, these studies both use robust epidemiology study designs and consider the role of copollutants. However, their findings are limited by the potential for exposure measurement error and confounding by factors not assessed by the investigators.

Crouse *et al*. (2015) reported that increased concentrations of ozone were associated with elevated mortality from cardiometabolic diseases and diabetes in both single and copollutant models. Similarly, Turner *et al*. (2016) reported positive associations between ozone and diabetes mortality in multipollutant models adjusted for $PM_{2.5}$ and NO_2. Similar to many studies, in both studies, ozone exposures were estimated based on residential postal codes and did not account for ozone exposure that could occur elsewhere, which can introduce exposure measurement error. In the Crouse *et al*. (2015) study, the authors used data from the Canadian air quality forecast to model the ozone surface. However, the authors did not provide information on model performance, so it is uncertain how well ozone exposure was assessed. An additional limitation to the studies is the lack of information on physical activity in both studies and a lack of information on preexisting conditions in Crouse *et al*. As a result, there is potential for unmeasured confounding.

4.2.4 There is only limited evidence from copollutant models.

As discussed in detail above in Section 4.2.1, the findings from Jerrett *et al*. suggest potential confounding by NO_2, and other studies considered as key evidence by the draft Ozone ISA did not account for copollutants at all.

4.2.5 Evidence does not support biological plausibility at ambient exposures.

For long-term ozone effects, similar to short-term effects, the draft Ozone ISA presents both a figure and text outlining the studies that provide evidence of biological plausibility (Figure 5-2, p. 5-28). EPA relies heavily on both short- and long-term experimental studies as evidence of biological plausibility. As discussed above in section 4.1.5, the draft Ozone ISA states ozone can act as an pulmonary irritant and activate the HPA axis. While notable that the draft Ozone ISA focuses on biological plausibility for the causality determinations, the evidence presented does not show a clear pathway from exposure to downstream effects (*i.e.*, metabolic endpoints). As discussed above, the experimental evidence from short-term ozone studies do not provide consistent, sufficient evidence for biological plausibility at ambient concentrations. The evidence cited by the draft Ozone ISA includes several of the key studies described above in Section 4.2.1. The results from these few long-term animal toxicity studies were inconsistent and significant effects only occurred in the highest exposure groups (*i.e.*, 500, 800, and 1,000 ppb) (Section 4.2.1).

4.3   Conclusions

The draft Ozone ISA indicates that there is limited evidence from epidemiology and controlled human exposure studies but that animal toxicity studies provide robust evidence of the effects of short-term ozone on metabolic effects. While the evidence presented in the draft Ozone ISA supports the effects of short-term ozone on glucose impairment at 800 and 1,000 ppb ozone, the evidence for ozone-induced effects on other metabolic endpoints is not consistent. Evidence regarding long-term effects is limited. Overall, the evidence presented in the draft Ozone ISA does not indicate that short- or long-term ozone exposure below the current ozone standard likely causes adverse effects on metabolic endpoints. Rather, it is inadequate to address causation.

5 Evidence for associations between short-term exposure and cardiovascular effects and total mortality is inadequate.

The causality determinations for short-term ozone and cardiovascular effects and total mortality were reduced from "likely to be causal" to "suggestive of, but not sufficient to infer, a causal relationship." The draft Ozone ISA states (p ES-9):

> The evidence that supports this change in the causality determinations includes: (1) a growing body of controlled human exposure studies providing less consistent evidence for an effect of short-term ozone exposure on cardiovascular health endpoints; (2) a paucity of positive evidence from epidemiology studies for more severe cardiovascular morbidity endpoints (i.e., heart failure, ischemic heart disease and myocardial, arrhythmia and cardiac arrest, and stroke); and (3) uncertainties due to a lack of control for potential confounding by pollutants in epidemiology studies. Although there is generally consistent evidence for a limited number of ozone-induced cardiovascular endpoints in animal toxicological studies and for cardiovascular mortality in epidemiology studies, these results are not coherent with results from controlled human exposure and epidemiology studies examining cardiovascular morbidity endpoints. There remains evidence for ozone-induced cardiovascular mortality from epidemiology studies. However, inconsistent results from a larger number of recent controlled human exposure studies that do not provide evidence of cardiovascular effects in response to short-term ozone exposure introduce additional uncertainties. (US EPA, 2019a)

Although it is true the lack of coherence argues against a likely causal association between short-term ozone exposure and cardiovascular effects and total mortality, the lack of coherence also argues against a "suggestive" association. Using the term, "suggestive causal relationship," implies that a causal association is more likely than not, when this is clearly not the case. For example, as discussed in Goodman *et al*. (2014), there were a few statistically significant associations reported in epidemiology studies of cardiovascular morbidity and mortality, and these were very small in magnitude and likely attributable to confounding, bias, or chance. In experimental animal studies, the reported statistically significant cardiovascular effects at high exposures were not observed at lower exposures. Taken together, the weight-of-evidence is not suggestive of a causal association between short-term ozone and cardiovascular effects below the ozone standards (Goodman *et al*., 2014).

As indicated in the Institute of Medicine (IOM) report, *Improving the Presumptive Disability Decision-making Process for Veterans* (IOM, 2008), in situations when there are multiple but inconsistent high-quality studies, the appropriate conclusion is that evidence is "below equipoise"; a classification of the evidence as "inadequate" would also be appropriate. The causality determinations for short-term ozone and cardiovascular effects and total mortality should be reduced to inadequate.

6 Evidence does not support causal or likely causal associations with other health effects.

On page IS-86, the draft Ozone ISA states:

> Older and recent studies examining short- or long-term ozone exposure and several other health effects (i.e., nervous system effects, reproductive effects, cancer) are few or report inconsistent evidence of an association with the health effect of interest. For these health effects, there is often limited coherence across studies from different scientific disciplines, and limited evidence for biologically plausible pathways by which effects could occur. Other sources of uncertainty, such as limited assessment of potential copollutant confounding, are inherent in these evidence bases. (US EPA, 2019a)

Based on the limited evidence and sources of uncertainty, the evidence for these health effects should be considered inadequate, not "suggestive" of a causal relationship.

7   Conclusions

There have been several improvements in the ISA process with regard to the review of the scientific literature on ozone-induced health effects, but several issues remain, particularly with respect to the literature search and study selection, study quality evaluations, biological plausibility evaluations, evidence integration, and causal conclusions. More specifically, there are inconsistencies in the selection and review of evidence, and the reliance on toxicity studies that evaluate high ozone concentrations. The ISA process could be improved by adding transparent criteria for assessing study quality in the systematic review and causal framework, as well as detailed methods for integrating evidence in a way that fully and systematically considers individual study quality and relevance, and considers the coherence of results across studies within and across scientific disciplines (see example in Appendix A).

Although there is evidence supporting short-term ozone exposure and glucose impairment, this was only consistent for high exposures (*i.e*., 800 and 1,000 ppb). At elevated ozone concentrations, different biological mechanisms may be activated that are not relevant to humans exposed to ambient ozone concentrations. Overall, evidence for short- and long-term ozone exposure fall short of causal and likely causal conclusions for respiratory effects and metabolic effects, respectively, at ambient ozone concentrations. While the evidence for short-term ozone exposure and cardiovascular effects and total mortality certainly does not support a likely causal relationship (as indicated in the draft Ozone ISA), it is not suggestive of a causal relationship, but rather it is inadequate to address causality, if not suggestive of a lack of association. Finally, we concur with the draft Ozone ISA that evidence for other endpoints does not support causal or likely causal associations; however, like the evidence for short-term ozone exposure and cardiovascular effects and total mortality, this evidence falls short of suggestive.

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Appendix A

Proposed NAAQS Systematic Review Framework