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Integrated Science Assessment for Ozone and Related Photochemical Oxidants

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Center for Public Health and Environmental Assessment
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INTEGRATED SYNTHESIS

Overall Conclusions of the Ozone Integrated Science Assessment (ISA)

Human Health Effects

- Recent studies support and expand upon the strong body of evidence, which has been accumulating over many decades, that short-term ozone exposure causes respiratory effects. The strongest evidence comes from controlled human exposure studies demonstrating ozone-induced decreases in lung function and inflammation in healthy, exercising adults at concentrations as low as 60 ppb after 6.6 hours of exposure. In addition, epidemiologic studies continue to provide strong evidence that ozone is associated with respiratory effects, including asthma and chronic obstructive pulmonary disease exacerbations, as well as hospital admissions and emergency department visits for respiratory diseases. The results from toxicological studies further characterize potential mechanistic pathways and provide continued support for the biological plausibility of ozone-induced respiratory effects.
- There is emerging evidence that short-term ozone exposure contributes to metabolic disease, including complications related to diabetes. Specifically, animal toxicological studies demonstrate that ozone exposure impaired glucose tolerance, increased triglycerides in serum, fasting hyperglycemia, and increased hepatic gluconeogenesis.
- The integration of recent evidence from controlled human exposure studies showing inconsistent evidence of ozone-induced cardiovascular effects with the overall body of evidence for an association of short-term ozone exposure with cardiovascular effects and total (nonaccidental) mortality available in the 2013 Ozone ISA, results in a change in the causality determinations for those outcome categories.

Welfare Effects

Ecological Effects

- A large body of scientific evidence spanning more than 60 years clearly demonstrates that ozone affects vegetation and ecosystems. The strongest evidence comes from vegetation-related endpoints; foliar injury, reduced growth, and decreased yield resulting from ozone exposure are well characterized in a variety of crop and noncrop species. Ecological effects of ozone are observed across several scales of biological organization (i.e., from the cellular level through individual organisms to the level of communities and ecosystems), ultimately affecting aboveground and belowground processes including productivity, carbon sequestration, biogeochemical cycling and hydrology. In most cases, new research strengthens the previously reached conclusions in the 2013 Ozone ISA. New endpoints included in this review result from emerging areas of study such as chemical ecology (e.g., plant-insect signaling) or new evidence enabling further refinement of previously understood ozone effects (e.g., plant reproduction, tree mortality, herbivore growth and reproduction, terrestrial community composition).

Effects on Climate

- New research builds on the evidence in the 2013 Ozone ISA and continues to support the previous findings of tropospheric ozone impacts on radiative forcing and climate variables, including temperature and precipitation (referred to as “climate change” in the 2013 Ozone ISA).

IS.1 Introduction

IS.1.1 Purpose and Overview

The Integrated Science Assessment (ISA) serves as the scientific foundation of the National Ambient Air Quality Standard (NAAQS) review process. [1] The ISA is a comprehensive evaluation and synthesis of the policy-relevant science "useful in indicating the kind and extent of all identifiable effects on public health or welfare[2] which may be expected from the presence of [a] pollutant in the ambient air," as described in Section 108 of the Clean Air Act (42 U.S. Code [U.S.C.] 7408).[3] This ISA reviews and synthesizes the air quality criteria for the health and welfare effects of ozone and related photochemical oxidants in ambient air. It draws on the existing body of evidence to evaluate and describe the current state of scientific knowledge on the most relevant issues pertinent to the current review of the ozone NAAQS,[4] to identify changes in the scientific evidence since the previous review, and to describe remaining or newly identified uncertainties and limitations in the evidence. In 2015, the U.S. EPA lowered the level of the primary and secondary ozone standards to 0.070 ppm and maintained the form of the standard as the annual fourth-highest daily max 8-hour concentration averaged over 3 years.[5] The ozone primary NAAQS is established to protect public health, including at-risk populations such as children and people with asthma, with an adequate margin of safety. The ozone secondary standard is intended to protect the public welfare from known or anticipated adverse effects associated with the presence of ozone and related photochemical oxidants in the ambient air.

This ISA identifies and critically evaluates the most policy-relevant current scientific literature published since the 2013 Ozone ISA across scientific disciplines including epidemiology, controlled human exposure studies, experimental animal toxicology, atmospheric science, exposure science, vegetation studies, agricultural science, ecology, and climate-related science. Key scientific conclusions (e.g., causality determinations; Section IS.1.2.4) are presented that provide the basis for developing risk

[1] Section 109(d)(1) of the Clean Air Act requires periodic review and, if appropriate, revision of existing air quality criteria to reflect advances in scientific knowledge on the effects of the pollutant on public health and welfare. Under the same provision, U.S. EPA is also to periodically review and, if appropriate, revise the NAAQS, based on the revised air quality criteria.

[2] Under CAA section 302(h), effects on welfare include, but are not limited to, "effects on soils, water, crops, vegetation, manmade materials, animals, wildlife, weather, visibility, and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being."

[3] The general process for developing an ISA, including the framework for evaluating weight of evidence and drawing scientific conclusions and causal judgments, is described in a companion document, Preamble to the Integrated Science Assessments (U.S. EPA, 2015).

[4] The "indicator" of a standard defines the chemical species or mixture that is to be measured in determining whether an area attains the standard. The indicator of the current NAAQS for photochemical oxidants is ozone.

[5] Final rule signed October 1, 2015 and effective December 28, 2015 (80 FR 65291).

and exposure analyses, evaluating policy, and making environmental health and welfare decisions. In characterizing the evidence for each of the health and welfare effects categories evaluated, this ISA draws conclusions about the causal nature of the relationships between ozone exposure and outcomes by integrating information across scientific disciplines and synthesizing evidence from previous and recent studies. As in previous reviews, the ISA for this review focuses mainly on the assessment of health and welfare effects resulting from exposure to concentrations of tropospheric ozone. Ozone is currently the NAAQS indicator for photochemical oxidants, and the primary literature evaluating the health and ecological effects of photochemical oxidants includes ozone almost exclusively as an indicator of photochemical oxidants.[1] This ISA thus provides the policy-relevant scientific information that supports the review of the current ozone NAAQS.

IS.1.2 Process and Development

Through iterative NAAQS reviews, ISAs build on evidence and conclusions from previous assessments. The previous ozone ISA was published in 2013 (U.S. EPA, 2013b) and included peer-reviewed literature published through July 2011. Prior assessments include the 2006 Air Quality Criteria Document (AQCD) for Ozone and Related Photochemical Oxidants (U.S. EPA, 2006a), the 1996 AQCD for Ozone (U.S. EPA, 1996a), the 1986 AQCD for Ozone (U.S. EPA, 1986), the 1978 Air Quality Criteria for Ozone and Other Photochemical Oxidants (U.S. EPA, 1978), and the 1970 Criteria Document (NAPCA, 1970). This ISA focuses on synthesizing and integrating the new evidence (i.e., studies published between January 2011 and March 2018, as well as more recent studies identified during peer review or by public comments) with the information and conclusions from previous assessments.

In the process of developing an ISA, systematic review methodologies are used to identify and evaluate relevant scientific information, which is synthesized into text and figures for the purpose of communicating the state of the science. The process begins with a "Call for Information" published in the Federal Register that announces the start of the NAAQS review and invites the public to assist in this process by identifying relevant research studies in the subject areas of concern. For this Ozone NAAQS review, the Federal Register notice was published on June 26, 2018 (83 FR 29785). The subsequent ISA development steps are described in greater detail in the Preamble to the Integrated Science Assessments (U.S. EPA, 2015), which provides a general overview of the process. The Preamble describes the general framework for evaluating scientific information, including criteria for assessing study quality and developing scientific conclusions. The U.S. EPA uses a structured and transparent process to evaluate scientific information and to determine the causal nature of relationships between air pollution and health

[1] Ozone is the only photochemical oxidant other than nitrogen dioxide (NO_2) that is routinely monitored in ambient air (i.e., U.S. EPA's AQS database; https://www.epa.gov/aqs). Data for other photochemical oxidants (e.g., PAN, H_2O_2, etc.) typically have been obtained only as part of special field studies. Consequently, no data on nationwide patterns of ambient air concentrations are available for these other photochemical oxidants; nor are extensive data available on the relationships of concentrations and patterns of these photochemical oxidants to those of ozone.

and welfare effects [see Preamble (U.S. EPA, 2015)]. Development of the ISA includes approaches for literature searches, application of criteria for selecting and evaluating relevant studies, and application of framework for evaluating the weight of evidence and forming causality determinations. As part of this process, the ISA is reviewed by the public and by the Clean Air Scientific Advisory Committee (CASAC), which is a formal, independent scientific committee (Section 10.4). The Preamble describes a science and policy workshop that often occurs at the beginning of the NAAQS review process; such a workshop was not convened for the current Ozone NAAQS review. Instead, the "Call for Information" published in the Federal Register requested public input on science and polity issues pertinent to the Ozone NAAQS review.

IS.1.2.1 Scope of the ISA and the Population, Exposure, Comparison, Outcome, and Study Design (PECOS) Tools

The Ozone ISA includes research relevant to characterizing ozone in ambient air (hereafter referred to as ambient ozone) and assessing the health and welfare effects of exposure to ambient ozone. Health effects evidence evaluated in the ISA includes experimental controlled human exposure and animal toxicological studies, and observational epidemiologic studies. Welfare-based evidence included in the Ozone ISA focuses specifically on ecological effects and effects on climate. The evidence connecting tropospheric ozone and UV-B (short-wave ultraviolet rays) shielding was evaluated in the 2013 Ozone ISA and determined to be inadequate to draw a causal conclusion. The current ISA concludes there was no new evidence since the 2013 Ozone ISA relevant to the question of UV-B shielding by tropospheric ozone, including the incremental effects of tropospheric ozone concentration changes on UV-B. (Section 9.1.3.4), and this topic is not discussed further in this synthesis.

The scope of the health and welfare effects evidence evaluated in this ISA is further refined by using the Population, Exposure, Comparison, Outcome, and Study Design (PECOS) tool. The PECOS tools provide structured frameworks for defining the scope of the ISA. There are discipline-specific PECOS tools for experimental and epidemiologic studies (Section 3.1.2, Section 3.2.2, Section 4.1.2, Section 4.2.1.1, Section 5.1.1, Section 5.2.1, Section 6.1.1.1, Section 6.2.1.1, Section 7.1.1.1, Section 7.2.1.1, Section 7.2.2.1, and Section 7.3.1.1), ecological studies (Table 8-2), and studies of the effects of tropospheric ozone on climate (Table 9-1). These PECOS criteria were developed with consideration of the evidence base available at the time of the last review (i.e., the causality determinations from the 2013 Ozone ISA) and the uncertainties and limitations associated with that evidence. The use of PECOS tools is a widely accepted and rapidly growing approach to systematic review in risk assessment, and their use is consistent with recommendations by the National Academy of Sciences for improving the design of risk assessment through planning, scoping, and problem formulation to better meet the needs of decision makers (NRC, 2009). The PECOS tools serve as guides for the inclusion or exclusion of studies in the ISA. Additional details on the development and use of these PECOS tools can be found in Appendix 10 (Section 10.3.1).

IS.1.2.2 Organization of the ISA

The ISA consists of the Preface (legislative requirements and history of the primary and secondary ozone NAAQS; and purpose and overview of the ISA along with the overall scope, and process for evaluating evidence), Executive Summary, Integrated Synthesis, and 10 Appendices. This Integrated Synthesis provides the key information for each topic area, encompassing a description of ozone concentrations in the U.S. (including background sources), conclusions regarding the health and welfare effects associated with ozone exposure (including causality determinations for relationships between exposure to ozone and specific types of health and welfare effects), identification of the human lifestages and populations at increased risk of the effects of ozone, and a discussion of the key strengths, limitations, and uncertainties inherent in this evidence base. The purpose of this Integrated Synthesis is not to summarize each of the Appendices; rather it is to synthesize the key findings on each topic considered in characterizing ozone exposure and relationships with health and welfare effects. This Integrated Synthesis also discusses additional policy-relevant issues. These include exposure durations, metrics, and concentrations eliciting health and welfare effects and the concentration-response (C-R) relationships for specific effects, including their overall shapes and the evidence with regard to discernibility of threshold exposures below which effects are unlikely to occur. Subsequent Appendix 1−Appendix 10 are organized by subject area, with the detailed assessment of atmospheric science (Appendix 1), exposure (Appendix 2), health (Appendix 3−Appendix 7), and welfare evidence (Appendix 8−Appendix 9). Each of the Appendices contain an evaluation of results from recent studies integrated with evidence from previous reviews. Appendices for each broad health effect category (e.g., respiratory effects) discuss potential biological pathways and conclude with a causality determination describing the strength of the evidence between exposure to ozone and the outcome(s) under consideration. Likewise, the Appendices devoted to ecological (Appendix 8) and climate evidence (Appendix 9) for welfare effects include causality determinations for multiple effects on ecosystems and climate, respectively. Appendix 10 describes the process of developing the ozone ISA, including aspects related to systematic review of the literature, evaluation of study quality, and quality assurance (QA) and quality control (QC) documentation.

IS.1.2.3 Quality Assurance Summary

The use of QA and peer review helps ensure that the U.S. EPA conducts high-quality science assessments that can be used to help policymakers, industry, and the public make informed decisions. Quality assurance activities performed by the U.S. EPA ensure that environmental data are of sufficient quantity and quality to support the Agency's intended use. The U.S. EPA has developed a detailed Program-level QA Project Plan (PQAPP) for the ISA Program to describe the technical approach and associated QA/QC procedures associated with the ISA Program. All QA objectives and measurement criteria detailed in the PQAPP have been employed in developing this ISA. Furthermore, the Ozone ISA is classified as a Highly Influential Scientific Assessment (HISA), which is defined by the Office of

Management and Budget (OMB) as a scientific assessment that is novel, controversial, or precedent-setting, or has significant interagency interest (OMB, 2004). OMB requires a HISA to be peer reviewed before dissemination. To meet this requirement, the U.S. EPA engages the Clean Air Scientific Advisory Committee (CASAC) as an independent federal advisory committee to conduct peer reviews. Both peer-review comments provided by the CASAC panel and public comments submitted to the panel during its deliberations about the external review draft were considered in the development of this ISA. For a more detailed discussion of peer review and quality assurance, see Section 10.4 and Section 10.5, respectively.

IS.1.2.4 Evaluation of the Evidence

This ISA draws conclusions about the causal nature of relationships between exposure to ozone and categories of related health and welfare effects (e.g., respiratory effects) by integrating recent evidence across scientific disciplines and building on the evidence from previous assessments. Determinations are made about causation, not just association, and are based on judgments of consistency, coherence, and biological plausibility of observed effects, and on related uncertainties. The ISA uses a formal causal framework to classify the weight of evidence using a five-level hierarchy [i.e., "causal relationship"; "likely to be causal relationship"; "suggestive of, but not sufficient to infer, a causal relationship"; "inadequate to infer the presence or absence of a causal relationship"; or "not likely to be a causal relationship" as described in Table II of the Preamble (U.S. EPA, 2015)] that is based largely on the aspects for causality proposed by Sir Austin Bradford Hill, as well as other frameworks to assess causality developed by other organizations.

IS.1.3 New Evidence Evaluation and Causality Determinations

In the 2013 Ozone ISA, the causality determinations communicated the extent of the then current knowledge of health and welfare effects. Updates to the causality determinations for ozone based on new evidence in this review are summarized below and are described in greater detail in Section IS.4 (Health) and Section IS.5 (Welfare).

IS.1.3.1 Human Health

The results from the health studies, supported by the evidence from atmospheric chemistry and exposure assessment studies, contribute to the causality determinations made for the health outcomes. The conclusions from the 2013 Ozone ISA and the causality determinations from this review are summarized in Table IS-1.

Table IS-1 Summary of causality determinations by exposure duration and health outcome.

Health Outcome[a]	Conclusions from 2013 Ozone ISA	Conclusions in the 2020 ISA
Short-term exposure to ozone		
Respiratory effects	Causal relationship	Causal relationship
Cardiovascular effects	Likely to be causal relationship	Suggestive of, but not sufficient to infer, a causal relationship[c]
Metabolic effects	No determination made	Likely to be causal relationship[b]
Total mortality	Likely to be causal relationship	Suggestive of, but not sufficient to infer, a causal relationship[c]
Central nervous system effects	Suggestive of a causal relationship[d]	Suggestive of, but not sufficient to infer, a causal relationship
Long-term exposure to ozone		
Respiratory effects	Likely to be causal relationship	Likely to be causal relationship
Cardiovascular effects	Suggestive of a causal relationship[d]	Suggestive of, but not sufficient to infer, a causal relationship
Metabolic effects	No determination made	Suggestive of, but not sufficient to infer, a causal relationship[b]
Total mortality	Suggestive of a causal relationship[d]	Suggestive of, but not sufficient to infer, a causal relationship
Reproductive effects	Suggestive of a causal relationship[d]	Effects on fertility and reproduction: suggestive of, but not sufficient to infer, a causal relationship[b]
		Effects on pregnancy and birth outcomes: suggestive of, but not sufficient to infer, a causal relationship[b]
Central nervous system effects	Suggestive of a causal relationship[d]	Suggestive of, but not sufficient to infer, a causal relationship
Cancer	Inadequate to infer a causal relationship[e]	Inadequate to infer the presence or absence of a causal relationship

[a]Health effects (e.g., respiratory effects, cardiovascular effects) include the spectrum of outcomes, from measurable subclinical effects (e.g., decrements in lung function, blood pressure) to observable effects (e.g., medication use, hospital admissions) and cause-specific mortality. Total mortality includes all-cause (nonaccidental) mortality, as well as cause-specific mortality.

[b]Denotes new causality determination.

[c]Denotes change in causality determination from 2013 Ozone ISA.

[d]Since the 2013 Ozone ISA, the causality determination language has been updated and this category is now stated as suggestive of, but not sufficient to infer, a causal relationship.

[e]Since the 2013 Ozone ISA, the causality determination language has been updated and this category is now stated as inadequate to infer the presence or absence of a causal relationship.

The strongest evidence for health effects due to ozone exposure continues to come from studies of short- and long-term ozone exposure and respiratory health. Consistent with conclusions from the 2013 Ozone ISA, it is determined that there is a "causal relationship" between short-term ozone exposure and respiratory effects, and there is a "likely to be causal relationship" between long-term ozone exposure and respiratory effects. For short-term ozone exposure, controlled human exposure studies provide experimental evidence for ozone-induced lung function decrements, respiratory symptoms, and respiratory tract inflammation. Epidemiologic studies continue to provide evidence that increased ozone concentrations are associated with a range of respiratory effects, including asthma exacerbation, chronic obstructive pulmonary disease (COPD) exacerbation, respiratory infection, and hospital admissions and ED visits for combined respiratory diseases. A large body of experimental animal toxicological studies demonstrates ozone-induced changes in measures of lung function, inflammation, increased airway responsiveness, and impaired lung host defense. These animal studies also inform the potential mechanisms underlying downstream respiratory effects (e.g., respiratory tract inflammation) and thereby provide strong support for the biological plausibility of epidemiologic associations between short-term ozone exposure and respiratory-related ED visits and hospital admissions. With respect to long-term ozone exposure, there is strong coherence between animal toxicological studies of changes in lung morphology and epidemiologic studies reporting positive associations between long-term ozone exposure and new-onset asthma, and respiratory symptoms in children with asthma. Furthermore, the experimental evidence provides biologically plausible pathways through which long-term ozone exposure could lead to the types of respiratory effects reported in epidemiologic studies.

Metabolic effects related to ozone exposure are evaluated as a separate health endpoint category for the first time in this ISA. Recent evidence from animal toxicological, controlled human exposure, and epidemiologic studies support a "likely to be causal relationship" between short-term ozone exposure and metabolic effects. The strongest evidence for this determination is provided by animal toxicological studies that demonstrate impaired glucose tolerance, fasting hyperglycemia, and increased serum triglycerides and free fatty acids in various stocks/strains of animals across multiple laboratories. Biological plausibility is provided by results from controlled human exposure and animal toxicological studies that demonstrate activation of sensory nerve pathways following ozone exposure that trigger the central neuroendocrine stress response, as indicated by increased corticosterone/cortisol and adrenaline production. These findings are coherent with epidemiologic studies that report associations between ozone exposure and perturbations in glucose and insulin homeostasis. In addition, these pathophysiological changes are often accompanied by increased inflammatory markers in peripheral tissues and by changes in liver biomarkers.

The strongest evidence for metabolic effects following long-term ozone exposure is provided by epidemiologic studies. In prospective cohort studies in the U.S. and Europe, increased incidence of type 2 diabetes was observed with long-term ozone exposure. In a study conducted in China, long-term ozone exposure was associated with the development and diagnosis of metabolic syndrome. Several long-term ozone exposure studies in China, one in adults and one in children, observed increased odds of obesity (a

risk factor for type 2 diabetes) in both adults and children. Positive associations between long-term exposure to ozone and diabetes-related mortality were observed in well-established cohorts in the U.S. and Canada. The results of mortality studies are supported by epidemiologic and experimental studies reporting effects on glucose homeostasis and serum lipids, as well as other indicators of metabolic function (e.g., peripheral inflammation and neuroendocrine activation). This evidence is "suggestive of, but not sufficient to infer, a causal relationship" between long-term ozone exposure and metabolic effects.

Notably, compared with the 2013 Ozone ISA, there are changes in the causality determinations for short-term ozone exposure and cardiovascular effects and total mortality. In both instances, the 2013 Ozone ISA concluded that the evidence was sufficient to conclude a "likely to be causal relationship," but after integrating the previous evidence with recent evidence, the collective evidence is "suggestive of, but not sufficient to infer, a causal relationship" in this ISA. The evidence that supports this change in the causality determination 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 epidemiologic studies for more severe cardiovascular morbidity endpoints (i.e., heart failure [HF], ischemic heart disease [IHD] and myocardial infarction [MI], arrhythmia and cardiac arrest, and stroke); and (3) uncertainties due to few studies evaluating the potential for confounding by copollutants in epidemiologic studies. Although there is consistent or generally consistent evidence for several ozone-induced cardiovascular endpoints in animal toxicological studies and for cardiovascular mortality in epidemiologic studies, these results are not coherent with those in controlled human exposure and epidemiologic studies examining cardiovascular morbidity endpoints.

IS.1.3.2 Welfare: Ecological Effects

The 2013 Ozone ISA (U.S. EPA, 2013b) concluded that the responses to ozone exposure occur across multiple biological scales and a broad array of ecological endpoints, with the strongest evidence for effects on vegetation. The focus of the current ISA and literature evaluated herein are those effects observed at the individual-organism level of biological organization and higher (e.g., population, community, ecosystem). New research largely strengthens the previous conclusions on the ecological effects of ozone. The types of ecological effects studies conducted since the 2013 Ozone ISA mostly fall into three categories: (1) empirical research that has refined/reinforced earlier studies, in some cases using new approaches, new species, or larger-scale systems; (2) meta-analyses that have provided a more statistically based understanding of patterns compiled from existing literature; and (3) modeling approaches that have increased in complexity and enabled examination of ozone effects at larger spatial scales (e.g., regional, national). There are 12 causality determinations for ecological effects of ozone (Table IS-2), generally organized from the individual-organism scale to the ecosystem scale. Similar to the findings of the 2013 Ozone ISA, five are causal relationships (i.e., visible foliar injury, reduced vegetation growth, reduced crop yield, reduced productivity, and altered belowground biogeochemical cycles) and two are likely to be causal relationships (i.e., reduced carbon sequestration, altered ecosystem

water cycling). One endpoint, alteration of terrestrial community composition, is now concluded to be a causal relationship, whereas this endpoint was classified as likely to be causal in the 2013 Ozone ISA. Three new endpoint categories (i.e., increased tree mortality, alteration of herbivore growth and reproduction, and alteration of plant-insect signaling) not evaluated for causality in the 2013 Ozone ISA all have a "likely to be causal relationship." Plant reproduction, previously considered as part of the evidence for growth effects, is now a stand-alone causal relationship.

IS.1.3.3 Welfare: Effects on Climate

Recent evidence continues to support a causal relationship between tropospheric ozone and radiative forcing and a likely to be causal relationship, via radiative forcing, between tropospheric ozone and temperature, precipitation, and related climate variables (referred to as "climate change" in the 2013 Ozone ISA; the revised title for this causality determination provides a more accurate reflection of the available evidence [Table IS-3]). The new evidence comes from the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report [AR5; Myhre et al. (2013)] and its supporting references—in addition to a few more recent studies—and builds on evidence presented in the 2013 Ozone ISA. The new studies further support the causality determinations included in the 2013 Ozone ISA.

Table IS-2 Summary of causality determinations for ecological effects.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions in the 2020 ISA
Visible foliar injury	Causal relationship	Causal relationship
Reduced vegetation growth	Causal relationship	Causal relationship
Reduced plant reproduction	No separate causality determination; included with plant growth	Causal relationship[a]
Increased tree mortality	Causality not assessed	Likely to be causal relationship[a]
Reduced yield and quality of agricultural crops	Causal relationship	Causal relationship
Alteration of herbivore growth and reproduction	Causality not assessed	Likely to be causal relationship[a]
Alteration of plant-insect signaling	Causality not assessed	Likely to be causal relationship[a]
Reduced productivity in terrestrial ecosystems	Causal relationship	Causal relationship
Reduced carbon sequestration in terrestrial ecosystems	Likely to be causal relationship	Likely to be causal relationship
Alteration of belowground biogeochemical cycles	Causal relationship	Causal relationship
Alteration of terrestrial community composition	Likely to be causal relationship	Causal relationship[b]
Alteration of ecosystem water cycling	Likely to be causal relationship	Likely to be causal relationship

[a]Denotes new causality determination.

[b]Denotes change in causality determination from 2013 Ozone ISA.

Table IS-3 Summary of causality determinations for tropospheric ozone effects on climate.

	Conclusions in 2013 Ozone ISA	Conclusions in the 2020 ISA
Radiative forcing	Causal relationship	Causal relationship
Temperature, precipitation, and related climate variables	Likely to be causal relationship	Likely to be causal relationship

IS.2 Atmospheric Chemistry, Ambient Air Ozone Concentrations, and Background Ozone

Scientific advances in atmospheric ozone research relevant to the Ozone NAAQS are reviewed in this section, with a primary focus on understanding the relative contribution of precursor emissions to ambient ozone concentrations from natural processes and anthropogenic activities. The section summarizes recent developments in measurement and modeling methods, atmospheric chemistry, and ambient air concentration trends (Section IS.2.1). The U.S. background (USB) ozone concentration is defined as the ozone concentration that would occur if all U.S. anthropogenic ozone precursor emissions were removed, as described in Section IS.2.2. This definition facilitates separate consideration of ozone that results from anthropogenic precursor emissions within the U.S. and ozone originating from natural and foreign precursor sources. This discussion is followed by a summary of recent observations and research related to USB ozone, with an emphasis on major sources (Section IS.2.2.1), estimation methods (Section IS.2.2.2), and geographic, seasonal, and long-term ozone concentration trends (Section IS.2.2.3).

IS.2.1 Ambient Air Ozone Anthropogenic Sources, Measurement, and Concentrations

The general photochemistry of tropospheric ozone is described in detail in previous assessments (U.S. EPA, 2013b, 2006a). Anthropogenic ozone in urban settings is produced primarily by the reaction of volatile organic compounds (VOCs) with oxides of nitrogen (NO_X) in the presence of sunlight. Carbon monoxide (CO) and methane (CH_4) also react with NO_X to form ozone in the absence of more reactive organic compounds (Section 1.4). The most abundant national and global sources of VOCs are biogenic (U.S. EPA, 2013b), and oxides of nitrogen are predominately emitted from a range of anthropogenic sources, including automobile exhaust, off-road vehicles and engines, electric power generation, industrial activities, and stationary fuel combustion (U.S. EPA, 2016). Recent developments in understanding ozone chemistry include observations of high ozone concentrations during the winter in some western U.S. mountain basins (Section 1.4.1). For example, wintertime ozone concentrations in the

Uintah Basin of Utah and Upper Green River Basin of Wyoming have been measured as high as 150 ppb (1-hour avg), with episodes driven by local concentrations of ozone precursor emissions from oil and gas extraction coinciding with strong mountain valley temperature inversions on cold winter days with snow cover. In addition, there is new research on the role of marine halogen chemistry in suppressing coastal ozone concentrations (Section 1.4.2). Incorporating marine halogen chemistry into atmospheric modeling methods for predicting ozone concentrations has improved agreement between model results and observed ozone near marine environments.

Extensive air monitoring data are obtained from the state and local air monitoring site (SLAMS) network for ozone, consisting of more than 1,300 monitors throughout the U.S. (Section 1.7). In the SLAMS network, ozone is measured by ultraviolet spectroscopy using a Federal Equivalency Method (FEM) at most sites (Section 1.6.1.1). A new Federal Reference Method (FRM) for ozone measurement was adopted in 2015 (Section 1.6.1.1) based on chemiluminescence resulting from the reaction of ozone with nitric oxide, and is used at some sites. In addition to network monitoring, satellite-based remote sensing methods are increasingly used to measure the total ozone column in the atmosphere, and satellite data are used to constrain model estimates of ground-level tropospheric ozone concentrations (Section 1.6.1.2). Because tropospheric concentration estimates based on satellite measurements can have much greater uncertainty than total column ozone measurements, these technologies are most suitable for investigating trends in total column ozone or in the upper troposphere. The 2013 Ozone ISA provided an overview of chemical transport models (CTMs), including the relevant processes, numerical approaches, relevant spatial scales, and methods for evaluation (U.S. EPA, 2013b). Since the 2013 Ozone ISA, numerous improvements to these models have been made. These include: the addition of a halogen chemistry mechanism; improvements in the representation of land cover and near surface meteorology; the inclusion of dry deposition and stomatal uptake, stratosphere-troposphere exchange, and biogenic emissions; and, the integration of meteorological models and CTMs (Section 1.6.2).

SLAMS network data for the period 2015−2017 show higher nationwide median "max daily 8-hour avg" (MDA8) ozone concentrations across all monitoring sites in spring (median = 46 ppb) and summer (median = 46 ppb) than in autumn (median = 38 ppb) and winter (median = 34 ppb). The highest values of annual 4th-highest MDA8 ozone concentration (>75 ppb) occur in central and southern California, Arizona, Colorado, Utah, Texas, along the shore of Lake Michigan, and in the Northeast Corridor, typically during the warm season between May and September (Section 1.2.1.1). These results are similar to those reported in the 2013 Ozone ISA (U.S. EPA, 2013b). The highest values of W126, an example of a cumulative index of plant exposure (Section IS.3.2 and Section 1.2.1.2), occurred in California and the southwestern U.S.

Several recent studies have documented a long-term decreasing trend in nationwide average ambient air MDA8 ozone concentration over several decades and a faster decline in the magnitude and frequency of high MDA8 ozone episodes (Section 1.7). Comparison of the difference between 5th and 95th percentile concentrations indicates a compression of the MDA8 ozone concentration distribution

occurring widely across the U.S. This compression results from a decrease in 95th percentile concentrations together with a general increase in 5th percentile concentrations. This is consistent with observed reductions in NO_X emissions (Section 1.3.1), because there is less NO available to react with ozone at low ozone concentrations, as well as less NO_2 available to form ozone at high ozone concentrations.

IS.2.2 Background Ozone

Use of the term "background ozone" varies within the air pollution research community. It has generally been used to describe ozone levels that would exist in the absence of anthropogenic emissions within a particular area and has been broadly applied to every geospatial scale: local, regional, national, continental, or global. For instance, on a local scale, ozone that originates from precursor emissions outside of a locality's municipal boundaries could be considered background ozone for that locality. Similarly, on a national scale, background ozone could be defined as ozone that is not formed from anthropogenic emissions within national boundaries.

The USB concentration is defined as the ozone concentration that would occur if all U.S. anthropogenic ozone precursor emissions were removed. It is a hypothetical construct that cannot be measured. The 2006 Ozone AQCD (U.S. EPA, 2006a) and 2013 Ozone ISA (U.S. EPA, 2013b) concluded that background ozone concentrations could not be determined solely from ozone measurements, even at the most remote monitoring sites, because of long-range transport of ozone originating from U.S. anthropogenic precursors. Since then, chemical transport models have been used as the primary tool for estimating USB ozone concentrations.

IS.2.2.1 Sources of U.S. Background Ozone

Major contributors to ground-level USB ozone concentrations are stratospheric exchange, international transport, wildfires, lightning, global methane emissions, and natural biogenic and geogenic precursor emissions. As the USB literature has evolved, much of the discussion has focused on the relative importance of stratospheric ozone and intercontinental transport as major sources.

Tropospheric ozone derived from stratosphere-troposphere dynamics was described in detail in the 2013 Ozone ISA (U.S. EPA, 2013b). Stratospheric air naturally rich in ozone can be transported into the troposphere under certain meteorological circumstances, with maximum contributions observed at midlatitudes during the late winter and early spring. This process, known as "tropopause folding," is characterized by episodic events typically lasting a few days from late winter through spring when deep stratospheric intrusions rich in ozone can quickly and directly well into the troposphere and, more rarely, reach ground level (U.S. EPA, 2013b). The 2013 Ozone ISA (U.S. EPA, 2013b) also discussed the potential importance of deep convection, another form of stratosphere-troposphere exchange that occurs

mainly in summer, as a mechanism for transporting stratospheric ozone into the upper troposphere. Stratospheric ozone contributions from deep intrusion between 17 and 40 ppb have been estimated at ground level for springtime model simulations in the western U.S. (Section 1.3.2.1). Stratospheric intrusion events related to frontal passage and tropopause folding that reach the surface have less influence on surface ozone during the summer months when total ground-level ozone concentrations tend to be highest.

Intercontinental transport from Asia has also been identified as a major source of precursors that can contribute 5 to 7 ppb to USB ozone concentrations over the western U.S. (U.S. EPA, 2013b, 2006a, b). Ozone precursor emissions from China and other Asian countries have been estimated to have more than doubled in the period 1990−2010 (Section 1.3.1.2), and an estimated increase of 0.3 to 0.5 ppb/year of midtropospheric ozone USB in spring over the western U.S. in the two decades after 1990 was largely attributed to a tripling of Asian NO_X emissions (Section 1.3.1). However, after this period, trends in NO_X emissions from China, the largest ozone precursor source in Asia, have declined as confirmed by rapidly decreasing satellite-derived tropospheric NO_2 column measurements over China since 2012. Stringent air quality standards implemented in 2013 within China have markedly reduced national emissions of NO_X (Section 1.3.1.2).

Other contributors to USB are either smaller or more uncertain than stratospheric and intercontinental contributions. Wildfires have been estimated to contribute a few ppb to seasonal mean ozone concentrations in the U.S., but episodic contributions may be as high as 30 ppb (Section 1.3.1.2). However, estimates of the magnitude of ozone formation from wildfires is highly uncertain with some work showing large overpredictions of modeled wildfire contributions (Section 1.3.1.3). Lightning was estimated to contribute 2 to 3 ppb to ground level ozone concentrations in the southeastern U.S. in the summer (U.S. EPA, 2013b). Eighty percent of the NO_X present in the upper troposphere is generated by lightning where it can have a longer atmospheric residence time than NO_X derived from ground sources (Section 1.3.1.3). There is an approximately linear relationship between anthropogenic methane emissions and tropospheric ozone, which is consistent with the contribution of anthropogenic methane emissions to global annual mean ozone concentration of ~4−5 ppb reported in the 2013 Ozone ISA (U.S. EPA, 2013b). Biogenic emissions of NO_X are estimated to contribute 0.3 Tg N/year, or about 7.5% of total NO_X emissions (Section 1.3.1.3).

IS.2.2.2 Methods for Estimating U.S. Background Ozone

Large uncertainties are associated with estimating USB ozone concentrations. Approaches for estimating USB ozone are described in Section 1.8.1. USB ozone is estimated using either zero-out simulations or source apportionment simulations. The most widely used approach to measuring USB or other measures of background ozone is the zero-out method, in which anthropogenic U.S. or other areas emissions are set to zero in a model simulation to estimate these ozone measures (Section 1.8.1.1). As an

alternative to model sensitivity approaches, source apportionment techniques track source contributions to ozone formation without perturbing emissions (Section 1.8.1.2). Tracking techniques use reactive tracer species to tag specific emissions source categories or source regions and then track the ozone produced by emissions from those source groups. Both approaches are essential and complementary for understanding and estimating USB ozone. The zero out approach is suited for estimating what ozone levels would have existed in recent modeled years in the absence of all U.S. emissions, while the source apportionment approach is suited for estimating the fraction of current ozone originating from background sources in recent modeled years. The difference between estimates from these approaches is small in remote areas that are most strongly affected by USB sources. However, the differences in the estimates given by these methods can be substantial in urban areas strongly affected by anthropogenic sources that influence both production and destruction of ozone.

USB ozone concentrations vary daily and by location and are a function of season, meteorology, and elevation. Quantification of USB ozone on days when MDA8 ozone concentrations exceed 70 ppb is more relevant to understanding USB ozone contributions on those days than are seasonal mean USB ozone estimates, but also more uncertain (Jaffe et al., 2018). Jaffe et al. (2018) reviewed recent modeling results and reported that USB ozone estimates contain uncertainties of about 10 ppb for seasonal average concentrations and 15 ppb for MDA8 avg concentrations on individual days. Because of uncertainty in model predictions of USB, model results are often adjusted using simple bias correction approaches. Because such approaches might not be reliable if the model has diverging errors in USB ozone and locally produced ozone, however, days with poor model performance have sometimes been excluded when using model results to estimate USB or other measures of background ozone. There have been continued efforts to improve model performance and better understand biases and uncertainties involved in the application of CTMs to estimating USB or other measures of background ozone (Section 1.8.1.5).

IS.2.2.3 U.S. Background Concentrations and Trends

A greater variety of approaches for estimating USB concentrations and other measures of background ozone used in recent years have led to a wider range of USB estimates than reported in the 2013 Ozone ISA (U.S. EPA, 2013b), although some of the basic patterns remain consistent. For example, higher USB concentrations (and related measures of background ozone) were estimated in the western U.S. than in the eastern U.S. in the 2013 Ozone ISA (U.S. EPA, 2013b), especially in the intermountain West and Southwest. Higher USB concentrations were also estimated at elevations higher than 1,500 m than at lower elevations (U.S. EPA, 2013b). New studies since the 2013 Ozone ISA confirm these findings (Section 1.8.2.1).

USB concentrations are relatively constant with increasing total ozone concentration, indicating that days with higher ozone concentrations generally occur because of higher U.S. anthropogenic contributions (Section 1.8.2.3). In the eastern U.S. and in urban and low-elevation areas of the western

U.S., there is consistent evidence across several studies that daily USB ozone concentrations are similar to or smaller than seasonal mean USB ozone concentrations on most high ozone concentration days (i.e., days with MDA8 ozone greater than 60 ppb). In contrast, for high elevation locations in the western U.S., USB concentration estimates have been consistently predicted to increase with total ozone concentration, consistent with a larger background contribution. Lower USB contributions on days of high ozone concentration can result from meteorological conditions that favor large ozone production from U.S. anthropogenic sources relative to USB sources (Section 1.5.2). The highest ozone concentrations observed in the U.S. have historically occurred during stagnant conditions when an air mass remains stationary over a region abundant in anthropogenic ozone precursor sources (U.S. EPA, 2013b, 2006a, 1996a), while the largest USB contributions often occur under the opposite conditions, when the atmosphere is well mixed and transport of USB ozone generated in the stratosphere or during long-range transport of Asian or natural precursors in the upper troposphere more readily occurs (Section 1.5.2).

Characterizing long-term trends in USB presents numerous challenges (Section 1.8.2.4). Research has mainly focused on high elevation sites in the western U.S. or measurements made aloft, where, until recently, increasing midtropospheric ozone was reported. The most recent analyses suggest that this trend has now slowed or reversed, and there is no evidence to suggest that USB is still increasing, even in the western U.S. (Section 1.8.2.4).

IS.3 Exposure to Ambient Ozone

IS.3.1 Human Exposure Assessment in Epidemiologic Studies

With regard to exposure assessment relevant to human health effects, the 2013 Ozone ISA (U.S. EPA, 2013b) primarily discussed personal exposure to ozone and its relationship to ambient air concentrations.

Its primary conclusions were that personal exposure to ozone is moderately correlated with ambient air concentration (Pearson $R = 0.3–0.8$) and indoor ozone concentrations were roughly 10−30% of ambient air concentrations. In addition, ozone exposure minimization efforts through public messaging (e.g., ozone action days) were effective in reducing exposures for people younger than 20 years old but did not make an appreciable difference in exposure among those ages 20−64 years old. The 2013 Ozone ISA noted that urban scale ozone concentrations often have low spatial variability except in the vicinity of roadways, where nitrogen oxides emitted from motor vehicles tend to scavenge ozone.

The 2013 Ozone ISA also found that exposure measurement error can bias epidemiologic associations between ambient ozone concentrations and health outcomes and widen confidence intervals around effect estimates. Recently published studies agree with these previous findings. Although ozone

concentrations measured at fixed-site ambient air monitors are still widely used as surrogates for ozone exposure in epidemiologic studies (Section 2.3.1.1), the availability and sophistication of models to predict ambient ozone concentrations for this purpose have increased substantially in recent years (Section 2.3.2). The greatest expansion in modeling capability has occurred in chemical transport modeling (CTM; Section 2.3.2.3), especially when incorporated into a hybrid spatiotemporal framework that integrates modeling output with monitoring and satellite data over time and space (Section 2.3.2.4). Hybrid methods have produced lower error predictions of ozone concentration compared with spatiotemporal models using land use and other geospatial data alone (Section 2.3.2.2) but may be subject to overfitting given the many different sources of data incorporated into the hybrid framework.

Use of an exposure surrogate in epidemiologic studies generally leads to underestimation of any association between short-term exposure to ozone and a health effect, with reduced precision. Although the magnitude of an association between ambient ozone and a health effect is uncertain, the evidence indicates that the true effect is typically larger than the effect estimate in these cases. Epidemiologic studies evaluating short-term ozone exposure examine how short-term (e.g., hourly, daily, weekly) changes in health effects are associated with short-term changes in exposure (Section 2.6.1). Accurate characterization of temporal variability is more important than accurate characterization of spatial variability for these studies. Use of an exposure surrogate may produce bias when temporal variability in the concentration at the location of the measurement or model prediction differs from temporal variability of the true exposure concentration. As a result, the correlation between the exposure surrogate and the incidence of the effect would decrease due to the additional scatter in that relationship, and the reduced correlation would also likely flatten the slope of the relationship between the effect and exposure surrogate.

For effects elicited by ozone, the use of exposure estimates that do not account for population behavior and mobility (e.g., via use of time-activity data) may underestimate the true effect and have reduced precision. Although the magnitude of association between ozone and such health effects are uncertain, the evidence suggests that the true effect of ambient ozone exposure is larger than the effect estimate when time-activity data are not considered in the analysis. Uncharacterized exposure variability due to omission of time-activity data for short-term studies (Section 2.4.1) creates uncertainty in the exposure estimate that could reduce the correlation between the exposure estimate and the health effect.

Depending on the exposure model and scenario being modeled for application in epidemiology studies, the true effect of long-term exposure to ambient ozone may be underestimated or overestimated when the exposure model respectively overestimates or underestimates ozone exposure. It is much more common for the effect to be underestimated, and the bias is typically small in magnitude. Long-term epidemiologic studies examine the association between the health effect endpoint and long-term average ambient ozone exposure (Section 2.6.2). For cohort studies of long-term ambient ozone exposure, ambient ozone concentration measured at monitors or estimated by a model is often used as a surrogate for ambient ozone exposure. These studies typically examine differences among cohorts in different

locations, at the scale of neighborhoods, cities, or states. Uncharacterized spatial variability in ozone exposure across the study area could lead to bias in the effect estimate if modeled or measured ambient concentration is not representative of ambient exposure. Bias can occur in either direction but more often has been reported to be towards the null in exposure measurement error studies. Uncertainties in time activity and residential patterns of exposed individuals and surface losses of ozone can reduce precision in the effect estimates.

IS.3.2 Ecological Exposure

The key conclusions from the 1996 and 2006 Ozone AQCDs, and the 2013 Ozone ISA regarding ozone exposure to vegetation, highlighted below, are still valid and most effects observed for nonvegetation biota are mediated through ozone effects on vegetation. Absorption of ozone from the atmosphere into leaves is controlled by the leaf boundary layer and stomatal conductance. Stomata provide the principal pathway for ozone to enter and affect plants, with subsequent oxidative injury to leaf tissue triggering a cascade of physical, biogeochemical, and physiological events that may scale up to responses at the whole-plant scale.

As described in previous ozone assessments, ozone-related injury is a function of flux (i.e., the amount of ozone taken up by the plant over time). Ozone flux is affected by modifying factors such as temperature, vapor pressure deficit, light, soil moisture, and plant growth stage (U.S. EPA, 2013b). Flux is very difficult to measure directly, requiring quantification of stomatal or canopy conductance. While some efforts have been made in the U.S. to calculate ozone flux into leaves and canopies, little information has been published relating these fluxes to effects on vegetation. The scarcity of flux data in the U.S. and lack of understanding of plant detoxification processes have made this technique less viable for risk assessments in the U.S. (U.S. EPA, 2013b). An alternative to flux-based exposure estimates are exposure indices. Exposure indices quantify exposure as it relates to measured plant response (e.g., growth) and only require ambient air quality data rather than more complex indirect calculations of dose to the plant. Cumulative indices summarize ozone concentrations over time to provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. For ecological studies in this ISA, emphasis is placed on studies that characterize exposures at concentrations occurring in the environment or experimental ozone concentrations within an order of magnitude of recent concentrations observed in the U.S. (Appendix 1).

It is well established that exposure duration influences the degree of plant response and that ozone effects on plants are cumulative. In previous ozone assessments, effects are clearly demonstrated to be related to the cumulative exposure over the growing season for crops and herbaceous plant species. For long-lived plants, such as trees, exposures occur over multiple seasons and years. Cumulative indices of exposure are, therefore, best suited to assess exposure. Since the 1980s, cumulative-type indices such as threshold weighted (e.g., SUM06, AOTx) and continuous weighted (e.g., W126) functions have been

applied to evaluate ozone exposure in plants (U.S. EPA, 2013b). The 2013 Ozone ISA primarily discussed SUM06, AOTx, and W126 exposure metrics. Below are the definitions of the three cumulative index forms:

- SUM06: Sum of all hourly ozone concentrations greater than or equal to 60 ppb observed during a specified daily and seasonal time window (U.S. EPA, 2013b).
- AOTx: Sum of the differences between hourly ozone concentrations greater than a specified threshold during a specified daily and seasonal time window. For example, AOT40 is the sum of the differences between hourly concentrations above 40 ppb during a specified period (U.S. EPA, 2013b).
- W126: Sigmoidally weighted sum of all hourly ozone concentrations observed during a specified daily and seasonal time window (Lefohn et al., 1988; Lefohn and Runeckles, 1987).

IS.4 Evaluation of the Health Effects of Ozone

IS.4.1 Connections among Health Effects

Broad health effect categories are evaluated separately in the Appendices of this ISA, though the mechanisms underlying disease progression may overlap and not be restricted to a single organ system. This section provides a brief overview of how the relationship between ozone and a variety of health outcomes may be related or affect one another.

Ozone-induced injuries can take place via complex pathways within the body. After inhalation, ozone reacts with lipids, proteins, and antioxidants in the respiratory tract epithelial lining fluid to create secondary oxidation products [U.S. EPA (2013b); Section 5.2.3]. The first steps (i.e., initial events) in the cascade of physiological events includes activation of sensory nerves in the respiratory tract and respiratory tract inflammation. These early physiological reactions to ozone may trigger a host of autonomic, endocrine, immune, and inflammatory responses throughout the body at the cellular, tissue, and organ level. Because the circulatory system is connected to all body systems, insults to multiple organ systems may contribute to a single health effect. The 2006 Ozone AQCD [U.S. EPA (2006a); Chapter 4] and the 2013 Ozone ISA [U.S. EPA (2013b); Section 5.3] provide extensive background on dosimetry and potential pathways underlying health effects for these responses.

Modulations of the autonomic nervous system, which consists of the sympathetic and parasympathetic systems, provide inhibitory or excitatory inputs to tissues to generate organ responses. Some examples of responses from alterations of the autonomic nervous system include changes to heart rate, bronchodilation/bronchoconstriction, altered blood glucose, glycogenolysis/gluconeogenesis, hormone release, and other organ functions (McCorry, 2007). Endocrine, immune, and inflammatory responses can send signals capable of altering multiple pathways and eliciting cardiovascular, respiratory, and metabolic health effects.

While all systems of the body are connected intrinsically, most research presented in the field of air quality examines specific health endpoints resulting from exposure to a pollutant. In an effort to bring together the scientific body of evidence in an easily understandable and relatable way, this document has separated the supporting Appendices into Respiratory (Appendix 3), Cardiovascular (Appendix 4), Metabolic (Appendix 5), Mortality (Appendix 6), and Other Health Effects (Appendix 7).

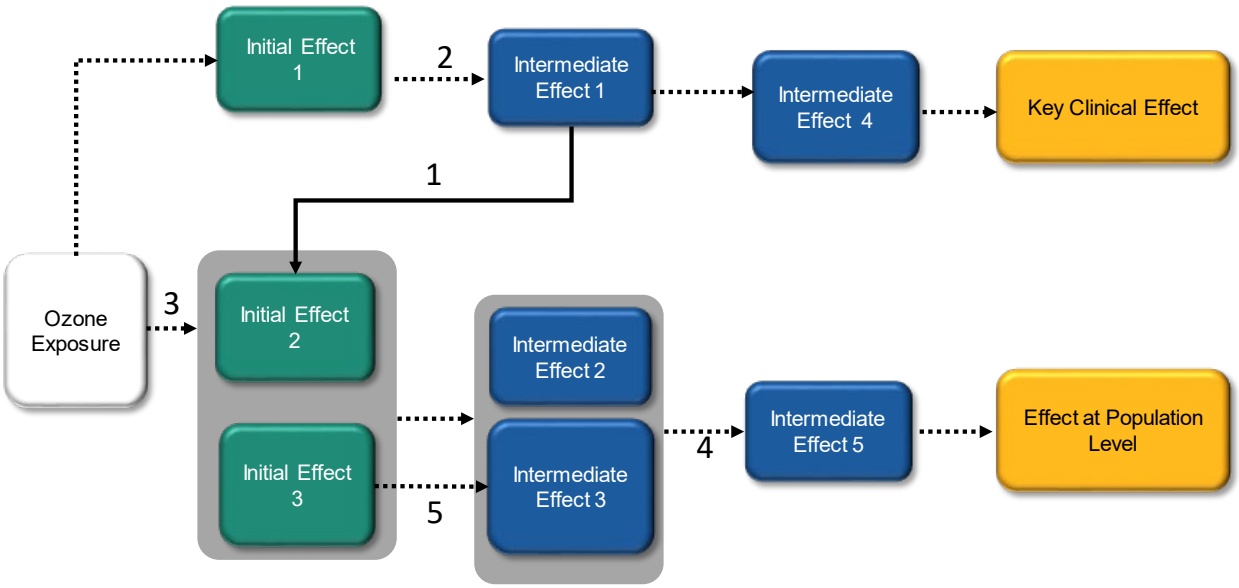
IS.4.2 Biological Plausibility

New to this Ozone ISA are biological plausibility sections for the broad health outcome categories that are included in the human health Appendices (Appendix 3−Appendix 7). These sections outline potential pathways along the exposure to outcome continuum and provide plausible links between inhalation of ozone and health outcomes at the population level. Biological plausibility can strengthen the basis for causal inference (U.S. EPA, 2015). In this ISA, biological plausibility is part of the weight-of-evidence analysis that considers the totality of the health effects evidence, including consistency and coherence of effects described in experimental and observational studies. Although there is some overlap in the potential pathways between the Appendices, each biological plausibility section is tailored to the specific broad health outcome category and exposure duration for which causality determinations are made.

Each of the biological plausibility sections includes a figure depicting potential biological pathways that is accompanied by text. The figures illustrate possible pathways related to ozone exposure that are based on evidence evaluated in previous assessments, both AQCDs and ISAs, as well as evidence from more recent studies. The text characterizes the evidence upon which the figures are based, including results of studies demonstrating specific effects related to ozone exposure and considerations of physiology and pathophysiology. Together, the figure and text portray the available evidence that supports the biological plausibility of ozone exposure leading to specific health outcomes. Gaps in the evidence base (e.g., health endpoints for which studies have not been conducted) are represented by corresponding gaps in the figures and are identified in the accompanying text.

In the model figure below (Figure IS-1), each box represents evidence that has been demonstrated in a study or group of studies for a particular effect related to ozone exposure. While most of the studies used to develop the figures are experimental studies (i.e., animal toxicological and controlled human exposure studies), some observational epidemiologic studies also contribute to the pathways. These epidemiologic studies are generally (1) panel studies that measure the same or similar effects as the experimental studies (and thus provide supportive evidence) or (2) emergency department and hospital admission studies or studies of mortality, which are effects observed at the population level. The boxes are arranged horizontally, with boxes on the left side representing initial effects that reflect early biological responses and boxes to the right representing intermediate (i.e., subclinical or clinical) effects

and effects at the population level. The boxes are color-coded according to their position in the exposure to outcome continuum.



Note: The boxes above represent the effects for which there is experimental or epidemiologic evidence related to ozone exposure, and the arrows indicate a proposed relationship between those effects. Solid arrows denote evidence of essentiality as provided, for example, by an inhibitor of the pathway or a genetic knockout model used in an experimental study involving ozone exposure. Shading around multiple boxes is used to denote a grouping of these effects. Arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes. Progression of effects is generally depicted from left to right and color coded (white, exposure; green, initial effect; blue, intermediate effect; orange, effect at the population level or a key clinical effect). Here, population-level effects generally reflect results of epidemiologic studies. When there are gaps in the evidence base, there are complementary gaps in the figure and the accompanying text below.

Figure IS-1 Illustrative figure for potential biological pathways for health effects following ozone exposure.

The arrows that connect the boxes indicate a progression of effects resulting from ozone exposure. In most cases, arrows are dotted (arrow 1), denoting a possible relationship between the effects. While most arrows point from left to right, some arrows point from right to left, reflecting progression of effects in the opposite direction or a feedback loop (arrow 2). In a few cases, the arrows are solid (arrow 2), indicating that progression from the upstream to downstream effect occurs as a direct result of ozone exposure. This relationship between the boxes, where the upstream effect is necessary for progression to the downstream effect, is termed *essentiality* (OECD, 2016). Evidence supporting essentiality is generally provided by experimental studies using pharmacologic agents (i.e., inhibitors) or animal models that are genetic knockouts. The use of solid lines, as opposed to dotted lines, reflects the

availability of specific experimental evidence that ozone exposure results in an upstream effect which is necessary for progression to a downstream effect.

In the figures, upstream effects are sometimes linked to multiple downstream effects. In order to illustrate this proposed relationship using a minimum number of arrows, downstream boxes are grouped together within a larger shaded box and a single arrow (arrow 3) connects the upstream single box to the outside of the downstream shaded box containing the multiple boxes. Multiple upstream effects may similarly be linked to a single downstream effect using an arrow (arrow 4) that connects the outside of a shaded box, which contains multiple boxes, to an individual box. In addition, arrows sometimes connect one individual box to another individual box that is contained within a larger shaded box (arrow 2) or two individual boxes both contained within larger shaded boxes (arrow 5). Thus, arrows may connect individual boxes, groupings of boxes, and individual boxes within groupings of boxes depending on the proposed relationships between effects represented by the boxes.

IS.4.3 Summary of Health Effects Evidence

This ISA evaluates the relationships between an array of health effects and short- and long-term exposure to ozone in epidemiologic, controlled human exposure, and animal toxicological studies. Short-term exposures are defined as those with durations of hours up to 1 month, with most studies examining effects related to exposures in the range of several hours to 1 week. Long-term exposures are defined as those with durations of more than 1 month, with many studies spanning a period of years. As detailed in the Preface, the evaluation of the health effects evidence from animal toxicological studies focuses on exposures conducted at concentrations of ozone that are relevant to the range of human exposures associated with ambient air (up to 2 ppm, which is one to two orders of magnitude above recent ambient air concentrations in the U.S.). Drawing from evidence related to the discussion of biological plausibility of ozone-related health effects and the broader health effects evidence spanning scientific disciplines described in detail in Appendix 3−Appendix 7, as well as issues regarding exposure assessment and potential confounding described in Appendix 2, the subsequent sections characterize the evidence that forms the basis of the causality determinations for health effect categories of a "causal relationship" or a "likely to be causal relationship," or describe instances where a causality determination has been changed (i.e., "likely to be causal" changed to "suggestive of, but not sufficient to infer a causal relationship"). The evidence that supports these causality determinations builds upon the potential biological pathways, which provide evidence of biological plausibility, as well as the broader health effects evidence spanning scientific disciplines for each health effects category, as well as issues related to dosimetry, exposure assessment, and potential confounding. Other relationships between ozone and health effects where the causality determinations are *"suggestive of, but not sufficient to infer a causal relationship"* or "*inadequate to infer the presence or absence of a causal relationship*" are noted in Table IS-1, and more fully discussed in the respective health effects Appendices.

IS.4.3.1 Short-Term Exposure and Respiratory Health Effects

The 2013 Ozone ISA concluded that there is a "causal relationship" between short-term ozone exposure and respiratory health effects (U.S. EPA, 2013b). This conclusion was based largely on controlled human exposure studies demonstrating ozone-related respiratory effects in healthy individuals (Table IS-4). Specifically, statistically significant decreases in group mean pulmonary function in response to 6.6-hour ozone exposures (which included six 50-minutes periods of moderate exertion) to concentrations as low as 60 ppb[1] were observed in young, healthy adults (Figure IS-2). Additionally, controlled human exposure and experimental animal studies demonstrated ozone-induced increases in respiratory symptoms, lung inflammation, airway permeability, and airway responsiveness. The experimental evidence was supported by strong evidence from epidemiologic studies demonstrating associations between ambient ozone concentrations and respiratory hospital admissions and ED visits across the U.S., Europe, and Canada. This evidence was further supported by a large body of individual-level epidemiologic panel studies that demonstrated associations of short-term ozone concentrations with respiratory symptoms in children with asthma. Additional support for a causal relationship was provided by epidemiologic studies that observed ozone-associated increases in indicators of airway inflammation and oxidative stress in children with asthma. Additionally, several multicity studies and a multicontinent study reported associations between short-term increases in ozone concentrations and increases in respiratory mortality.

Table IS-4 Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the respiratory effects of short-term exposure to ozone.

	Conclusions from 2013 Ozone ISA	Results and Conclusions from 2020 ISA[a]
Respiratory effects	Evidence integrated across controlled human exposure, epidemiologic, and animal toxicological studies and across the spectrum of respiratory health endpoints demonstrated that **there was a causal relationship between short-term ozone exposure and respiratory health effects.**	Recent evidence from controlled human exposure, epidemiologic, and animal toxicological studies support and extend the conclusions from the 2013 Ozone ISA that **there is a causal relationship between short-term ozone exposure and respiratory effects.**

[1] Concentrations from controlled human exposure studies are target concentrations, unadjusted for study-specific measurement information.

Table IS-4 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the respiratory effects of short-term exposure to ozone.

	Conclusions from 2013 Ozone ISA	Results and Conclusions from 2020 ISA[a]
Lung function	Controlled human exposure studies of young, healthy adults demonstrate group mean decreases in FEV_1 in the range of 2 to 3% with 6.6-h exposures, while exercising, from concentrations as low as **60 ppb** ozone. The collective body of epidemiologic evidence demonstrate associations between short-term ambient ozone concentrations and decrements in lung function, particularly in children with asthma, children, and adults who work or exercise outdoors.	Controlled human exposure studies of young, healthy adults demonstrate ozone-induced decreases in FEV_1 at concentrations as low as **60 ppb** and the combination of FEV_1 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).
Airway responsiveness	A limited number of studies observe increased airway responsiveness in rodents and guinea pigs after being exposed for 72 h to ozone concentrations ranging from less than **300 ppb** up to **1,000 ppb**. As previously reported in the 2006 O_3 AQCD, increased airway responsiveness demonstrated at **80 ppb** in young, healthy adults, and at **50 ppb** in certain strains of rats.	Controlled human exposure studies provide evidence of increased airway responsiveness with exposures as low as **80 ppb**. Baseline airway responsiveness does not appear predictive of changes in lung function following ozone exposure. Recent animal toxicological studies demonstrate increases in airway responsiveness following ozone exposures as low as **800 ppb**. A recent animal toxicological study showed increased airway responsiveness to a greater degree in allergic mice than in naïve mice at **1,000 ppb** for 8 h.
Pulmonary inflammation, injury, and oxidative stress	Epidemiologic studies provide evidence for associations of ambient ozone with mediators of airway inflammation and oxidative stress and indicated that higher antioxidant levels may reduce pulmonary inflammation associated with ozone exposure. Generally, these studies had mean 8-h daily max ozone concentrations less than **66 ppb**. Controlled human exposure studies show ozone-induced inflammatory responses at **60 ppb**, the lowest concentration evaluated.	Controlled human exposure studies demonstrate ozone-induced increases in pulmonary inflammation at concentrations as low as **60 ppb** after 6.6 h of exposure. Studies show interindividual variability in inflammatory responses with some individuals reproducibly experiencing intrinsically greater responses than average. Animal toxicological studies demonstrate inflammation, injury, and oxidative stress following ozone exposures as low as **300 ppb** for up to 72 h. Epidemiologic studies observe associations with pulmonary inflammation in studies of healthy children (mean 8-h daily max ozone concentrations as low as **53 ppb**).
Respiratory symptoms and medication use	The collective body of epidemiologic evidence demonstrate positive associations between short-term exposure to ambient ozone and respiratory symptoms (e.g., cough, wheeze, and shortness of breath) in children with asthma. Generally, these studies had mean 8-h daily max ozone concentrations less than **69 ppb**.	Controlled human exposure studies provide evidence of increased respiratory symptoms following 6.6-h exposures to **70 ppb and greater**. Limited data suggests that lung function responses to ozone in individuals with asthma may depend on baseline lung function and medication use. The large body of epidemiologic evidence from the 2013 Ozone ISA continues to provide the strongest support for these outcomes.

Table IS-4 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the respiratory effects of short-term exposure to ozone.

	Conclusions from 2013 Ozone ISA	Results and Conclusions from 2020 ISA[a]
Lung host defenses	Controlled human exposure studies demonstrate the increased expression of cell surface markers and alterations in sputum leukocyte markers related to innate adaptive immunity with short-term ozone exposures of **80−400 ppb**. Animal toxicological studies demonstrate increased susceptibility to infectious disease with short-term ozone exposures as low as **80 ppb**. Altered macrophage function was reported with exposures as low as **100 ppb**. Other effects on the immune system (i.e., adaptive immunity and natural killer cells) are seen with exposures as low as **500 ppb**.	A limited number of recent controlled human exposure studies report results that are consistent with studies evaluated in the 2013 Ozone ISA that demonstrated impaired lung host defense following acute ozone exposure. A limited number of recent animal toxicological studies demonstrate susceptibility to infectious disease at **2,000 ppb** ozone for 3 h. Recent epidemiologic studies of ED visits for respiratory infection provide the strong support for these outcomes.
Allergic and asthma-related responses	Controlled human exposure studies in atopic individuals with asthma demonstrate increased airway eosinophils, enhanced allergic cytokine production, increased IgE receptors, and enhanced markers of innate immunity and antigen presentation with short-term exposure **to 80−400 ppb** ozone, all of which may enhance allergy and/or asthma. Increased airway responsiveness is seen in atopic individuals with asthma at **120−250 ppb** ozone. In allergic rodents, enhanced goblet cell metaplasia is seen using exposure concentrations as low as **100 ppb, and enhanced responses to allergen challenge is seen with short-term exposure to 1,000 ppm ozone.**	A limited number of recent controlled human exposure and animal toxicological studies demonstrate enhanced type 2 immune responses following acute ozone exposures as low as **200 ppb** in atopic adults with asthma and **800 ppb** (8 h a day for 3 days) in healthy rodents. Exacerbated bronchoconstriction (airway resistance) and lung injury is seen in allergic rodents at **1,000 ppb**. These results support and expand upon evidence from the 2013 Ozone ISA that ozone enhances allergic and asthma related responses.
Respiratory hospital admissions, ED visits, and physician visits	Consistent, positive associations of ambient ozone concentrations with respiratory hospital admissions and ED visits in the U.S., Europe, and Canada are observed with supporting evidence from single-city studies. Generally, these studies had mean 8-h max ozone concentrations less than **60 ppb**.	Evidence from many recent, large multicity epidemiologic studies provide further support for an association between ozone and ED visits and hospital admissions for asthma; associations are generally strongest in magnitude for children between the ages of 5 and 18 years in studies with mean 8-h daily max ozone concentrations between **31 and 54 ppb**. Additional epidemiologic evidence for associations between ozone and hospital admissions and ED visits for combinations of respiratory diseases (**31** to **50 ppb** as the study mean 8-h daily max), ED visits for COPD (**33** to **55 ppb** as the study mean daily 1-h max), and ED visits for respiratory infection (**33** to **55 ppb** as the study mean daily 1-h max).

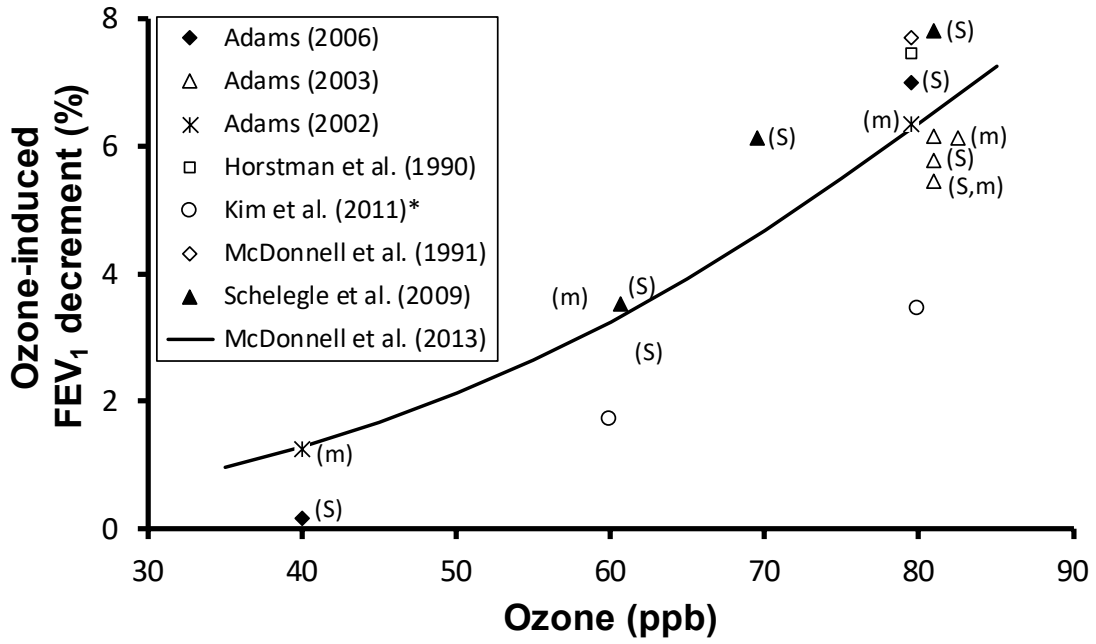
Table IS-4 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the respiratory effects of short-term exposure to ozone.

	Conclusions from 2013 Ozone ISA	Results and Conclusions from 2020 ISA[a]
Respiratory mortality	Multicity time-series studies and a multicontinent study consistently demonstrated associations between ambient ozone concentrations and respiratory-related mortality across the U.S., Europe, and Canada with supporting evidence from single-city studies. Generally, these studies had mean 8-h max ozone concentrations less than **63 ppb**.	Recent epidemiologic evidence for respiratory mortality is limited, but there remains evidence of consistent, positive associations, specifically in the summer months, with mean daily 8-h max ozone concentrations between **8.7 and 63 ppb**. When recent evidence is considered in the context of the larger number of studies evaluated in the 2013 Ozone ISA, there remains consistent evidence of an association between short-term ozone exposure and respiratory mortality.

[a]Conclusions from the 2020 ISA include evidence from recent studies integrated with evidence included in previous Ozone ISAs and AQCDs.

Evidence from recent controlled human exposure studies augment the evidence from 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.

Evidence from epidemiologic studies of healthy populations is generally coherent with experimental evidence, with most of the evidence coming from panel studies that were previously evaluated in the 2013 Ozone ISA (U.S. EPA, 2013b). Several panel studies of healthy children reported decreases in FEV_1 and increases in markers of pulmonary inflammation associated with increases in short-term ozone exposure. While there is coherence between epidemiologic and experimental evidence of ozone-induced lung function decrements and pulmonary inflammation, respiratory symptoms were not associated with ozone exposure in a limited number of epidemiologic studies. However, these studies generally relied on parent-reported outcomes that may have resulted in under- or over-reporting of respiratory symptoms.



Note: All studies used constant exposure concentrations in a chamber unless designated as stepwise (S) and/or facemask (m) exposures. All responses at and above 70 ppb (targeted concentration) were statistically significant. Adams (2006) found statistically significant responses to square-wave chamber exposures at 60 ppb based on the analysis of Brown et al. (2008) and Kim et al. (2011). During each hour of the exposures, subjects were engaged in moderate quasi-continuous exercise (20 L/minute per m^2 BSA) for 50 minutes and rest for 10 minutes. Following the 3rd hour, subjects had an additional 35-minute rest period for lunch. The data at 60 and 80 ppb have been offset along the *x*-axis for illustrative purposes. The curved solid line from McDonnell et al. (2013) illustrates the predicted FEV_1 decrements using Model 3 coefficients at 6.6 hours as a function of ozone concentration for a 23.8-year-old with a BMI of 23.1 kg/m^2.

*80 ppb data for 30 health subjects were collected as part of the Kim et al. (2011) study, but only published in Figure 5 of McDonnell et al. (2012).

Source: Adapted from Figure 6-1 of 2013 Ozone ISA (U.S. EPA, 2013b). Studies appearing in the figure legend are: Adams (2006), Adams (2003), Adams (2002), Horstman et al. (1990), Kim et al. (2011), McDonnell et al. (2013), McDonnell et al. (1991), and Schelegle et al. (2009).

Figure IS-2 Cross-study comparisons of mean ozone-induced forced expiratory volume in 1 second (FEV_1) decrements in young healthy adults following 6.6 hours of exposure to ozone.

Evidence from numerous recent, large, multicity epidemiologic studies conducted in the U.S. among people of all ages also expands upon evidence from the 2013 Ozone ISA (U.S. EPA, 2013b) to further support an association between ozone exposure and ED visits and hospital admissions for asthma (Section 3.1.5.1 and Section 3.1.5.2). Reported associations were generally highest for children between the ages of 5 and 18 at mean daily 8-hour concentrations of 31−54 ppb. Additionally, consistent, positive associations were reported across models implementing measured and modeled ozone concentrations. A large body of evidence from the 2013 Ozone ISA (U.S. EPA, 2013b) reported ozone associations with markers of asthma exacerbation (e.g., respiratory symptoms, medication use, lung function) that support the ozone-related increases in asthma hospital admissions and ED visits observed in recent studies. Few

recent epidemiologic studies in the U.S. or Canada have examined respiratory symptoms and medication use, lung function, and subclinical effects in people with asthma. Recent experimental studies in animals, along with similar studies summarized in the 2013 Ozone ISA (U.S. EPA, 2013b), provide coherence with and biological plausibility for the epidemiologic evidence of asthma exacerbation, indicating respiratory tract inflammation, oxidative stress, injury, allergic skewing, goblet cell metaplasia, and upregulation of mucus synthesis and storage in allergic mice exposed to ozone (Section 3.1.5.4, Section 3.1.5.5, and Section 3.1.5.6).

In addition to epidemiologic evidence of asthma exacerbation, a number of recent epidemiologic studies continue to provide evidence of an association of ozone concentrations with hospital admissions and ED visits for combined respiratory diseases (Section 3.1.8), ED visits for respiratory infection (Section 3.1.7.1), and ED visits for COPD (Section 3.1.6.1.1). Recent epidemiologic evidence for respiratory mortality is limited, but there remains evidence of consistent, positive associations, specifically in the summer months (Section 3.1.9). A limited number of recent controlled human exposure and animal toxicological studies are consistent with studies evaluated in the 2013 Ozone ISA (U.S. EPA, 2013b) that demonstrate altered immunity and impaired lung host defense following acute ozone exposure (Section 3.1.7.3). These findings support the epidemiologic evidence of an association between ozone concentrations and respiratory infection. Additionally, results from recent animal toxicological studies provide new evidence that chronic inflammation enhances sensitivity to ozone exposure, providing coherence for ozone-related increases in ED visits for COPD (Section 3.1.6.2.1.2).

Copollutant analyses were limited in epidemiologic studies evaluated in the 2013 Ozone ISA, but they did not indicate that associations between ozone concentrations and respiratory effects were confounded by copollutants or aeroallergens. Copollutant analyses have been more prevalent in recent studies and continue to suggest that observed associations are independent of coexposures to correlated pollutants or aeroallergens (Section 3.1.10.1 and Section 3.1.10.2). Despite expanded copollutant analyses in recent studies, determining the independent effects of ozone in epidemiologic studies is complicated by the high copollutant correlations observed in some studies and the possibility for effect estimates to be overestimated for the better measured pollutant in copollutant models (Section 2.5). Nonetheless, the consistency of associations observed across studies with different copollutant correlations, the generally robust associations observed in copollutant models, and evidence from controlled human exposure studies demonstrating respiratory effects in response to ozone exposure in the absence of other pollutants, provide compelling evidence for the independent effect of short-term ozone exposure on respiratory symptoms.

Several controlled human exposure studies provided evidence on the C-R relationship for FEV_1 decrements in young healthy adults exposed during moderate exercise for 6.6. hours to ozone concentrations between 40 and 120 ppb. The lack of any studies at lower ozone concentrations and the small decrements observed at 40 ppb preclude characterization of the C-R relationship at lower concentrations. A model-predicted C-R function is described in a recent study presenting a mechanistic

model based on these [and other controlled human exposure data; McDonnell et al. (2013); Figure IS-1; Section 3.1.4.1.1].

Epidemiologic studies examining the shape of the relationship between ambient air concentrations and the studied health outcome and/or the presence of a threshold in this relationship have been inconsistent (Section 3.1.10.1.4). While most studies assume a no-threshold, log-linear C-R shape, a limited number of studies have used more flexible models to test this assumption. Results from some of these studies indicate approximately linear associations between ozone concentrations and hospital admissions for asthma, while others indicate the presence of a threshold ranging from 20 to 40 ppb 8-hour max ozone concentrations.

Most epidemiologic studies that examine the relationship between short-term concentrations of ozone in ambient air and health effects rely primarily on a 1-hour max, 8-hour max, or 24-hour avg averaging times. Epidemiologic time-series and panel studies evaluated in the 2013 Ozone ISA do not provide any evidence to indicate that any one averaging time is more consistently or strongly associated with respiratory-related health effects (U.S. EPA, 2013b). Recent epidemiologic studies examining respiratory effects continue to show evidence of positive associations for each of these averaging times (see Figure 3-4, Figure 3-5, Figure 3-6, and Figure 3-7). For example, Darrow et al. (2011), as detailed in the 2013 Ozone ISA, demonstrated a similar pattern of associations between short-term ozone exposure and respiratory-related ED visits for 1-hour max, 8-hour max, and 24-hour avg exposure metrics (Section 3.1.10.3.2). Similarly, a recent panel study focusing on respiratory symptoms in children reported positive associations when using both a 1-hour max and 8-hour max averaging time [Lewis et al. (2013); Section 3.1.5.3.2]. The combination of evidence from studies evaluated in the 2013 Ozone ISA, along with the results across recent studies that demonstrate positive associations using either a 1-hour max, 8-hour max, or 24-hour avg averaging time, further supports the conclusion that no one averaging time is more consistently or strongly associated with respiratory effects and that each of these averaging times could be surrogates for the exposure conditions that elicit respiratory health effects**.**

The evaluation of the lag structure of associations is an important consideration when examining the relationship between short-term ozone exposure and respiratory effects. With respect to ozone exposure, epidemiologic studies often examine associations between short-term exposure and health effects over a series of single-day lags, multiday lags, or by selecting lags *a priori* (Section 3.1.10.3). For respiratory health effects, when examining more overt effects, such as respiratory-related hospital admissions and ED visits (i.e., asthma, COPD, and all respiratory outcomes), epidemiologic studies reported strongest associations occurring within the 1st few days of exposure (i.e., in the range of 0 to 3 days). The effects of ozone exposure on subclinical respiratory endpoints, including lung function, respiratory symptoms, and markers of airway inflammation, similarly occur at lags of 0 and 1 day. This finding is consistent with the evidence from controlled human exposure and experimental animal studies of respiratory effects occurring relatively soon after ozone exposures.

In summary, recent studies evaluated since the completion of the 2013 Ozone ISA (U.S. EPA, 2013b) support and expand upon the strong body of evidence indicating a "causal relationship" between short-term ozone exposure and respiratory effects. Controlled human exposure studies demonstrate ozone-induced FEV_1 decrements and respiratory tract inflammation at concentrations as low as 60 ppb after 6.6 hours of exposure with exercise among young, healthy adults. The combination of lung function decrements and respiratory symptoms has been observed following exposure to 70 ppb and greater ozone concentrations over 6.6-hours and combined with exercise. Epidemiologic studies continue to provide evidence that increased ozone concentrations are associated with a range of respiratory effects, including asthma exacerbation, COPD exacerbation, respiratory infection, and hospital admissions and ED visits for combined respiratory diseases. A large body of animal toxicological studies demonstrate ozone-induced changes in lung function measures, inflammation, increased airway responsiveness, and impaired lung host defense. Additionally, mouse models indicate enhanced ozone-induced inflammation, oxidative stress, injury, allergic skewing, goblet cell metaplasia, and upregulation of mucus synthesis and storage in allergic mice compared with naïve mice. These toxicological results provide further information on the potential mechanistic pathways that underlie downstream respiratory effects. They also provide continued support for the biological plausibility of the observed epidemiologic results. Thus, the recent evidence integrated across disciplines, along with the total body of evidence evaluated in previous assessments, is sufficient to conclude that there **is a "causal relationship" between short-term ozone exposure and respiratory effects.**

IS.4.3.2 Long-Term Exposure and Respiratory Effects

The 2013 Ozone ISA concluded that there was "likely to be causal relationship" between long-term exposure to ozone and respiratory health effects (U.S. EPA, 2013b). The epidemiologic evidence for a relationship between long-term ozone exposure and respiratory effects in the 2013 Ozone ISA was provided by epidemiologic studies that typically evaluated the association between the annual average of daily ozone concentrations and new-onset asthma, respiratory symptoms in children with asthma, and respiratory mortality. Notably, associations of long-term ozone concentrations with new-onset asthma in children and increased respiratory symptoms in individuals with asthma were primarily observed in studies that examined interactions between ozone and exercise or different genetic variants. The evidence relating new-onset asthma to long-term ozone exposure was supported by toxicological studies of allergic airways disease in infant monkeys exposed to biweekly cycles of alternating filtered air and ozone (i.e., 9 consecutive days of filtered air and 5 consecutive days of 0.5 ppm ozone, 8 hours/day). This evidence from a nonhuman primate study of ozone-induced changes in the airways provided biological plausibility for early-life exposure to ozone contributing to asthma development in children. Generally, the consistent evidence from epidemiologic and animal toxicological studies formed the basis of the conclusions that there is "likely to be causal relationship" between long-term exposure to ambient ozone and respiratory effects. Uncertainties in the evidence base included limited assessment of potential copollutant confounding and the potential for exposure measurement error

relating to exposure assignment from fixed site monitors in epidemiologic studies. Although potential copolluant confounding was examined in a limited number of epidemiologic studies, results suggested that the reported associations were robust to adjustment for other pollutants, including $PM_{2.5}$. Building upon the evidence from the 2013 Ozone ISA, more recent epidemiologic evidence, combined with toxicological studies in rodents and nonhuman primates, provides coherence and biological plausibility to support that there is a "likely to be causal relationship" between long-term exposure to ozone and respiratory effects.

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 (Section 3.1.4.2).

Table IS-5 Summary of evidence from epidemiologic and animal toxicological studies on the respiratory effects associated with long-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA[a]
Respiratory effects	Epidemiologic evidence, combined with toxicological studies in rodents and nonhuman primates, provided biologically plausible evidence that there **is likely to be causal relationship between long-term exposure to ozone and respiratory effects.**	Epidemiologic evidence, combined with toxicological studies in rodents and nonhuman primates, continue to provide biologically plausible evidence for respiratory effects due to long-term ozone exposure. **Overall, the collective evidence is sufficient to conclude that there is a likely to be causal relationship between long-term ozone exposure and respiratory effects.**

Table IS-5 (Continued): Summary of evidence from epidemiologic and animal toxicological studies on the respiratory effects associated with long-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA[a]
New onset asthma	Animal toxicological studies provided evidence that perinatal exposure to ozone compromises airway growth and development in infant monkeys (**500 ppb;** 6 h a day, 5 days a week for 20 weeks). Animal toxicological studies also demonstrate increased airway responsiveness, allergic airways responses, and persistent effects on the immune system, which may lead to the development of asthma. There is evidence that different genetic variants (HMOX, GST, ARG), in combination with ozone exposure, are related to new-onset asthma. These associations were observed when subjects living in areas where the mean annual 8-h daily max ozone concentration was **55.2 ppb**, compared with those who lived in areas with a mean of **38.4 ppb**.	Recent epidemiologic studies provide generally consistent evidence for associations of long-term ozone exposure with the development of asthma in children. Associations observed in locations with mean annual concentrations of **32.1 ppb** in one study that reported study mean concentrations (community-specific annual average concentrations ranged from **26** to **76 ppb**). Recent animal toxicological studies demonstrate effects on airway development in rodents (**500 ppb**; 6 h a day for 3−22 weeks) and build on and expand the evidence for long-term ozone exposure-induced effects that may lead to asthma development.
Asthma hospital admissions	Epidemiologic studies provided evidence that long-term ozone exposure is related to increased hospital admissions in children and adults, and first childhood asthma hospital admissions in a linear concentration-response relationship. Generally, these studies had mean annual 8-h daily max ozone concentrations less than **41 ppb.**	Long-term exposure is associated with hospital admissions and ED visits for asthma in study locations with mean annual ozone concentrations between **30.6** and **47.7 ppb,** although uncertainties remain because most studies do not adjust for short-term ozone concentrations.
Pulmonary structure and function	Evidence for pulmonary function effects was inconsistent, with some epidemiologic studies observing positive associations (mean annual 8-h daily max ozone concentrations less than **65 ppb**). Results from toxicological studies demonstrated that long-term exposure of adult monkeys and rodents (**>120 ppb;** 6 h a day, 5 days a week for 20 weeks) can result in irreversible morphological changes in the lung, which in turn can influence pulmonary function.	Recent animal toxicological studies provide evidence that postnatal ozone exposure may affect processes in the developing lung, including impaired alveolar morphogenesis, a key step in lung development, in infant monkeys (**500 ppb**; 6 h a day for 3−22 weeks). Notably, the impairments in alveolar morphogenesis were reversible (reversibility of the other effects was not studied). A limited number of recent epidemiologic studies continue to provide inconsistent support for an association between long-term ozone exposure and lung function development in children.
Pulmonary inflammation, injury, and oxidative stress	Several epidemiologic studies (mean 8-h max ozone concentrations less than **69 ppb**) and animal toxicological studies (as low as **500 ppb**) added to existing evidence of ozone-induced inflammation and injury.	Recent experimental studies in animals provide evidence that postnatal ozone exposure may affect the developing lung (**500 ppb**). Results from studies of neonatal rodents demonstrate ozone-induced changes in injury and inflammatory and oxidative stress responses during lung development (**1,000 ppb**).
Lung host defenses	Evidence demonstrated a decreased ability to respond to pathogenic signals in infant monkeys exposed to **500 ppb** ozone and an increase in severity of post-influenza alveolitis in rodents exposed to **500 ppb.**	A recent study demonstrates decreased ability to respond to pathogenic signals in infant monkeys exposed to **500 ppb.**

Table IS-5 (Continued): Summary of evidence from epidemiologic and animal toxicological studies on the respiratory effects associated with long-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA[a]
Allergic responses	Evidence demonstrated a positive association between allergic response and ozone exposure, but the magnitude of the association varied across studies; exposure to ozone may increase total IgE in adult asthmatics. Allergic indicators in infant monkeys and adult rodents were increased by exposure to ozone concentrations of **500 ppb.**	Cross-sectional epidemiologic studies provide generally consistent evidence that ozone concentrations (mean annual concentration less than **51.5 ppb**) are associated with hay fever/rhinitis and serum-markers of allergic response, although uncertainties related to study design and potential confounding by pollen remain. A recent animal toxicological study provides evidence of ozone-induced airway eosinophilia in a mouse model of allergic sensitization (**100 ppb**; 0.33 h per day for 5 days per week for 2 weeks and once weekly for 12 weeks).
Development of COPD	Animal toxicological studies provided evidence that long-term ozone exposure could lead to persistent inflammation and interstitial remodeling in adult rodents and monkeys, potentially contributing to the development of chronic lung disease such as COPD. The 2013 Ozone ISA did not evaluate any epidemiologic studies that examined the relationship between long-term exposure to ozone and the development of COPD.	One recent epidemiologic study provides evidence of an association between long-term ozone concentrations and incident COPD hospitalizations (mean annual concentrations **39.3 ppb**). Recent animal toxicological studies provide consistent evidence that subchronic ozone exposure (**500−1,000 ppb**) can lead to airway injury and inflammation. In adult animals, these changes may underlie the progression and development of chronic lung disease and provide biological plausibility for ozone-induced development of COPD.
Respiratory mortality	A single study demonstrated that exposure to ozone (long-term mean ozone less than **104 ppb**) elevated the risk of death from respiratory causes. This effect was robust to the inclusion of $PM_{2.5}$ in a copollutant model.	Recent epidemiologic studies provide some evidence of an association with respiratory mortality, but the evidence is not consistent (mean annual ozone concentrations **25.9−57.5 ppb**). New evidence from one study reports an association with COPD mortality.

[a]Conclusions from the 2020 ISA include evidence from recent studies integrated with evidence included in previous Ozone ISAs and AQCDs.

In support of evidence from recent epidemiologic studies, a number of recent animal toxicological studies expand the evidence base for long-term ozone exposure-induced effects leading to asthma development (Section 3.2.4.1.2). Specifically, both older and more recent long-term ozone exposure studies in nonhuman primates show that postnatal ozone exposure can compromise airway growth and development, promote the development of an allergic phenotype, and cause persistent alterations to the immune system (Section 3.2.4.6.2). In addition, findings that ozone exposure enhances injury, inflammation, and allergic responses in allergic rodents provide biological plausibility for the relationship between ozone exposure and the exacerbation of allergic asthma.

In addition to studies of asthma, several new or expanded lines of evidence from epidemiologic and animal toxicological studies published since the completion of the 2013 Ozone ISA provide evidence of associations between long-term ozone exposure and the development of COPD (Section 3.2.4.3) and allergic responses (Section 3.2.4.6). A recently available epidemiologic study provides limited evidence that long-term ozone exposure is associated with incident COPD hospitalizations in adults with asthma. This finding is supported by recent animal toxicological studies that provide consistent evidence of airway injury and inflammation resulting from subchronic ozone exposures. These results are coherent with animal toxicological studies reviewed in the 2013 Ozone ISA, which demonstrated that chronic ozone exposure damages distal airways and proximal alveoli, resulting in persistent inflammation and lung tissue remodeling that leads to irreversible changes including fibrotic- and emphysematous-like changes in the lung. Respiratory tract inflammation and morphologic and immune system-related changes may underlie the progression and development of chronic lung disease like COPD.

A larger body of epidemiologic studies also supports an association between long-term ozone exposure and allergic responses, including hay fever/rhinitis and serum allergen-specific IgE. While recent studies demonstrate generally consistent results, potential confounding by pollen exposure remains an uncertainty. However, there is supporting evidence from animal toxicological studies demonstrating enhanced allergic responses in allergic rodents (Section 3.2.4.6.2). In addition, animal toxicological studies reviewed in the short-term exposure section show type 2 immune responses in nasal airways of rodents exposed repeatedly to ozone, indicating that ozone exposure can trigger allergic responses (Section 3.1.4.4.2). These findings are characteristic of induced nonatopic asthma and rhinitis and provide biological plausibility for the observed epidemiologic associations with hay fever/rhinitis.

Taken together, previous and more recent animal toxicological studies of long-term exposure to ozone provide biological plausibility for the associations reported in the recent epidemiologic studies. Specifically, there is strong evidence of ozone-induced inflammation, injury, and oxidative stress in adult animals. These effects represent initial events through which ozone may lead to a number of downstream respiratory effects, including altered morphology in the lower respiratory tract and the development of COPD. Furthermore, there is evidence of a range of ozone-induced effects on lung development in neonatal rodents and infant monkeys, including altered airway architecture, airway sensory nerve innervation, airway cell death pathways, increased serotonin-positive airway cells, and immunomodulation. An infant monkey model of allergic airway disease also demonstrated effects on lung development, including compromised airway growth, impaired alveolar morphogenesis, airway smooth muscle hyperreactivity, an enhanced allergic phenotype, priming of responses to oxidant stress, and persistent effects on the immune system. These various upstream effects provide a plausible pathway through which ozone may act on downstream events. These events include altered immune function leading to altered host defense and allergic responses, as well as morphologic changes leading to the development of asthma. A more thorough discussion of the biological pathways that potentially underlie respiratory health effects resulting from long-term exposure to ozone can be found in Section 3.2.3.

Recent epidemiologic studies provide some evidence that long-term ozone exposure is associated with respiratory mortality, but the evidence is not consistent across studies (Section 3.2.4.9). A recent nationwide study in the U.S. reported associations between ozone and the underlying causes of respiratory mortality, including COPD. This finding is supported by the new lines of evidence from animal toxicological and epidemiologic studies on the development of COPD, as discussed previously. Results from epidemiologic studies of ozone-related respiratory mortality in populations outside the U.S. are inconsistent.

A notable source of uncertainty across the reviewed epidemiologic studies is the lack of examination of potential copollutant confounding. A limited number of studies that include results from copollutant models suggest that ozone associations may be attenuated but still positive after adjustment for NO_2 or $PM_{2.5}$. However, the few studies that include copollutant models examine different outcomes, making it difficult to draw strong conclusions about the nature of potential copollutant confounding for any given outcome. Importantly, in addition to studies that explicitly address potential copollutant confounding through modeling adjustments, many studies report modest copollutant correlations, suggesting that strong confounding due to copollutants is unlikely. Another source of uncertainty common to epidemiologic studies of air pollution is the potential for exposure measurement error. The majority of recent epidemiologic studies of long-term ozone exposure use concentrations from fixed-site monitors as exposure surrogates. Exposure measurement error relating to exposure assignment from fixed-site monitors has the potential to bias effect estimates in either direction, although it is more common that effect estimates are underestimated, and the magnitude of the bias is likely small relative to the magnitude of the effect estimate, given that ozone concentrations do not vary over space as much as other criteria pollutants, such as NO_2 or SO_2 (Section 2.3.1.1).

Strong coherence from animal toxicological studies supports the observed epidemiologic associations related to respiratory morbidity. Experimental evidence also provides biologically plausible pathways through which long-term ozone exposure may lead to respiratory effects. **Overall, the collective evidence supports a "likely to be causal relationship" between long-term ozone exposure and respiratory effects.**

IS.4.3.3 Short-Term Exposure and Metabolic Effects

The metabolic effects reviewed in this ISA include the risk factors and related endpoints for metabolic syndrome, complications due to diabetes, and indicators of metabolic function. Metabolic syndrome is a clinical diagnosis used to describe a collection of risk factors that include high blood pressure (elevated systolic and/or diastolic blood pressure), dyslipidemia (elevated triglycerides and low levels of high-density lipoprotein [HDL] cholesterol), obesity (central obesity), and increased fasting blood glucose (Alberti et al., 2009). Diagnosis of metabolic syndrome in humans is based on the presence of three of these five risk factors (Alberti et al., 2009). The presence of these risk factors may predispose

individuals to an increased risk of type 2 diabetes and cardiovascular disease. Diabetes is characterized by hyperglycemia (i.e., elevated glucose level) resulting from defects in insulin signaling, secretion, or both. Indicators of metabolic function include adipose tissue inflammation, altered liver function, and alterations in adrenal hormones, among other endpoints.

The evidence was not sufficient to evaluate metabolic effects as a separate health effect category in the 2013 Ozone ISA. As a result, there were no causality determinations for metabolic effects in the 2013 Ozone ISA (U.S. EPA, 2013b). Since the completion of the 2013 Ozone ISA, the number of studies examining the relationship between short-term ozone exposure and metabolic effects has expanded substantially (Table IS-6). This recent evidence, primarily from experimental animal studies, demonstrates that short-term ozone exposure triggers a stress response that leads to a cascade of transient metabolic effects. Consistent animal toxicological evidence from multiple laboratories demonstrates that short-term ozone exposure increases circulating levels of adrenaline and corticosterone, released from the adrenal medulla and adrenal cortex, respectively (Section 5.1.5.3.2). This evidence is coherent with results of a controlled human exposure study demonstrating that short-term ozone exposure to 300 ppb resulted in increased circulating cortisol and corticosterone. In animals, the metabolic effects that follow short-term ozone exposure are similar to those that are used in the clinical diagnosis of metabolic syndrome in humans. The strongest and most consistent evidence is for glucose and insulin homeostasis. Several high-quality animal toxicological studies from multiple laboratories demonstrate that short-term ozone exposure impairs glucose tolerance and causes insulin resistance (Section 5.1.3). Some but not all animal toxicological studies show ozone-induced fasting hyperglycemia, with inconsistencies between studies potentially caused by differences in rodent stock, strain, sex, or diet. Multiple animal toxicological studies in several rodent strains demonstrate that short-term ozone exposure increases serum levels of triglycerides and free fatty acids, results that are consistent with the mobilization of energy stores and increased glucose (Section 5.1.3.2). Coherent with results in animal models, the controlled human exposure study reported increases in medium- and long-chain circulating free fatty acids following short-term exposure to 300 ppb ozone. However, this study did not find ozone-induced changes in serum insulin, nonfasting glucose, insulin resistance, or triglyceride levels. Some epidemiologic studies examining changes in glucose and lipids provide support for effects associated with short-term ozone exposure.

There is additional evidence for ozone-induced metabolic effects from experimental animal toxicological studies that are the same effects used for the clinical diagnosis of metabolic syndrome in humans. This generally consistent evidence demonstrates that short-term ozone exposure affects obesity-relevant endpoints and causes adipose tissue inflammation. Some, but not all, animal toxicological studies reported that short-term ozone exposure reduces body-weight gain[1] in rodent models of diabetes and of spontaneous hypertension (Section 5.1.5). In addition, multiple animal toxicological studies from different laboratories consistently reported that short-term ozone exposure affected levels of

[1] Reductions or increases in body-weight gain can indicate altered metabolic function in animal models of disease, such as those used in these studies.

leptin, a hormone that regulates food intake. In coherence with these results, an epidemiologic study reported trends for an association between short-term ozone exposure and changes in the obesity-related hormones. In addition to changes in hormone levels, multiple animal toxicological studies in both healthy and disease-prone rodent models showed that short-term ozone exposure can induce adipose tissue inflammation (Section 5.1.5.1). Furthermore, while several studies reported null effects, others reported that short-term ozone exposure can affect levels of HDL, LDL, and total cholesterol, with the directionality of the effect varying by the rodent model and exposure duration (Section 5.1.5.1). Finally, some animal toxicological studies provide evidence that short-term ozone exposure affects blood pressure (Section 5.1.3.5).

Table IS-6 Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the metabolic effects of short-term exposure to ozone.

	Results and Conclusions from 2020 ISA
Metabolic effects	Recent evidence from controlled human exposure, epidemiologic, and animal toxicological studies support a **likely to be causal relationship between short-term ozone exposure and metabolic effects.**
Effects contributing to the clinical diagnosis of metabolic syndrome in humans	Animal toxicological studies provide evidence for elevated triglycerides and fasting hyperglycemia. Evidence is present, though less consistent, for low HDL cholesterol, high blood pressure, and central adiposity.
Complications from diabetes	An epidemiologic study provides evidence of associations between increases in short-term ozone exposure and hospital admissions for diabetic ketoacidosis and diabetic coma in older population subgroups.
Other indicators of metabolic function	Multiple metabolic indicators provide evidence that ozone exposure induces changes within the liver, affecting glucose homeostasis. Healthy volunteers who exercised with ozone exposure in controlled human exposure studies had increased ketone body formation. In animal toxicological studies, ozone exposure induced changes to the liver, including hepatic gluconeogenesis, altered bile acid profile, alterations to β-oxidation, and alterations to proteins in hepatic metabolic pathways. In addition, elevated circulating stress hormones were consistently observed in animal models and in a single controlled human exposure study. Removal of the adrenal glands prevented the release of adrenaline and corticosterone, and furthermore, prevented ozone-induced metabolic effects in animal toxicological studies. Thus, neuroendocrine stress activation may be a primary mechanism through which adverse metabolic outcomes develop from short-term ozone exposure.

Recent studies of short-term ozone exposure and metabolic effects evaluated associations between different age groups. One epidemiologic study observed increased risk among older adults (e.g., 75−84 years and 85+ years) compared with other age groups (<65 years) for hospital admissions for diabetic coma (Section 5.1.7.1) with a 24-hour avg ozone concentration across study areas of 64.4 ppb. In

addition, an animal toxicological study demonstrated increases in metabolic indicators (i.e., increased triglycerides and serum insulin) in aged animals.

Despite limited controlled human exposure and epidemiologic evidence, the expanding body of animal toxicological studies shows robust evidence for short-term ozone exposure contributing to an array of metabolic effects. These outcomes follow a biologically plausible pathway whereby ozone exposure results in release of adrenaline and cortisol/corticosterone from the adrenal glands. These hormones act on multiple organs and tissues of the metabolic system to mobilize energy reserves, including glucose and lipids. In summary, based on evidence from animal toxicological and epidemiologic studies, as well as some support from one controlled human exposure study, short-term ozone exposure consistently impairs glucose and insulin homeostasis and increases triglycerides and fatty acids. In line with this, animal toxicological studies show that inhibiting adrenaline and/or corticosterone, through either removal of the adrenal glands or adrenal medulla, or by blocking the synthesis of corticosterone, prevents ozone-induced metabolic effects, including hyperglycemia, glucose intolerance, and elevated circulating triglycerides. In addition, there are generally consistent effects from animal toxicological studies showing that short-term ozone exposure affects obesity-relevant endpoints and causes inflammation in adipose tissue. Further supporting evidence comes from a limited number of animal toxicological studies providing some evidence for alterations in HDL, LDL, and total cholesterol and changes in blood pressure following short-term ozone exposure. **Overall, the collective evidence is sufficient to conclude that the relationship between short-term ozone exposure and metabolic effects is likely to be causal**.

IS.4.3.4 Short-Term Exposure and Cardiovascular Effects

The 2013 Ozone ISA concluded that there is a "likely to be causal" relationship between relevant short-term exposures and cardiovascular effects, but it also identified important uncertainties (U.S. EPA, 2013b). The available animal toxicological studies demonstrated ozone-induced impaired vascular and cardiac function, as well as changes in heart rate (HR) and heart rate variability (HRV). The controlled human exposure studies provided additional evidence but had limited coherence with the evidence from animal studies. The epidemiologic evidence, while reporting associations between short-term ozone exposure and cardiovascular mortality, did not show associations between short-term ozone exposure and cardiovascular morbidity. This lack of coherence between the results for studies investigating associations of cardiovascular morbidity with cardiovascular mortality was recognized as a complication in interpreting the overall evidence for ozone-induced cardiovascular effects.

More recent animal toxicological studies published since the 2013 Ozone ISA provide generally consistent evidence for impaired heart function and endothelial dysfunction, but limited evidence for indicators of arrhythmia, HRV, and markers of oxidative stress and inflammation in response to ozone exposure. Additional controlled human exposure studies have been published in recent years, although they show little evidence for ozone-induced effects on cardiovascular endpoints. Specifically, some recent

studies do not indicate an effect of ozone on cardiac function, ST segment, endothelial dysfunction, or HR, while some evidence from a small number of controlled human exposure studies indicates ozone exposure can result in changes in blood pressure, indicators of arrhythmia, HRV, markers of coagulation, and inflammatory markers. The number of epidemiologic studies evaluating short-term ozone concentrations and cardiovascular effects has grown somewhat, but overall, remains limited and continues to provide little, if any, evidence for associations with heart failure, heart attack, arrhythmia and cardiac arrest, or stroke. Recent epidemiologic evidence for short-term ozone exposure and cardiovascular mortality is limited to one multicity study, but the collective body of evidence spanning multicity studies evaluated in the 2013 Ozone ISA provides evidence of consistent positive associations. Overall, many of the same limitations and uncertainties that existed in the body of evidence in the 2013 Ozone ISA continue to exist. However, the number of controlled human exposure studies evaluating short-term ozone exposure and cardiovascular endpoints has grown, and now includes studies at concentrations closer to those likely to be encountered in U.S. ambient air. When evaluated in the context of the studies available for the 2013 Ozone ISA, the controlled human exposure study evidence, overall, is less consistent and less indicative of a relationship (Table IS-7).

Table IS-7 Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the cardiovascular effects of short-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA
Cardiovascular effects	Evidence from animal toxicological studies demonstrated ozone-induced impaired vascular and cardiac function, as well as changes in HR and HRV. This evidence was supported from a limited number of controlled human exposure studies in healthy adults demonstrating changes in HRV, as well as in blood markers associated with an increase in coagulation. There was limited or no evidence from epidemiologic studies for short-term ozone exposure and cardiovascular morbidity, such as effects related to HF, IHD, and MI, arrhythmia and cardiac arrest, or thromboembolic disease. There was consistent evidence from epidemiologic studies reporting positive associations between short-term ozone exposure and cardiovascular-related mortality. Overall, there **is likely to be causal relationship between long-term exposure to ozone and cardiovascular effects.**	Recent animal toxicological studies continue to provide evidence for impaired heart function and endothelial dysfunction, with limited evidence from a small number of studies for indicators of arrhythmia, HRV, and markers of oxidative stress and inflammation in response to ozone exposure. Recent controlled human exposure studies provide little evidence for ozone-induced effects on a number of cardiovascular endpoints. No effect of ozone was reported for indicators of cardiac function, IHD, endothelial dysfunction, or changes in HR. There is limited or inconsistent evidence from a small number of studies for changes in cardiac electrophysiology, HRV, blood pressure, markers of coagulation, and inflammatory markers. Epidemiologic studies remain few and continue to provide little, if any, evidence for associations with HF, IHD, and MI, arrhythmia and cardiac arrest, or stroke. Overall, the evidence is **suggestive of, but not sufficient to infer, a causal relationship.**

Table IS-7 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the cardiovascular effects of short-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA
Heart failure, impaired heart function	A limited number of animal toxicological studies demonstrated ozone-induced cardiovascular effects, including decreased cardiac function. Epidemiologic studies generally did not observe associations between short-term ozone exposure and cardiovascular morbidity; studies of cardiovascular-related hospital admissions and ED visits did not find consistent evidence of a relationship with ozone exposure.	Multiple animal toxicological studies report some indicators of impaired cardiac function following short-term ozone exposure (**~200−300 ppb** for 3−4 h). However, a recent controlled human exposure study (100 and 200 ppb for 3 h) reported no changes in measures of cardiac function. There is a limited number of recent studies of hospital admissions and ED visits that analyzed associations with heart failure, and they continue to report inconsistent associations with short-term exposure to ozone.
Ischemic heart disease	Animal toxicological studies, although few, demonstrated ozone-induced cardiovascular effects, including enhanced ischemia/reperfusion (I/R) injury. Epidemiologic studies generally did not observe associations between short-term ozone exposure and cardiovascular morbidity; studies of cardiovascular-related hospital admissions and ED visits did not find consistent evidence of a relationship with ozone exposure.	An animal toxicological study in SH rats demonstrates ST segment depression following an **800**- but not **200-ppb** exposure to ozone for 4 h. However, no such changes are observed in the single controlled human exposure study (**70 and 120 ppb** for 3 h). Recent epidemiologic studies consistently report null or weak positive effect estimates in analyses of MI, including for STEMI and NSTEMI.
Cardiac and endothelial dysfunction	Animal toxicological studies, although limited in number, demonstrated ozone-induced cardiovascular effects, including vascular disease and injury.	Recent animal toxicological studies demonstrate generally consistent evidence for impaired cardiac and endothelial function in rodents following short-term ozone exposure of **400−1,000 ppb** for 4 h. However, coherence with controlled human exposure and epidemiologic studies is lacking.
Cardiac electrophysiology, arrhythmia, cardiac arrest	Animal toxicological studies, although few, demonstrated ozone-induced cardiovascular effects, including disrupted nitric oxide-induced vascular reactivity. Epidemiologic studies reported generally positive associations for hospital admissions or ED visits due to arrythmia or dysrhythmia.	A small number of recent animal toxicological studies demonstrate some evidence for changes in indicators of conduction abnormalities (**800 but not 200 ppb** for 3−4 h). Multiple controlled human exposure studies report little effect of short-term ozone exposure on conduction abnormalities (**70 and 120 ppb** for 2−3 h). Increases in out-of-hospital cardiac arrests associated with 8-h max or 24-h avg increases in ozone concentrations were reported by a few case-crossover studies; however, analyses of other endpoints (e.g., dysrhythmia, arrhythmia, or atrial fibrillation) generally report null results.

Table IS-7 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the cardiovascular effects of short-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA
Blood pressure changes and hypertension	A limited number of epidemiologic studies reported inconsistent associations with measures of blood pressure. Two studies observed increases in DBP associated with ozone concentration, but the association was attenuated to null after adjusting for $PM_{2.5}$ concentrations.	Recent animal toxicological studies demonstrate inconsistent effects of ozone-induced effects on changes in blood pressure (**300 and 500 ppb** for 3−8 h). Multiple controlled human exposure studies report no evidence of an ozone-induced effect on blood pressure (**120−700 ppb** for 1−3 h), while a single controlled human exposure study reported a decrease in DBP. Few epidemiologic panel studies evaluated blood pressure, and the results were inconsistent.
Heart rate and heart rate variability	Animal toxicological studies, although few, demonstrated ozone-induced cardiovascular effects, including increased HRV. Controlled human exposure studies provided some coherence with the evidence from animal toxicological studies, by demonstrating increases and decreases in HRV following relatively low (**120 ppb** during rest) and high (**300 ppb** with exercise) ozone exposures, respectively.	Evidence is inconsistent for changes in HR in animals (**~200−800 ppb** for 3−8 h) and lacking for changes in HR in healthy adults from multiple controlled human exposure studies (**70−300 ppb** for 1−4 h). With respect to HRV, there is limited evidence for changes in animal toxicological (**200−800 ppb** for 3−4 h) and controlled human exposure (**70−300 ppb** for 1−4 h) studies. Similarly, recent epidemiologic panel studies have reported inconsistent associations between short-term exposure to ozone and both HR and HRV.
Coagulation and thrombosis	A controlled human exposure study demonstrated changes in markers of coagulation following short-term ozone exposure. Specifically, there were decreases in PAI-1 and plasminogen levels and a trend toward an increase in tPA. There was very limited animal toxicological evidence that short-term exposure to ozone could result in an increase in factors related to coagulation. Epidemiologic studies observed inconsistent results for coagulation biomarkers such as PAI-1, fibrinogen, and vWF.	Recent animal toxicological studies provide limited evidence for changes in factors that may promote coagulation (**250−1,000 ppb** for 4 h). Similarly, there is limited additional evidence from recent controlled human exposure studies that short-term ozone exposure can result in changes to markers of coagulation that may promote thrombosis (**100−300 ppb** for 1−2 h). Epidemiologic studies continue to observe inconsistent associations with changes in biomarkers of coagulation.
Systemic inflammation and oxidative stress	Controlled human exposure studies demonstrated ozone-induced effects on blood biomarkers of systemic inflammation and oxidative stress.	There is inconsistent evidence from recent animal toxicological studies for an increase in markers associated with systemic inflammation and oxidative stress (**300−800 ppb** for 2−24 h) and some evidence for increases in markers of systemic inflammation from CHE studies (**100−300 ppb** for 0.5−4 h). Additionally, the newly available epidemiologic panel study did not observe an association between short-term ozone concentrations and myeloperoxidase.
Stroke	A limited number of epidemiologic studies observed inconsistent associations with stroke.	Inconsistent results were observed in several recent epidemiologic studies that analyzed hospital admissions and ED visits for stroke and stroke subtypes.

Table IS-7 (Continued): Summary of evidence from epidemiologic, controlled human exposure, and animal toxicological studies on the cardiovascular effects of short-term ozone exposure.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA
Cardiovascular hospital admissions and ED visits	With few exceptions, studies of ozone concentrations and cardiovascular hospital admissions and ED visits for all CVD diagnoses combined did not report positive associations.	Recent studies that reported a risk ratio for combined cardiovascular disease outcomes show a similar inconsistent pattern to those studies included in the 2013 Ozone ISA.
Cardiovascular mortality	Multicity epidemiologic studies observed positive associations for cardiovascular mortality in all-year and summer/warm season analyses. Lack of coherence with epidemiologic studies of cardiovascular morbidity remains an important uncertainty.	A recent multicity study is consistent with the evidence examining cardiovascular mortality evaluated in the 2013 Ozone ISA.

[a]Conclusions from the 2020 ISA include evidence from recent studies integrated with evidence included in previous Ozone ISAs and AQCDs.

When considered as a whole, the evidence is "suggestive of, but not sufficient to infer, a causal relationship" between short-term exposure to ozone and cardiovascular effects. This causality determination represents a change from the conclusion in the 2013 Ozone ISA. This change is largely because the number of controlled human exposure studies showing little evidence of ozone-induced cardiovascular effects has grown substantially, while the epidemiologic evidence for ozone effects on endpoints other than mortality continues to be limited. Consequently, the plausibility for a relationship between short-term ozone exposure to cardiovascular health effects is weaker than it was in the previous review, leading to the revised causality determination.

IS.4.3.5 Short-Term Exposure and Total Mortality

Recent multicity epidemiologic studies conducted in the U.S. and Canada continue to provide evidence of consistent, positive associations between short-term ozone exposure and total mortality in both all-year and summer/warm season analyses across different averaging times (i.e., max daily 1-hour max, max daily 8-hour avg, 8-hour avg, and 24-hour avg; Table IS-8). Cause-specific mortality (e.g., respiratory mortality, cardiovascular mortality) was assessed in a limited number of recent studies. The evidence from these recent studies is consistent with the pattern of positive associations reported for studies evaluated in the 2013 Ozone ISA. Lastly, most of the recent multicity studies examined associations between short-term ozone exposure and mortality using ozone data collected before the year 2000, with only Di et al. (2017) including more recent ozone concentration data.

Recent studies continue to assess the influence of important potential confounders on the ozone-mortality relationship, including copollutants, temporal/seasonal trends, and weather covariates. Overall, these studies report that associations remain relatively unchanged across the different approaches

used to control for each confounder. The assessment of potential copollutant confounding in recent studies demonstrates that associations between short-term ozone concentrations and mortality remain positive in copollutant models with PM_{10} or NO_2. Importantly, the issues surrounding the assessment of potential copollutant confounding that complicate interpretation of the ozone-mortality relationship (as detailed in the 2013 Ozone ISA) persist, specifically within studies that relied on PM data collected using every 3rd- and 6th-day sampling schedules (U.S. EPA, 2013b).

Building upon the 2013 Ozone ISA, there remains strong evidence for respiratory effects due to short-term ozone exposure (Appendix 3) that is consistent within and across disciplines and which provides coherence and biological plausibility for the positive respiratory mortality associations reported across epidemiologic studies. Although there remains epidemiologic evidence for ozone-induced cardiovascular mortality along with animal toxicological evidence of cardiovascular effects, recent controlled human exposure studies do not provide evidence that is consistent with the controlled human exposure studies presented in the 2013 Ozone ISA showing cardiovascular effects. Additionally, there is limited evidence from epidemiologic studies of relationships between short-term ozone exposure and more severe cardiovascular effects, such as emergency department visits and hospital admissions. The limited experimental evidence, in combination with the lack of coherence between experimental and epidemiologic studies of cardiovascular morbidity, does not allow for an understanding of potential biological pathways leading to cardiovascular mortality (Appendix 4) or other causes of mortality.

Overall, the recent multicity studies conducted in the U.S. and Canada provide additional support for the consistent, positive associations with total mortality reported across multicity studies evaluated in the 2006 Ozone AQCD (U.S. EPA, 2006a) and 2013 Ozone ISA (U.S. EPA, 2013b). These results are supported by studies that further examine uncertainties in the ozone-mortality relationship, such as potential confounding by copollutants and other variables, modification by temperature, and the C-R relationship and whether a threshold exists. Although there continues to be strong evidence from studies of respiratory morbidity to support respiratory mortality, there remains relatively limited biological plausibility and coherence within and across disciplines to support the epidemiologic evidence for cardiovascular mortality, the largest contributor to total mortality. Collectively, evidence is "suggestive of, but not sufficient to infer, a causal relationship" between short-term ozone exposure and total mortality.

Table IS-8 Summary of evidence from epidemiologic studies on the association of short-term ozone exposure with mortality.

	Conclusions from 2013 Ozone ISA	Results and Conclusions from 2020 ISA[a]
Mortality	Consistent, positive associations were reported across multicity and multicontinent studies in combination with strong evidence from studies of respiratory morbidity. There was evidence from a limited number of studies of cardiovascular morbidity, providing coherence and biological plausibility. **Evidence demonstrated that there was a likely to be causal relationship between short-term ozone exposure and mortality**.	Recent multicity studies continue to provide evidence of consistent, positive associations, which is supported by strong evidence from studies of respiratory morbidity, providing coherence and biological plausibility. Recent studies of cardiovascular morbidity do not provide coherence between experimental and epidemiologic studies, and therefore, biological plausibility for cardiovascular mortality is limited. **Evidence is suggestive of, but not sufficient to infer, a causal relationship between short-term ozone exposure and mortality**.
Epidemiologic evidence	Multicity and multicontinent studies provided evidence of consistent positive associations for total (nonaccidental), respiratory, and cardiovascular mortality.	Recent multicity studies continue to provide evidence of consistent, positive associations with total (nonaccidental), respiratory, and cardiovascular mortality, but the cause-specific mortality evidence is limited to one recent multicity study.
Copollutant confounding	Ozone-mortality associations remained positive and relatively unchanged in copollutant models with PM and $PM_{2.5}$ components, but analyses of $PM_{2.5}$ components are limited by the every-3rd and 6th-day sampling schedule.	Recent multicity studies have conducted a limited assessment of potential copollutant confounding, but report that ozone-mortality associations remain positive and relatively unchanged in copollutant models with PM_{10} and NO_2, the only pollutants assessed.
Biological plausibility	The strong and consistent evidence within and across scientific disciplines for respiratory morbidity provided coherence and biological plausibility for respiratory mortality. For cardiovascular mortality, controlled human exposure and animal toxicological studies provided initial evidence supporting a biologically plausible mechanism by which short-term ozone exposure could lead to cardiovascular mortality, but there was inconsistency in results between experimental and epidemiologic studies of cardiovascular morbidity.	There continues to be strong and consistent evidence within and across disciplines for respiratory morbidity, which provides coherence and biological plausibility for respiratory mortality. Although there remains evidence of cardiovascular mortality, recent controlled human exposure studies do not report evidence of cardiovascular effects in response to short-term ozone exposure and epidemiologic studies provide limited evidence of associations with more sever cardiovascular effects, such as emergency department visits and hospital admissions. Collectively, there is a lack of coherence between experimental and epidemiologic studies providing limited evidence of a biologically plausible pathway to cardiovascular mortality or to other causes of mortality.

[a]Conclusions from the 2020 ISA include evidence from recent studies integrated with evidence included in previous Ozone ISAs and AQCDs.

IS.4.3.6 Other Health Endpoints

The evidence for the other health endpoints not discussed in previous sections, including long-term ozone exposure and cardiovascular and metabolic effects and mortality, and short- and long-term ozone exposure and reproductive effects, nervous system effects, and cancer, is limited or inconsistent, resulting in causality determinations of either "suggestive of, but not sufficient to infer, a causal relationship" or "inadequate to infer the presence or absence of a causal relationship." The evidence for these health effects is summarized here, with more details of the evidence that formed the basis for these conclusions in Appendix 4, Appendix 5, Appendix 6, and Appendix 7.

IS.4.3.6.1 Long-Term Ozone Exposure and Cardiovascular Effects

Collectively, **the body of evidence for long-term ozone exposure and cardiovascular effects is "suggestive of, but not sufficient to infer, a causal relationship."** Recent animal toxicological and epidemiologic studies add to the body of evidence that formed the basis of the conclusions in the 2013 Ozone ISA for cardiovascular health effects. This body of evidence is limited, however, with some experimental and observational evidence for subclinical cardiovascular health effects and little evidence for associations with outcomes such as IHD or MI, HF, or stroke. The strongest evidence for the association between long-term ozone exposure and cardiovascular health outcomes continues to come from animal toxicological studies of impaired cardiac contractility and epidemiologic studies of blood pressure changes and hypertension and cardiovascular mortality. Recent epidemiologic studies observed positive associations with changes in blood pressure or hypertension, but animal toxicological studies do not report effects of ozone on blood pressure changes. In conclusion, the results observed across both recent and older experimental and observational studies conducted in various locations provide limited evidence for an association between long-term ozone exposure and cardiovascular health effects.

IS.4.3.6.2 Long-Term Exposure and Metabolic Effects

In the 2013 Ozone ISA, evidence was insufficient to evaluate metabolic effects as a separate health effect category. Therefore, no causality determinations for metabolic effects were made in that document (U.S. EPA, 2013b). Since then, the epidemiologic and experimental literature investigating long-term ozone exposure and outcomes related to metabolic effects has expanded substantially. Positive associations between long-term exposure to ozone and diabetes-related mortality were observed in recent evaluations of well-established cohorts in the U.S. and Canada. The mortality results are supported by epidemiologic and experimental studies reporting effects on glucose homeostasis and serum lipids, as well as other indicators of metabolic function (e.g., peripheral inflammation and neuroendocrine stress response). Findings from an epidemiologic study of metabolic disease demonstrate increases in the clinical diagnosis of metabolic syndrome. Additionally, in prospective cohort studies in the U.S. and

Europe, increased incidence of type 2 diabetes is observed in association with long-term ozone exposure. Despite an increased number of studies, many uncertainties remain regarding the metabolic effects related to long-term ozone exposure. Most studies from the epidemiologic literature did not evaluate potential copollutant confounding. There were a very limited number of studies available for review from the animal toxicological literature; these studies had few overlapping endpoints, and furthermore, they were primarily conducted by the same set of authors. Overall, considering the positive epidemiologic studies and limited support from animal toxicological studies, the collective evidence is "**suggestive of, but not sufficient to infer, a causal relationship" between short-term exposure to ozone and metabolic effects.**

IS.4.3.6.3 Ozone Exposure and Reproductive Effects

Overall, the evidence is "suggestive of, but not sufficient to infer, a causal relationship" between ozone exposure and (1) male and female reproduction and fertility and (2) pregnancy and birth outcomes. Separate conclusions are made for these groups of reproductive effects because they are likely to have different etiologies and critical exposure windows over different lifestages. The 2013 Ozone ISA concluded that the evidence was "suggestive of a causal relationship"[1] between ozone exposure and the inclusive category for all reproductive and developmental outcomes.

The strongest evidence in the 2013 Ozone ISA for effects on reproduction and fertility came from epidemiologic and animal toxicological studies of sperm. Recent studies of sperm quality are consistent with this evidence but remain limited. Uncertainties that contribute to the determination include a lack of evaluation of copollutant confounding or multiple potential sensitive windows of exposure, and the generally small sample size of studies in human subjects.

The strongest evidence in the 2013 Ozone ISA for effects on pregnancy and reproduction came from epidemiologic studies of birth weight. Recent studies of birth weight are consistent with this evidence but remain limited. There are several well-designed, well-conducted studies that indicate an association between ozone and poorer birth outcomes, particularly for outcomes of continuous birth weight and preterm birth. In particular, studies of preterm birth that examine exposures in the first and second trimesters show fairly consistent positive associations (increased ozone exposures associated with increased odds of preterm birth). In addition, some animal toxicological studies demonstrate decreased birth weight and changes in uterine blood flow. Epidemiologic studies of continuous birth weight and preterm birth did not generally adjust for potential copollutant confounding, although studies that did appeared to show limited impacts. There is also inconsistency across exposure windows for associations with continuous birth weight. Also, the magnitude of effect estimates varies.

[1] Since the 2013 Ozone ISA, the causality determination language has been updated and this category is now stated as suggestive of, but not sufficient to infer, a causal relationship.

IS.4.3.6.4 Short-Term Ozone Exposure and Nervous System Effects

Overall, the evidence is "suggestive of, but not sufficient to infer, a causal relationship" between short-term exposure to ozone and nervous system effects. The 2013 Ozone ISA concluded that the evidence was "suggestive of a causal relationship"[1] between short-term ozone exposure and nervous system effects. The strongest evidence supporting this causality determination came from experimental animal studies of CNS structure and function. Most of the recent experimental animal studies demonstrate that short-term exposure to ozone induces oxidative stress and inflammation in the central nervous system (Section 7.2.1.3). In some cases, these effects are associated with changes in brain morphology and effects on neurotransmitters. In some instances, the effects of short-term ozone exposure on the nervous system were exacerbated in aged animals. No epidemiologic studies of short-term ozone exposure and nervous system effects were reviewed in the 2013 Ozone ISA, and the epidemiologic evidence remains limited. Recent epidemiologic evidence consists only of a study reporting an association between short-term ozone exposure and depressive symptoms, and several studies of hospital admissions or ED visits for symptoms related to a range of nervous system diseases or mental disorders (e.g., multiple sclerosis, Alzheimer's disease, Parkinson's disease, depression, psychiatric disorders). These findings for depressive symptoms are coherent with experimental animal studies showing depression-like behaviors in rodents. Biological plausibility of these effects is supported by multiple toxicological studies in laboratory animals showing inflammation and morphological changes in the brain following short-term ozone exposure (Section 7.2.1.2).

IS.4.3.6.5 Long-Term Ozone Exposure and Nervous System Effects

Overall, the evidence is "suggestive of, but not sufficient to infer, a causal relationship" between long-term ozone exposure and nervous system effects. This conclusion is consistent with that of the 2013 Ozone ISA. The strongest evidence supporting the causality determination for long-term ozone exposure and nervous system effects from the 2013 Ozone ISA came from animal toxicological studies demonstrating effects on CNS structure and function, with several studies indicating the potential for neurodegenerative effects similar to Alzheimer's or Parkinson's diseases in a rat model. The body of evidence has grown since the 2013 Ozone ISA. Recent epidemiologic studies have examined nervous system effects, including cognitive effects, depression, neurodegenerative disease, and autism. Although the epidemiologic evidence remains limited, the strongest evidence is for effects on cognition in adults. Recent experimental animal studies continue to provide coherence for these effects. Several recent animal toxicological studies report increased markers of oxidative stress and inflammation, including lipid peroxidation, microglial activation, and cell death following long-term exposure to ozone. There was some evidence to support that aged and young populations may have increased sensitivity to ozone exposure. Uncertainties that contribute to the causality determination include the limited number of epidemiologic studies, the lack of consistency across the available studies of Alzheimer's and Parkinson's disease, and the limited evaluation of copollutant confounding in these studies. In addition, the evidence

supporting the biological plausibility of the associations with autism or ASD in epidemiologic studies is limited.

IS.4.3.6.6 Long-Term Ozone Exposure and Cancer

The evidence describing the relationship between exposure to ozone and cancer remains "inadequate to infer the presence or absence of a causal relationship." In the 2013 Ozone ISA, very few studies were available to assess the relationship between long-term ozone exposure and cancer. The few available epidemiologic and animal toxicological studies indicated that ozone exposure may contribute to DNA damage. However, given the overall lack of studies, the 2013 Ozone ISA concluded that the evidence was inadequate to determine whether a causal relationship existed between long-term ozone exposure and cancer. More recent studies provide some additional animal toxicological evidence of DNA damage. In addition, several, but not all, recent cohort and case-control studies have observed positive associations between long-term ozone exposure and lung cancer incidence or mortality. Several of the studies evaluating lung cancer mortality were conducted in populations that had already been diagnosed with cancer in a different organ system. Associations between ozone exposure and other types of cancer were generally null. Given the limited evidence base, the lack of an evaluation of copollutant confounding in epidemiologic studies reporting associations, and the evaluation of study populations that had already been diagnosed with cancer in several of the epidemiologic studies, the evidence is not sufficient to draw a conclusion regarding causality.

IS.4.3.6.7 Long-Term Ozone Exposure and Mortality

Collectively, this body of evidence is "suggestive of, but not sufficient to infer, a causal relationship" between long-term ozone exposure and total mortality. Recent epidemiologic studies add to the limited body of evidence that formed the basis of the conclusions of in 2013 Ozone ISA for total mortality. This body of evidence is generally inconsistent, with some U.S. and Canadian cohorts reporting modest positive associations between long-term ozone exposure and total mortality, while other recent studies conducted in the U.S, Europe, and Asia reporting null or negative associations. The strongest evidence for the association between long-term ozone exposure and total (nonaccidental) mortality continues to come from analyses of patients with pre-existing disease from the Medicare cohort and from recent evidence demonstrating positive associations with cardiovascular mortality. The evidence from the assessment of ozone-related respiratory disease, with more limited evidence from cardiovascular and metabolic morbidity, provides some biological plausibility for mortality due to long-term ozone exposures. In conclusion, the inconsistent associations observed across both recent and older cohort and cross-sectional studies conducted in various locations provide limited evidence for an association between long-term ozone exposure and mortality.

IS.4.4 At-Risk Populations

Interindividual variation in exposure to or human responses to ambient air pollution exposure can result in some groups or lifestages being at increased risk for health effects. The NAAQS are intended to protect public health with an adequate margin of safety. In so doing, protection is provided for both the population as a whole and those potentially at increased risk for health effects in response to exposure to a criteria air pollutant [e.g., ozone; see Preamble to the ISAs (U.S. EPA, 2015)]. There is interindividual variation in both physiological responses, and exposure to ambient air pollution. The scientific literature has used a variety of terms to identify factors and subsequently populations or lifestages that may be at increased risk of an air pollutant-related health effect, including *susceptible, vulnerable, sensitive, at risk,* and *response-modifying factor* [Vinikoor-Imler et al. (2014); see Preamble to the ISAs (U.S. EPA, 2015)]. Acknowledging the inconsistency in definitions for these terms across the scientific literature and the lack of a consensus on terminology in the scientific community, "at-risk" is the all-encompassing term used in ISAs for groups with specific factors that increase the risk of an air pollutant (e.g., ozone)-related health effect in a population, as initially detailed in the 2013 Ozone ISA (U.S. EPA, 2013b). Therefore, this ISA takes an inclusive and all-encompassing approach and focuses on identifying those populations or lifestages potentially "at risk" of an ozone-related health effect.

As discussed in the Preamble to the ISAs (U.S. EPA, 2015), the risk of health effects from exposure to ozone may be modified as a result of intrinsic (e.g., pre-existing disease, genetic factors) or extrinsic factors (e.g., sociodemographic or behavioral factors), differences in internal dose (e.g., due to variability in ventilation rates or exercise behaviors), or differences in exposure to air pollutant concentrations (e.g., more time spent in areas with higher ambient concentrations). Some factors may lead to a reduction in risk and are recognized as such during the evaluation. However, in order to inform decisions on the NAAQS, this ISA focuses on identifying those populations or lifestages at greater risk. While a combination of factors (e.g., residential location and socioeconomic status [SES]) may increase the risk of ozone-related health effects in portions of the population, information on the interaction among factors remains limited. Thus, this ISA characterizes the individual factors that potentially result in increased risk for ozone-related health effects [see Preamble to the ISAs (U.S. EPA, 2015)].

IS.4.4.1 Approach to Evaluating and Characterizing the Evidence for At-Risk Factors

The ISA takes a pragmatic approach to identifying and evaluating factors that may increase the risk of a population or specific lifestage to an ambient air ozone-related health effect; this approach is described in detail in the Preamble to the ISAs (U.S. EPA, 2015) and illustrated in Table IS-9. While Appendix 3–Appendix 7 include a discussion of some populations and lifestages in order to explicitly characterize the causal nature between ozone exposure and health effects based on the body of evidence (e.g., children, individuals with asthma), this section focuses on summarizing evidence that can inform the identification of such populations and lifestages. Those populations and lifestages explicitly

considered in this ISA include those with pre-existing asthma, children, older adults, and outdoor workers, for which there was adequate evidence of increased risk in the 2013 Ozone ISA.

The evidence evaluated in this section includes relevant studies discussed in Appendix 3−Appendix 7 of this ISA and builds on the evidence presented in the 2013 Ozone ISA (U.S. EPA, 2013b). Based on the approach developed in previous ISAs (U.S. EPA, 2016, 2013a, b), recent evidence is integrated across scientific disciplines and health effects, and where available, with information on exposure and dosimetry. In evaluating factors and population groups, greater emphasis is placed on the evidence for those health outcomes for which a "causal" or "likely to be causal" relationship is concluded in Appendix 3−Appendix 7 of this ISA.

Table IS-9 Characterization of evidence for factors potentially increasing the risk for ozone-related health effects.

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

As discussed in the Preamble to the ISAs (U.S. EPA, 2015), consideration of at-risk populations includes evidence from epidemiologic, controlled human exposure, and animal toxicological studies, in addition to relevant exposure-related information. *Regarding epidemiologic studies, the evaluation focuses on those studies that include stratified analyses to compare populations or lifestages exposed to similar air pollutant concentrations within the same study design along with consideration of the strengths and limitations of each study*. Other epidemiologic studies that do not stratify results but instead examine a specific population or lifestage can provide supporting evidence for the pattern of associations observed in studies that formally examine effect measure modification. Similar to the characterization of

evidence in Appendix 3–Appendix 7, the greatest emphasis is placed on patterns or trends in results across studies. Experimental studies in human subjects or animal models that focus on factors, such as genetic background or health status, are evaluated because they provide coherence and biological plausibility of effects observed in epidemiologic studies. Also evaluated are studies examining whether factors may result in differential exposure to ozone and subsequent increased risk of ozone-related health effects. Conclusions are made with respect to whether a specific factor increases the risk of an ozone-related health effect based on the characterization of evidence using the framework detailed in Table III of the Preamble (U.S. EPA, 2015), and presented in Table IS-9.

IS.4.4.2 Summary of At-Risk Populations

The 2013 Ozone ISA (U.S. EPA, 2013b) concluded that there was adequate evidence to classify individuals with pre-existing asthma, children and older adults, individuals with reduced intake of certain nutrients (i.e., vitamins C and E), and outdoor workers as populations at increased risk to the health effects of ozone. These conclusions were based on the consistency in findings across studies, as well as on coherence of results from different scientific disciplines. Recent studies provide additional evidence that individuals with pre-existing asthma and children are at increased risk of the effects of ozone. There is relatively little recent evidence to add to the evidence presented in the 2013 Ozone ISA for older adults, individuals with reduced intake of certain nutrients, and outdoor workers.

Recent, large multicity epidemiologic studies conducted in the U.S. expand upon evidence from the 2013 Ozone ISA to provide further support the relationship between ozone and ED visits and hospital admissions for asthma among individuals with pre-existing asthma (Table IS-10; Section IS.4.4.3.1).

Generally, studies comparing age groups also reported higher magnitude associations for respiratory hospital admissions and ED visits for children (Section IS.4.4.4.1) than for adults. In addition, recent evidence from studies of nonhuman primates and rodents demonstrate ozone-induced respiratory effects and support the biological plausibility of associations observed in epidemiologic studies between long-term exposure to ozone and the development of asthma in children. Specifically, these experimental studies indicate that early-life ozone exposure can cause structural and functional changes that could potentially contribute to airway obstruction and increased airway responsiveness. Also, children have both higher exposure (due to increased time spent outdoors) and dose (due to their greater ventilation rate). Childrens' respiratory systems are also still undergoing lung growth.

The majority of evidence for older adults being at increased risk of health effects related to ozone exposure comes from studies of short-term ozone exposure and mortality evaluated in the 2013 Ozone ISA (Section IS.4.4.4.2).

Table IS-10 Summary of evidence for populations at increased risk to the health effects of ozone.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA
Adequate evidence		
Pre-existing asthma	Collective evidence from controlled human exposure studies is supported by animal toxicological studies. Some, but not all, epidemiologic studies report greater risk of health effects among individuals with asthma.	Evidence from controlled human exposure and animal toxicological studies provide biological plausibility for the associations observed in epidemiologic studies of short-term ozone exposure and asthma exacerbation. Results from experimental studies in humans demonstrate that ozone exposures lead to increased respiratory symptoms, lung function decrements, increased airway responsiveness, and increased lung inflammation in individuals with asthma.
Children	Controlled human exposure and animal toxicological studies provide evidence of increased risk from ozone exposure for younger ages, which is coherent with findings from epidemiologic studies that report larger associations for respiratory ED visits and hospital admissions for children than adults.	Recent, large multicity epidemiologic studies conducted in the U.S. expand upon previous evidence and support an association between ozone and ED visits and hospital admissions for asthma, which are strongest in children between the ages of 5 and 18; animal toxicological studies in infant monkeys and neonatal rats show that early-life ozone exposure can cause structural and functional changes that could potentially contribute to airway obstruction and increased airway responsiveness.
Older adults	Epidemiologic studies report consistent positive associations between short-term ozone exposure and mortality in older adults.	Controlled human exposure studies demonstrate changes in FEV_1 and FVC among older adults at a relatively light activity level and brief duration of ozone exposure, though these responses are not greater than in other age groups; evidence from studies of metabolic effects is inconsistent.
Outdoor workers	Strong evidence from 2006 Ozone AQCD, which demonstrated increased exposure, dose, and ultimately risk of ozone-related health effects in this population supports that there is adequate evidence to indicate that increased exposure to ozone through outdoor work increases the risk of ozone-related health effects.	No recent information has been evaluated that would inform or change prior conclusions.
Genetic factors	Multiple genetic variants have been observed in epidemiologic and controlled human exposure studies to affect the risk of ozone-related respiratory outcomes and support is provided by animal toxicological studies of genetic factors.	No recent information has been evaluated that would inform or change prior conclusions.
Diet	Individuals with reduced intake of vitamins E and C are at risk for ozone-related respiratory effects based on substantial, consistent evidence both within and among disciplines.	No recent information has been evaluated that would inform or change prior conclusions.

Table IS-10 (Continued): Summary of evidence for populations at increased risk to the health effects of ozone.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA
Suggestive evidence		
Sex	Evidence for increased risk for ozone-related health effects present for females in some studies and males in other studies; some indication that females are increased risk of ozone-related respiratory hospital admissions and ED visits.	No recent information has been evaluated that would inform or change prior conclusions.
Pre-existing obesity	Multiple epidemiologic, controlled human exposure, and animal toxicological studies report increased ozone-related respiratory health effects among obese individuals.	Recent animal toxicological studies expand upon previous evidence and continue to indicate that, compared to lean mice, obese mice exhibit enhanced airway responsiveness and pulmonary inflammation in response to acute ozone exposures.
SES	Most studies report that individuals with low SES and those living in neighborhoods with low SES are more at risk for ozone-related respiratory hospital admissions and ED visits; inconsistent results for mortality and reproductive outcomes.	No recent information has been evaluated that would inform or change prior conclusions.
Inadequate evidence		
Race/ethnicity	A small number of studies provide inadequate evidence that there may be race-related increase in risk of ozone-related health effects for some outcomes.	No recent information has been evaluated that would inform or change prior conclusions.
Pre-existing COPD	Epidemiologic studies indicate that persons with COPD may have increased risk of ozone-related cardiovascular effects, but little information is available on whether COPD leads to an increased risk of ozone-induced respiratory effects.	Small number of recent studies provided inadequate evidence to determine whether COPD results in an increased risk of ozone-related health effects.
Pre-existing CVD	Most short-term exposure studies did not report increased ozone-related cardiovascular morbidity for individuals with pre-existing CVD. Limited number of studies examined whether CVD modifies the association between ozone and respiratory effects. Some evidence that CVD increases risk of ozone-related total mortality.	Some studies provide evidence that cardiovascular disease exacerbates the respiratory effects of ozone exposure; a limited number of recent epidemiologic cohort studies observed increased risk estimates for incident diabetes among those with pre-existing hypertension or among subjects that had some pre-existing condition (MI, COPD, hypertension, or hyperlipidemia) compared to those without pre-existing disease.
Pre-existing diabetes	There are a limited number of epidemiologic studies and lack of controlled human exposure studies or toxicological studies to determine whether pre-existing diabetes modifies ozone effects on health.	A small number of studies provide inadequate evidence that individuals with pre-existing metabolic disease may be at greater risk of mortality associated with long-term ozone exposure.

Table IS-10 (Continued): Summary of evidence for populations at increased risk to the health effects of ozone.

	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA
Smoking	There are a limited number of studies and insufficient coherence for differences in ozone-related health effects by smoking status.	No recent information has been evaluated that would inform or change prior conclusions.

COPD = chronic obstructive pulmonary disease; CVD = cardiovascular disease; ED = emergency department; FEV_1 = forced expiratory volume in 1 second; FVC = forded vital capacity; MI = myocardial infarction; SES = socioeconomic status.

IS.4.4.3 Pre-existing Disease

Individuals with some pre-existing diseases may be at greater risk of an air pollution-related health effect because they may be in a compromised biological state that can vary depending on the disease and severity. The 2013 Ozone ISA (U.S. EPA, 2013b) concluded that there was adequate evidence that those with pre-existing respiratory disease, specifically asthma, were at greater risk for the health effects associated with exposure to ozone, but that evidence was inadequate to determine whether those with COPD, cardiovascular disease, or diabetes were at increased risk of ozone-related health effects. Of the recent epidemiologic studies evaluating effect measure modification by pre-existing disease or condition, most focused on asthma, COPD, or cardiovascular disease. Table IS-11 presents the prevalence of these diseases according to the Centers for Disease Control and Prevention's (CDC's) National Center for Health Statistics (Blackwell et al., 2014), including the proportion of adults with a current diagnosis categorized by age and geographic region. The large proportions of the U.S. population affected by many chronic diseases, including various respiratory and cardiovascular diseases, indicates the potential public health impact, and thus, the importance of identifying populations that may be at increased risk for ozone-related health effects.

Table IS-11 Prevalence of respiratory diseases, cardiovascular diseases, diabetes, and obesity among adults by age and region in the U.S. in 2012.

Chronic Disease/Condition	Adults (18+)	Age (%)[a]				Region (%)[b]			
	N (in thousands)	18−44	45−64	65−74	75+	North-east	Midwest	South	West
All (N, in thousands)	234,921	111,034	82,038	23,760	18,089	42,760	53,378	85,578	53,205
Selected respiratory diseases									
Asthma[c]	18,719	8.1	8.4	7.8	6.0	9.2	8.1	7.3	7.8
COPD—chronic bronchitis	8,658	2.5	4.7	4.9	5.2	3.2	4.4	3.9	2.4
COPD—emphysema	4,108	0.3	2.3	4.7	4.7	1.3	2.0	1.9	1.0
Selected cardiovascular diseases/conditions									
All heart disease	26,561	3.8	12.1	24.4	36.9	10.0	11.6	11.6	9.3
Coronary heart disease	15,281	0.9	7.1	16.2	25.8	5.3	6.5	7.0	5.1
Hypertension	59,830	8.3	33.7	52.3	59.2	21.4	24.1	26.6	21.5
Stroke	6,370	0.6	2.8	6.3	10.7	1.8	2.5	3.0	2.5
Metabolic disorders/conditions									
Diabetes	21,391	2.4	12.7	21.1	19.8	7.6	8.4	10.0	7.3
Obesity (BMI ≥30 kg/m^2)	64,117	26	33.7	29.7	18	25.1	29.9	29.9	25.2
Overweight (BMI 25−30 kg/m^2)	78,455	31.4	36.8	40.7	38.6	34.3	34.1	34.2	35.3

BMI = body mass index; COPD = chronic obstructive pulmonary disease.

[a]Percentage of individual adults within each age group with disease, based on N (at the top of each age column).

[b]Percentage of individual adults (18+) within each geographic region with disease, based on N (at the top of each region column).

[c]Asthma prevalence is reported for “still has asthma.”

Source: Blackwell et al. (2014); National Center for Health Statistics: Data from Tables 1−4, 7, 8, 28, and 29 of the Centers for Disease Control and Prevention report.

IS.4.4.3.1 Pre-existing Asthma

Asthma is the leading chronic illness affecting children. Approximately 8% of adults and 9% of children (age <18 years) in the U.S. currently have asthma (Blackwell et al., 2014; Bloom et al., 2013). Regarding consideration of those with asthma potentially being at increased risk for an ozone-related health effect, it is important to note that individuals with asthma, and children in general, tend to have a higher degree of oronasal breathing, which can result in greater penetration of ozone into the lower respiratory tract.

The 2013 Ozone ISA concluded that there is adequate evidence that individuals with asthma are at increased risk of health effects related to ozone exposure; this conclusion is based on a number of controlled human exposure, epidemiologic, and animal toxicological studies. Consistent with this evidence, recent, large multicity epidemiologic studies conducted in the U.S. expand upon evidence from the 2013 Ozone ISA to provide further support for an association between ozone and ED visits and hospital admissions for asthma. Hospital admission and ED visit studies that presented age-stratified results reported the strongest associations in children between the ages of 5 and 18 years. Additionally, associations were observed across a range of ambient ozone concentrations and were consistent in models where exposure was assigned using either measured or modeled ozone concentrations. While there is a lack of recent epidemiologic studies conducted in the U.S. or Canada that have examined respiratory symptoms and medication use, lung function, and subclinical effects in people with asthma, a large body of evidence from the 2013 Ozone ISA (U.S. EPA, 2013b) reported ozone associations with these less severe indicators of asthma exacerbation that provide support for the ozone-related increases in asthma hospital admissions and ED visits observed in recent studies.

Evidence from controlled human exposure and animal toxicological studies provide biological plausibility for the associations observed in epidemiologic studies of short-term ozone exposure and asthma exacerbation. Results from experimental studies in humans demonstrate that ozone exposures lead to increased respiratory symptoms, decrements in lung function, increased airway responsiveness, and increased lung inflammation in individuals with asthma. However, observed responses across the range of endpoints did not generally differ due to the presence of asthma. Animal toxicological studies similarly found that ozone exposures altered lung function measures, increased airway responsiveness, and increased pulmonary inflammation and bronchoconstriction in allergic animals. In contrast to controlled human exposure studies, there was some evidence from studies of rodents that the observed respiratory effects were enhanced in allergic animals compared to naïve animals.

Overall, recent evidence expands upon evidence available in the 2013 Ozone ISA and is adequate to conclude that individuals with pre-existing asthma are at greater risk of ozone-related health effects based on the substantial and consistent evidence within epidemiologic studies and the coherence with toxicological studies.

IS.4.4.3.2 Pre-existing Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) comprises chronic bronchitis and emphysema and affects approximately 8.6 million adults in the U.S. (Table IS-11). In the U.S., over 4% of adults report having chronic bronchitis and almost 2% report having emphysema (Pleis et al., 2009). Chronic lower respiratory disease, including COPD, was ranked as the third leading cause of death in the U.S. in 2011 (Hoyert and Xu, 2012). Given that people with COPD have compromised respiratory function and underlying respiratory tract inflammation, it is plausible that they could be at increased risk for an array of ozone-related health effects.

Epidemiologic studies evaluated in the 2013 Ozone ISA indicate that individuals with COPD may have increased risk of ozone-related cardiovascular effects, but little information was available on whether COPD leads to an increased risk of ozone-induced respiratory effects. A limited number of recent epidemiologic studies provide inconsistent evidence that individuals with pre-existing COPD could be at greater risk for respiratory health effects associations with ozone exposure. Overall, a limited number of recent studies add to the scarce evidence available in the 2013 Ozone ISA and, collectively, is inadequate to conclude whether or not individuals with pre-existing COPD are at greater risk of ozone-related health effects.

IS.4.4.3.3 Pre-existing Obesity

Obesity, defined as a BMI of 30 kg/m^2 or greater, is an issue of increasing importance in the U.S., with self-reported obesity at 39.8% of the general population in 2016, up from 26.7% in 2009 (Hales et al., 2017). BMI may affect ozone-related health effects through multiple avenues, including systematic inflammation, increased pre-existing disease, and poor diet. Increased risk of air pollution-related health effects has been observed among obese individuals compared with nonobese individuals (U.S. EPA, 2009). The 2013 Ozone ISA concluded that there was suggestive evidence for increased ozone-related respiratory health effects among obese individuals. This conclusion was based on evidence from controlled human exposure studies and epidemiologic studies reporting greater lung function decrements in obese compared with nonobese individuals, as well as enhanced pulmonary inflammation in genetically and dietarily obese mice (U.S. EPA, 2013b).

Recent animal toxicological studies expand the body of evidence evaluated in the 2013 Ozone ISA and continue to indicate that, compared with lean mice, obese mice exhibit enhanced airway responsiveness and pulmonary inflammation in response to acute ozone exposures. In contrast, a recent controlled human exposure study reported evidence of ozone-related increases in pulmonary inflammation in both obese and normal-weight adult women during exercise, but inflammatory responses did not differ between the groups. Overall, recent studies contribute some additional support to the evidence available in the 2013 Ozone ISA and there is suggestive evidence indicating that individuals with pre-existing obesity are at potentially increased risk of ozone-related health effects based on the

limited evidence within epidemiologic studies and some coherence from controlled human exposure and animal toxicological studies.

IS.4.4.3.4 Pre-existing Metabolic Syndrome

Metabolic syndrome is a clinical diagnosis used to describe a collection of risk factors that include high blood pressure, dyslipidemia (elevated triglycerides and low levels of high-density lipoprotein [HDL] cholesterol), obesity (particularly central obesity), and increased fasting blood glucose (Alberti et al., 2009). The presence of these risk factors may predispose an individual to an increased risk of type 2 diabetes and cardiovascular disease. In the 2013 Ozone ISA, a limited number of epidemiologic studies provided inadequate evidence to indicate whether individuals with metabolic syndrome (generally indicated by a diabetes diagnosis) were at an increased risk of ozone-related health effects compared with those without diabetes.

In recent studies of a diabetes-prone mouse model, subacute ozone exposure increased airway inflammation and proinflammatory genes in lung tissue (Section 3.1.6.2). In contrast, an epidemiologic panel study observed a negative association between increased ozone exposure and pulmonary inflammation in adults with type 2 diabetes mellitus. This inverse association may be explained by negative correlations with copollutants that demonstrated strong positive associations with pulmonary inflammation in the same population. Overall, a limited number of recent studies add to the small body of evidence available in the 2013 Ozone ISA and, collectively, the evidence is inadequate to conclude that individuals with pre-existing metabolic disease are at greater risk of ozone-related health effects.

IS.4.4.3.5 Pre-existing Cardiovascular Disease

Cardiovascular disease has become increasingly prevalent in the U.S., with about 12% of adults aged 45−64 years reporting a diagnosis of heart disease (Table IS-11). This number doubles to 24% among adults aged 65−74 years and is even higher for adults aged 75 years and older. A high prevalence of other cardiovascular-related conditions has also been observed, such as hypertension which is prevalent among more than 50% of older adults. In the 2013 Ozone ISA, most epidemiologic studies evaluating short-term ozone exposure did not report increased risk of cardiovascular morbidity for individuals with pre-existing cardiovascular disease. There was some evidence from a limited number of epidemiologic studies that those with pre-existing cardiovascular disease were at greater risk of ozone-related mortality compared with those without pre-existing cardiovascular disease. Overall, the 2013 Ozone ISA concluded that the evidence was inadequate to classify pre-existing cardiovascular disease as a potential at-risk factor for ozone-related health effects.

Several recent studies evaluated respiratory effects of acute ozone exposure (0.2−1 ppm, 3−6 hours) in rodents with cardiovascular disease. Some of the studies provide evidence that

cardiovascular disease exacerbates the respiratory effects of ozone exposure. Injury, inflammation, and oxidative stress measured in the respiratory system, lung function changes, and increased airway responsiveness were documented in animals with cardiovascular disease in response to ozone exposure. Acute ozone exposure in animal models of hypertension resulted in enhanced injury and inflammation measured in the respiratory system, and airway responsiveness compared with healthy animals. A limited number of recent epidemiologic cohort studies evaluated the potential for pre-existing cardiovascular disease to modify associations between long-term ozone exposure and metabolic effects. These studies observed increased risk estimates for incident diabetes among those with pre-existing hypertension or among subjects that had some pre-existing condition (MI, COPD, hypertension, or hyperlipidemia) compared with those without pre-existing disease. Overall, a limited number of recent studies add to the evidence available in the 2013 Ozone ISA and, collectively, are inadequate to conclude whether individuals with pre-existing cardiovascular disease are at greater risk of ozone-related health effects.

IS.4.4.4 Lifestage

Lifestage refers to a distinguishable time frame in an individual's life characterized by unique and relatively stable behavioral and/or physiological characteristics that are associated with development and growth (U.S. EPA, 2014). Differential health effects of ozone across lifestages could be due to several factors. With regard to children, the human respiratory system is not fully developed until 18−20 years of age; therefore, it is biologically plausible for children to have increased intrinsic risk for respiratory effects if exposures are sufficient to contribute to potential perturbations in normal lung development. Moreover, children in general may experience higher exposure to ozone than adults based on more time spent outdoors while exercising during afternoon hours when ozone concentrations may be highest. The ventilation rates also vary between children and adults, particularly during moderate/heavy activity. Children have higher ventilation rates relative to their lung volume, which tends to increase the dose normalized to lung surface area. Older adults, typically considered those 65 years of age or greater, have weakened immune function, impaired healing, decrements in pulmonary and cardiovascular function, and greater prevalence of chronic disease [Table IS-11; Blackwell et al. (2014)], which may contribute to, or worsen, health effects related to ozone exposure. Also, exposure or internal dose of ozone may differ across lifestages due to varying ventilation rates, increased oronasal breathing at rest, and time-activity patterns.

For decades, children, especially those with asthma, and older adults have been identified as populations at increased risk of health effects related to ozone exposure (U.S. EPA, 2013b, 2006a, 1996a). Long-standing evidence from controlled human exposure studies demonstrated that children have greater spirometric responses to ozone compared with middle-aged or older adults (U.S. EPA, 1996a). In addition, epidemiologic studies reported larger associations for respiratory hospital admissions and ED visits for children than for adults, and animal toxicological studies demonstrated ozone-induced health effects in immature animals, including infant monkeys (U.S. EPA, 2013b). Compared with other age

groups, there was evidence for an increased risk of mortality associated with ozone exposure among older adults (U.S. EPA, 2013b, 2006a). The 2013 Ozone ISA concluded that there was adequate evidence that children and older adults are at increased risk of ozone-related health effects.

IS.4.4.4.1 Children

Recent, large multicity epidemiologic studies conducted in the U.S. expand on evidence from the 2013 Ozone ISA and provide further support for an association between short-term ozone exposure and ED visits and hospital admissions for asthma. Hospital admission and ED visit studies that presented age-stratified results reported the strongest associations in children between the ages of 5 and 18 years. The evidence relating new-onset asthma to long-term ozone exposure is supported by toxicological studies in infant monkeys, which indicate that postnatal ozone exposures can lead to the development of asthma. This nonhuman primate evidence of ozone-induced respiratory effects supported the biological plausibility of associations between long-term exposure to ozone and the development of asthma in children observed in epidemiologic studies. Specifically, these experimental studies indicate that early-life ozone exposure can cause structural and functional changes that could potentially contribute to airway obstruction and increased airway responsiveness.

Overall, recent evidence expands upon evidence available in the 2013 Ozone ISA and is adequate to conclude that children are at greater risk of ozone-related health effects based on the substantial and consistent evidence within epidemiologic studies and the coherence with animal toxicological studies.

IS.4.4.4.2 Older Adults

Collectively, the majority of evidence for older adults being at increased risk of health effects related to ozone exposure comes from studies of short-term ozone exposure and mortality. Many of these were evaluated in the 2013 Ozone ISA. As reported in the 1996 and 2006 Ozone AQCDs (U.S. EPA, 2006a, 1996a), decrements in lung function and increases in respiratory symptoms in response to ozone exposure decreased with increasing age. However, whether inflammatory responses persisted with increasing age remained unstudied at the time of the 2013 Ozone ISA (U.S. EPA, 2013b). Two recent controlled human exposure studies demonstrate inflammatory responses in older adults, but it is not possible to quantify inflammatory response as a function of age because of differences in experimental protocols (i.e., duration of exposure to ozone, ozone concentration, activity level, and post-exposure time of sputum collection). A recent controlled human exposure study also demonstrates changes in FEV_1 and FVC among adults aged 55−70 years at a relatively light activity level and brief duration of exposure, but a statistically significant interaction with age was not observed. This is generally consistent with studies evaluated in previous assessments that showed ozone-associated lung function decrements declining with age, but still being present in adults 50−60 years of age. This recent study was conducted at a lower ozone delivery rate, which is more representative of that likely to occur in the ambient environment and shows

small lung function decrements occurring in groups of older adults ranging up to 70 years of age. These recent studies demonstrate that inflammatory responses and lung function changes following ozone exposure can occur in older adults, but do not indicate greater responses in older adults than other age groups.

Overall, recent studies add little to the evidence available in the 2013 Ozone ISA. This evidence is adequate to conclude that older adults are at greater risk of ozone-related health effects.

IS.5 Evaluation of Welfare Effects of Ozone

The scientific evidence for welfare effects of ozone is largely for effects on vegetation and ecosystems and effects on climate. Appendix 8 presents the most policy-relevant information related to this review of the NAAQS for ecological effects of ozone. Appendix 9 presents the most policy-relevant information related to this review of the NAAQS for effects on climate. The framework for causal determinations [see Preamble (U.S. EPA, 2015)] has been applied to the body of scientific evidence to examine effects attributed to ozone exposure. Conclusions from the 2013 Ozone ISA and key findings that inform the current causality determinations for welfare effects of ozone are summarized in Table IS-12.

Table IS-12 Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA[a]
Visible foliar injury Section 8.2	**Causal relationship** Visible foliar injury from ozone exposure was well characterized and documented over several decades of research prior to the 2013 Ozone ISA on sensitive tree, shrub, herbaceous, and crop species in the U.S. Some sensitive species that show visible injury identified in field surveys are verified in controlled exposure settings. Ozone concentrations are high enough to induce visible symptoms in sensitive vegetation.	**Causal relationship** Studies published since the 2013 Ozone ISA strengthen previous conclusions that there is strong evidence that ozone causes foliar injury in a variety of plant species. The use of bioindicators to detect phytotoxic levels of ozone is a longstanding and effective methodology and is supported by more information on sensitive species.

Table IS-12 (Continued): Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA[a]
Reduced vegetation growth Section 8.3	**Causal relationship** Studies added to the evidence from the 2006 AQCD and earlier assessments and indicated that ozone reduced growth of vegetation. Studies from the Aspen FACE experiment showed reduction in total biomass in aspen, paper birch, and sugar maple, findings which were overall consistent with OTC studies in previous NAAQS reviews. Meta-analysis showed ambient ozone concentrations (approx. 40 ppb avg across all hours of exposure) decreased annual total biomass growth of forest species by an avg of 7% with potentially greater exposures with elevated ozone. Studies also demonstrated that ozone alters biomass allocation, generally reducing C allocated to roots.	**Causal relationship** New evidence from controlled exposure experiments and illustration of potential impacts using models built with empirical data strengthen previous conclusions that ozone reduces plant growth and biomass. Additional studies find that ozone significantly changes patterns of carbon allocation below and aboveground.
Reduced plant reproduction Section 8.4	**No separate causality determination; included with plant growth** Evidence from studies that ozone alters reproduction in herbaceous and woody plant species adds to evidence from the 2006 AQCD (primarily in herbaceous and crop species) for ozone effects on metrics of plant reproduction.	**Causal relationship** A new meta-analysis published since the 2013 Ozone ISA provides strong and consistent evidence for negative effects of ozone on plant reproduction. For all exposure categories evaluated, including the lowest exposure category of <40 ppb, between one and eight metrics of reproduction significantly decreased. In addition, more evidence is available that plant reproductive tissues are directly affected by ozone exposure.
Increased tree mortality Section 8.4.3	**Causality not assessed** Evidence built on observations from the 2006 Ozone AQCD of decline of conifer forests over time observed in several regions affected by elevated ozone along with other factors (Valley of Mexico, southern France, Carpathian Mountains). At the Aspen FACE site, there was reduced growth and increased mortality of a sensitive aspen clone.	**Likely to be causal relationship** In a new large-scale multivariate analysis evaluating tree mortality over a 15-year period ozone significantly increased tree mortality in 7 out of 10 plant functional types in the eastern and central U.S. An Aspen FACE study shows that sensitive aspen genotypes have increased mortality compared to tolerant genotypes.
Reduced yield and quality of agricultural crops Section 8.5	**Causal relationship** Detrimental effects of ozone on crop production were recognized since the 1960s. There are well-documented yield losses in a variety of agricultural crops with increasing ozone concentration. Ozone also decreased crop quality. Modeling studies at large geographic scales showed ozone generally reduced crop yield, but effects vary across regions and species.	**Causal relationship** Greenhouse, OTC, FACE, and modeling studies published since the 2013 Ozone ISA strengthen previous conclusions that ozone reduces yield in major U.S. crops including wheat, soybean, and other non-soy legumes. Advances in characterization of ozone effects on U.S. crop yield include further geographic and temporal refinement of ozone sensitivity. For soybean, there are updated exposure-response curves.

Table IS-12 (Continued): Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA[a]
Altered herbivore growth and reproduction Section 8.6	**Causality not assessed** A meta-analysis of 16 studies found that elevated ozone decreased development time and increased pupal mass in insect herbivores. Other field and laboratory studies reported species-level and community-level responses in insects yet the directionality of response to ozone was mixed. This is congruent with findings from the 2006 AQCD and 1996 AQCD, where statistically significant effects on herbivorous insects were observed, but did not provide any consistent pattern of response across growth, reproduction, and mortality endpoints.	**Likely to be causal relationship** There is a large body of evidence showing altered growth and reproduction in insect herbivores. More research has since been published on a range of species and at varying levels of ozone exposure although there is no clear trend in the directionality of response for most metrics. The most commonly measured responses are fecundity, development time, and growth.
Alteration of plant-insect signaling Section 8.7	**Causality not assessed** A few experimental and modeling studies reported altered chemical signaling in insect-plant interactions due to ozone exposure. The effect of ozone on chemical signaling is an emerging area of study that may result in further elucidation of effects with more empirical data.	**Likely to be causal relationship** Laboratory, greenhouse, OTC, and Finnish FACE experiments expand the evidence for altered/degraded emissions of chemical signals from plants and reduced detection of volatile plant signaling compounds by insects, including pollinators, in the presence of ozone. Affected plant-insect interactions include plant defense against herbivory and insect attraction to plants. New evidence includes consistent effects in multiple insect species.
Reduced productivity in terrestrial ecosystems Section 8.8.1	**Causal relationship** Studies from long-term FACE experiments provided evidence of the association of ozone exposure and reduced productivity at the ecosystem scale. Results across different ecosystem models were consistent with the FACE experimental evidence. Models consistently found that ozone exposure negatively impacted indicators of ecosystem productivity. Studies at the leaf and plant scales show that ozone decreased photosynthesis and plant growth, providing coherence and plausibility for reported decreases in ecosystem productivity. Magnitude of response varied among plant communities.	**Causal relationship** Modeling studies and controlled exposure experiments (including Aspen FACE), published since the 2013 Ozone ISA strengthen previous conclusions. Much of the research is confirmatory, with some work providing new mechanistic insight into the effects of ozone on productivity and creating a more nuanced understanding of how these effects vary among species, communities, and environmental conditions.
Reduced carbon sequestration in terrestrial ecosystems Section 8.8	**Likely to be causal relationship** Studies add to the strong and consistent evidence in the 2006 AQCD that ozone decreases plant photosynthesis. Most assessments of the effects of ozone on terrestrial C are from model simulations.	**Likely to be causal relationship** Several new model simulations strengthen previous conclusions from the 2013 Ozone ISA by providing further support for regional and global scale decreases in terrestrial C sequestration from ozone pollution; however, these relationships are spatially and temporally dependent. One empirical study from the Aspen FACE experiment adds to the evidence base for reduced ecosystem C content.

Table IS-12 (Continued): Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA[a]
Alteration of belowground biogeochemical cycles Section 8.9	**Causal relationship** It has been documented since the 2006 Ozone AQCD that while belowground roots and soil organisms are not exposed directly to ozone, belowground processes could be affected by ozone through alterations in the quality and quantity of carbon supply to the soils from photosynthates and litterfall. The 2013 Ozone ISA presented evidence that ozone was found to alter multiple belowground endpoints including root growth, soil food web structure, soil decomposer activities, soil respiration, soil carbon turnover, soil water cycling, and soil nutrient cycling.	**Causal relationship** New evidence confirms conclusions from the 2013 Ozone ISA on effects on soil decomposition, soil carbon, and soil nitrogen. The direction and magnitude of these changes often depends on the species, site, and time of exposure.
Alteration of terrestrial community composition Section 8.10	**Likely to be causal relationship** The body of evidence is for effects on community composition shifts in terrestrial plant communities. For broadleaf forests, the ozone-tolerant aspen clone was the dominant clone at the Aspen FACE site. In grasslands, evidence generally showed shifts from grass-legume mix to grass species. A shift in community composition of bacteria and fungi was observed in both natural and agricultural systems, although no general pattern could be discerned.	**Causal relationship** Recent evidence builds upon the conclusions of the 2013 Ozone ISA by strengthening the understanding of effects of ozone on forest and grassland communities and confirming that effects upon soil microbial communities are diverse. New observational and experimental studies of ozone effects on tree species extend to regional forest composition in the eastern U.S. In grasslands, new studies are consistent with previous research that ozone shifts grassland community composition.
Alteration of ecosystem water cycling Section 8.11	**Likely to be causal relationship** Ozone can affect water use in plants and ecosystems through several mechanisms including damage to stomatal functioning and loss of leaf area. Several field and modeling studies showed an association of ozone exposure and the alteration of water use and cycling in vegetation and ecosystems. Direction of response varied among studies.	**Likely to be causal relationship** New evidence is consistent with the findings in the 2013 Ozone ISA. New evidence identifies a relationship between ozone and wood anatomy associated with water transport. Additional studies add to the evidence base for decreased root growth and density. New empirical and modeling studies continue to show reduced sensitivity of stomatal closing in response to ozone. There are a few studies that scale-up these changes to effects on ecosystem scales including a study linking ozone effects on tree growth and water use to ecosystem stream flow in six watersheds in eastern U.S. forests and from Aspen FACE.
Radiative forcing (RF) Section 9.2	**Causal relationship** The 2013 Ozone ISA reported an RF of 0.35 W/m^2 from tropospheric ozone from preindustrial times to the present (1750 to 2005) based on multimodel studies as reported in the AR4 IPCC assessment.	**Causal relationship** New evidence is consistent with the findings in the 2013 Ozone ISA. The most recent IPCC assessment, AR5, reports tropospheric ozone RF as 0.40 (0.20 to 0.60) W/m^2, which is within range of previous assessments (i.e., AR4). There have also been a few individual modeling studies of tropospheric ozone RF since AR5 which reinforce the AR5 estimates and the causal relationship between tropospheric ozone and RF.

Table IS-12 (Continued): Summary of evidence for welfare effects of ozone.

Endpoint	Conclusions from 2013 Ozone ISA	Conclusions from 2020 ISA[a]
Temperature, precipitation and related climate variables Section 9.3	**Likely to be Causal Relationship** The increase of tropospheric ozone abundance has contributed an estimated 0.1−0.3°C warming to the global climate since 1750 based on studies included in the AR4 IPCC assessment.	**Likely to be Causal Relationship** Consistent with previous estimates, the effect of tropospheric ozone on global surface temperature continues to be estimated at roughly 0.1−0.3°C since preindustrial times, with larger effects regionally. In addition to temperature, ozone changes have impacts on other climate metrics such as precipitation and atmospheric circulation patterns. Current limitations in climate modeling tools, variation across models, and the need for more comprehensive observational data on these effects represent sources of uncertainty in quantifying the precise magnitude of climate responses to ozone changes, particularly at regional scales.

AQCD = Air Quality Criteria Document; AR4 = IPCC Fourth Assessment Report; AR5 = IPCC Fifth Assessment Report; FACE = free-air carbon dioxide enrichment; IPCC = Intergovernmental Panel on Climate Change; NAAQS = National Ambient Air Quality Criteria; OTC = open-top chamber; RF = radiative forcing.

[a]Conclusions from the 2020 ISA include evidence from recent studies integrated with evidence included in previous Ozone ISAs and AQCDs.

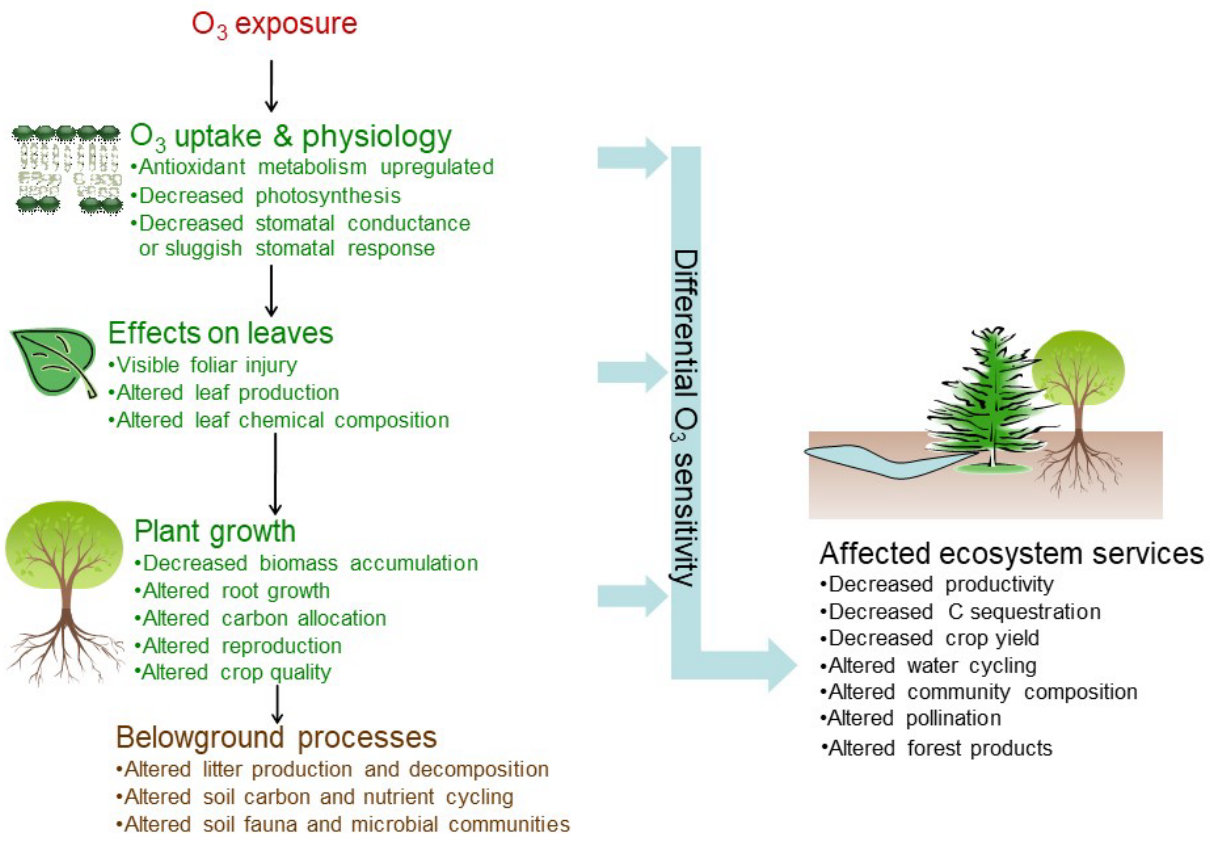
IS.5.1 Ecological Effects

The evidence for ozone effects on vegetation and ecosystems is best understood in the context of some general concepts within ecology. Ecosystems[1] are inherently complex and inter-connected. Ecosystem structure may be described by a variety of measurements used to assess ozone response at different levels of biological organization [i.e., suborganismal, organism, population,[2] community;[3] Suter et al. (2005)]. For example, ozone effects on sensitive species at the whole-plant scale of biological organization (i.e., reduced growth and biomass, reduced plant reproduction, decreased yield) cascade up to effects on population and community structure and ecosystem function (Figure IS-3). "Function" refers to the suite of processes and interactions among the ecosystem components that involve energy or matter. Examples include water dynamics and the flux of trace gases from processes such as photosynthesis, decomposition, or carbon cycling. Ecosystem changes are often considered undesirable if important structural or functional components of the ecosystems are altered following pollutant exposure (U.S. EPA, 2013a, 1998). Methods to assess effects of ozone on ecological structure and function range from indoor controlled environment laboratory and greenhouse studies to field observational studies where biological changes are measured in uncontrolled situations with high natural variability (U.S. EPA, 2015). Free-air carbon dioxide/ozone enrichment (FACE) systems are a more natural way of estimating ozone effects on aboveground and belowground processes. Research conducted at the SoyFACE facility in Illinois (to study responses in soybean fields) and the Aspen FACE (in operation from 1998 to 2011) system in Wisconsin (to study responses in broadleaf forest) have contributed a substantial body of robust evidence that supports the characterization of ozone effects at multiple scales. Experimental methodologies and approaches are summarized in Section 8.1.2.

[1] A functional unit consisting of living organisms (biota), their nonliving environment and the interactions within and between them (IPCC, 2014).

[2] An ecological population consists of interbreeding groups of individuals of the same species that occupy a defined geographic space. Metrics to assess response in ecological populations include changes over time in abundance or density (number of individuals in a defined area), age or sex structure, and production or sustainable rates of harvest (Barnthouse et al., 2008).

[3] Interacting populations of different species occupying a common spatial area form a community (Barnthouse et al., 2008). Community level attributes affected by pollutants include species richness, species abundance, composition, evenness, dominance of one species over another, or size (area) of the community (U.S. EPA, 2013a).



Source: Adapted from U.S. EPA (2013b).

Figure IS-3 Illustrative diagram of ozone effects cascading up through scales of biological organization from the cellular level to plants and ecosystems.

Ozone effects on ecosystems are also inter-connected to human health and well-being. The term "ecosystem services" refers to a concept that ecosystems provide benefits to people, directly or indirectly (Costanza et al., 2017), and these benefits are socially and economically valuable goods and services deserving of protection, restoration, and enhancement (Boyd and Banzhaf, 2007). The concept of ecosystem services recognizes that human well-being and survival are not independent of the rest of nature and that humans are an integral and inter-dependent part of the biosphere. Preservation of ecosystem structure and function contributes to the sustainability of ecosystem services that benefit human welfare and society. Ecosystem services affected by ozone include productivity, carbon sequestration, crop yield, water cycling, pollination, and production of forest commodities (Figure IS-3).

Tropospheric ozone affects terrestrial ecosystems across the entire continuum of biological organization from the cellular and subcellular level to the individual organism up to ecosystem level

processes and services (Figure IS-3). For ozone, the majority of evidence for ecological effects is for vegetation. Damage to terrestrial ecosystems caused by ozone is largely a function of damage to plants, which starts with uptake of ozone into the leaf via stomata (gas exchange openings on leaves). Subsequent reactions with plant tissues produce reactive oxygen species that affect cellular function (Section 8.1.3 and Figure 8-2). Reduced photosynthesis, altered carbon allocation, and impaired stomatal function lead to observable responses in plants. Observed vegetation responses to ozone include visible foliar injury (Section IS.5.1.1); and whole-plant level responses (Section IS.5.1.2) including reduction in aboveground and belowground growth, altered reproduction, and decreased yield. Plant-fauna linkages affected by ozone include herbivores that feed on ozone-damaged plants and interactions mediated by volatile plant signaling compounds (Section IS.5.1.3). Ozone can result in broad changes in ecosystems such as productivity and carbon sequestration (Section IS.5.1.4), belowground processes (Section IS.5.1.5), terrestrial community composition (Section IS.5.1.6), and water cycling (Section IS.5.1.7). Effects of ozone exposure on aboveground and belowground ecosystem components, across trophic levels, and on carbon allocation at multiple scales of biological organization are described for forests (Section IS.5.1.8.1) and grasslands (Section IS.5.1.8.2).

IS.5.1.1 Visible Foliar Injury

In the 2013 Ozone ISA the evidence was sufficient to conclude a causal relationship between ozone exposure and visible foliar injury on sensitive vegetation across the U.S. Visible foliar injury (Figure IS-4) resulting from exposure to ozone has been well characterized and documented in over six decades of research on many tree, shrub, herbaceous, and crop species using both long-term field studies and laboratory approaches (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978; NAPCA, 1970; Richards et al., 1958). Recent experimental evidence continues to show a consistent association between visible injury and ozone exposure (Section 8.2). In a recent global-scale synthesis documenting foliar injury from ozone exposure in the field, across gradients, or in controlled ozone experiments, at least 179 of the identified plant species have populations in the U.S. (Table 8-4). The use of sensitive species as biological indicators to detect phytotoxic levels of ozone is a longstanding and effective methodology. More recently, ozone-sensitive species planted in ozone gardens serve as a source of data on plant responses and as an educational outreach tool. Although visible injury is a bioindicator of the presence of phytotoxic concentrations of ozone in ambient air, it is not always a reliable predictor of other negative effects on vegetation (e.g., growth, reproduction), and foliar injury can vary considerably between and within taxonomic groups (U.S. EPA, 2013b). Since the 2013 Ozone ISA, new sensitive species showing visible foliar injury continue to be identified and the role of modifying factors such as soil moisture and time of day in visible foliar injury symptoms are further characterized (Section 8.2 and Section 8.12). New information is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a "causal relationship" between ozone exposure and visible foliar injury.**

Note: Tulip poplar (*Liriodendron tulipifera*) on the left and black cherry (*Prunus serotina*) on the right.
Source: USDA Plants Database. Forest Service Forest Inventory and Analysis Program.

Figure IS-4 Representative ozone foliar injury in two common tree species in the U.S.

IS.5.1.2 Whole-Plant Effects

The phytotoxicity of tropospheric ozone has been documented for over 50 years in a variety of plant species (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978). Ozone-induced oxidative damage at the biochemical and leaf-level (Figure IS-3) lead to changes in photosynthesis and carbon allocation which scale up to reduced growth and impaired reproduction in individual plants. Plant growth is assessed by quantification of biomass, and analysis of patterns in carbon allocation to aboveground and belowground plant parts. Direct exposure of reproductive tissues to ozone or indirect effects due to injury of vegetative tissues results in fewer total available resources to invest in flowers or seeds. In plants cultivated for agricultural production, damage due to ozone is assessed as reduced crop yield and quality. The evidence supports causal relationships between ozone and plant growth, plant reproduction, and crop yield, and a likely to be causal relationship between ozone and tree mortality. Such relationships indicate detrimental effects of ozone at the individual-organism scale of biological organization.

In the 2013 Ozone ISA the evidence was sufficient to conclude a causal relationship between ozone exposure and reduced growth of native woody and herbaceous vegetation. As reported in previous assessments, ozone has long been known to cause decreases in growth which is documented in many species including herbaceous plants, grasses, shrubs, and trees (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978). In an analysis conducted in the 2013 Ozone ISA, effects on growth from the Aspen FACE site closely agreed with exposure-response functions based on data from earlier OTC experiments (U.S. EPA, 2013b). New controlled exposure experiments consistently demonstrate reduced plant growth, and models

built with empirical data illustrate potential larger-scale impacts (Section 8.3). In support of findings in the 2013 Ozone ISA and prior AQCDs, a recent international synthesis of studies published over the past five decades documents reductions in biomass due to ozone exposure. At least 69 plant species of those documented in the study have populations in the U.S. (Table 8-7). In addition to reduced growth, numerous studies from different ecosystems find ozone significantly changes patterns of carbon allocation below- and aboveground. New evidence from Aspen FACE for effects on growth and biomass of vegetation includes shifts in wood anatomy (e.g., vessel size and density) and altered distribution of roots across the soil profile following long-term exposure to elevated ozone. Biomass allocation within an individual plant is relevant to whole plant growth and function. New studies provide context for scaling up long-known detrimental effects of ozone on photosynthesis and growth on numerous plant species to changes at the community and ecosystem level (Section 8.3.3). New information is consistent with the conclusions of the 2013 Ozone ISA that the **evidence is sufficient to infer a "causal relationship" between ozone exposure and reduced vegetation growth.**

Ozone effects on metrics of plant reproduction (e.g., flower number, fruit number, fruit weight, seed number, rate of seed germination) in multiple experimental settings (e.g., *in vitro*, whole plants in the laboratory, whole plants and/or reproductive structures in the green house, and whole plant communities in the field) reported in the 2006 Ozone AQCD, the 2013 Ozone ISA, and this ISA clearly show ozone reduces plant reproduction [Section 8.4; U.S. EPA (2013b, 2006a)]. A qualitative review in the 2006 Ozone AQCD showed that plant reproductive organs may be particularly sensitive to ozone injury (Black et al., 2000). The biological mechanisms underlying ozone's effect on plant reproduction are twofold. They include both direct negative effects on reproductive tissues and indirect negative effects that result from decreased photosynthesis and other whole-plant physiological changes. Since the 2013 Ozone ISA, a quantitative meta-analysis of >100 independent studies of crop and noncrop species (published from 1968 to 2010) showed statistically significant and sometimes large decreases in reproduction (Leisner and Ainsworth, 2012). Two metrics of plant reproduction, fruit number and fruit weight, show greater reductions under increased ozone when combined across species for ozone concentrations that span 40 to >100 ppb; other metrics do not show such reductions or do so across a narrower range of ozone concentrations. In addition, there is more recent evidence that plant reproductive tissues are directly affected by ozone exposure. There are a few new studies on the effects of ozone on phenology (i.e., timing of germination and flowering), and similar to previously reviewed studies, they have less consistent results than the studies on plant reproduction. In the 2013 Ozone ISA, plant reproduction was considered with plant growth. Increased research and synthesis on ozone effects on plant reproduction (Table 8-9) warrants a separate causality category and evidence is now **sufficient to infer a "causal relationship" between ozone exposure and reduced plant reproduction.**

Multiple studies from different research groups show the co-occurrence of ozone exposure and increased mortality of trees (Section 8.4.3 and Table 8-10). Evidence for plants other than trees is currently lacking. Studies linking ozone and tree mortality are consistent with known and well-established individual plant-level mechanisms that explain ozone phytotoxicity, including variation in sensitivity and

tolerance based on age class, genotype, and species. Increased mortality is also consistent with effects at higher levels of biological organization, including changes in vegetation cover and altered community composition (Section 8.10). Since the 2013 Ozone ISA, a large-scale empirical analysis was conducted of factors contributing to annual mortality of trees using over three decades of Forest Inventory and Analysis data. This U.S. Forest Service data showed a significant positive correlation between 8-hour max ozone concentration and tree mortality. Ozone significantly increased tree mortality in 7 out of 10 plant functional types in the eastern and central U.S. (Dietze and Moorcroft, 2011). Experimentally, elevated ozone exposure has been shown to increase mortality in sensitive aspen genotypes (Moran and Kubiske, 2013). This evidence is considered with studies from the 2006 AQCD and 2013 Ozone ISA where decline of conifer forests under ozone exposure was continually observed in several regions [Valley of Mexico, southern France, Carpathian Mountains; U.S. EPA (2013b, 2006a)]. Previous evidence and new evidence evaluated here **is sufficient to infer a "likely to be causal relationship" between ozone exposure and tree mortality.**

In the 2013 Ozone ISA, the evidence was sufficient to conclude a causal relationship between ozone exposure and reduced yield and quality of agricultural crops. The detrimental effect of ozone on crop production has been recognized since the 1960s, and a large body of research has subsequently characterized decreases in yield and quality of a variety of agricultural and forage crops (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978). The 1986 Ozone AQCD and 1996 Ozone AQCD reported new OTC experiments on growth and yield, including U.S. EPA's National Crop Loss Assessment Network (NCLAN), that served at the basis for exposure-response functions for agricultural crop species (U.S. EPA, 1996b, 1986). As in noncrop plants, the concentrations at which damage is observed vary from species to species and sometimes between genotypes of the same species.

There is a considerable amount of new research on major U.S. crops, especially soybean, wheat, and other non-soy legumes at concentrations of ozone occurring in the environment (Section 8.5). For soybean, further refinement of exposure-response curves and analysis of yield data identified a critical level of 32 ppb (7-hour seasonal mean) at which a 5% loss can occur (Osborne et al., 2016). At SoyFACE, a linear decrease in yield at the rate of 37 to 39 kg per hectare per ppb ozone exposure over 40 ppb (AOT40) was observed across two growing seasons (Betzelberger et al., 2012). Meta-analyses published since the 2013 Ozone ISA provide further supporting evidence that current levels of ambient ozone decrease wheat growth and yield and affect reproductive and developmental plant traits important to agricultural and horticultural production (Section 8.5). Recent advances in characterizing ozone's effects on U.S. crop yield include further geographic and temporal refinement of ozone sensitivity and national-scale estimates of crop losses attributable to ozone. Previous research highlighted in the 2013 Ozone ISA and previous AQCDs show ozone effects on crop yield and crop quality (U.S. EPA, 2013b, 2006a, 1996a, 1986, 1978). New information is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a "causal relationship" between ozone exposure and reduced yield and quality of agricultural crops.**

IS.5.1.3 Effects on Plant-Fauna Interactions

In addition to detrimental effects on plants, elevated ozone can alter ecological interactions between plants and other species, including (1) herbivores consuming ozone-exposed vegetation, (2) pollinators and seed dispersers, and (3) predators and parasitoids of insect herbivores. Many of these interactions are mediated through volatile plant signaling compounds (VPSCs), which plants use to signal to other community members (Section 8.7). Elevated tropospheric ozone has been shown to alter the production, emission, dispersion, and lifespan of VPSCs thereby reducing the effectiveness of these signals. VPSCs play an important role in attracting pollinators, and their alteration can affect the crucial ecosystem service of pollination of wild plants and crops. Ozone exposure also modifies chemistry and nutrient content of leaves (U.S. EPA, 2013b), which may affect the physiology and behavior of herbivores (Section 8.6).

Previous ozone assessments have evaluated studies examining ozone-insect-plant interactions and found information on a wide range of insect species studied in the orders Coleoptera (weevils, beetles), Hemiptera (aphids), and Lepidoptera [moths, butterflies; U.S. EPA (2013b, 2006a, 1996b)]. The majority of studies focused on growth and reproduction while fewer studies considered herbivore survival and population- and community-level responses to ozone. Although statistically significant effects were frequently observed, they did not provide any consistent pattern of response across growth, reproduction, and mortality endpoints. Research has since been published on additional species and at varying levels of ozone exposure, although there is no clear trend in the directionality of response for most effects (Section 8.6). The most commonly measured responses are fecundity, development time, growth, and feeding preferences (Table 8-14). The strongest evidence of ozone effects is from herbivorous insects with limited evidence from vertebrate feeding studies. Changes in nutrient content and leaf chemistry following ozone exposure likely account for observed effects in herbivores. The body of evidence is **sufficient to infer a "likely to be causal relationship" between ozone exposure and alteration of herbivore growth and reproduction.**

In the 2013 Ozone ISA, a few experimental and modeling studies reported altered insect-plant interactions that are mediated through chemical signaling (U.S. EPA, 2013b). New empirical research from laboratory, greenhouse, OTC, and FACE experiments expand the evidence for altered/degraded emissions of chemical signals from plants and reduced detection of volatile plant signaling compounds by insects, including pollinators, in the presence of ozone (Section 8.7 and Table 8-17). New evidence includes consistent effects in multiple insect species, although this research has examined only a small fraction of the total number of chemical-signaling responses potentially affected by ozone. Elevated ozone (≥50 ppb) degrades some plant VPSCs, changing the floral scent composition and reducing floral scent dispersion. Preference studies in a few insect species show reduced pollinator attraction, decreased plant host detection, and altered plant host preference in the presence of elevated, yet environmentally relevant ozone concentrations. Exposure to elevated ozone had variable effects on VPSCs emissions and on the stability of individual volatile compounds with potentially important ecological implications for

plant-insect signaling involved in defense against herbivory. To attract predators and parasitoids that target phytophagous insects, plants emit more VPSCs. Parasitoid-host attraction was either reduced, enhanced, or unaffected by elevated ozone. **The body of evidence is sufficient to infer a "likely to be causal relationship" between ozone exposure and alteration of plant-insect signaling.**

IS.5.1.4 Reduced Productivity and Carbon Sequestration

The evidence in the 2013 Ozone ISA was sufficient to conclude a causal relationship between ozone exposure and reduced plant productivity (U.S. EPA, 2013b). Studies at the leaf and plant scale show that ozone decreases plant growth, providing biological plausibility for decreases in ecosystem productivity. Evidence of decreased ecosystem productivity from ozone exposure comes from many different experiments with different study designs in a variety of ecosystems: OTC experiments; long-term, ecosystem-manipulation, chamberless exposure experiments (Aspen FACE, SoyFACE, FinnishFACE); empirical models using eddy covariance measures; forest productivity models parameterized with empirical physiological and tree life history data; and various well-studied ecosystem models and scenario analysis (Section 8.8.1). New information is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a "causal relationship" between ozone exposure and reduced productivity in terrestrial ecosystems.**

The evidence in the 2013 Ozone ISA was sufficient to conclude a likely causal relationship between ozone exposure and decreased terrestrial carbon sequestration (U.S. EPA, 2013b). Ozone-mediated changes in plant carbon budgets result in less carbon available for allocation to various pools: reproductive organs, leaves, stems, storage, and roots as well as maintenance, defense, and repair. Changes in allocation (Section 8.8.3) can scale up to population- and ecosystem-level effects, including changes in soil biogeochemical cycling (Section 8.9), increased tree mortality (Section 8.4.3), shifts in community composition (Section 8.10), changes to species interactions (Section 8.6), declines in ecosystem productivity and carbon sequestration (Section 8.8), and alteration of ecosystem water cycling (Section 8.11). The relationship between ozone exposure and terrestrial C sequestration is difficult to measure at the landscape scale. Most of the evidence regarding this relationship is from model simulations, although this endpoint was also examined in a long-term manipulative chamberless ecosystem experiment (Aspen FACE). For example, experiments at Aspen FACE found ozone exposure caused a 10% decrease in cumulative (Net Primary Production) and an associated 9% decrease in ecosystem C storage, although the effects of ozone gradually disappeared towards the end of the 10-year exposure (Talhelm et al., 2014; Zak et al., 2011) possibly due to loss of ozone-sensitive individuals and lower ozone exposures in the last 3 years. Additional studies at this research site suggests that the effects of ozone on plant productivity will be paralleled by large and meaningful decrease in soil C, but the experimental observations reviewed did not find a direct link between ozone, NPP, and soil C pools. It is likely that stand age and development and disturbance regimes are complicating factors in the partitioning of ecosystem-level effects of ozone exposure on carbon sequestration. Even with these limitations, the

results from the Aspen FACE experiment and the model simulations provide further evidence that is consistent with the conclusions of the 2013 Ozone ISA that **the body of evidence is sufficient to infer a "likely to be causal relationship" between ozone exposure and reduced carbon sequestration in ecosystems.**

IS.5.1.5 Belowground Processes/Biogeochemical Cycles

In the 2013 Ozone ISA, the evidence was sufficient to conclude that there is a causal relationship between ozone exposure and the alteration of belowground biogeochemical cycles (U.S. EPA, 2013b). It has been documented since the 2006 Ozone AQCD (U.S. EPA, 2006a) that while belowground roots and soil organisms are not exposed directly to ozone, below-ground processes can be affected by ozone through alterations in the quality and quantity of carbon supply to the soils from photosynthates and litterfall (Andersen, 2003). The 2013 Ozone ISA presented evidence that ozone was found to alter multiple belowground endpoints including root growth, soil food web structure, soil decomposer activities, soil respiration, soil carbon turnover, soil water cycling, and soil nutrient cycling. The new evidence since the 2013 Ozone ISA (U.S. EPA, 2013b) included in this assessment confirms ozone effects on soil decomposition (Section 8.9.1), soil carbon (Section 8.9.2), and soil nitrogen (Section 8.9.3), although the direction and magnitude of these changes often depends on the species, site, and length of exposure. As in the 2013 Ozone ISA, the evidence is **sufficient to conclude that there is a "causal relationship" between ozone exposure and the alteration of belowground biogeochemical cycles.**

IS.5.1.6 Terrestrial Community Composition

In the 2013 Ozone ISA, the evidence was sufficient to conclude that there is a likely causal relationship between ozone exposure and the alteration of community composition of some ecosystems, including conifer forests, broadleaf forests, and grasslands, and altered fungal and bacterial communities in the soil in both natural and agricultural systems (U.S. EPA, 2013b). Ozone effects on individual plants can alter the larger plant community as well as the belowground community of microbes and invertebrates, which depend on plants as carbon sources. Ozone may alter community composition by having uneven effects on co-occurring species, decreasing the abundance of sensitive species and giving tolerant species a competitive advantage. Key new studies (Wang et al., 2016; Gustafson et al., 2013) model ozone effects on regional forest composition in the eastern U.S. Additionally, a global-scale synthesis of decades of research on an array of ozone effects on plants confirms that some plant families (e.g., Myrtaceae, Salicaceae, and Onagraceae) are more susceptible to ozone damage than others (Bergmann et al., 2017). This lends biological plausibility to a mechanism by which elevated ozone alters terrestrial community composition by inhibiting or removing ozone-sensitive plant species or genotypes, which alters competitive interaction to favor the growth or abundance of ozone-tolerant species or genotypes. In grasslands, previous evidence included multiple studies from multiple research groups to

show that elevated ozone shifts the balance among grasses, forbs, and legumes (Section 8.10.1.2). There are new studies with findings consistent with earlier research (Section 8.10), including new studies from European grasslands that found exposure-response relationships between ozone and community composition. The 2013 Ozone ISA presented multiple lines of evidence that elevated ozone alters terrestrial community composition, and recent evidence strengthens our understanding of the effects of ozone upon plant communities, while confirming that the effects of ozone on soil microbial communities are diverse (Table 8-20). The evidence is **sufficient to conclude that there is a "causal relationship" between ozone exposure and the alteration of community composition of some ecosystems.**

IS.5.1.7 Ecosystem Water Cycling

In the 2013 Ozone ISA, the evidence was sufficient to conclude a likely causal relationship between ozone exposure and the alteration of ecosystem water cycling (U.S. EPA, 2013b). Ozone can affect water use in plants and ecosystems through several mechanisms, including damage to stomatal functioning and loss of leaf area, which may affect plant and stand evapotranspiration and lead, in turn, to possible effects on hydrological cycling. Although the 2013 Ozone ISA found no clear universal consensus on leaf-level stomatal conductance response to ozone exposure, many studies reported incomplete stomatal closure and loss of stomatal control in several plant species, which result in increased plant water loss [Section 9.4.5; U.S. EPA (2013b)]. Additionally, ozone has been found to alter plant water use through decreasing leaf area index, accelerating leaf senescence, and by causing changes in branch architecture, which can significantly affect stand-level water cycling. There is mounting biologically relevant, statistically significant, and coherent evidence from multiple studies of various types about the mechanisms of ozone effects on plant water use and ecosystem water cycling (reduced leaf area, reduced leaf longevity, changes in root and branch biomass and architecture, changes in vessel anatomy, stomatal dysfunction, reduced sap flow; [Section 8.11]). Additionally, there are a few strong studies that scale up these changes to effects on ecosystem scales and show significant effects. The most compelling evidence is from six watersheds in eastern forests and from Aspen FACE (Kostiainen et al., 2014; Sun et al., 2012). This new information adds to the evidence base in the 2013 Ozone ISA and supports the conclusion that **the body of evidence is sufficient to infer a "likely to be causal relationship" between ozone exposure and the alteration of ecosystem water cycling.**

IS.5.1.8 Integration of Ozone Effects in Ecosystems

IS.5.1.8.1 orests

The effects of ozone exposure on U.S. forests have been an active area of research for over 50 years; evaluation of the role of ozone in forest health declines in the mixed conifer forest of the San

Bernardino Mountains began in the early 1960s (Miller and McBride, 1999). Since that time, studies have confirmed variation in sensitivity to ozone exposure in trees and plants based on age class, genotype, and species (U.S. EPA, 2013b, 2006a, 1996b). There has been strong and consistent evidence from multiple studies that ozone-induced oxidative damage leads to declines in photosynthesis and carbon gain, which scale up to reduced growth in individual plants [Section 8.3; U.S. EPA (2013b, 2006a, 1996b)]. For example, studies from the Aspen FACE experiment have shown that ozone caused reduction in total biomass in quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), and sugar maple [*Acer saccharum*; U.S. EPA (2013b)]. These findings were overall consistent with open-top chamber studies that established ozone exposure-response relationships on growth in a number of native U.S. tree species detailed in previous NAAQS reviews (U.S. EPA, 2013b); these species include aspen, black cherry (*Prunus serotina*), tulip poplar (*Liriodendron tulipifera*), white pine (*Pinus strobus*), and ponderosa pine (*Pinus ponderosa*). In addition to overall reductions in growth, there is evidence that ozone changes plant growth patterns by significantly reducing root growth in some tree species. New information reviewed in the current document support earlier conclusions that ozone reduces photosynthesis, growth, and carbon allocation in a number of plant species found in forest ecosystems.

In addition to declines in root carbon allocation, results from Aspen FACE and other experimental studies reviewed in the 2013 Ozone ISA consistently found that ozone exposure reduced litter production and altered leaf chemistry in trees (U.S. EPA, 2013b). These direct effects of ozone on plants may lead to changes in soil properties and processes in forests, but these changes are dependent on species and genotype of community members, and potentially on other factors like the stage of stand development.

Ozone effects on tree water use can also scale up to significant and measurable effects on ecosystem water cycling in forests. Ozone-mediated impairment of stomatal function in plants has been documented for decades (Keller and Häsler, 1984), although impairment seems to be species specific. Studies continue to show reduced sensitivity of stomatal closing in response to various factors (light, vapor pressure deficit, temperature, soil moisture) when exposed to ozone ("sluggish stomata") in a number of species. A recent meta-analysis of ozone effects on stomatal response in 68 species (including trees, crops, and grassland) found that trees were the most adversely affected, with 73% showing an altered stomatal response. In this synthesis, 4 tree species exhibited sluggish stomata and 13 showed stomatal opening in response to ozone (Mills et al., 2016; Mills et al., 2013). Ozone exposure has also been linked to decreased water use efficiency and changes in sap flow (Mclaughlin et al., 2007a; Mclaughlin et al., 2007b) and to reduced late-season stream flow in eastern forest ecosystems (Sun et al., 2012).

Differences between species in ozone sensitivity leads to significant changes in forest community composition, as ozone sensitive trees decline and are replaced by less sensitive ones (Section 8.10.1.1). Species-specific responses to ozone in terms of plant growth reductions and biomass allocation are a possible mechanism for these community shifts. In a model simulation of long-term effects of ozone on a

typical forest in the southeastern U.S. involving different tree species with varying ozone sensitivity, Wang et al. (2016) found that ozone significantly altered forest community composition and decreased plant biodiversity. Models using Aspen FACE data confirm that ozone effects on tree biomass and productivity scale to affect community composition at the genotype and species level (Moran and Kubiske, 2013). In simulations using Aspen FACE data of northern forests at the landscape level over centuries, elevated ozone altered species abundance and the speed of replacement and succession (Gustafson et al., 2013). Multiple studies from different research groups show the co-occurrence of ozone exposure and increased mortality of trees (Section 8.4.3). In a Bayesian empirical model built with field measurement data from the U.S. Forest Service's Forest Inventory and Analysis program, ozone significantly increased tree mortality in 7 out of 10 plant functional types in the eastern and central U.S. (Dietze and Moorcroft, 2011).

IS.5.1.8.2 Grasslands

In grassland ecosystems, herbaceous plants and grasses in particular are the dominant vegetation rather than shrubs or trees. There is a wide range of sensitivity to ozone in grassland plant communities. For example, studies going back to the 1996 Ozone AQCD show varying ozone sensitivity within the genus *Trifolium* (clover) and general shifts in community biomass that favors grass species (U.S. EPA, 1996a). Evidence reviewed in the 2013 Ozone ISA from a large-scale ozone fumigation experiment in grasslands demonstrated ozone decreases gross primary productivity in these systems (Volk et al., 2011). Experiments reviewed in the 2013 Ozone ISA and previous AQCDs and the current Ozone ISA generally show ozone associated with biomass loss, and a decrease in nutritive quality of forage species. Further, ozone responses differed across species of grassland plants (Volk et al., 2006). Ozone effects on seed production, germination, and flower number and date of peak flowering have been demonstrated in representative grassland species (Section 8.4).

In grasslands, ozone effects on biodiversity or species composition may result from competitive interactions among plants in mixed-species communities. Studies from mesocosm, OTC, and FACE experiments generally show a shift in the biomass from grass-legume mixtures over time, in favor of grass species. There are also new studies from European grasslands that found exposure-response relationships for community composition (Section IS.5.1.9) that included some species that also grow in the U.S. In the 2013 Ozone ISA, a review of ozone sensitive plant communities [identified as sensitive if they had six or more species that exhibited significant ozone-caused changes in biomass in peer-reviewed controlled experiments; Mills et al. (2007)] found that the largest number of these sensitive communities were associated with grassland ecosystems (U.S. EPA, 2013b). Among grassland ecosystems, alpine grassland, subalpine grassland, woodland fringe, and dry grassland were identified as the most ozone-sensitive communities. Ozone effects on grassland ecosystems extend belowground to the associated soil microbial communities (Section 8.10.2), which show changes in proportions of bacteria or fungi in response to elevated ozone and to fauna that feed on grassland vegetation.

IS.5.1.9 Exposure-Response Relationships

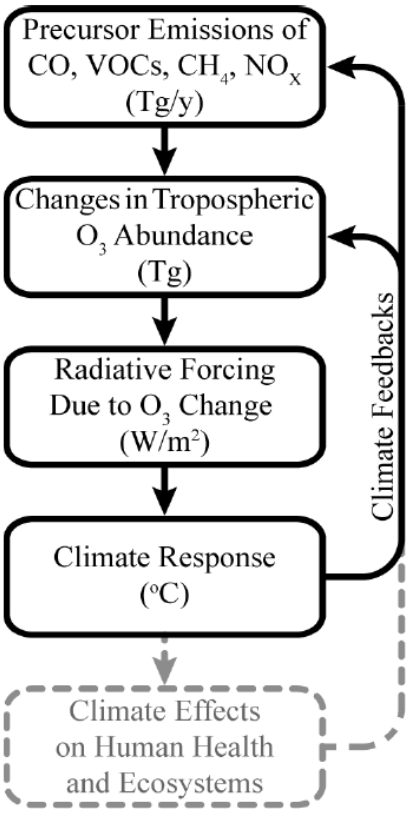
For over 40 years, controlled ozone exposure experiments have yielded a wealth of information on exposure-response relationships. Ozone exposure response has been demonstrated in many tree and herbaceous species, including crops (U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978). As described in Section IS.3.2, various indices have been used to quantify ozone exposure in plants, including threshold-weighted (e.g., SUM06) and continuous sigmoid-weighted (e.g., W126) functions. Weighting of cumulative indices takes into account the greater effects of ozone on vegetation with elevated ozone concentrations. As ozone concentrations increase, plant defense mechanisms are overwhelmed and the capacity of the plant to detoxify reactive oxygen species is compromised (U.S. EPA, 2013b). For decades, it has also been well characterized that plant sensitivity varies by time of day and development stage. Growth responses vary depending on the growth stage of the plant. Furthermore, the time of highest ozone concentrations may not occur at the time of maximum plant uptake. Weighted hourly concentrations during the daylight hours and during the growing season are the most important variables in a cumulative exposure index (U.S. EPA, 2013b). For vegetation, quantifying exposure with indices that accumulate the ozone hourly concentrations and preferentially weight the higher concentrations improves the explanatory power of exposure for effects on growth and yield, compared with using indices based on mean and peak exposure values.

None of the information on the effects of ozone on vegetation published since the 2013 Ozone ISA has modified conclusions on quantitative exposure-response relationships. Since the 2013 Ozone ISA, there have been a few new experimental studies that add more exposure-response relationship information to the large historical database available on U.S. plants (Section 8.13.2). In a new experimental study, Betzelberger et al. (2012) studied seven cultivars of soybean at the SoyFACE experiment in Illinois. They found that the cultivars showed similar responses in a range of ozone exposures expressed as AOT40 (Section IS.3.2). These results support conclusions of previous studies (Betzelberger et al., 2010) and the 2013 Ozone ISA that sensitivity of current soybean genotypes is not different from early genotypes; therefore, soybean response functions developed in the NCLAN program remain valid. A study by Neufeld et al. (2018) provided information on foliar injury response on two varieties of cutleaf coneflower (*Rudbeckia laciniata*). For example, one variety had statistically detectable foliar injury when the 24-hour W126 index reached 23 ppm hour (12-hour AOT40 = 12 ppm hour). Although recent U.S. exposure-response studies in experimental systems are limited, U.S. and international syntheses have highlighted response function information (e.g., biomass growth, foliar injury, yield) for grassland and other plant species that occur in the U.S. (see Section 8.13.2). For example, in a synthesis of previously published studies, linear relationships of biomass growth in response to ozone were found using AOT40 for 87 grassland species that occur in Europe (van Goethem et al., 2013). Seventeen of these species are native to the U.S. and 65 additional species have been introduced to the U.S. and may have significant ecological, horticultural, or agricultural value (USDA, 2015). This study has the most significant amount of new exposure response information for plants in the U.S.

IS.5.2 Effects on Climate

Changes in the abundance of tropospheric ozone perturb the radiative balance of the atmosphere by interacting with incoming solar radiation and outgoing longwave radiation. This effect is quantified by the radiative forcing metric. Radiative forcing is the perturbation in net radiative flux at the tropopause (or top of the atmosphere) caused by a change in radiatively active forcing agent(s) after stratospheric temperatures have readjusted to radiative equilibrium (stratospherically adjusted radiative forcing). Through this effect on the Earth's radiation balance, tropospheric ozone plays a major role in the climate system, and increases in its ozone abundance contribute to climate change (Myhre et al., 2013).

For ozone effects on climate (Appendix 9), there are inter-connections to human health and ecosystems. As discussed in the 2013 Ozone ISA, the Earth's atmosphere-ocean system responds to changes in radiative forcing with a climate response, including a change in near-surface air temperature with associated impacts on precipitation and atmospheric circulation patterns. This climate response causes downstream climate-related health and ecosystem effects, such as the combined health effects of both climate (e.g., heat waves) and ozone air quality or redistribution of diseases or ecosystem characteristics. Feedbacks from both the direct climate response and such downstream effects can, in turn, affect the abundance of tropospheric ozone and ozone precursors through multiple mechanisms (Figure IS-5). Variations in climate can potentially alter the conditions that lead to the formation, transport, and persistence of ozone in the troposphere (Appendix 1), as well as increase the vulnerability of plants and ecosystems. The degree to which climate and weather alter the effects of ozone is context and species specific because damage to terrestrial ecosystems caused by ozone is largely a function of plant uptake. Factors that modify the effects of ozone in ecosystems, including carbon dioxide, weather, and climate are discussed in Section 8.12.



Source: U.S. EPA (2013b).

Figure IS-5 Schematic illustrating the effects of tropospheric ozone on climate; including the relationship between precursor emissions, tropospheric ozone abundance, radiative forcing, climate response and climate impacts.

Characterization of ozone impacts on radiative forcing (Section 9.2) builds on the findings in the 2013 Ozone ISA and draws heavily on the IPCC Assessment Reports. In the 2013 Ozone ISA, the evidence was sufficient to conclude a causal relationship between tropospheric ozone and radiative forcing (U.S. EPA, 2013b). The 2013 Ozone ISA reported a radiative forcing (RF) of 0.35 W/m^2 from the change in global tropospheric ozone abundance from preindustrial times to the present (1750 to 2005) based on multimodel studies (Forster et al., 2007). The most recent IPCC assessment, AR5, reports global tropospheric ozone RF as 0.40 (0.20 to 0.60) W/m^2 (Myhre et al., 2013), which is within range of previous assessments (i.e., AR4). There have also been a few individual studies of tropospheric ozone RF (Section 9.2) since AR5, including the study of tropospheric ozone RF based on the Coupled Model Intercomparison Project Phase 6 (CMIP6) data set, and the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP) multimodel study of tropospheric chemistry, all of which reinforce

the AR5 estimates and continues to support **a "causal relationship" between tropospheric ozone and RF.**

In the 2013 Ozone ISA, the evidence was sufficient to conclude a likely to be causal relationship, via radiative forcing, between tropospheric ozone and climate change (now referred to as "temperature, precipitation, and related climate variables"; the revised title for this causality statement provides a more accurate reflection of the available evidence) (U.S. EPA, 2013b). New studies reviewed in Section 9.3 are consistent with previous estimates and the effect of global, total tropospheric ozone increases on global mean surface temperature continues to be estimated at roughly 0.1−0.3°C since preindustrial times (Xie et al., 2016; Myhre et al., 2013), with larger effects regionally. In addition to temperature, ozone changes affect other climate metrics such as precipitation and atmospheric circulation patterns (Macintosh et al., 2016; Allen et al., 2012; Shindell et al., 2012). All of this evidence reinforces **a "likely to be causal relationship" between temperature, precipitation, and related climate variables.**

IS.6 Key Aspects of Health and Welfare Effects Evidence

There is extensive scientific evidence that demonstrates health and welfare effects from exposure to ozone. In assessing the older and more recent evidence, the U.S. EPA characterizes the key strengths and remaining limitations of this evidence. In the process of assessing the evidence across studies and scientific disciplines and ultimately forming causality determinations, the U.S. EPA takes into consideration multiple aspects that build upon the Hill criteria (Hill, 1965) and include, but are not limited to, consistency in findings, coherence of findings, and evidence of biological plausibility [see U.S. EPA (2015)]. As documented by the extensive evaluation of evidence throughout the subsequent Appendices to this ISA, the U.S. EPA carefully considers uncertainties in the evidence, and the extent to which recent studies have addressed or reduced uncertainties from previous assessments, as well as the strengths of the evidence. Uncertainties considered in the epidemiologic evidence, for example, include potential confounding by copollutants or covarying factors and exposure error. The U.S. EPA evaluates many other important considerations (not uncertainties) such as coherence of evidence from animal and human studies, heterogeneity of risk estimates, and the shape of the concentration-response relationships. All aspects are considered along with the degree to which chance, confounding, and other biases affect interpretation of the scientific evidence in the process of drawing scientific conclusions and making causality determinations. Uncertainties do not necessarily change the fundamental conclusions of the literature base. In fact, some conclusions may be robust to such uncertainties. Where there is clear evidence linking ozone with health and welfare effects with or despite minimal remaining uncertainties, the U.S. EPA makes a determination of a causal or likely to be causal relationship.

IS.6.1 Health Effects Evidence: Key Findings

A large body of scientific evidence spanning many decades clearly demonstrates there are health effects related to both short- and long-term ozone exposure (Figure IS-6). The strongest evidence supports a relationship between ozone exposure and respiratory health effects. The collective body of evidence for each health outcome category evaluated in this ISA is systematically considered and assessed, including the inherent strengths, limitations, and uncertainties in the overall body of evidence, resulting in the causality determinations detailed in Table IS-1. Through identification of the strengths and limitations in the evidence, this ISA may help in the prioritization of research efforts to support future ozone NAAQS reviews.

An inherent strength of the evidence integration in this ISA is the extensive amount (in both breadth and depth) of available evidence resulting from decades of scientific research that describes the relationship between both short- and long-term ozone exposure and health effects. The breadth of the enormous database is illustrated by the different scientific disciplines that provide evidence (e.g., controlled human exposure, epidemiologic, animal toxicological studies), the range of health outcomes examined (e.g., respiratory, cardiovascular, metabolic, reproductive, and nervous system effects, as well as cancer and mortality), and the large number of studies within several of these outcome categories. The depth of the literature base is exemplified by the examination of effects that range from biomarkers of exposure, to subclinical effects, to overt clinical effects, and even mortality. Depth is further demonstrated through the variety of the study designs used across the scientific disciplines and exposure duration periods.

In this ISA, systematic review methodologies are applied to identify and characterize this expansive evidence base (see Appendix 10 for details). The evidence is integrated from (1) a variety of study designs within the same scientific discipline, (2) different scientific disciplines, and (3) a span of different health endpoints within a health effect category. Finally, a formal framework is applied systematically to draw conclusions about the causal nature of the relationship between ozone exposure and health effects (U.S. EPA, 2015).

A first step in integrating evidence for a health effect category is to consider the biological plausibility of health responses observed in association with ozone exposure. The process for characterizing biological plausibility is described in Section IS.4.2. Recent studies in humans and animals expand on findings from prior assessments (U.S. EPA, 2013b, 2006a, 1996a) to further understand plausible pathways that may underlie the observed respiratory health effects related to short-term exposure to ozone (Figure 3-1). Consistent evidence for several respiratory endpoints within a large number of animal toxicological, controlled human exposure, and epidemiologic studies, as well as coherent evidence across these studies contribute to a large degree of certainty in assessing the relationship between short-term ozone exposure and this health effect category. Furthermore, uncertainty is addressed by epidemiologic studies that examine potential copollutant confounding, examine different model specifications, and account for potential confounders.

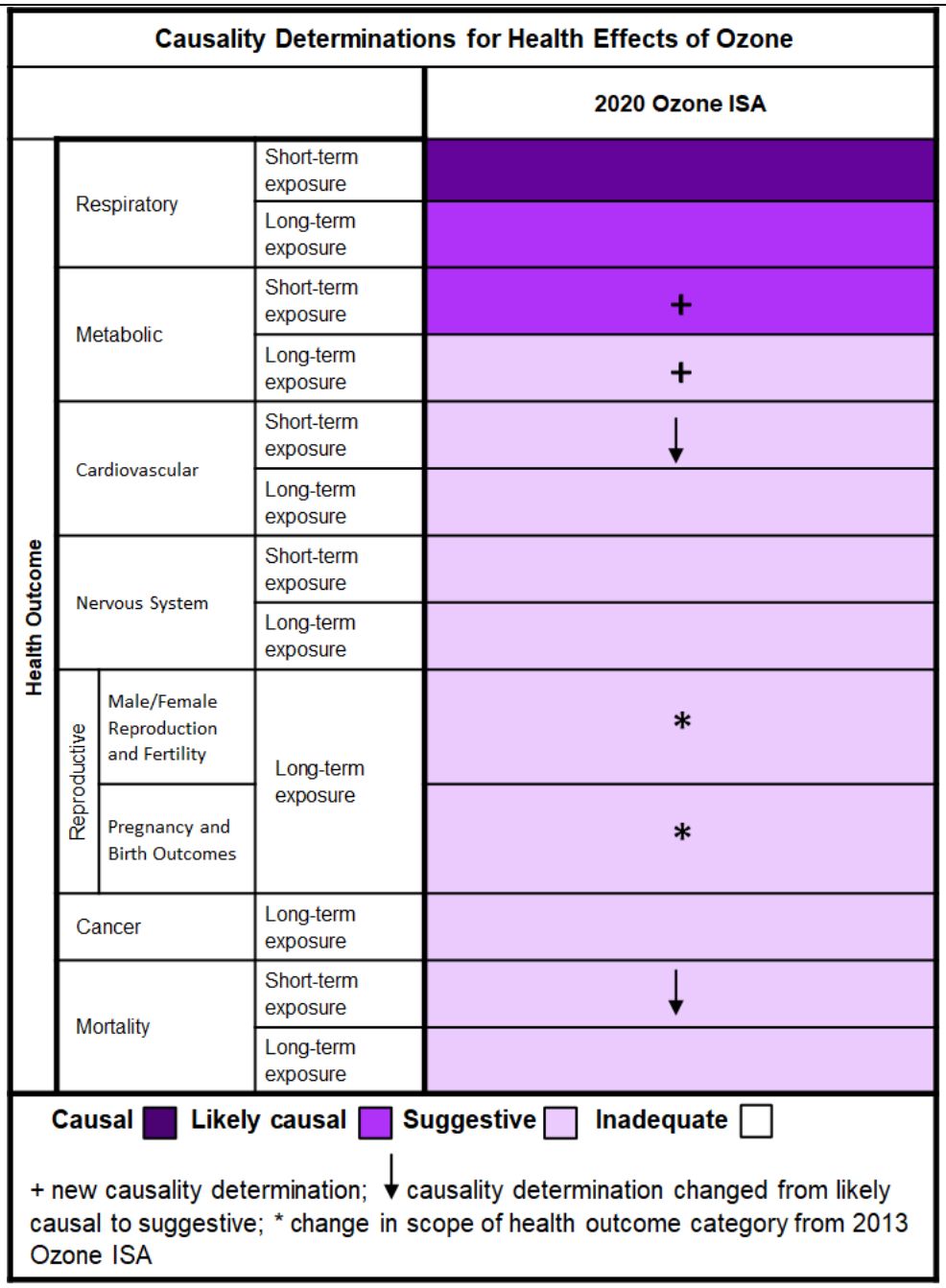


Figure IS-6 Causality determinations for health effects of short- and long-term exposure to ozone.

Both older and more recent studies provide evidence for biologically plausible pathways that may underlie respiratory effects related to long-term ozone exposure, and metabolic effects related to short-term exposure. Epidemiologic studies of long-term ozone exposure and respiratory effects are supported by numerous animal toxicological studies examining related endpoints. This coherence reduces some of the uncertainty related to the independence of the ozone effect, though there are some remaining uncertainties for these health effects. For example, there are still relatively few studies evaluating the effect of ozone exposure on metabolic effects in human populations (i.e., controlled human exposure or epidemiologic studies).

With regard to short-term ozone exposure and cardiovascular health effects, there is some evidence for biologically plausible pathways for the worsening of IHD or HF, the development of heart attack or stroke, and cardiovascular-related ED visits and hospital admissions (Figure 4-1). However, the evidence comes mainly from animal toxicological studies, is generally not supported by controlled human exposure studies, and is limited for epidemiologic studies. While there is some epidemiologic evidence that short-term ozone concentrations are associated with total mortality, the evidence of plausible steps that could lead to death (e.g., IHD, HF) are lacking in epidemiologic studies that examined these types of endpoints (e.g., hospital admissions for IHD or HF). Furthermore, controlled human exposure studies in healthy adults generally do not show that short-term ozone exposure leads to the types of intermediate health effects (e.g., impaired vascular function, changes in ECG measures) that could lead to IHD or stroke. Most of the studies supporting the biological plausibility of epidemiologic studies of mortality are from animal studies that are not generally supported by studies in humans.

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.

There is strong and consistent animal toxicological evidence linking short- and long-term ozone exposure with respiratory, cardiovascular, and metabolic health effects. However, several uncertainties should be considered when evaluating and synthesizing evidence from these studies. Experimental studies are often conducted at ozone concentrations higher than those observed in ambient air (i.e., 250 to >1,000 ppb) to evoke a response within a reasonable study length. These studies are informative and the conduct of studies at these concentrations is commonly used for identifying potential human hazards. There are also substantial differences in exposure concentrations and exposure durations between animal toxicological and controlled human exposure studies. For example, animal toxicological studies generally expose rodents to 250 to >1,000 ppb, while controlled human exposure studies generally expose humans to 60 to 300 ppb. Additionally, a number of animal toxicological studies were performed in rodent disease models, while controlled human exposure studies generally are conducted in healthy individuals. This

difference could explain some of the inconsistencies across studies between these scientific disciplines. Controlled human exposure studies do not typically include unhealthy or diseased individuals for ethical reasons; therefore, this represents an important uncertainty to consider in interpreting the results of these studies. Additional animal toxicological studies conducted at lower concentrations could help to reduce this uncertainty. Finally, in addition to exposure concentration and disease status differences in physiology (e.g., rodents are obligate nose breathers), differences in the duration and timing of exposure (e.g., rodents are exposed during the day, during their resting cycle, while humans are exposed during the day when they are normally active), and differences in the temperature at which the exposure was conducted may contribute to the lack of coherence between results of experimental animal and human studies. Dosimetric studies of animals and humans might inform understanding of the potential role of such differences.

Controlled human exposure studies provide the strongest evidence for the effects of short-term ozone exposure on respiratory effects. There are, however, several limitations of controlled human exposure studies. These include the study of generally healthy individuals and the measurement of relatively minor health effects (or indices of health effects) for ethical reasons (unhealthy or very sick people are studied rarely). Therefore, individuals that may be at greater risk are not included in controlled human exposure studies. However, controlled human exposure studies offer several strengths for studying human health effects from ozone exposure. The experimental nature of controlled human exposure studies allows them to virtually eliminate the chance, bias, and other potential confounding factors inherent in observational epidemiologic studies. In addition, controlled human exposure studies are not susceptible to some of the uncertainties commonly attributed to animal toxicological studies, such as the need to extrapolate between animal models and humans, and the use of relatively high ozone concentrations compared with concentrations typically encountered in ambient air.

Though susceptible to chance, bias, and other potential confounding due to their observational nature, epidemiologic studies have the benefit of evaluating real-world exposure scenarios and can include populations that cannot typically be included in controlled human exposure studies, such as children, pregnant women, and individuals with pre-existing disease. In addition, innovations in epidemiologic study designs and methods have substantially reduced the role of chance, bias, and other potential confounders in well-designed, well-conducted epidemiologic studies. Many epidemiologic studies have been conducted in diverse geographic locations, encompassing different population demographics, and using a variety of exposure assignment techniques. They continue to report consistent, positive associations between short-term ozone exposure and health effects. When combined with coherent evidence from experimental studies, the epidemiologic evidence can support and strengthen determinations of the causal nature of the relationship between health effects and exposure to ozone at relevant ambient air concentrations.

The most common source of uncertainty in epidemiologic studies of ozone is exposure measurement error. The majority of recent epidemiologic studies of long-term ozone exposure use

concentrations from fixed-site monitors as exposure surrogates. Some recent epidemiologic studies incorporate new ozone exposure assignment methods that integrate several sources of available data (i.e., satellite observations, CTM predictions, and ambient monitors) into a spatiotemporal model. These hybrid methods are well validated by ozone monitors in areas with moderate to high population density, and they better allow for the inclusion of populations from less urban areas, where monitor density is lower. Relatively low spatial variability of ozone (compared with UFP, CO, NO_2, or SO_2) in most locations increases confidence in application of these methods for predicting ozone exposure. Furthermore, disentangling the effects of short-term ozone exposure from those of long-term ozone exposure (and vice-versa) is an inherent uncertainty in the evidence base.

Additionally, the populations included in epidemiologic studies have long-term, variable, and uncharacterized exposures to ozone and other ambient pollutants. Epidemiologic studies evaluate the relationship between health effects and specific ozone concentrations during a defined study period. The generally consistent and coherent associations observed in these epidemiologic studies contribute to the causality determinations and the conclusions regarding the causal nature of the effect of ozone exposure on health effects, However, they do not provide information about which averaging times or exposure metrics may be eliciting the health effects under study.

Each of the exposure assignment methods used in short- and long-term ozone exposure epidemiologic studies have inherent strengths and limitations, and exposure measurement errors associated with those methods contribute bias and uncertainty to health effect estimates. For short-term exposure studies, exposure measurement error generally leads to underestimation and reduced precision of the association between short-term ozone concentrations and health effects. For long-term exposure studies, exposure measurement error can bias effect estimates in either direction, although it is more common that effect estimates are underestimated. Underestimation of health effect associations in short- and long-term ozone exposure studies implies that true health effect associations are even larger than what is reported in epidemiologic studies. The magnitude of bias in the effect estimate is likely small for ozone, because ozone concentrations do not vary over space as much as other criteria pollutants, such as NO_X or SO_2 (Section 2.6).

Copollutant analyses were limited in epidemiologic studies evaluated in the 2013 Ozone ISA but indicated that associations between ozone concentrations and health effects were not confounded by copollutants or aeroallergens (U.S. EPA, 2013b). Copollutant analyses are more prevalent in recent studies and continue to suggest that observed associations are independent of coexposures to correlated pollutants or aeroallergens. Despite expanded copollutant analyses in recent studies, determining the independent effects of ozone in epidemiologic studies is complicated by the high copollutant correlations observed in some studies, and the possibility for effect estimates to be overestimated for the pollutant measured with less error in copollutant models (Section 2.5). That said, some studies report modest copollutant correlations, which suggests that strong confounding due to copollutants is unlikely. In addition, evidence from copollutant models is available for a small subset of all the pollutants that

co-occur with ozone in the air. Nonetheless, the consistency of associations observed across studies with different copollutant correlations, the generally robust associations observed in copollutant models, and evidence from other scientific disciplines generally provide compelling evidence for an independent effect of ozone exposure on human health and reduce the uncertainties associated with potential copollutant confounding.

The 2013 Ozone ISA noted that multicity epidemiologic studies, particularly examining short-term ozone exposure and mortality, reported evidence of heterogeneity in the magnitude and precision of risk estimates across cities. There are few recent multicity studies of short-term ozone exposure and health effects that could allow an evaluation of such heterogeneity; thus, the uncertainty identified in the 2013 Ozone ISA remains.

Examination of the concentration-response (C-R) relationship has primarily been conducted in studies of short-term ozone exposure and respiratory health effects or mortality, with some more recent studies characterizing this relationship for long-term ozone exposure and mortality. Across recent studies that used a variety of statistical methods to examine potential deviations from linearity, evidence continues to support a linear C-R relationship, but with less certainty in the shape of the curve at lower concentrations (i.e., below 30−40 ppb). In addition, some studies evaluate the potential for a population-level threshold, below which health effects would unlikely be observed. Generally, these studies conclude that if a population-level threshold exists, it would occur at lower concentrations (i.e., below 30−40 ppb) where there is less certainty in the ozone-health effect relationship due to few observations at these lower concentrations. Similar to the uncertainty mentioned previously, the populations included in epidemiologic studies have long-term, variable, and uncharacterized exposures to ozone and other ambient pollutants. Epidemiologic studies evaluate the C-R relationship between health effects and specific ozone concentrations during a defined study period. The generally consistent C-R relationships observed in these epidemiologic studies do not indicate which averaging times or exposure metrics may be eliciting the health effects under study.

IS.6.2 Welfare Effects Evidence: Key Findings

The collective body of evidence for each welfare endpoint evaluated in this ISA was carefully considered and assessed, including the inherent strengths, limitations, and uncertainties in the overall body of evidence, resulting in the causality determinations for ecological effects detailed in Table IS-2 and effects on climate in Table IS-3.

IS.6.2.1 Ecological Effects

A large body of scientific evidence spanning more than 60 years clearly demonstrates there are effects on vegetation and ecosystems attributed to ozone exposure resulting from anthropogenic activities

(U.S. EPA, 2013b, 2006a, 1996b, 1986, 1978; NAPCA, 1970; Richards et al., 1958). There is high certainty in ozone effects on impairment to leaf physiology as mechanisms for cascading effects at higher levels of biological organization (e.g., plant growth, ecosystem productivity; Section 8.1.3; Figure IS-7). The overwhelming strength of many of the studies is that they consist of controlled ozone exposure to plants, plots of forests, and crop fields to eliminate any confounding factors (Section 8.12). For example, for ozone effects on plants, there are robust exposure response functions (i.e., from carefully controlled experimental conditions, involving multiple concentrations and based on multiple studies) for about a dozen important tree species and ten major commodity crop species.

The use of visible foliar injury to identify phytotoxic levels of ozone is an established and widely used methodology. However, foliar injury is not always a reliable indicator of other negative effects on vegetation (e.g., growth, reproduction), and there is a lack of quantitative exposure-response information that accounts for the important role of soil moisture in foliar injury. As documented in the 2013 Ozone ISA (Table IS-12) and retained in the current Ozone ISA (Figure IS-7), there are causal relationships between ozone exposure and visible foliar injury at the individual-organism level, and causal relationships between ozone exposure and reduced plant growth and crop yield from the individual to population levels. Since the 2013 Ozone ISA (U.S. EPA, 2013b), a meta-analysis of existing literature on plant reproductive metrics and new research support a causal relationship between ozone exposure and reduced plant reproduction. In the previous ISA, plant reproduction was considered within the broader category of growth but the current body of evidence for this endpoint warrants a separate causality category.

While the effect of ozone on vegetation is well established in general, there are some knowledge gaps regarding precisely which species are sensitive and what exposures elicit adverse responses for many species. Currently there are over 40,000 plants and lichens occurring in the U.S. as documented by the USDA PLANTS database (USDA, 2015). It not feasible to know what the effects are on all U.S. species and what the ecological consequences of the differential sensitivities are of these species. However, there have been many important trees, crops, and other plants studied to indicate the potential array of ecological effects in the U.S. The exposure-response relationships for a subset of individual plants are discussed in Section 8.13. Within and between these species there is a range of sensitivities, and it is difficult to identify the representativeness of these relationships within the wider population of plants that occur in the U.S. There are also uncertainties about how plant responses change with age and size. The technique of meta-analysis is one approach that can be used to consolidate and extract a summary of significant responses from a selection of previously published studies. These meta-analyses can show patterns of cause and effect relationships between ecological endpoints and ozone exposure; they are robust enough to overcome individual variation and are useful for looking at trends in plant response across, for example, geographic locations, environmental conditions, plant functional groups, and ecosystems.

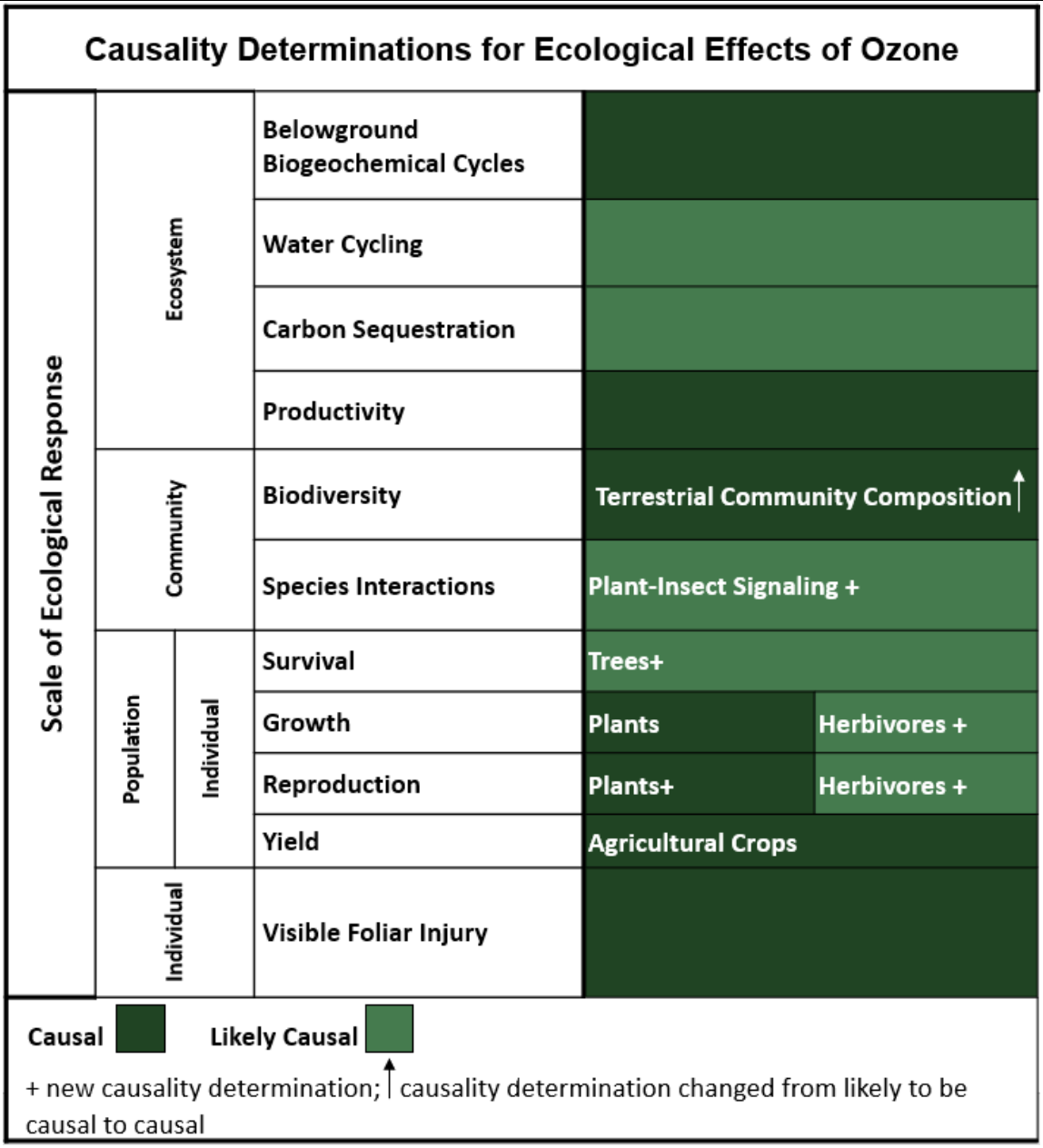


Figure IS-7 Causality determinations for ecological effects of ozone across biological scales of organization and taxonomic groups.

The majority of evidence for ecological effects of ozone is for vegetation. Fewer studies examine plant-ozone-insect interactions. There are multiple, statistically significant findings showing ozone effects on fecundity and growth in insect herbivores. However, no consistent directionality of response is observed across the literature, and uncertainties remain in regard to different plant consumption methods across species and the exposure conditions associated with particular severities of effects. There is also variation in study designs and endpoints used to assess ozone responses. Most responses observed in insects appear to be indirect (i.e., mediated through ozone effects on vegetation, although direct effects of ozone exposure on insects could also play a role). New research in chemical ecology has provided clear evidence of ozone modification of VPSCs and behavioral responses of insects to these modified chemical signatures; however, most of these studies have been carried out in laboratory conditions rather than in natural environments. Characterization of airborne pollutant effects on chemical signaling in ecosystems is an emerging area of research with information available on a relatively small number of insect species and plant-insect associations and knowledge gaps in the mechanisms and consequences of modulation of VPSCs by ozone.

There are some uncertainties in characterizing how ozone damage to leaves and individual plant species scale up to ecological communities and ecosystem processes. Although estimating ozone effects to the ecosystem level remains a challenge, there is a large body of knowledge of how ecosystems work gained through ecological observations and models that simulate processes at multiple scales. The models attempt to capture interactive effects of multiple stressors in ecosystems in the field. Studies of ozone effects beyond the plant scale use a combination of empirical studies and statistical modeling, or large controlled exposure ecosystem experiments, or field observations along ozone gradients. Interactive effects in natural ecosystems with multiple stressors (e.g., drought, disease) are difficult to study, but can be addressed through different statistical methods. For example, multivariate models and mechanistic models have been used for studying ozone with other environmental factors [e.g., Dietze and Moorcroft (2011)] and for scaling up ozone effects on tree growth and water use to ecosystem stream flow [e.g., Sun et al. (2012)]. Another approach is to use meta-analysis techniques to examine trends across large geographic areas or at higher biological levels of organization (e.g., plant functional groups, forest types). More research on ecosystem-level responses will strengthen understanding of how effects at lower levels of biological organization influence higher level responses.

In general, the most promising approaches to estimating or characterizing ozone effects at the ecosystem level include evaluation of ecological response using a suite of parameters and exposure-response functions, both empirical and modeled. The quantitative uncertainty of empirically observed variables in ecology is determined by the use of statistics. In general, ecological endpoints affected by ozone were reported in the ISA if they were statistically significant. In addition, models of chemical and ecological processes provide representations of biological interactions through mathematical expressions. The models used can be complex, including many interacting variables. Each of the input variables in a model has some uncertainty. Models can also be evaluated on the basis of the

mechanistic understanding of how ecological systems work and how ozone effects may propagate through ecological systems.

IS.6.2.2 Effects on Climate

Ozone is an important greenhouse gas and increases in its abundance have affected the Earth's climate. Over the last century, global average surface air temperature has increased by approximately 1.0°C, and emissions of greenhouse gases are the dominant cause (Wuebbles et al., 2017; IPCC, 2013). There are many other aspects of the global climate system that are changing in addition to this warming, including melting glaciers, reductions in snow cover and sea ice, sea level rise, ocean acidification, and increases in the frequency or intensity of many types of extreme weather events (Wuebbles et al., 2017). The magnitude of future climate change, globally and regionally, and in terms of both temperature increases and these other types of associated impacts, will depend primarily on the amount of greenhouse gases emitted globally (Wuebbles et al., 2017; IPCC, 2013). The most recent IPCC report, AR5, which is a comprehensive assessment of the peer-reviewed literature, reported global tropospheric ozone RF as 0.40 (0.20 to 0.60) W/m^2 (Myhre et al., 2013). In the 2013 Ozone ISA, there was a causal relationship between tropospheric ozone and RF and a likely to be causal relationship between tropospheric ozone and climate change (U.S. EPA, 2013b). None of the new studies support a change to either causality determination (Figure IS-8).

While the warming effect of tropospheric ozone in the climate system is well established in general, various uncertainties render the precise magnitude of the overall effect of tropospheric ozone on climate more uncertain than that of the well-mixed greenhouse gases (Myhre et al., 2013). These include several uncertainties associated with estimating the magnitude of RF attributed to tropospheric ozone increases, such as uncertainties in estimating preindustrial ozone concentrations. In addition, precisely quantifying changes in surface temperature due to tropospheric ozone changes, along with related climate effects, requires complex climate simulations that include all relevant feedbacks and interactions. For example, trends in free tropospheric ozone and upper tropospheric ozone (where RF is particularly sensitive to changes in ozone concentrations) are not captured well by models. Substantial variation also exists across models. Such modeling uncertainties make it especially difficult to provide precise quantitative estimates of the climate effects of regional-scale ozone changes.

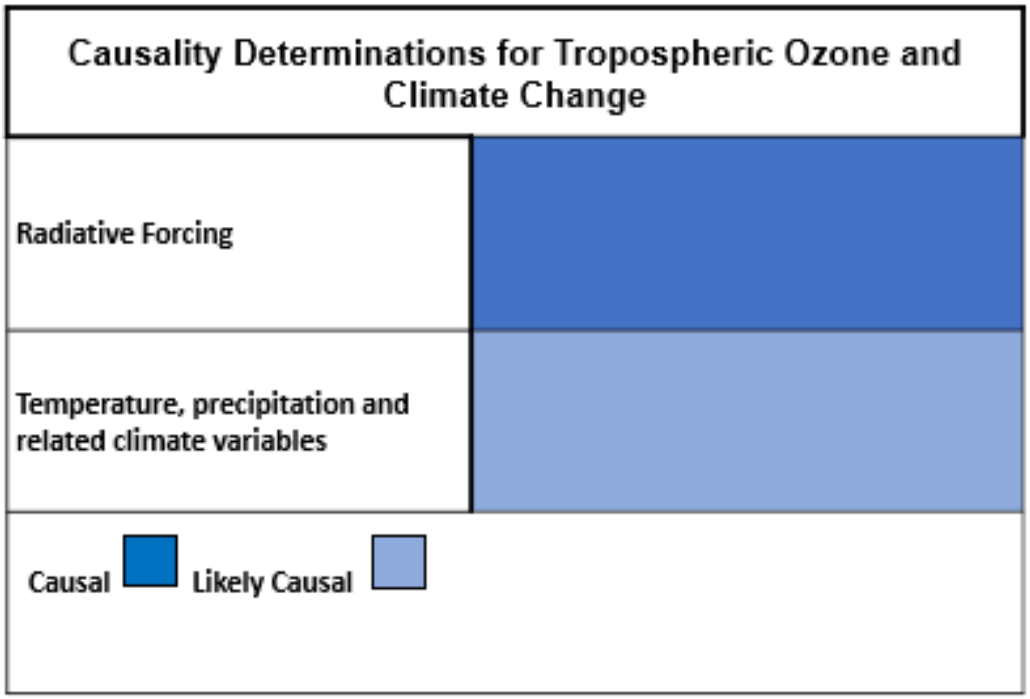


Figure IS-8 Causality determinations for tropospheric ozone and climate change.

IS.7 References for Integrative Synthesis

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