

Summary Information on Selected Science Topics Related to Ozone

**Background document prepared for the Independent Workshop on
Ozone NAAQS Science and Policy by members of the Ozone
Workshop Steering Committee.**

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Introduction

This document contains summaries of important topics for understanding ozone science. It includes discussions about ozone mode of action, human clinical studies, adverse effects, epidemiology, exposure, a history of the ozone National Ambient Air Quality Standard (NAAQS), and information about the NAAQS causal framework. These particular topics are included in this document because, in the authors opinion, these data are important to inform the choice of the level of the ozone NAAQS. This document was prepared by Sabine Lange from the Texas Commission on Environmental Quality (TCEQ), and by Julie Goodman and Sonja Sax of Gradient. It was edited for content and clarity by Michael Honeycutt of TCEQ, and Jacqueline Patterson and Michael Dourson of Toxicology for Excellence in Risk Assessment (TERA).

1 Summary of Ozone Mode of Action

This information is derived largely from the EPA Ozone Integrated Science Assessment (US EPA, 2013), Chapter 5 (Dosimetry, Mode of Action and Species Homology). The following mode-of-action (MOA) summary is based primarily on human studies.

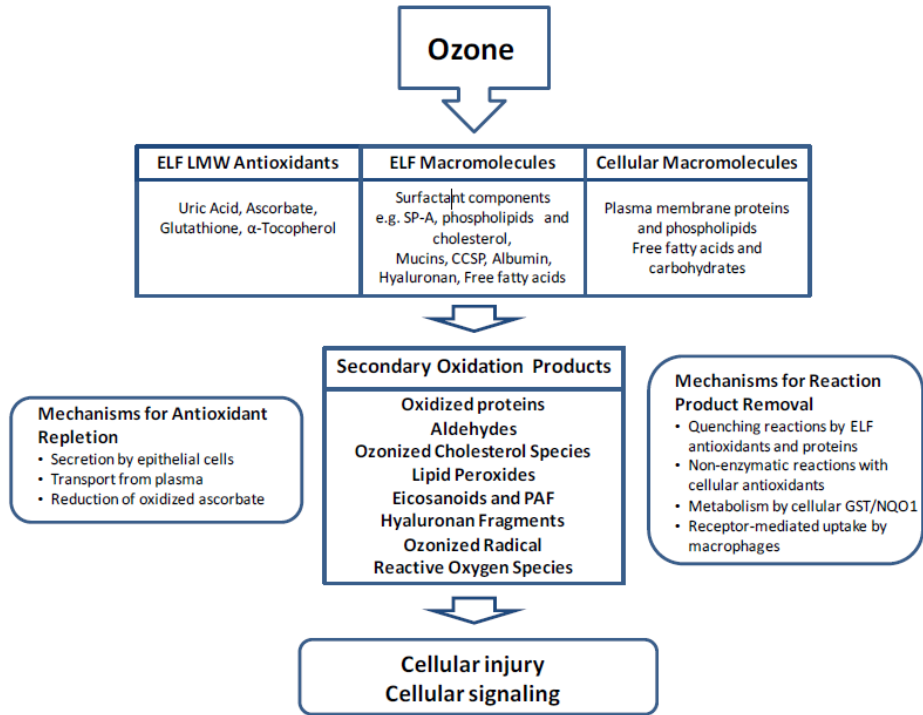
Ozone dosimetry and uptake

- Ozone is a poorly water soluble, highly reactive gas at room temperature.
- Inhaled dose is the dose of ozone that enters the respiratory tract, and is calculated by multiplying concentration x time x minute ventilation (V_E).
- Tissue dose is the amount of ozone or its secondary reaction products that are available to react with the tissues, and is rarely measured. Inhaled dose is used as a surrogate for tissue dose. Because ozone is so reactive, the tissue dose likely exists in the form of oxidation products such as aldehydes and peroxides.
- Humans uptake 80-95% of ozone in inhaled air into the entire respiratory tract (Hu *et al.*, 1992). About 50% of that reacts in the head (mouth, nose, pharynx), ~7% in the larynx/trachea, and ~43% in the lungs. In the lung, the primary area where ozone is absorbed is the centriacinar region – this is the junction between the tracheobronchial region and the gas exchange region. In particular, the respiratory bronchioles absorb the highest dose in the lung (Plopper *et al.*, 1998).
- While breathing at rest, very little ozone reaches the alveolae (Postelthwait *et al.*, 1994), but with an increased ventilation rate, less ozone reacts with the upper respiratory tract (particularly with a switch to oral breathing) and more makes it into the alveolar regions (Hu *et al.*, 1994; Nodelman & Ultman, 1999).

- There is a 10-50% inter-individual variability in ozone uptake measurements (Santiago *et al.*, 2001, Rigas *et al.*, 2000). This could contribute to the inter-individual variability observed in response to ozone (Taylor *et al.*, 2006).

Ozone reaction products

- Ozone moves from the airways and into the tissues by a process called reactive absorption, which are the chemical reactions and coupled diffusion of ozone in the epithelial lining fluid (ELF) of the respiratory tract.
- Of the molecules in the ELF, ozone will react with phospholipids, neutral lipids (cholesterol), free fatty acids, proteins and antioxidants. Ozone reaction products from reactions with unsaturated fatty acids include reactive ozonide, aldehydes, hydroperoxides, and free radicals; and those from reactions with cholesterol include oxysterols, β -epoxide and 6-oxo-3,5-diol.
- The primary antioxidants present in the ELF include uric acid, ascorbic acid and glutathione, and they are present in relatively high concentrations (Mudway & Kelly 1998; Mudway *et al.*, 1996). Experimental evidence indicates that uric acid is the principal antioxidant responsible for scavenging ozone in the nasal ELF, whereas ascorbic acid plays a larger role in the broncho-alveolar region (Mudway *et al.*, 1996, 1999). Antioxidants that are depleted in the ELF can be replenished from various sources, depending on the particular antioxidant. Experimental evidence suggests that this replenishment is time-dependent (Mudway *et al.*, 1999).
- In order to reach the epithelial lining of the respiratory tract, ozone must diffuse across the air-liquid interface and move through the ELF layer. Because of the reactivity of ozone, likely little, if any, ozone reaches the underlying cellular layer, particularly in the nasal cavities where the ELF is thicker (Pryor 1992, Santiago *et al.*, 2001). It is possible that ozone could come into direct contact with the alveolar epithelial cells because the ELF layer there is thinner (Bastacky *et al.*, 1995). In contrast, secondary reaction products can generally reach the cellular layer regardless of ELF thickness.



Note: Contents of this figure not discussed in [Section 5.2](#) will be discussed in [Section 5.3](#). Low molecular weight, LMW; Clara cell secretory protein, CCSP; Surfactant Protein-A, SP-A; Platelet activating factor, PAF. Ozone will react with components of the ELF to produce reaction products that may lead to cellular injury and cell signaling as discussed in [Section 5.3](#).

Figure 5-7 Details of the O₃ interaction with the airway ELF to form secondary oxidation products.

Figure 1: Details of the O₃ interaction with the airway ELF to form secondary oxidation products.
Source: US EPA (2013).

Mode of action

Ozone affects a number of pathways in the respiratory tract. These include the activation of neural reflexes, which leads to symptoms and spirometric responses; inflammation; alteration of epithelial barrier function; sensitization of bronchial smooth muscle reactivity; alteration of immune responses; and airways remodeling. The salient information about each of these health effects is discussed below.

- Activation of neural reflexes:
 - The vagal afferent pathway, likely initiated by bronchial C-fibers, causes ozone-mediated spirometric and symptomatic responses in humans (Passannante *et al.*, 1998, Hazucha *et al.*, 1989). The affected spirometric responses include forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), and airway resistance (sRaw).
 - TRPA1 receptors in bronchial C-fibers are sensitive to lipid reaction products of ozone, particularly nonenals (Taylor-Clark *et al.*, 2008, Trevisani *et al.*, 2007), and likely stimulate the ozone response.
 - Evidence suggests that ozone reacts with the ELF, causing a time-dependent depletion of ELF antioxidants. Neural reflexes are only activated after the antioxidant defenses are overwhelmed (Schelegle *et al.*, 2007).

- Inflammation:
 - Respiratory inflammation is considered to be a more detrimental result of ozone exposure than the spirometric responses (Balmes *et al.*, 1996). It occurs at comparable doses as spirometric responses, but at later times after ozone exposure (Kim, 2011).
 - The most utilized and consistent marker of respiratory inflammation is influx of neutrophils, and this occurs more slowly than the spirometric responses to ozone. There is a linear dose-response relationship, with a threshold concentration, between total inhaled ozone dose and bronchoalveolar lavage neutrophils (Mudway & Kelly, 2004).
 - At later time points after ozone exposure, lymphocytes, monocytes, and macrophages have also been observed, but not as consistently as the neutrophils (Mudway & Kelly, 2004, Blomberg *et al.*, 1999, Mudway *et al.*, 1999, Alexis *et al.*, 2010).
 - Increases in inflammatory cytokines have also been measured in lavage fluid, including eicosanoids, IL-8, and TNF- α (Kafoury *et al.*, 1998, Mudway & Kelly, 2000).
 - Cellular and biochemical measures of inflammation are not correlated with spirometric responses in individuals, suggesting that these are activated by independent pathways (Aris *et al.*, 1993, Schelegle *et al.*, 1991). However, the protein Substance P is related to both, and may provide a bridge between spirometric responses and inflammation in response to ozone (Krishna *et al.*, 1997).
- Alteration of Epithelial Barrier Function:
 - Impaired epithelial barrier function has been observed after ozone exposure, as indicated by histological observation of damaged tight junctions, by increased flux of small molecules into the ELF, or by increased protein content in lavage fluids (Costa *et al.*, 1985, Hu *et al.*, 1982, Kehrl *et al.*, 1987, Mudway & Kelly, 2004).
 - This was not correlated with spirometry or with airway hyper-responsiveness (Kehrl *et al.*, 1987, Que *et al.*, 2011), suggesting different mechanisms of ozone action between these different endpoints.
- Sensitization of Bronchial Smooth Muscle Reactivity:
 - Ozone increases airway hyper-responsiveness (measured as changes in sRaw or FEV₁) to bronchoconstrictive drugs such as metacholine and histamine.
 - The mechanism by which ozone affects smooth muscle reactivity is not entirely known, but it may be through direct effects on smooth muscles or on the sensory nerves (O'Byrne *et al.*, 1983, O'Byrne *et al.*, 1984, Holtzman *et al.*, 1979).
 - These effects may be worse in those with compromised airways (Kehrl *et al.*, 1999, Jorres *et al.*, 1996, Molfino *et al.*, 1991).
- Modification of Immune System Responses:
 - As described in the inflammation section, ozone exposure can lead to recruitment of activated innate immune cells such as neutrophils, monocytes and dendritic-like cells (Alexis *et al.*, 2010, Lay *et al.*, 2007).
 - Evidence suggests that the activation of innate immune cells can trigger adaptive immunity (specifically T cells) to modify airway response to pathogens and allergens.

- Airways Remodeling:
 - Remodeling of airways in response to ozone has not been directly tested in humans, but with higher than ambient concentrations of ozone and chronic exposures, airways remodeling has been observed in animal models (Mudway & Kelly, 2000).

MOA uncertainties

There are several different types of uncertainties associated with MOA information.

- The MOA discussed in this document is derived primarily from human studies. When MOA is derived from animal studies, there are two primary considerations when applying the information to humans: dose and species similarities.
 - Typical animal studies are conducted at doses that are much higher than ambient pollutant concentrations, and as such, the information gained from these studies must be carefully considered before it is applied to average human exposures. Because of the concepts of dose-dependent transitions in mechanisms of toxicity (Slikker *et al.*, 2004), mechanisms that are relevant at high doses of exposure may not be applicable at low doses. In addition, because ozone is an inhaled pollutant, there are three components of dose: concentration, time of exposure and ventilation rate. In animal studies, ventilation rate is rarely properly accounted for, making it difficult to calculate dose.
 - Uncertainty exists that responses in animal studies will not be relevant to people. This uncertainty can be diminished by using data from species that have similar physiology and mechanistic pathways as humans (mammals, particularly non-human primates). In addition, similar MOAs observed after exposure in different species increases the likelihood that a particular MOA is also applicable to humans.
- MOAs derived from human studies are directly applicable. However, there can still be problems with environmentally-relevant doses, as well as limitations in the mechanistic investigations that can be completed.
 - For ozone, the exposure concentrations that have been used for the human clinical studies are typically much higher than ambient concentrations – up to 750 ppb (typical range: 80-200 ppb). Ambient concentrations are considerably lower (the highest 8-hour average in 2013 in the US was 141 ppb). While in the past ambient concentrations of ozone were much higher, current concentrations are lower than the amounts used in many ozone exposure studies. Similarly, because ozone dose is affected by the ventilation rate of the study participants, exposures that are conducted at high ventilations (*i.e.*, heavy exercise) for extended periods of time may also produce doses that are high enough to be of questionable relevance to the general population. For example, the high end ventilation rate used to determine Reference Concentrations (RfCs) for air contaminants by EPA is 20 m³/day (14 liters/minute for a 24 hour period). This ventilation rate is much lower than many of the human clinical studies. Similarly, a standard worker ventilation is 10 m³/8 hours (22 L/min; US EPA 1994), which is also lower than the human exposures studies (which are typically conducted at ~32 L/min for 6.6 hours).
 - Human clinical studies are restricted by the types of interventions and investigations that can be done to examine the health effects of ozone. Therefore, the information that can be obtained from human studies is not as extensive as from animal studies, and more indirect measures of endpoints (biomarkers) are used. For example, there are animal studies that investigate the long-term effects of ozone on lung development in infant non-human primates (US EPA 2013, Table 7-1). These studies cannot be repeated in humans.

2 Summary of Human Clinical Ozone Studies and Ozone Dose-Response

Clinical studies of human responses to ozone have been conducted since the 1970s. These studies have looked at many endpoints, including those for spirometric responses, inflammation, airway hyper-responsiveness, and changes in epithelial permeability. The conclusions about the effects of ozone on these pathways are summarized in Section 1 above.

There is an expansive literature of human clinical exposure studies and ozone dose-response modeling. In this section we summarize the few studies that have been done at ozone concentrations that are at or below the current ozone NAAQS (at or below 75 ppb). Also included is a discussion of ozone dose-response models. With the exception of one study, these models have all used FEV₁ as the health endpoint. This is because investigators have found this to be a sensitive indicator of effect that can be consistently measured and has been consistently reported. One study assessed the dose-response characteristics of ozone exposure on neutrophil infiltration into bronchoalveolar lavage (BAL) fluid (Mudway & Kelly, 2004).

Human clinical ozone exposure studies

- The ozone NAAQS, first set in 1970, has two elements that are directly applicable to human clinical exposures: level (concentration) and averaging time (Goodman *et al.*, 2015). Until 1997, the averaging time of the ozone NAAQS was 1 hour, and the level was 0.12 parts per million (ppm). In 1997, the averaging time was changed to the daily maximum 8-hour average and the level was changed to 0.08 ppm. In 2008 the level was decreased to 0.075 ppm (8-hour average).
- Controlled human exposure studies of measured doses of ozone have been done for decades. Ozone dose is a function of concentration, duration of exposure and minute ventilation (*i.e.*, exercise level). The early studies were done at high ozone concentrations (e.g., 120–500 ppb), for shorter time periods (e.g., 1–3 hours) and with a variety of ventilation rates (mimicking resting all the way up to intense exercise). These studies observe effects in the human subjects and the results could be directly related to the 1 hour averaging time of the NAAQS.
- Over time, as ambient ozone levels and NAAQS levels decreased and the averaging time changed, investigators began exposing humans to ozone doses that were more relevant to current conditions and to the averaging time of the NAAQS. Therefore, the experimental ozone concentrations decreased (e.g. 40–120 ppb), and the exposure time increased (6-8 hours). The exercise levels (and therefore the ventilation rates) of the human subjects were chosen in order to mimic a day of heavy manual labor (Folinsbee *et al.*, 1988, McDonnell *et al.*, 1991).
- In addition to varying the dose in different clinical studies, the studies varied the exposure regimen (Adams, 2006, Schelegle *et al.*, 2009). Historically, most regimens exposed study participants to a single concentration of ozone for the entire study period (square wave exposure). However, in order to more closely mimic the diurnal variations in ozone concentrations (ozone increases and then decreases during the day), some investigators exposed subjects to increasing and then decreasing levels of ozone that would average out to the same concentration as the square wave exposures (called stepped or ramped triangular exposure). Intermediate time points

in these triangular exposures tended to show greater responses than the square exposures, but by the last time point, the two exposures tended to show equal responses (Adams, 2006).

- We have plotted intermediate time points of exposure from square wave and triangular wave regimens by dose instead of by time. Our work shows that the differences in the intermediate time points between the two exposure regimens can be fully accounted for by differences in the doses received (webinar presentation).

Human clinical studies with exposures below the current NAAQS level

There are four human clinical studies that have been completed at ozone concentrations below the level of the current ozone NAAQS (0.075 ppm): Adams (2002, 2006) Schelegle *et al.* (2009), and Kim *et al.* (2011). They all use the same exposure/exercise regimen: the study participants were exposed to filtered air or ozone while exercising for 50 minutes of every hour for 6 hours, with a 35 minute lunch break between the 3rd and 4th hour. The exercise ventilation rate varied from 33 – 39 liters/minute (L/min), and total (exercising and resting) ventilation was 28 – 33 L/min. This can be compared to a worker ventilation used in RfC calculations of 10 m³/8 hours (which is 22 L/min in 8 hours; US EPA 1994). In addition to measuring FEV₁ decrements, these studies also measured symptoms, which are often quantified as total subjective symptoms.

- Adams (2002) exposed 30 young adults (15 male and 15 female) for 6.6 hours to filtered air or ozone at concentrations of 40, 80 or 120 ppb. These were square wave exposures and were done either in an exposure chamber or via facemask. Adams showed that the study subjects experienced statistically significant decreases in FEV₁, which were associated with significant increases in symptoms, after exposure to 80 and 120 ppb ozone (mean FEV₁ changes of -13% and -4%, respectively), but not after exposure to 40 ppb ozone (FEV₁ change of +1.1%), compared to filtered air (FEV₁ change of +2.4%). There was no difference in response between exposures via chamber or facemask.
- Adams (2006) exposed 30 young adults (15 male and 15 female) for 6.6 hours to filtered air or ozone at concentrations of 40, 60 or 80 ppb. These exposures were conducted in an exposure chamber and were either square wave or stepped triangular wave exposures. Both exposure regimens caused a significant FEV₁ decrement with a significant increase in symptoms at 80 ppb ozone (change in FEV₁ of -4.7% for square wave or -5.6% for triangular) but not at 60 ppb (FEV₁ change of -1.5% for square wave or -1.4% for triangular), or 40 ppb (FEV₁ change of +1.2%), compared to filtered air (FEV₁ change of +1.4%). There were no significant differences in the final mean FEV₁ changes when comparing the same total dose of ozone using the square or triangular wave exposure regimens. The triangular regimen caused a greater decrease in FEV₁ response earlier during the exposure period, likely because the study subjects were exposed to the ozone dose faster in this protocol.
- Schelegle (2009) exposed 31 young adults (15 male and 16 female) for 6.6 hours to filtered air or ozone at concentrations of 63, 72, 81 or 88 ppb. Subjects were exposed to ozone in an exposure chamber in a stepped triangular wave manner. For the 63 and 72 ppb exposures, the exposure scenarios chosen were the most extreme (*i.e.*, with the largest hourly changes in ozone concentration) of ten hourly 6-hour sequences measured in several cities (Lefohn & Hazucha, 2005). Exposures to 72, 81 and 88 ppb, but not 63 ppb ozone, caused significant decrements in FEV₁ (FEV₁ mean changes of -5.3%, -7%, -11.4%, and -2.7%, respectively), compared to filtered air (FEV₁ change of +0.8%). All exposures, except 63 ppb, were also associated with significant increases in total symptom severity score.
- Kim (2011) exposed 59 young adults (27 male and 32 female) for 6.6 hours to filtered air or ozone at a concentration of 60 ppb. These were square wave exposures conducted in an exposure

chamber. Exposure to 60 ppb ozone caused a significant decrement in FEV₁ (FEV₁ change of -1.8%) compared to filtered air (FEV₁ change of 0%), that was not associated with significant increases in symptoms. Kim *et al.* (2011) also demonstrated a significant increase in sputum neutrophils after ozone exposure (a 1.4-fold increase compared to filtered air).

Data used for ozone dose-response analysis

There are a number of ozone-FEV₁ dose-response analyses that use data from ozone exposure studies. Here we will be discussing the data and analysis used in the studies by: Adams *et al.* (1981), McDonnell & Smith (1994) McDonnell *et al.* (1991, 1997, 2007, 2010 and 2012) and Schelegle *et al.* (2012). This is not all of the studies that have analyzed ozone-FEV₁ dose-response, but they represent many of the core findings from these types of analyses, and the latest papers (McDonnell *et al.* 2012 and Schelegle *et al.* 2012) have been incorporated into the EPA's ozone risk assessment (US EPA 2014b).

- The magnitude of FEV₁ decrements caused by ozone is a function of concentration, minute ventilation and duration of exposure. For most dose-response data, the exposure times ranged from 1-8 hours, with a range of ozone concentrations of 0-400 ppb and ventilations from rest to heavy exercise.
- The FEV₁ response variable was often the filtered air response subtracted from the ozone response (McDonnell *et al.*, 2012, Schelegle *et al.*, 2012). Typically, all of the data were used in generating the dose-response models, although McDonnell *et al.* (2012) also restricted their analysis to just those individuals with FEV₁ decrements > 10%, 15%, or 20%. This was the basis of the EPA's ozone MSS model used in the most recent ozone health risk assessment (US EPA, 2014b).
- Many of the studies used the majority of the data to generate the models, and a small portion of the data to test the goodness-of-fit of the model (McDonnell *et al.*, 1997, 2007, 2012; Schelegle *et al.*, 2012).
- Most models used individual data from many ozone exposure studies with sample sizes, for example, of 473 (McDonnell and Smith, 1994), 485 (McDonnell *et al.*, 1997), 541 (McDonnell *et al.*, 2007), 741 (McDonnell *et al.*, 2012), and 704 (Schelegle *et al.*, 2012). Most of the data were from young (age 18-36) Caucasian males, although the more recent studies also included women. McDonnell *et al.* (2012) also used the population means of the data and demonstrated that using these data slightly over-predicted the individual and group mean responses.

Dose-response models

In this section we discuss multiple features of the ozone-FEV₁ dose-response models, including the shape of the dose-response curve, the importance of each of the three facets of dose (time, concentration and ventilation) in predicting response, how other factors (gender, age, etc) affected ozone-FEV₁ dose-response, the biological theory that was used to inform the mathematical modeling, evidence for thresholds of effect, and an ozone-inflammatory biomarker dose-response model.

- The shape of the ozone-FEV₁ dose-response curves varied among the studies. Some demonstrated linear responses (Silverman *et al.*, 1976, Adams *et al.*, 1981, Colucci, 1983), while most studies demonstrated sigmoidal curves (McDonnell *et al.*, 1994, 1997, 2007, 2012; Folinsbee *et al.*, 1978, Larsen *et al.*, 1991, Schelegle *et al.*, 2012).

- The dose-response models do not agree on which ozone dose variable (concentration, time or ventilation) is a greater predictor of FEV₁ response. Adams *et al.* (1981) and Folinsbee *et al.* (1978) both showed that concentration is a greater predictor of FEV₁ response than ventilation or time. However, these studies had relatively narrow ranges of time and ventilation rates, compared to concentration, as was noted by McDonnell *et al.* (1994). More recent models derived from data with a greater range of values for both time and ventilation have shown no difference between the predictive contributions of the concentration and ventilation variables (McDonnell *et al.*, 1997), or small differences (McDonnell *et al.*, 2007).
- The models also investigated the effects of additional variables on the ozone-FEV₁ dose-response. There has been no observed effect with gender (McDonnell *et al.*, 2012, Schelegle *et al.*, 2012), mixed results with body surface area (BSA) or body size (McDonnell *et al.*, 1997, McDonnell *et al.*, 2007, Schelegle *et al.*, 2012), as well as with the observation of lesser ozone-induced FEV₁ decrements with increasing age (McDonnell *et al.*, 1997, McDonnell *et al.*, 2007, McDonnell *et al.*, 2012).
- Various studies have proposed two and three compartment models to explain the ozone-FEV₁ response. The most recent McDonnell model (2007, 2010) is a two-compartment model where compartment one describes the concentration of an unnamed factor (X) that activates afferent nerves; and compartment two describes the decrement in FEV₁ increasing as a sigmoid function of X with age and BMI. The Schelegle (2012) model also has two compartments, where the first compartment takes into account the ozone dose associated with onset of response, and the second compartment is the same as the first McDonnell (2007, 2010) compartment.
- Multiple groups have found evidence of threshold doses of ozone at which there was no effect on FEV₁ [also called dose of onset by Schelegle *et al.* (2012)]. Adams *et al.* (1981) showed that responses do not begin to occur until an ozone dose of 400 parts per million x liters (ppm x L; or 780 µg). Similarly, McDonnell *et al.* (2012) postulated a threshold of response of 59 ppm x L/m² BSA (~ 118 ppm x L, assuming an average BSA of 2 m²), and Schelegle modeled a dose of onset of responses of 1078 ± 668 µg (~553 ± 342 ppm x L). Schelegle (2012) also noted that the dose of onset decreases as minute ventilation increases. We think that this is consistent with a mechanism whereby the faster the exposure occurs, the less time the respiratory tract has to replenish the antioxidants that are being consumed by ozone, and therefore the greater the response.
- One study by Mudway & Kelly (2004) investigated the dose-response of ozone and markers of respiratory inflammation and epithelial permeability. They found evidence of a relatively simple linear relationship between total inhaled ozone dose and neutrophilia in BAL fluid samples. Using regression analysis, they identified threshold doses of ozone where the ozone response deviated significantly from the control response. These thresholds were at 645 µg/m² BSA (~658 ppm x L) for neutrophilia occurring 0-6 hours after exposure, and at 810 µg/m² BSA (~827 ppm x L) for neutrophil infiltration at 18-24 hours after ozone exposure. Note that we have decreased the doses reported in Mudway & Kelly's paper (2004) by 1000-fold because of a mistake we found that was made by those authors in converting between liters and m³. No significant relationship was found between total ozone dose and neutrophils in bronchial lavage. The authors also investigated epithelial permeability (by total protein levels), which showed no relationship with total dose, unless several studies were excluded. They also reported that there was little evidence of an increase in lavage total protein or albumin below a dose of 800 µg/m² (~ 820 ppm x L).

Uncertainties in clinical studies and implications for dose-response modeling

There are uncertainties associated with interpreting clinical studies and applying them to dose-response modeling. These include sample size, responses of subpopulations, comparing experimental exposure regimens to ambient exposure regimens, determining adverse effects thresholds, and the consistency of results between different evidence streams.

- The ozone clinical studies are used to test the effects of ozone on various respiratory endpoints in humans. The purpose of obtaining such data is to eventually make judgments about what exposures could be harmful to the general population. Each individual study exposed only about 30 people. The dose-response models have pooled subjects from multiple clinical studies (*e.g.*, the McDonnell *et al.* 2012 data set uses 741 people), but the total number of individuals is still small. Therefore, there is uncertainty in applying the results from this relatively small group to the general population.
- Another uncertainty to consider is how well these ozone-FEV₁ dose-response models capture the responses of sensitive subpopulations. The majority of the human clinical ozone exposures have used healthy young people (mostly Caucasian males) as study subjects. However, ozone may have greater effects on certain subpopulations, for example those with respiratory diseases. Some respiratory disease groups have been tested for ozone-induced FEV₁ responses, such as asthmatics [most studies showed no difference between asthmatics and healthy individuals for FEV₁ response (Holz *et al.*, 1999, Stenfors *et al.*, 2002, Linn *et al.*, 1994, Nightingale *et al.*, 1999), although there are some studies that do show a greater response of asthmatics to ozone (Horstman 1995)], but there are other groups which may not respond in the same way to ozone as the study population.
- As noted above, these experiments are used to understand how ozone will affect the general population. However, the application of the total dose (not just the concentration) to the population must be considered before these data can be directly applied to the general population. This means considering real world ozone concentrations, as well as ventilation rates and outdoor exposure times. This is discussed in the Lange *et al.* (2015) white paper.
- Determining at what point a response to ozone becomes adverse is discussed in section 3. However, it is worth noting here that there is uncertainty in determining the critical effect, that is, the first adverse response or its known and immediate precursor in the adversity spectrum of responses, and there is great importance in assessing the biological significance, as well as the statistical significance, of the health effects caused by ozone.
- Some uncertainty about conclusions drawn from modeling ozone effects can be mitigated by considering data from multiple evidence streams. For example, when choosing models that do or do not incorporate thresholds, the known mechanism of action of antioxidants scavenging ozone in the respiratory tract should be taken into account.

3 Adversity of Ozone Effects

As is described in the mode of action section, ozone can cause many effects on the respiratory tract, and systemic effects are also attributable to ozone exposure. Most of the risk analyses for ozone exposure focus on a single endpoint – decrements in FEV₁ attributable to ozone. The discussion below focuses primarily on adversity of FEV₁ effects.

Definitions of adverse effects and other types of effects

A number of documents have provided definitions of adversity, and have commented on the distinction between adaptation and adversity (US EPA, 1980, 2009, Renwick *et al.*, 2003, Sergeant, 2002, Goodman *et al.*, 2010). However, it is difficult to judge at what point, and in what population, a biological response goes from being adaptive or homeostatic, to being adverse. The question of adversity of a response is important when considering decisions regarding whether exposure to a substance should be reduced in order to prevent health impacts for individuals in the population of interest.

Several authors have defined “adverse effect” within the context of human health risk assessment:

- US EPA (1980, 2009): “Adverse effect: A biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism’s ability to respond to an additional environmental challenge”
- Renwick (2003): “Adverse effect: Change in the morphology, physiology, growth, development or life span of an organism, system or (sub) population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other external influences”
- Sergeant (2002): “...adverse effects are changes that are undesirable because they alter valued structural or functional attributes of the entities of interest.... The nature and intensity of effects help distinguish adverse changes from normal...variability or those resulting in little or no significant change”

In the interests of distinguishing adverse effects from adaptive and compensatory effects, Goodman *et al.* (2010) wrote that:

- “Adaptive changes are an organism’s response to stresses in the environment that maintain normal function, or homeostasis. These effects enhance the organism’s ability to respond to additional environmental stressors.”
- “Compensatory effects are generally neutral effects that maintain overall function without enhancement or substantial cost to the organism”. Goodman *et al.* consider compensatory effects to be non-adverse as long as they are short-term.

Together these different authors effectively define an adverse effect (versus adaptive or compensatory effects) as one that causes a change resulting in impaired function in a system or an organism that is intense enough to distinguish from normal variability, or that has the ability to increase the susceptibility of that organism or system to other external influences.

When assessing biological effects, there are other aspects to consider besides whether it is adverse, adaptive or compensatory (Goodman *et al.*, 2010):

- Transient effects – initial responses to an exposure that is not maintained for the duration of the exposure.
- Precursors to an apical effect – early responses that occur before a known overt, adverse effect.
- Reversible effects – effects that the organism recovers from completely at some point after the end of the exposure.
- Severe effects – adverse effects that alter functional capacity of an organism. The severity of an effect is the “degree or magnitude of change in functional capacity that occurs in an organism after a chemical exposure.” This means that the intensity of the effect can determine whether an effect is judged as adverse, and how adverse it is.

In fact, a continuum of effects is potentially associated with any chemical exposure and reflects a sequence of effects of differing severity. From least to most severe these effects include: adaptive, compensatory, critical, adverse, and frank. Definitions of the bolded terms in the following bullet points can be found at:

http://ofmpub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=&glossaryName=IRIS%20Glossary

- At low dose, adaptive effects first occur where the organism’s ability to withstand a challenge is enhanced. Doses associated with such effects are often referred to as No Observed Adverse Effect Levels (**NOAELs**). The NOAEL is defined as an exposure level at which there is no statistically or biologically significant increase in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may be produced at this level, but they are considered neither adverse, nor precursors to specific adverse effects. In an experiment with several NOAELs, the regulatory focus is primarily on the NOAEL seen at the highest dose. This leads to the common usage of the term NOAEL to mean the highest exposure without adverse effect. As dose increases, compensatory effects occur, which enable the organism to maintain overall function without further enhancement or significant cost. Doses associated with such effects are also often NOAELs.
- As dose further increases, the **critical effect**, the first adverse effect, or its known precursor, occurs to the most [relevant or] sensitive species. Note that the bracketed phrase “relevant or” is important since the most relevant species is always preferred over the most sensitive species (e.g., if data shows that the rat is more sensitive than the human, the human data are still preferred). Also the term “precursor” in this definition is singular, meaning the immediate precursor, not just any prior effect. This restriction is important both because it ties the concept of critical effect into common medical practice and because the resulting dose response, such as a reference dose (RfD), is more meaningful--without this restriction many lower RfDs can be estimated. Many examples of appropriate choice of critical effect by different groups are found on International Toxicity Estimates for Risk (*ITER*) database available at <http://toxnet.nlm.nih.gov>.
- Doses associated with such critical effects are generally considered to be the Lowest Observed Adverse Effect Levels (**LOAELs**). The LOAEL is defined as the lowest exposure level at which there is statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control group.
- As dose further increases, the critical effect is exceeded, and **adverse effects** are manifested as biochemical changes, functional impairments, or pathologic lesions. These progressively more severe effects impair the performance of the organism, and/or reduce its ability to respond to

additional challenges. At some point these adverse effects become manifestly overt and irreversible, and **frank effects** or disease ensues.

Adverse effects from exposure to ozone

Many health effects of ozone have been observed, including activation of neural reflexes, which leads to symptoms and spirometric responses; inflammation; alteration of epithelial barrier function; sensitization of bronchial smooth muscle reactivity; alteration of immune responses; and airways remodeling. Other endpoints are potentially associated with ozone (e.g., hospitalizations, emergency department visits, premature mortality); the evidence for these comes from epidemiology studies, which are discussed in sections 5 and 6.

Adversity of biomarker changes:

- For many of the respiratory effects attributable to ozone, biomarkers are used as surrogates for the apical effects (e.g., neutrophil infiltration for inflammation; monocyte activation for alteration of immune responses). However, the American Thoracic Society (ATS, 2000) notes that these biomarkers may not have been validated against established measures of effect, and so “the committee cautions that not all changes in biomarkers related to air pollution should be considered as indicative of injury that represents adverse effect.”
- The EPA has a different view of this as relates to the inflammatory biomarkers, stating that “any initiation of inflammation can be considered as evidence that injury has occurred.” (US EPA 2014a).

Adversity of FEV₁ response:

Hazard assessments of overt respiratory effects associated with ozone exposure focus primarily on changes in forced expiratory volume in one second (FEV₁), which is a measure of lung function. Multiple groups (ATS 2000, Pellegrino 2005, USEPA 1989) have postulated on the degree to which an FEV₁ decrement constitutes an adverse effect.

- The ATS (2000) states in their guidance that “reversible loss of lung function in combination with the presence of symptoms should be considered adverse.” This criterion has been used to judge the adversity of ozone-induced lung function decrements, although there is poor correlation between FEV₁ decrements and symptomatic responses in individuals exposed to ozone (Schelegle *et al.*, 2009, Frampton *et al.*, 1997, McDonnell *et al.*, 1999).
- An ATS/European Respiratory Society document provides the following FEV₁ adversity criteria: “two-point, short-term changes of >12% and >0.2 L in the FEV₁ are usually statistically significant and may be clinically important” (Pellegrino *et al.*, 2005). This document also notes that daily variation in FEV₁ in healthy individuals is ≥ 5%, weekly variation is ≥ 12%, and yearly variation is ≥ 15%. This normal variation can provide guidance when judging the adversity of FEV₁ decrements caused by ozone exposure.
- The EPA has stated that an FEV₁ decrement between 10-20% is of moderate severity, and this level of FEV₁ decrement with or without symptoms would be adverse (USEPA, 1989). However, the ATS notes that this definition has not been validated against other measures (ATS, 2000). In the most recent ozone risk assessment, the EPA states that “...a focus on the mid- to upper-end of the range of moderate levels of functional responses and higher (FEV₁ decrements ≥ 15%) is appropriate for estimating potentially adverse lung function decrements in active healthy adults,

while for people with asthma or lung disease, a focus on moderate functional responses (FEV₁ decrements down to 10%) may be appropriate” (US EPA, 2014b).

As noted above, there are other criteria to consider when judging an adverse effect, including transiency, reversibility and severity of effects. The FEV₁ responses are not transient – they are maintained throughout the exposure. However, at the doses used in human clinical studies, they are reversible – typically the FEV₁ returns to baseline within several hours of the end of the exposure (e.g., Schelegle *et al.*, 2009). At ambient concentrations of ozone (i.e., < 120 ppb for ~ 8 hour exposures at moderate exercise levels), most FEV₁ group mean decrements are < 20%, and as such the EPA would consider these to be moderate effects (US EPA, 1989).

Uncertainties associated with adverse effect judgments

There are several uncertainties associated with adverse effect judgments. Two major uncertainties are the decisions about where the line between adaptation and adversity lies, and if this line is different in different individuals or populations.

- As is suggested by the discussion about the adversity of FEV₁ decrements and inflammatory biomarkers, there can be differing opinions about what degree of change constitutes an adverse effect. Evidence-based professional judgment is often used to decide on a level of adversity.
- There is always a concern that some members of the population will experience adverse health effects at lower doses than those study subjects from whom the adversity level has been derived. For a common health endpoint such as FEV₁, information is available for human subpopulations, including those that may be more sensitive to ozone exposure than the subjects in the clinical studies (i.e., young healthy adults). These data can be used to decide if it is appropriate to set a different adverse effect level. For endpoints that use biomarkers to measure responses in humans, such as inflammation, it is difficult to define an adverse biomarker measurement, even for those populations that were studied (e.g., healthy adults). It is even more difficult to discern an adverse effect level for a sensitive population using biomarker information.

4 History of the Ozone NAAQS

Table 1 History of the Ozone National Ambient Air Quality Standard

Year	Indicator	Averaging Time	Level (ppm)	Form
1971	total photochemical oxidants	1-hour	0.08	Not to be exceeded more than once per year
1979	ozone	1-hour	0.12	Not to be exceeded more than once per year
1997	ozone	8-hour	0.08	Annual fourth-highest daily max, averaged over 3 years
2008	ozone	8-hour	0.075	Annual fourth-highest daily max, averaged over 3 years

Levels are identical for primary and secondary ozone standards.

ppm = parts per million.

Adapted from http://www.epa.gov/ttn/naaqs/standards/ozone/s_o3_history.html

- National Ambient Air Quality Standards (NAAQS) have four elements: indicator, averaging time, level and form.
- From 1971 to 1979, the indicator was photochemical oxidants; since then, it has been ozone, the most prevalent oxidant.
- Until 1997, the primary ozone standard was a daily 1-hour maximum concentration of 0.12 parts per million (ppm) that was not to be exceeded more than once in a year. Attainment of the 1-hour standard varied from year-to-year in a given area, depending primarily on meteorological conditions (NRC, 1991).
- In 1997, EPA determined that an 8-hour averaging time for ozone would provide greater stability for meeting the standard and more accurately reflect the way humans respond to ozone (Anderson and Bell, 2010). With the change in the averaging time from a 1-hour daily maximum to a daily 8-hour average maximum, the level of the standard was reduced from 0.12 to 0.08 ppm (equivalent to 0.084 ppm using standard rounding conventions).
- In 2008, the ozone NAAQS was revised so that the annual fourth-highest daily maximum 8-hour concentration, averaged over three years, should not exceed 0.075 ppm. The EPA Administrator has now recommended lowering the level of the standard to between 0.065 and 0.070 ppm (US EPA, 2014c).

5 Ozone Epidemiology Studies

Epidemiology studies evaluate associations between air quality (usually measured by central air monitors) and health effects in the population. Health effects associated with short-term ozone exposure are often estimated using time-series or panel studies. Health effects associated with long-term exposure are usually evaluated using a longitudinal cohort study design.

- In time-series studies, associations between time-varying ozone exposure and health for a defined population are assessed using aggregate estimates of exposure and outcomes. Health outcomes analyzed include daily population-average rates of acute health events, such as hospital admissions, emergency department visits, and deaths. Exposure assessment is usually based on ozone measurements made at one or more central air monitors. Time-series studies allow investigators to analyze health and exposure in a large study population, often using existing health databases, and are relatively insensitive to confounding by time-invariant subject characteristics, such as socio-economic status and smoking history.
- In panel studies the health status of each subject is repeatedly assessed (in some cases, hundreds of times) in association with time-varying ozone exposures. Health outcomes commonly analyzed in ozone panel studies include lung function and asthma symptoms. Because individual-level information on time-varying health status is collected, each subject effectively serves as his-or-her own control in the analysis. For some panel studies, estimates of personal ozone exposure are also measured, greatly reducing the amount of exposure measurement error compared to use of central site monitoring.
- In longitudinal cohort studies, a large cohort of individuals is followed for several years, and researchers evaluate health outcomes that occur in the cohort over time, such as death or asthma development. Researchers can control for some individual-level characteristics that may be associated with ozone exposures in these studies.

As in all air pollution epidemiology, individual studies that investigate the association between ozone exposure and health outcomes may be subject to methodological limitations that can impact the interpretation of results.

- Confounding by co-pollutants, such as particulate matter less than or equal to 2.5 micrometers in diameter (PM_{2.5}), and other factors can contribute to uncertainty in effect estimates observed in ozone epidemiology studies of every design. Results of time series studies are especially vulnerable to confounding by time-varying factors associated with ozone and health outcomes, including temperature, epidemics of respiratory infections, outdoor pollen and other aeroallergens, and long-term temporal trends in population health and air quality. If these variables are not adequately controlled for in the analysis, confounding can impact the observed associations between ozone and health effects (Lumley and Sheppard, 2000).
- Exposure measurement error can bias the results of air pollution epidemiology studies either towards or away from the null, especially in those studies that are dependent on ozone concentrations measured at central site monitors (Rhombert 2011).

- Other aspects of study design and statistical methods, such as self-reported health outcomes, model specification, and model selection bias can add to the uncertainty in interpreting the results from air pollution epidemiology.

In its Integrated Science Assessment (ISA) and Proposed Rule (PR), EPA highlighted epidemiology studies that were conducted since the previous ozone review. This substantial body of literature included studies of long-term and short-term exposure to ozone, as well as multi-city and single-city studies. A few examples of these key studies are described below.

- Strickland *et al.* (2010) analyzed over 90,000 emergency department (ED) visits for pediatric asthma in Atlanta in relation to 3-day moving averages of ozone measured at a network of monitoring stations across the study area, where ambient 8-h ozone concentrations averaged 0.0454 ppm.
 - The strengths of this study include its large sample size and rigorous methods, including a sensitivity analysis to assess the possibility of model misspecification.
 - Crude associations between ozone and ED visits were estimated as well as associations adjusted for carbon monoxide, nitrogen dioxide, elemental carbon, PM_{2.5}, and sulfate in two-pollutant models. In crude models, significantly elevated risks were observed in the warm season but not the cold season (relative risk [RR] = 1.082, 95% CI: 1.043-1.123 and RR = 1.044, 95% CI: 0.992-1.098, respectively). Adjustment of the warm season rates for PM_{2.5} and nitrogen dioxide in two-pollutant models resulted in attenuated risk estimates that were not statistically significant.
 - In the sensitivity analyses, the authors included daily counts of ED visits for upper respiratory infections and found it to be an "extremely strong" predictor of ED visit rates. Adjustment for this factor attenuated many associations between air pollutants and ED visits, including associations with ozone. We think that this indicates that this factor potentially confounds relationships measured in time series studies of ozone and ED visits.
- Most of the recent evaluations of new onset asthma were conducted as part of the Children's Health Study (CHS). The CHS is a prospective study that evaluates chronic effects of air pollution on the health of children living in 12 communities in Southern California (Peters *et al.*, 2004). A recent analysis of the CHS by McConnell *et al.* (2010) specifically investigated new onset asthma in the most recent cohort of kindergarten and first graders who were followed for three years.
 - McConnell *et al.* (2010) reported no significant difference in new onset asthma in children living in communities with the highest ozone concentrations (8-hour, 10 am to 6 pm annual average maximum, 0.0598 ppm) vs. the lowest ozone concentration (0.0295 ppm), over the range of ozone concentrations across the California communities studied (0.0303 ppm) [hazard ratio (HR) = 0.76, 95% confidence interval (CI): 0.38, 1.54]. Similarly, no ozone effects were reported when estimates were adjusted for exposures to modeled traffic-related air pollutants (HR = 1.01, 95% CI: 0.49, 2.11).
- Jerrett *et al.* (2009) evaluated the mortality risks associated with long-term ozone exposure in single- and two-pollutant models with PM_{2.5}. The authors evaluated the American Cancer Society cohort data from 1977 through 2000 in 96 and 86 cities, for single- and two-pollutant models, respectively, and included separate evaluations for respiratory and cardiovascular-related mortality.

- Jerrett *et al.* (2009) reported some increases in mortality, but the results varied by specific cause of death, including all-cause, cardiopulmonary, respiratory, cardiovascular, and ischemic heart disease (IHD) mortality. In single-pollutant models, exposure to ozone was not associated with all-cause mortality, but was associated with an increase in the risk of cardiopulmonary death per .010 ppm ozone (RR = 1.016, 95% CI: 1.008-1.024).
- In addition, Jerrett *et al.* (2009) observed statistically significant effects for subsets of cardiopulmonary disease, including cardiovascular (RR = 1.014, 95% CI: 1.005-1.023), ischemic heart disease (RR = 1.017, 95% CI: 1.006-1.029), and respiratory (RR = 1.027, 95% CI: 1.007-1.046) mortality in single-pollutant models. All of these estimates, with the exception of respiratory mortality, were null in two-pollutant models. Adjusted for PM_{2.5}, a RR of 1.040 (95% CI: 1.013-1.067) for respiratory mortality was observed.
- Katsouyanni *et al.* (2009) conducted a large multi-continent study that combined data from existing multi-city study databases from Canada, Europe (APHEA2), and the US (NMMAPS) to "develop more reliable estimates of the potential acute effects of air pollution on human health [and] provide a common basis for [the] comparison of risks across geographic areas" (Katsouyanni *et al.*, 2009).
 - The percent increase in standardized all-cause mortality ranged from 1.66-5.87 per 0.040 ppm increase in ozone, with the lowest estimates found in Europe and the highest in Canada. Results also varied by choice of model and lag period. Katsouyanni *et al.* (2009) assessed potential co-pollutant confounding in both all-year analyses and seasonal (summer-only) analyses, presenting estimates of mortality with and without adjustment for PM₁₀.
 - All-year mortality estimates were largely not statistically significant; however, in the few cases where statistically significant estimates were observed for ozone-only single-pollutant models (e.g., all-cause mortality in Canadian and US cities, and CV mortality in those ≥75 years old in Canadian cities), the PM₁₀-adjusted effects were not statistically significant. The notable exception was all-cause mortality across European cities, where the effect estimate was essentially unchanged and remained significant with PM₁₀ adjustment.
 - We note that, depending on the model used, US cities were found to have considerably lower ozone mortality effect estimates compared to Canada, and even negative associations, despite having the highest reported ozone concentrations (50th percentile of 0.035-0.060 ppm).
- Smith *et al.* (2009) examined regional variability of the ozone-mortality relationship and found statistically significant differences across regions, with significant effects reported in the Midwest and Northeast; small, and largely null, effect estimates in the Southeast; and negative or null estimates in the rest of the US, including southern California (which has cities with the highest reported ozone concentration).
 - Smith *et al.* (2009) also evaluated several factors that can potentially act as mortality effect modifiers, including temperature and PM₁₀. For temperature, effect modification was only significant in the summertime. Regional analyses showed temperature effect modification to be significant only in the Northeast and Industrial Midwest regions. Similarly, PM₁₀ was found to modify the ozone-mortality effect. The authors demonstrated reduced ozone effects of 22-33% across the various models when including PM₁₀.
 - Using the constrained distributed lag model of Bell *et al.* (2004) to estimate national average effects for ozone, Smith *et al.* (2009) reported an increase in non-accidental

mortality of 0.40% per .01 ppm increase in 24-hour ozone when fitted to days in which lag 1 PM₁₀ data were available (but not included in the model), and an increase of 0.31% per 10 ppb increase in 24-hour ozone when the analysis was repeated with PM₁₀ included as a co-pollutant. Smith *et al.* (2009) also considered an alternative model using a distributed lag to control for meteorology, which resulted in reduced mortality estimates.

- Zanobetti and Schwartz (2008) conducted a time-series study using generalized linear models that controlled for season, day of the week, and temperature to evaluate the association between mortality and ozone in 48 US cities during the summer months (June-August) between 1989 and 2000. The aim of the study was to determine if there was evidence of mortality displacement, *i.e.*, that deaths associated with exposure to ozone occurred in people that were dying, and exposure merely hastened but did not cause death.
 - The authors compared the mortality effect estimates at lag 0, lag 0-3, lag 4-20, and a distributed lag model (0-20). The standardized estimate was a 0.3% (95% CI: 0.2, 0.4) increase in all-cause mortality for a .01 ppm increase in 8-hour maximum ozone concentrations at lag 0 vs. 0.5% (95% CI: 0.05, 0.96) increase for the unconstrained distributed lag model (lag 0-20).
 - Similar results were reported for CV- and respiratory-cause mortality. The effects were statistically significant only for the lags up to 3 days, with negative effects (*i.e.*, mortality decreases) observed for the sum of lags 4-20. One way to interpret this is that ozone hastens death within 3 days but prevents it between 4 and 20 days. Since this is unlikely, the authors concluded that, based on these results, there was no evidence of mortality displacement.

6 Ozone Exposure Measurements in Epidemiology Studies

Ozone chemistry is complex, and important for understanding human exposure to ozone, as well as the implementation challenges of decreasing ozone levels.

- Ozone is a secondary air pollutant that is formed by photochemical reactions between precursor gases, primarily NO_x and volatile organic compounds (VOCs), in the presence of ultraviolet (UV) rays from the sun.

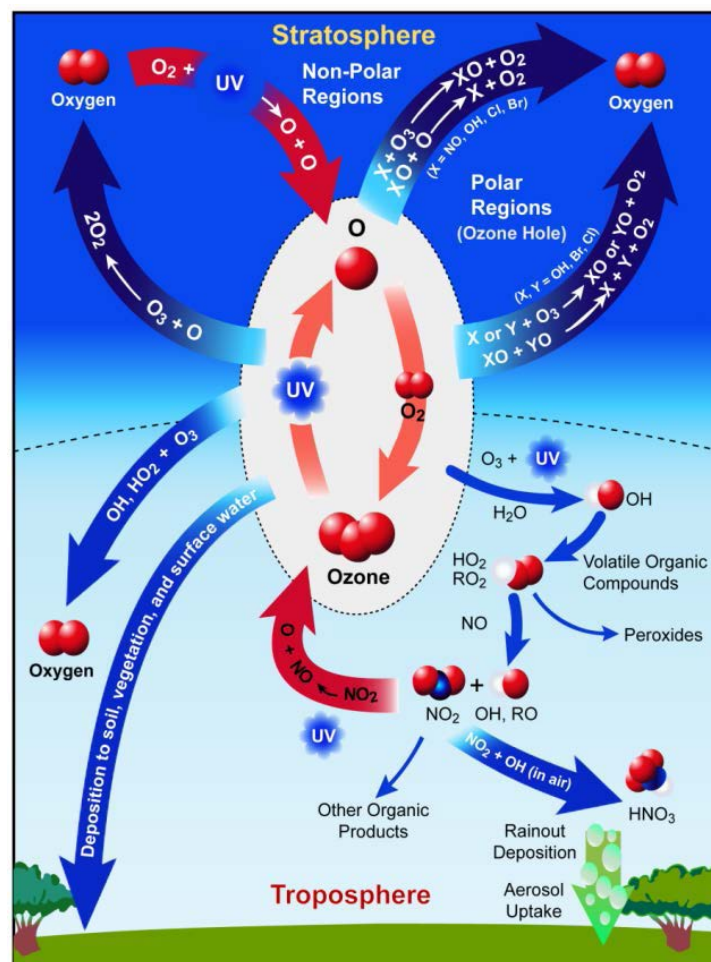


Figure 1. Overview of the photochemical processes influencing ozone formation. Source: US EPA (2013a).

- Mean background ozone concentrations across the US range from 0.027-0.040 ppm during the spring and summer, and sometimes reach higher than 0.060 ppm in the intermountain West (US EPA, 2013).
- Ozone formation and breakdown are complex and depend on many factors, such as the relative concentrations of precursor gases and meteorological factors (*e.g.*, sunlight intensity and atmospheric mixing).
- As a result of the variable factors influencing ozone formation and breakdown, ambient ozone concentrations vary widely both spatially and temporally (US EPA, 2013).
- The majority of ozone epidemiology studies rely on data from central ambient monitoring sites to provide community-average ambient ozone exposure concentrations. The accuracy of ozone epidemiology associations is predicated upon the assumption that these ambient measurements reflect actual personal exposures.
- Personal exposures are often lower than concentrations measured at ambient monitoring sites because people spend a large proportion of time indoors and there is a high deposition rate of ozone onto indoor surfaces. Indoor/outdoor ratios of ozone concentrations are typically about 0.10 to 0.30, and vary by season, region and ventilation practices (McClellan *et al.* 2009).
- During the prior ozone review process, CASAC highlighted exposure measurement error (*i.e.*, when ambient concentrations don't accurately reflect personal exposures) as a key uncertainty affecting the ozone epidemiology evidence, concluding that central site ambient monitors that measure ozone in the ambient air are generally poor measures of true personal exposures (US EPA, 2006). US EPA (2006) reported that personal ozone exposures are typically much lower than ambient ozone levels and often show little or no correlation with concentrations measured at the central ambient sites.
- We note that few studies that evaluated the correlation between personal and ambient concentrations have been published since the 2006 review. Nonetheless, based on a review of the available evidence, EPA concluded in the ozone ISA that "personal exposures are moderately well correlated with ambient concentrations" (US EPA 2013).
- Exposure measurement error may bias measured relationships between air pollutant and health either towards or away from the null (Rhombert *et al.*, 2011). Meng *et al.* (2005) showed that seasonal differences in infiltration behavior not only coincide with fluctuations in ambient particle concentrations, they also vary with location, and the magnitude of infiltration behavior can differ between communities and differentially impact the personal-ambient relationships. We think that this scenario is also applicable to ozone because ozone has relatively weak personal-ambient ozone correlations and low attenuation factors due to discrepancies between personal exposure and ambient concentrations and due to the interplay of a number of individual-, season-, and city-specific factors, including time-activity patterns, building characteristics, and ventilation practices.
- Exposure measurement error is also important because it complicates interpretation of multi-pollutant analyses, the method used by most air pollution researchers to address potential confounding by co-pollutants. When two pollutants are included in a statistical model at the same time, the one measured with less error will often display stronger associations with health outcomes (Vedal and Kaufman, 2011). Results of multi-pollutant models should be evaluated with careful consideration of relative amounts of measurement error associated with each pollutant; however, appropriate data required to assess the magnitude of measurement error is often not available for individual studies.

7 The NAAQS Causal Framework

One of the key challenges when evaluating causality is the selection and implementation of a framework that allows for a systematic, objective, and transparent assessment of all of the available evidence. Several organizations and regulatory bodies, including US EPA, have developed standardized methods for conducting systematic reviews of chemicals. These efforts have been fueled, at least in part, by the National Research Council's (NRC's) recent reviews of the regulatory risk assessment process, which identified the need for objective and standardized methods for evaluating the quality of individual studies and integrating evidence streams to develop scientifically supportable conclusions.

- EPA's NAAQS causal framework uses a five-level hierarchy intended to classify the weight of evidence (WoE) in support of causation for human health, ecological, and welfare effects. The levels are: 1) causal relationship, 2) likely to be a causal relationship, 3) suggestive of a causal relationship, 4) inadequate to infer a causal relationship, and 5) not likely to be a causal relationship (See Table 2).
- In the preamble of the ISA (US EPA, 2013), EPA stated that the NAAQS causal framework is largely based on the Institute of Medicine's (IOM, 2008) WoE framework and that its application relies heavily on the postulates put forth by Sir Austin Bradford Hill in his address to the British Royal Academy of Medicine in 1965 (Hill, 1965). The Hill postulates include strength of association, consistency, specificity, temporality, dose-response, biological plausibility, coherence, experiment, and analogy.
- We think that there are several instances in which the NAAQS causal framework deviates from both the Institute of Medicine (IOM, 2008) framework and Hill's postulates.
 - The NAAQS causal framework states that only one positive study is sufficient to establish a suggestive causal relationship when other results are inconsistent, whereas the IOM has much stricter guidance for concluding that evidence meets the criteria for classification as "equipoise and above" (*i.e.*, at least as likely as not).
 - EPA stated that an association is likely to be causal if "chance and bias can be ruled out with reasonable confidence but potential issues remain." EPA indicated that "potential issues" can include possible co-pollutant effects and limited or inconsistent findings from other lines of evidence. We think that it is difficult to rule out bias and confounding with "reasonable confidence" for most epidemiology studies because of the inherent limitations with observational study designs and authors' methodological choices (Dominici *et al.*, 2014). These can lead to a statistical association between a pollutant and a health effect that is not indicative of a causal relationship.
- There are several areas in which we think that the NAAQS framework is not sufficiently specific.
 - There is no explicit guidance for literature search strategies or for determining study exclusion criteria.
 - There is no guidance detailing the specific ways in which EPA should evaluate the strengths and weakness of the studies it reviews and how study quality characteristics affect evidence integration.

- It does not clearly describe how to apply the Hill postulates (*e.g.*, it does not define what a "strong association" means) and does not provide guidance for evaluating alternative hypotheses for observed associations.
- While there are many valuable features of the current NAAQS causal framework (*e.g.*, a hierarchy for categorization of the strength of the evidence), there are many well-developed WoE frameworks that EPA could have used or drawn upon, such as those by Adami *et al.* (2011), Suter and Cormier (2011), and Rhomberg *et al.* (2010). Although there are some differences among these frameworks, they all follow the same principles.
 - These include a transparent review protocol and literature search strategy, and clear criteria for evaluating individual studies (including how methodological limitations and uncertainties will be evaluated) and the body of studies as a whole (and how results from different lines of evidence should inform the interpretation of each other).
 - These frameworks all require evidence to be evaluated in a consistent manner, meaning that positive results should not be given more weight than null results simply because they are positive; results should only be given more weight if they are from more rigorous studies.
 - These frameworks indicate that one should consider both biological and statistical significance of results.
 - These methods are consistent with NRC's recommendations (NRC, 2011; 2014) for assessing causation, which include providing transparent descriptions regarding study inclusion criteria, a consistent method for evaluating studies' strengths and weaknesses, and a clear framework for evaluating the WoE in establishing causation.
- Although this was not in the ozone review, it is notable that in the Second External Review Draft of the Nitrogen Oxides (NO_x) Integrated Science Assessment (US EPA, 2015), EPA presents a new set of criteria for evidence integration. Specifically, EPA provides guidance on how to assess the study quality of epidemiology, controlled human exposure, and toxicology studies (detailed in US EPA, 2015, Table 5-1). Although EPA suggests individual studies be reviewed using these criteria, it states that they are "not criteria for a particular determination of causality in the five-level hierarchy" (US EPA, 2015).

Table 2. NAAQS Causal Framework

	Health Effects	Ecological and Welfare Effects
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (i.e., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in health effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. For example: a) controlled human exposure studies that demonstrate consistent effects; or b) observational studies that cannot be explained by plausible alternatives or are supported by other lines of evidence (e.g., animal studies or mode of action information). Evidence includes multiple high-quality studies	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures i.e., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. Controlled exposure studies (laboratory or small- to medium-scale field studies) provide the strongest evidence for causality, but the scope of inference may be limited. Generally, determination is based on multiple studies conducted by multiple research groups, and evidence that is considered sufficient to infer a causal relationship is usually obtained from the joint consideration of many lines of evidence that reinforce each other.
Likely to be a causal relationship	Evidence is sufficient to conclude that a causal relationship is likely to exist with relevant pollutant exposures, but important uncertainties remain. That is, the pollutant has been shown to result in health effects in studies in which chance and bias can be ruled out with reasonable confidence but potential issues remain. For example: a) observational studies show an association, but copollutant exposures are difficult to address and/or other lines of evidence (controlled human exposure, animal, or mode of action information) are limited or inconsistent; or b) animal toxicological evidence from multiple studies from different laboratories that demonstrate effects, but limited or no human data are available. Evidence generally includes multiple high-quality studies.	Evidence is sufficient to conclude that there is a likely causal association with relevant pollutant exposures. That is, an association has been observed between the pollutant and the outcome in studies in which chance, bias, and confounding are minimized, but uncertainties remain. For example, field studies show a relationship, but suspected interacting factors cannot be controlled, and other lines of evidence are limited or inconsistent. Generally, determination is based on multiple studies in multiple research groups.
Suggestive of a causal relationship	Evidence is suggestive of a causal relationship with relevant pollutant exposures, but is limited. For example, (a) at least one high-quality epidemiologic study shows an association with a given health outcome but the results of other studies are inconsistent; or (b) a well-conducted toxicological study, such as those conducted in the National Toxicology Program (NTP), shows effects in animal species,	Evidence is suggestive of a causal relationship with relevant pollutant exposures, but chance, bias and confounding cannot be ruled out. For example, at least one high-quality study shows an effect, but the results of other studies are inconsistent.
Inadequate to infer a causal relationship	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quantity, quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.	The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.
Not likely to be a causal relationship	Evidence is suggestive of no causal relationship with relevant pollutant exposures. Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter and considering at-risk populations, are mutually consistent in not showing an effect at any level of exposure.	Several adequate studies, examining relationships with relevant exposures, are consistent in failing to show an effect at any level of exposure.

Source: US EPA (2013), Table II.

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