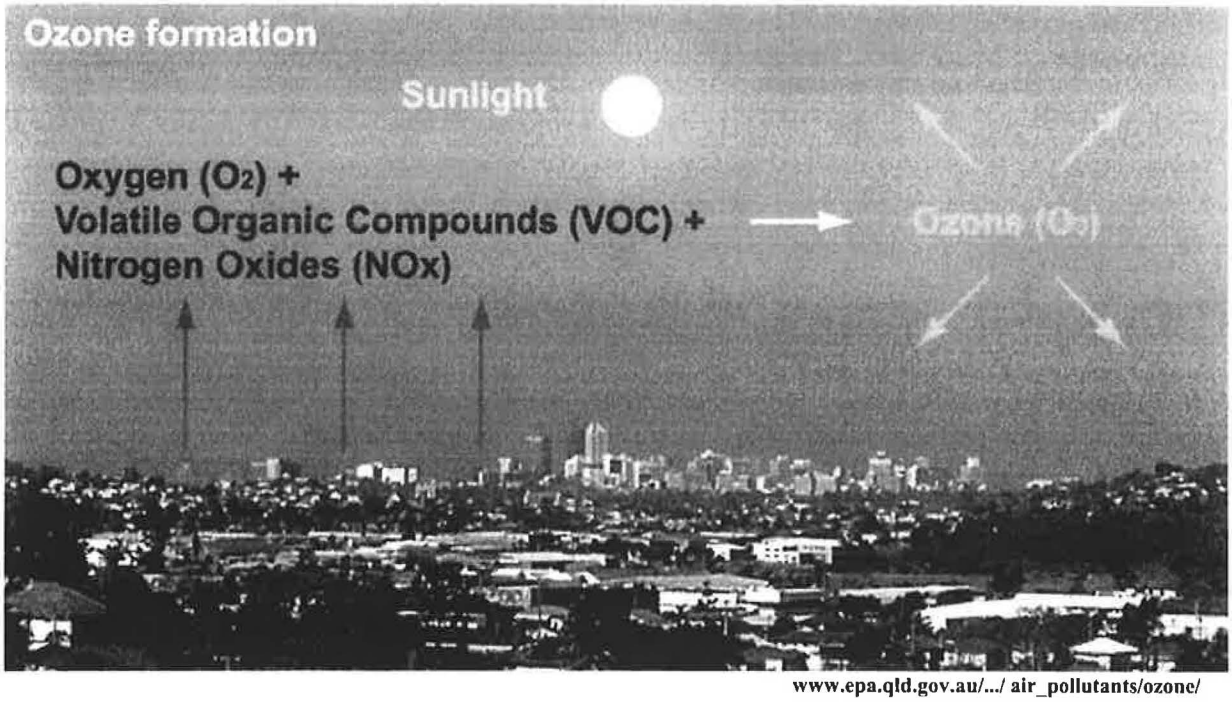
Ozone and the Lung:

Interactions with a Malicious Molecule



www.epa.qld.gov.au/.../ air_pollutants/ozone/

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Internal Medicine Grand Rounds
April 22, 2004

This is to acknowledge that Carlos E. Girod, M.D. has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this Program. Dr. Girod will not be discussing off-label uses in his presentation.

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Small airway diseases

I- INTRODUCTION

Air pollution has strong health, political and economic impact in our lives. It is also a topic of much speculation and controversy. An average person consumes about 10-20,000 liters of air each day[13]. Inevitably, some of this inspired air will be contaminated with airborne pollutants. Ozone, the main component of smog, is one of the most widely-known and studied pollutants[14]. During the hot summer days, news reports inform the public of poor air quality through "Ozone and Air Quality Index" alerts (Figure 1). Although well intentioned, this information often leads to either undue fear or complacency. The purpose of this Protocol is to clarify the effect of ozone exposure on overall lung health by reviewing ozone chemistry, lung epithelial interactions, and epidemiological studies identifying populations at risk.

Figure 1: Air quality index alert.[2]

II- AIR POLLUTION AND OZONE

a. History:

Anthropogenic (man-made) air pollution has existed since the discovery of fire and intensified during the industrialization era. In the 1940-1950's, three highly-publicized toxic episodes, the Los Angeles smog episode (1943), the SO_2 fog of Donora, PA (1948), and the London "Killer Fog" (1952), rapidly heightened public awareness of the detrimental effects of air pollution[1,14,16,17]. In the London smog incident of December 1952, climate changes led to the dense accumulation of fog and black smoke generated by the burning of sea coal for household heating. This incident was blamed for more than 4,000 deaths, mostly from respiratory disease (Figure 2)[16,18]. These sentinel episodes led to worldwide environmental policies that significantly reduced black smoke, smoke particulates, and sulfur dioxide to non-toxic levels[14]. In the United States, the Federal Clean Air Act was passed in 1967 requiring that all states meet air quality standards for these pollutants[1].

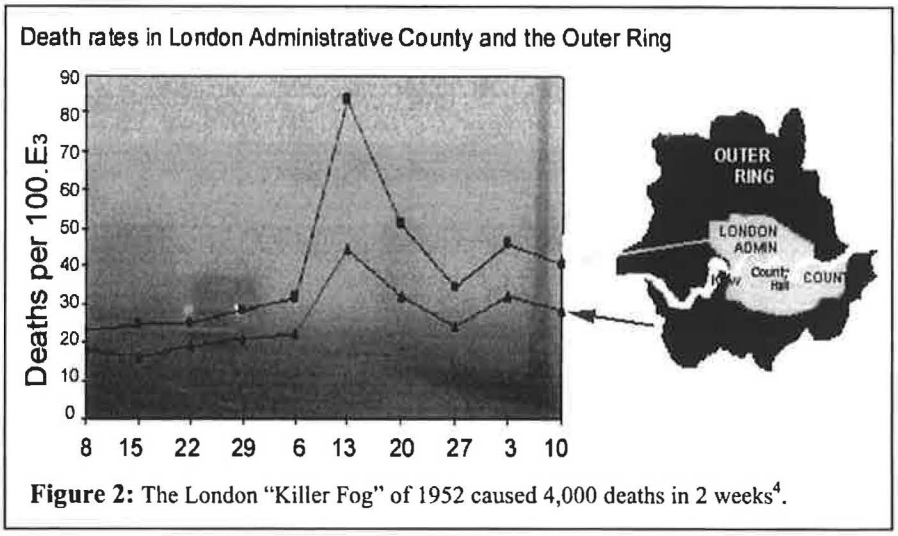


Figure 2: The London "Killer Fog" of 1952 caused 4,000 deaths in 2 weeks[4].

By the 1980's, ambient air pollution was no longer a topic of interest or high priority. Nevertheless, an expanding body of research identified new pollutants and their

association with increased respiratory and cardiovascular morbidity and mortality[14]. In 1990, the Clean Air Act was amended to address these pollutants. In 1997, the Environmental Protection Agency (EPA) set the current National Ambient Air Quality Standards (NAAQS) for six key pollutants: ozone, lead, NO_2, particulate matter (PM_{10} and $PM_{2.5}$), carbon monoxide, and sulfur oxides (Table 1)[3,19]. Of these pollutants, particulate matter and ozone have been associated with increased morbidity and mortality[13,20].

Table 1: National Ambient Air Quality Standards (NAAQS)[3]

Pollutant	Primary Standards	Averaging times
Ozone	**85 ppb** **120 ppb**	**8-hour** **1-hour**
Lead	1.5 $\mu g/m^3$	Quarterly Average
Nitrogen Dioxide	0.053 ppm	Annual (Arithmetic Mean)
Particulate Matter (PM_{10})	50 $\mu g/m^3$ 150 ug/m^3	Annual (Arith. Mean) 24-hour
Particulate Matter ($PM_{2.5}$)	15 $\mu g/m^3$ 65 ug/m^3	Annual (Arith. Mean) 24-hour
Carbon Monoxide	9 ppm 35 ppm	8-hour 1-hour
Sulfur Oxides	0.03 ppm 0.14 ppm	Annual (Arith. Mean) 24-hour

On April 15, 2004, the EPA announced that approximately 159 million people (54% of the population) in the U.S. lives in counties with ozone levels above the 8-hour average NAAQS standard[1,21].

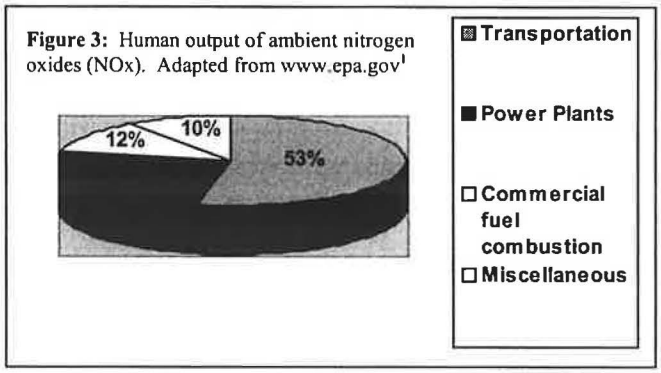
b. Ambient ozone generation:

Ozone as a pollutant needs to be distinguished from "healthy" ozone located in the earth's stratosphere approximately 10-50 kilometers from the earth's surface. This "Ozone Layer" protects earth from harmful UV rays and its depletion is directly responsible for global warming[22]. Pollutant ozone, better known as smog, is a by-product of man-made pollution and concentrates in the first layer of the atmosphere, the troposphere[14].

Ozone is a colorless gas generated by a photochemical reaction. It is a potent oxidant[16,23] with toxic effects not only on humans but also on vegetation[17,23]. Ozone is a

secondary pollutant and depends on the generation of primary pollutants, such as nitrogen oxides (NO_x), hydrocarbons, and volatile organic compounds (VOC's). These precursors are derived from fuel combustion, automobiles, power plant emissions, and small area sources (barbeques and fireplaces). In the U.S., nitrogen oxides (NO_x) constitute the most important ozone-generating primary pollutant. In 2003, NO_x was produced at an annual rate of 5 million tons (Figure 3)[1]. Natural sources (trees, volcanoes, forests) produce other volatile organic compounds (VOC's) that also generate ozone through photochemical oxidation[13,23]. This natural production contributes ~10-15 ppb of the 20-40 ppb baseline ambient ozone levels[7,17].

Figure 3: Human output of ambient nitrogen oxides (NOx). Adapted from www.epa.gov[1]



c. Atmospheric chemistry and ozone:

The initial step in ozone production in the troposphere depends on a photochemical reaction in which NO_2 from emissions absorbs energy from UV light ($\lambda \leq$ 410 nm wavelength) to form nitric oxide ($NO^{\cdot}$) and a molecule of atomic oxygen[7].

Reaction 1: $NO_2 + h\nu\ (\lambda \leq 410\ nm) \rightarrow NO^{\cdot} + O^{\cdot}$

Atomic oxygen is a highly reactive intermediate that combines with oxygen to produce ozone (O_3).

Reaction 2: $O^{\cdot} + O_2 \rightarrow O_3$

During normal ambient conditions, ozone (O_3) is scrubbed by available $NO^{\cdot}$ (Reaction 3).

Reaction 3: $O_3 + NO^{\cdot} \rightarrow O_2 + NO_2$

During episodes of smog, ozone accumulates when the scrubbing of O_3 by nitric oxide ($NO^{\cdot}$) (Reaction 3) is inhibited by the presence of the hydroxyl radical ($HO^{\cdot}$) converting nitric oxide ($NO^{\cdot}$) to NO_2. The hydroxyl radical ($HO^{\cdot}$) also reacts with organic compounds, especially hydrocarbons originating from inefficient fuel burning[7,24]. This is demonstrated in Reaction 4 (RH represents any organic molecule with a fragment containing carbon and hydrogen):

Reaction 4: $RH + HO^{\cdot} \rightarrow R^{\cdot} + H_2O$

The reactive organic radical ($R^{\cdot}$) reacts with atmospheric oxygen and any third molecule M to form a radical molecule, $RO_2^{\cdot}$. This radical then reacts with nitric oxide ($NO^{\cdot}$) reducing the amount of $NO^{\cdot}$ available for ozone scrubbing (Reaction 5):

Reaction 5: $R^{\cdot} + O_2 + M \rightarrow RO_2^{\cdot} + M$

$RO_2^{\cdot} + NO^{\cdot} \rightarrow RO^{\cdot} + NO_2$

$O_3 + \cancel{NO^{\cdot}} \rightarrow O_2 + NO_2$

Thus, the production of ozone in the troposphere is dependent on sunlight and the presence of both nitrogen oxides (NOx) and hydrocarbon organic emissions[7,24].

III. LUNG EPITHELIAL-OZONE INTERACTIONS

Ozone inhalation induces a variety of cellular and cytokine responses ultimately resulting in epithelial permeability, airway inflammation, bronchial hyperreactivity, and diminished lung function[13,25,26]. The toxic effects of ozone depend on the total dose, its distribution within the lung, inactivation by anti-oxidants, and the generation of oxygen radical species[26].

a. Dosimetry and pattern of distribution:

Inspired ozone has been demonstrated to deposit in the nasal epithelium, upper airway, and proximal bronchial airways[13,27]. Ozone also distributes in the peripheral airways and alveoli as demonstrated in the bronchoalveolar lavage (BAL) of normal subjects exposed to labeled O_3[28]. In animal models, ozone deposition and cellular injury occurs in the respiratory bronchioles (distal small airways) and centriacinar regions[10,29]. Inhaled ozone reacts avidly with molecules of the respiratory epithelial lining fluid and cell membranes of epithelial and resident cells so that residual ozone is not detected in exhaled air[7,13].

b. The lung lining fluid:

Extending from the nose to the alveoli, the lung lining fluid compartment serves as the first defense against ozone. It is composed of two layers: a thin aqueous layer covering the epithelium and a top mucous layer. The mucous layer is highly effective in capturing large inhaled particles and is constantly cleared by mucociliary action. The aqueous layer contains abundant anti-oxidant molecules that scrub ozone by a process called "reactive absorption"[7,13,30]. The major anti-oxidants in this lining fluid are urate,

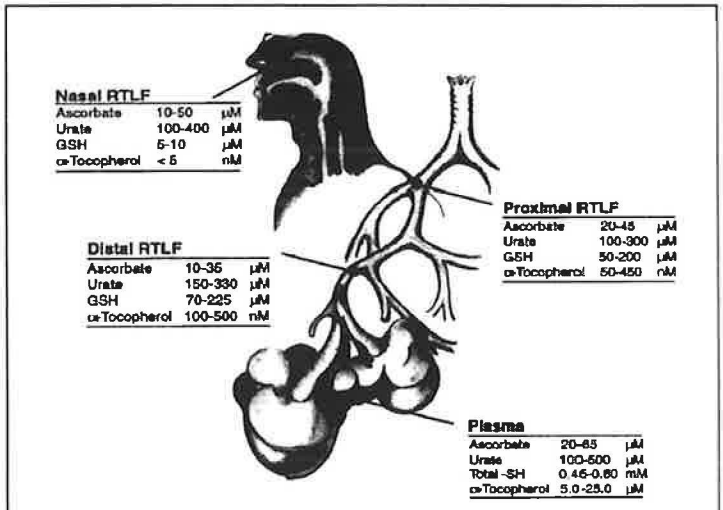


Figure 4: Distribution and concentration of anti-oxidant molecules in the respiratory tract lining fluid. From Mudway I.S. [7]

ascorbic acid, glutathione, and alpha-tocopherol. The local concentrations of some of these anti-oxidants is 100-fold those seen in plasma (Figure 4)[7]. Human subjects exposed to ozone (dose of 220 ppb) demonstrate a fall in airway antioxidants, especially glutathione peroxidases. Furthermore, high baseline levels of antioxidants correlate with decreased inflammation[31].

c. Epithelial cell interactions:

Ozone that is not scrubbed by anti-oxidants will trigger a variety of chemical reactions within the extracellular airway compartment. Ozone is not a radical species. Its cellular toxicity is mediated by production of reactive intermediary oxidants (H_2O_2, organic radicals, and aldehydes) through peroxidation (ozonation) of polyunsaturated fatty acids[7,13,32-34].

Epithelial cells and alveolar macrophages react with these secondary oxidants and express cytokines[13,16,25,35] through the activation of the transcription nuclear factor, NFκB[36]. NFκB binds to promoter regions that regulate expression of genes important in the inflammatory response: cytokines (GM-CSF, TNF-α, IL-1β), neutrophil chemoattractants (IL-8), and adhesion molecules (ICAM-1)[13,16,37]. These pro-inflammatory molecules promote neutrophil accumulation and activation (Figure 5)[38]. Mice overexpressing superoxide dismutase (SOD), a potent cellular oxygen radical scavenger, have blunted NFκB activation and demonstrate less lung injury during high-dose ozone exposure[39].

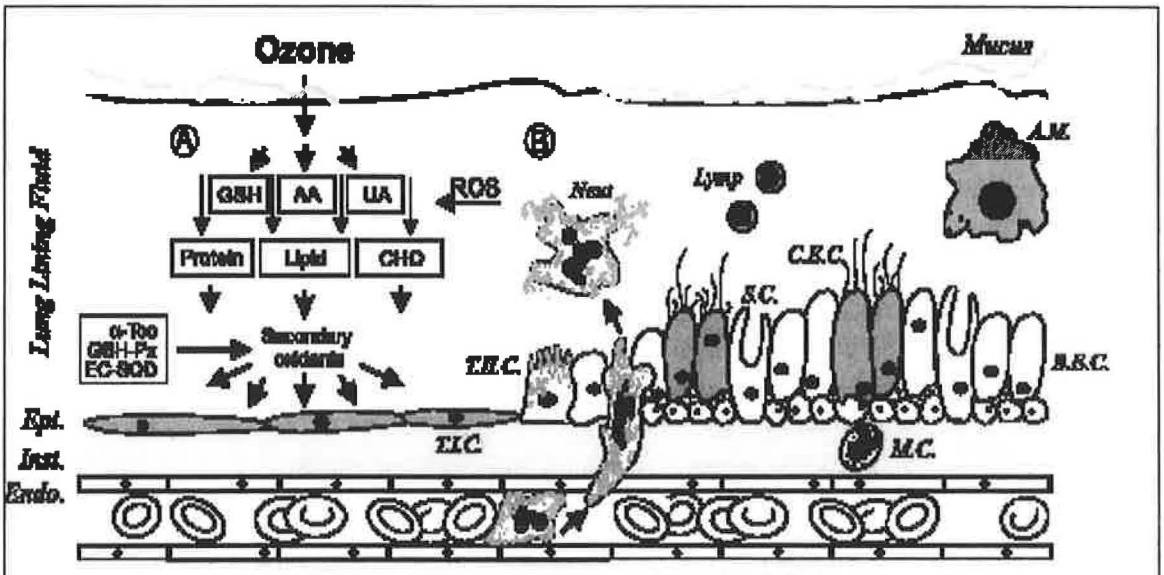


Figure 5: Diagram demonstrating the interaction of ozone with the respiratory epithelium. From Mudway I.S. (2000)[7]

Upon activation of neutrophils and macrophages, proteolytic enzymes and reactive oxygen species (H_2O_2 and ·OH) are secreted[26]. Finally, cytopathic changes are seen with necrosis of bronchial epithelial cells and alveolar type I cells[7,40-42]. A loss of intercellular tight junctions leads to a disruption of the airway epithelial barrier with

subsequent protein and fluid exudation into the airways and alveoli. It is hypothesized that this breach in the epithelial barrier results in enhanced tissue penetration of inhaled carcinogens, allergens, and infection[26].

IV. ANIMAL MODELS OF OZONE EXPOSURE

Animal studies have demonstrated the detrimental physiologic and cellular responses to acute and chronic ozone exposure. The first animal models utilized ozone concentrations that are significantly higher than those detected in polluted cities. The most recent animal models utilize exposure doses that are 5-10 fold above ambient ozone pollution[13]. This section will review only those animal models that mimic acute and chronic ambient ozone exposure in humans.

a. Interspecies variation:

Due to differences in lung anatomy and sensitivity, animal species vary widely in the location and degree of ozone injury. Rodents and rabbits have fewer terminal airways and lack respiratory bronchioles[43]. Therefore, the inflammatory response is focused in the nasal epithelium and the centriacinar regions of peripheral lung adjacent to terminal bronchioles[10]. Humans, monkeys, and carnivores have various generations of respiratory bronchioles and the inflammation focuses in these distal airways[10]. There is also significant interspecies variation in ozone sensitivity. For example, rats fail to demonstrate peripheral lung inflammation even with exposure to ozone concentrations 3-fold above ambient pollution levels (300 ppb)[26,44,45]. An ozone exposure of 500 ppb in rats induces the same inflammatory response seen in primates at 100 ppb[26].

b. Lessons learned from animal models of acute ozone exposure:

Despite these differences, ozone-induced lung injury is universal across species and results in the following inflammatory and cellular injury cascade[26]:

- Loss of integrity of respiratory mucosal barrier:
  - Precedes the inflammatory cell influx[46]
  - Characterized by the detection of serum proteins and albumin in the airway lumen[47]
  - Caused by the loss of intercellular tight junctions[26]
- Cytokine and chemokine release by epithelial cells and resident macrophages:
  - Induced by activation of NFκB
  - Arachidonic acid metabolites[48]
  - Prostaglandins (PGE_2 and PGF_2)[49]
  - Macrophage inflammatory protein 2 (MIP-2)[50]
  - IL-1 and TNF-α
- Inflammatory cell alterations[26]:
  - Immediate decline in alveolar macrophages followed by accumulation 4 days post-exposure

- Neutrophil influx peaking at 8-12 hours post-exposure with a return to baseline at 24 hours
- Enhanced neutrophil attachment and migration:
  - Increased expression of adhesion molecules: ICAM-1[51]
  - Fibronectin expression[52]
- Epithelial cell injury:
  - Necrosis of ciliated bronchial epithelial cells and alveolar type I cells[13]
  - Metaplasia of alveolar type II cells[53]
  - Clearance of necrotic epithelial cells dependent on neutrophil influx[41]

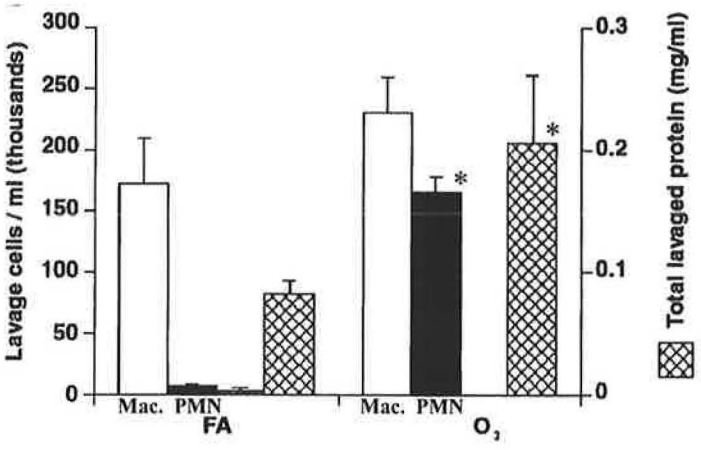


Figure 6: Ozone-exposed rhesus monkeys demonstrate a significant increase in neutrophils and total protein in bronchoalveolar lavage than controls exposed to filtered air (FA). From Sterner-Kock (2000)[10].

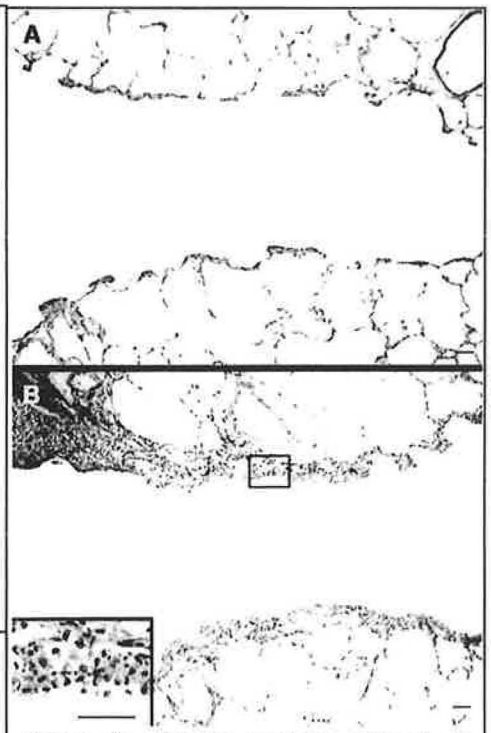


Figure 7: Rhesus monkeys exposed to filtered air (Panel A) with normal respiratory bronchioles. Animals exposed to 1000 ppb O_3 for 8 hours (Panel B) developed a respiratory bronchiolitis with neutrophil influx and epithelial sloughing[10].

Recent animal studies have focused on the rhesus monkey, the closest correlate to human lung anatomy and ozone responsiveness[8,11,54]. Rhesus monkeys exposed to 800 ppb ozone for 4-50 hours demonstrate alveolar type I cell necrosis and a respiratory bronchiolitis with accumulation of alveolar macrophages and epithelial metaplasia[55]. Sterner-Kock also demonstrated ozone-induced neutrophil influx and epithelial sloughing within respiratory bronchioles (Figures 6 and 7)[10].

c. Chronic ozone exposure leads to impaired post-natal lung development:

Post-natal lung maturation is complete by the age of 8-10 y/o in humans and by 3 years of age in rhesus monkeys. In both species, lung development occurs in the distal lung at the epithelial-mesenchymal "trophic" unit, which consists of epithelial and mesenchymal cells separated by a basement membrane. In the mesenchymal extracellular matrix, growth factors guide cellular interactions that lead to differentiation of the epithelium, smooth muscle, and nerve structures within the airways[56].

In 2003-2004, a group of researchers from the University of California-Davis reported blunting of post-natal lung development in rhesus monkeys exposed to ozone[11,54,56]. Infant monkeys were sensitized to house dust mite allergen (HDMA) and exposed to filtered air (FA), HDMA, ozone, or HDMA + ozone. Ozone exposure consisted of intermittent 500 ppb concentration for up to 6 months, a dose that resembles peak ambient ozone levels in cities like Mexico City and Los Angeles[8]. Ozone combined with an antigen challenge led to aberrant post-natal development with decreased airway and alveolar differentiation, altered basement membranes[56], diminished epithelial innervation[11], and abnormal smooth muscle bundles (Figures 8 and 9)[8].

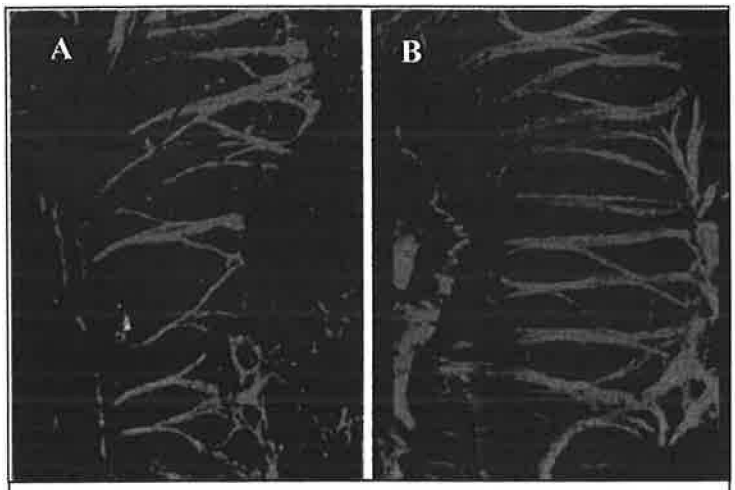


Figure 8: Smooth muscle fibers in ozone + HDMA exposed animals (Panel B) are thicker, more numerous, and have altered orientation in comparison to control animals exposed to filtered air (FA) (Panel A)[8].

Ozone + HDMA exposed monkeys had a decrease in functional distal airways (up to 3-5 generations of airway branching[8]). This developmental arrest could serve as a substrate for chronic airflow obstruction and hyperreactivity. Although further longitudinal studies are needed to evaluate for reversibility of this lesion[11], this animal model provides compelling evidence that seasonal ozone exposure could potentially stun lung development in young atopic children[8].

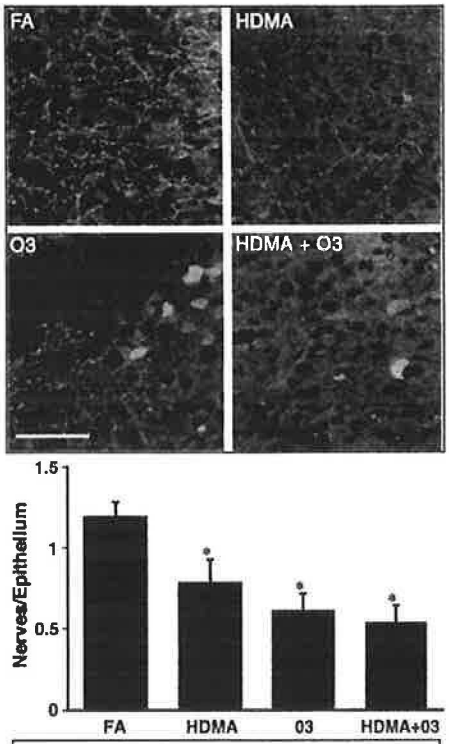


Figure 9A and 9B: Immunoflouresent staining of distal airways (Figure A) and morphometry (Figure B) demonstrate a dramatic reduction in nerve bundles for all treatment groups when compared to filtered air (FA)[11]. HDMA= house dust mite

V. HUMAN STUDIES OF CONTROLLED OZONE EXPOSURE:

To estimate the impact of ambient ozone on lung health, investigators have exposed normal subjects to controlled acute exposures. Unequivocally, this data reveals that ozone exposure at "ambient" levels causes pulmonary symptoms, physiologic dysfunction, cellular inflammation, and epithelial damage[13].

a. Dose response:

In resting adults, ozone doses above 400 ppb (above ambient pollution) are required in order to elicit symptoms or reduce pulmonary function. In 1983, McDonnell and colleagues demonstrated that brief exercise during exposure to ozone leads to significant reductions in FEV_1 even at concentrations below ambient pollution (Figure 10)[9]. No significant changes were observed in exercising subjects exposed to filtered air. Subsequent studies have confirmed that in exercising adults significant decrements in FEV_1 are seen with a 6-8 hour exposure to ozone at 80 ppb[57]. In 2003, the World Health Organization (WHO) concluded that the safe threshold for short-term (6-hour) ozone exposure is less than 60-80 ppb (below the current EPA NAAQS 8-hour ozone standard)[58].

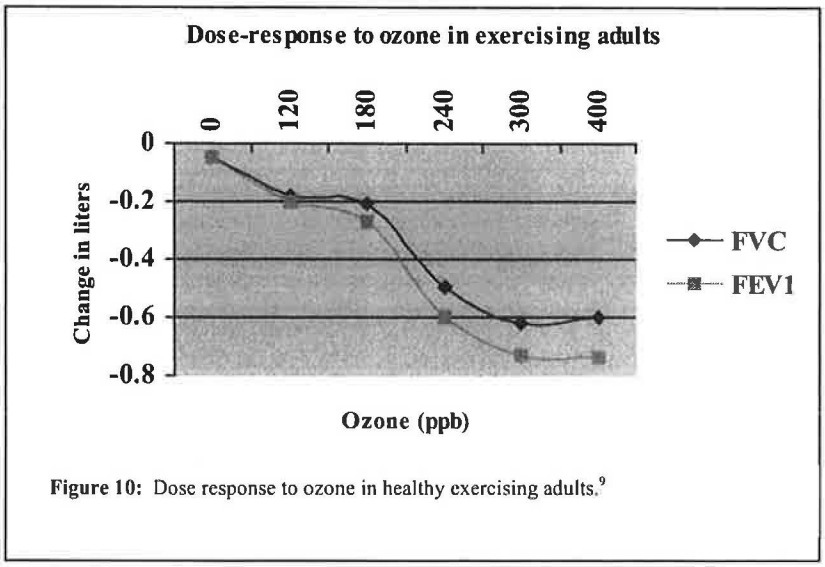


Figure 10: Dose response to ozone in healthy exercising adults.[9]

b. Symptoms and physiologic changes:

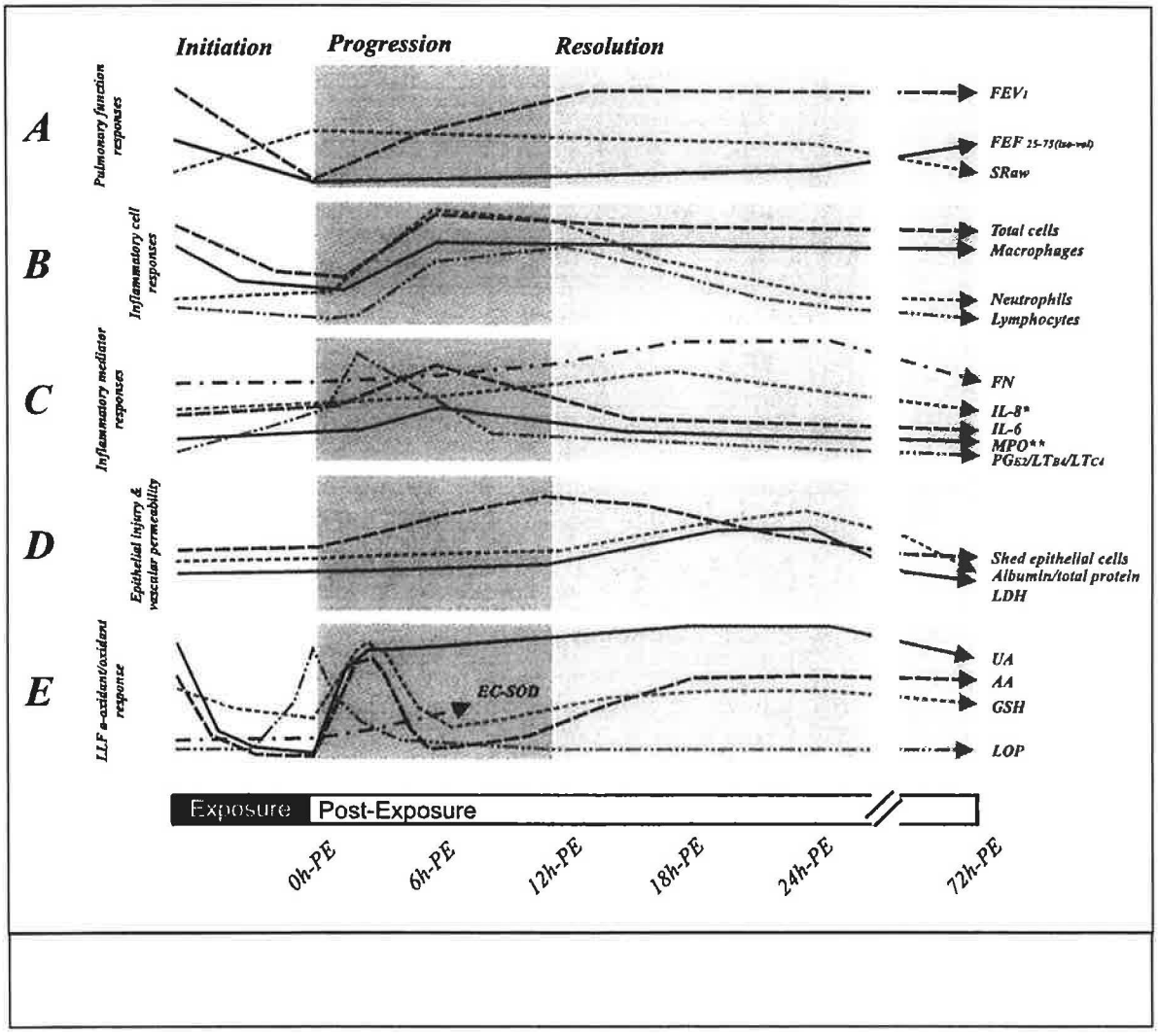
Subjects exposed to high ozone levels report increased cough, substernal chest pain, wheezing, and inability to sustain exercise[9,13,14,59,60]. Children develop cough, nasopharyngeal irritation, and chest pain in association with headaches and malaise[61].

Exposure to ozone causes equal reduction in FEV_1, FVC, and FEF25-75% suggesting a restrictive process. This is due to a reduction in the inspiratory capacity (IC) as documented by unchanged functional residual capacity (FRC) with an increase in residual volume (RV)[62]. The reduced lung function is not influenced by the administration of atropine or beta-2 agonists and appears to be independent of pulmonary compliance or inspiratory muscle strength[13]. It is suspected that ozone irritates afferent nerves located in the tracheobronchial tree (C-fibers) with activation of a "neurally-mediated" reflex leading to involuntary inhibition of inspiration and the onset of rapid shallow breathing[13,63]. This effect can be blocked with the use of sufentanil, a potent opioid[64].

An increase in small airways resistance and hyperreactivity has also been documented after ozone exposure. Frank (2001) demonstrated persistent small airway dysfunction 5 days post-exposure[65]. Furthermore, increased airway hyperreactivity, as measured by methacholine challenge, has been demonstrated in non-asthmatics[66].

c. Inflammation and epithelial permeability:

Controlled ozone exposure in humans leads to lung inflammation at ozone concentrations below those leading to a decline in lung function[13,67-70]. The inflammatory response is similar to that seen in animal models but the timecourse appears to be somewhat different (Figure 11)[7,26].



Ozone-induced inflammation has been demonstrated in nasal and bronchial biopsies[68,71]. Endobronchial biopsies have revealed increased submucosal mast cells[70] and neutrophil infiltration[68,72]. Increased epithelial permeability has been demonstrated by an increase in BAL total protein and albumin[26]. Recently, CC16, a protein specific for the Clara cell, a bronchial epithelial cell, has been measured in the serum of exercising adults after exposure to ozone doses as low as 60-84 ppb (below NAAQS standards)[73].

In summary, controlled human exposure models have confirmed ozone's toxicity. Although these physiologic and inflammatory changes are modest, they are in part

responsible for increased respiratory morbidity, hospital visits, asthma and COPD exacerbations, and lung function decline.

VI. EPIDEMIOLOGICAL STUDIES OF AMBIENT OZONE EXPOSURE:

a. Study design:

Population-based epidemiological studies have significant advantages over controlled human exposure studies. These studies follow sample populations numbering in the thousands, thus allowing for powerful statistical associations between ozone and a particular outcome, such as spirometric indices, hospitalization rate, and mortality[16]. The study of a large population allows for removal of individual confounding characteristics, such as smoking, socioeconomic status, education level, age, and gender. Most importantly, the cohort population serves as its own control since it is also exposed to low levels of pollution over time[6,16]. These epidemiological studies utilize powerful statistical methods that allow the separation of ozone from the effects of other variables, such as seasonality, influenza epidemics, ambient temperature, and co-pollutants: NO_2, PM, CO, and SO_2[6,16]. Of note, various studies have demonstrated a weak association between these index pollutants and ozone in ambient air[16,74].

c. Effects on mortality:

There is a direct association between ozone pollution and increased mortality from respiratory deaths[75]. A recent meta-analysis published in 1999 reported an estimated relative risk (RR) for death of 1.036 (95% CI, 1.023-1.050) for an increase of 100 ppb in daily 1-hour maximum ozone level (0.36% increase in mortality for each 10 ppb). The overall RR was increased to 1.056 when only those studies adjusting for temperature and PM (particulate matter) were analyzed (Figure 12)[6].

In 1997, the APHEA (Air Pollution and Health: a European Approach) study included a large population living in 6 large European cities. A 1.2% increase in mortality was associated with a 10 ppb increase in daily ozone levels[76]. In 2000, Samet et al. published a longitudinal population study of 20 U.S. cities from 1987-1994[74]. Five major outdoor pollutants were studied: PM10, ozone, nitrogen dioxide, sulfur dioxide, and carbon monoxide. Particulate matter (PM10) had the strongest association with increased mortality from respiratory and cardiovascular deaths. A correlation with ozone did not reach statistical significance. This group expanded these observations to 90 U.S. cities (NMMAPS-National Morbidity, Mortality, and Air Pollution Study- Part II) demonstrating an increase in mortality of 0.25% for each 10 ppb increase in ozone levels[77]. The combination of these large studies and meta-analysis has confirmed the impact of ozone on overall mortality.

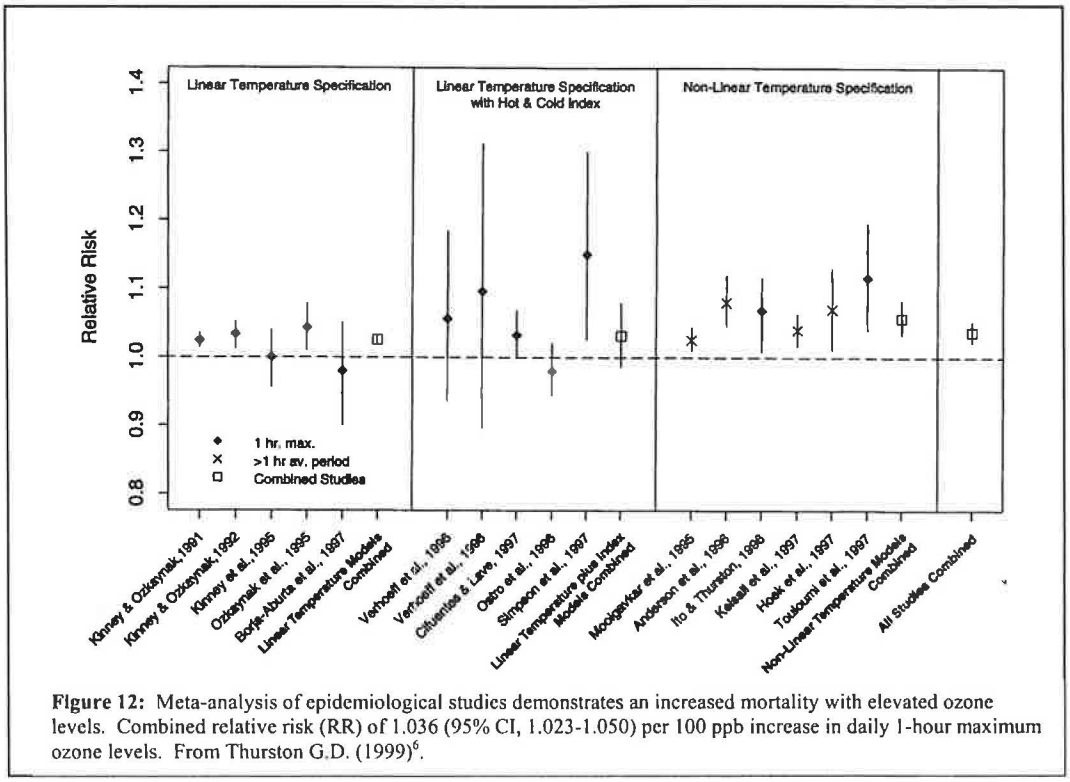


Figure 12: Meta-analysis of epidemiological studies demonstrates an increased mortality with elevated ozone levels. Combined relative risk (RR) of 1.036 (95% CI, 1.023-1.050) per 100 ppb increase in daily 1-hour maximum ozone levels. From Thurston G.D. (1999)[6].

c. Effect of ozone on hospitalization rate and Emergency Department visits:

In 1992 and 1994, Thurston evaluated the effects of ambient ozone on hospital admissions in New York State and Toronto[6]. Rising ozone levels were predictive of increased hospitalization rates for asthma and respiratory illnesses regardless of temperature and other pollutants (Figure 13)[6]. This association persisted even when the ozone levels fell below 80 ppb for 1-hour maximum level, a level below the EPA standards[6,78]. In 1997, the APHEA project published the results on the effects of five major pollutants (SO_2, black smoke, total suspended particles, NO_2, and O_3) on admissions for COPD in a

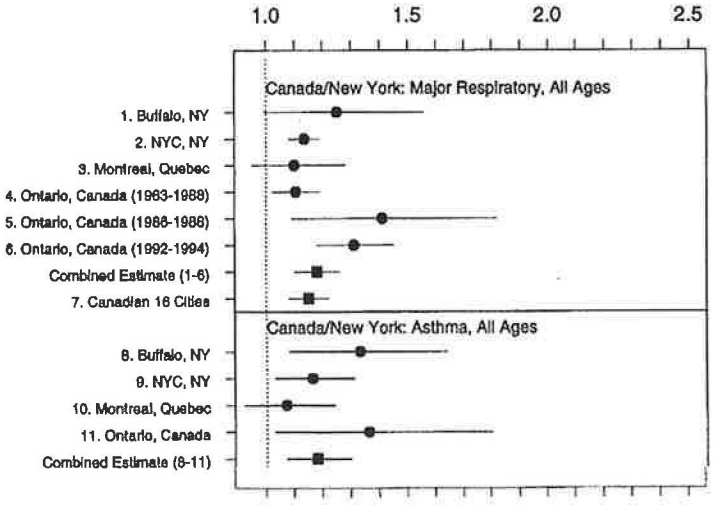


Figure 13: Combined RR for hospitalization for each 100 ppb increase in daily 1-hour maximum ozone levels. Data from various epidemiological studies of major metropolitan areas in New York State and Canada. Adapted from Thurston G.G. and Ito K. (1999)[6].

cohort population of five major European cities: London, Paris, Milan, Amsterdam, and Rotterdam. A relative risk RR of 1.038 (95% CI, 1.018-1.058) for hospitalization was associated with a 25 ppb increase in the 8-hour average ozone level[76].

Similar effects have been demonstrated with ozone and increased ED visits[6]. In a study published by Delfino in 25 Montreal area hospitals, a 15% increase in ED visits for respiratory illness (95% CI, 6-24%) was observed for a 25 ppb increase in the 1-hour maximum ozone level[79].

d. Effects on respiratory symptoms and lung function:

Six summer camp population studies prospectively evaluated pulmonary function tests in a total of 616 healthy children[6]. The results demonstrate a decline in afternoon FEV_1 with increasing ozone levels (Table 2). Although these changes are small, they could account for the observed increase in respiratory symptoms and healthcare utilization in children exposed to ozone pollution.

Table 2: Reduced slopes of afternoon FEV_1 correlate with increasing ozone levels. Data from 6 summer camp studies. Adapted from Thurston (1999)[6].

Study	Slope (ml/ppb)	SE (slope)	p-value
Fairview Lake, 1984	-0.50	0.16	0.002
Fairview Lake, 1988	-1.29	0.27	0.0001
Lake Couchiching	-0.19	0.44	0.66
CARES, 1986	-0.29	0.10	0.003
San Bernardino, 1987	-0.84	0.20	0.0001
Pine Springs, 1988	-0.32	0.13	0.013
Overall	-0.50	0.07	0.0001

An increase in respiratory complaints in association with rising ozone levels also transmits to school absenteeism. In a study of 4th graders in southern California, Gilliland found a 63% increase (95% CI, 18-124%) in illness-related school absences for an elevation of 20 ppb in the 8-hour average ozone level[80]. Furthermore, absences due to a respiratory illness increased by 83% (95% CI, 4-222%) for each 20 ppb elevation in the 8-hour average ozone level[80].

e. Chronic effects of ozone on the incidence of newly-diagnosed asthma:

Although few long-term cohort studies have been conducted, ozone has been associated with an increased incidence of asthma and decline in lung function[6,58,81]. The incidence of asthma was studied by the ASHMOG (Adventist Health Smog) study in California, a 15-year study of 3,091 adults completed in 1992. In males, a RR of 2.09 (95% CI, 1.03-4.16) for new-onset asthma (diagnosed by a physician) was seen for a 27 ppb increase in the 8-hour average ambient ozone concentration[82]. Recently, McConnell conducted a 5-year cohort study evaluating the incidence of asthma in 3535 children from 12 communities in southern California with varying levels of ambient ozone[83]. Physician diagnosis of new-onset asthma was made in 7.5% of the children during follow-up. In the communities with high ozone concentrations, a child playing three sports had a relative risk (RR) for new-onset asthma of 3.3 (95% CI, 1.9-5.8) when compared to a

child not playing sports. This ozone effect was independent of other co-pollutants and not due to exercise-induced bronchospasm. The authors suggest the possibility that ozone might not be causative for new-onset asthma but exacerbate previously unrecognized asthma[83].

There are few cohort studies examining the effect of ozone on the decline in lung function in children. In 1999, a three-year prospective study of 1,150 school children in Austria evaluated pulmonary function twice a year before and after the summer season. Ozone levels were associated with a reduction in pre- and post-summertime FEV_1 by 0.029 cc/d/ppb ($p<0.001$) and FVC by 0.018 cc/d/ppb ($p<0.001$). It is estimated that a child living in a high-ozone community could lose 49 ml in FVC (a 2% decrement in predicted FVC)[84] over a three year period. Most recently, Gauderman (2002) published a 4-year cohort study of 1,678 fourth-graders from 12 communities of southern California (Children Health Study). A variety of index pollutants were studied. Although acid vapor, NO_2, and PM2.5 had more influence on longitudinal lung function, ozone had a negative effect on annual expiratory peak flow rate (PEFR) at –1.21% (95% CI, -2.06, -0.36) for a 37 ppb increase in the annual average ozone level[85].

VII. OZONE EFFECTS ON CHRONIC LUNG DISEASE:

a. Effects of ozone on asthmatics:

In asthmatic children, high ozone levels correlate with increased respiratory symptoms (wheezing, shortness of breath, chest tightness)[13,86], bronchitic symptoms[87], use of rescue inhalers[86,88,89], asthma attacks[90], respiratory infections[90], and reduction in peak flows[88,90,91]. These studies are summarized in Table 3. In adult asthmatics, population-based studies have demonstrated increased ED visits[92] with increasing ambient ozone levels, especially in those who are heavy smokers, RR 1.72 (95% CI, 1.13-2.62) per 50 ppb ozone[93].

Increased ambient ozone levels could precipitate asthma exacerbations by augmenting lung inflammation[26]. An increase in nasal neutrophils is observed when exercising asthmatics are exposed to ozone at 120-240 ppb but not to filtered air[94]. Elevated IL-8, total protein, and other inflammatory markers have also been demonstrated in bronchoalveolar lavage of asthmatics exposed to ozone[95].

Furthermore, ozone seems to amplify the allergic response to allergen challenge. In 1999, Kehrl studied mild allergic asthmatics with controlled exposure to ozone 160 ppb or filtered air for about 8 hours in combination with light exercise. The concentration of house dust mite antigen (HDMA) needed to generate a decrease in FEV_1 of 20% (PC20) was significantly lower when the subjects were exposed to ozone before the antigen challenge[96]. Vagaggini demonstrated statistically-significant increase in eosinophils in the sputum of mild allergic asthmatics exposed to ozone followed by an allergen challenge[97]. It is suspected that ozone primes the airway for antigen challenges

by increasing epithelial permeability and decreasing the dose of antigen needed to elicit the late allergic response and airway hyperreactivity[91].

Table 3: Summary of studies demonstrating the effects of ozone on asthmatics:

Location	Ozone levels (ppb)	Duration of study	Study Design	Age	Observation	Reference
New York State	84-160 ppb 1-hour daily maximal level	One week in June 1991-1993	Cohort n=166 Summer camp	7-13 y/o	40% increase in asthma exacerbation or chest symptoms	Thurston 1997[88]
Netherlands	<65 ppb 1-hour maximum level	Summer 1995	Cohort n=61	7-13 y/o	Increase in acute respiratory symptoms and medication use. Black smoke and PM10 > ozone	Gielen 1997[98]
Inner City U.S.	48 ppb mean 8-h average; 5% of days exceeded EPA standard	3 months	Cohort n=846	4-9 y/o	Children born prematurely had decline in AM peak flows of 1.8% per 15 ppb ($p<0.05$) and increased AM symptoms	Mortimer 2000[91]
Los Angeles	62 ppb mean 8-h average	>3 months	Cohort n=138	6-13 y/o	Increase risk for the use of rescue medication: OR 1.10 (95% CI, 1.03-1.19). No effect on respiratory symptoms	Ostro 2001[89]
Paris, France	30 ppb overall mean; Maximum 60 ppb	3 months	Cohort n=82	8-13 y/o	Increase in asthma attacks, changes in lung function, and respiratory infections	Just 2002[90]
So. California	14-33 ppb 4-year average range	4 year	Cohort n=475	9-10 y/o 12-13 y/o	Bronchitic symptoms OR 1.06 (95% CI, 1.00-1.12)	McConnell 2003[87]
Southern New England	51 ppb mean 8-hour average (range 21-100)	6 months	Cohort n=271	< 12 y/o	Wheezing increased by 35% and chest tightness by 47% for a 50 ppb increase in 1-hour ozone. Increased SOB and use of rescue inhalers	Gent 2003[86]

b. Effects of ozone on COPD:

COPD patients exposed to high ozone pollution demonstrate an increase in respiratory symptoms[99], ED visits, and hospitalization rates (Table 4)[7,100]. Interestingly, PM10 (particulate matter $\leq$ 10 μm) pollution has a stronger correlation with COPD exacerbation, symptoms, and ED visits than the effects observed with ozone[20,100,101].

Cigarette smokers do not appear to have increased ozone susceptibility when compared to healthy subjects even at high ozone exposures (500 ppb for 6 hours)[102]. Furthermore, a recent mouse emphysema model failed to demonstrate a synergistic effect of ozone and cigarette smoke in the development of emphysema[103].

Table 4: Ambient ozone levels correlate with increased ED visit for COPD exacerbation.

City	% Increase in ER visits for COPD exacerbation per 25 ppb increase in ozone levels (95% CI)	References
Birmingham, AL	3.0% (-2.5-10%)	Schwartz (1994)[104]
Detroit, MI	**6.0% (1.5-10%)**	Schwartz (1994)[105]
Minneapolis, MN	3.0% (-3.9-7.0%)	Schwartz (1994)[106]
Spokane, WA	**13 % (1.1-25%)**	Schwartz (1996)[107]
APHEA	**4.3% (1.1-6.5%)**	Anderson (1997)[76]
Sydney, Australia	1.5% (-7.6-12%)	Morgan (1998)[108]
Valencia, Spain	**30.1% (11-50.5%)**	Tenias (2002)[109]

Adapted from Sunyer (2001)[100] with addition of current references by Dr. Girod.

X. INTERVENTION:

What is the magnitude of ozone pollution in the U.S.?

On April 15, 2004, the EPA announced that 474 counties located in 31 states (total population of 159 million) have ozone levels that exceed the 85 ppb NAAQS 8-hour average standard[1,21]. Most of these counties are located in the eastern U.S. (Figure 14)[1]. This had led to major efforts from the U.S. Government to generate new strategies to decrease all six major index pollutants, especially PM (particle matter) and ozone.

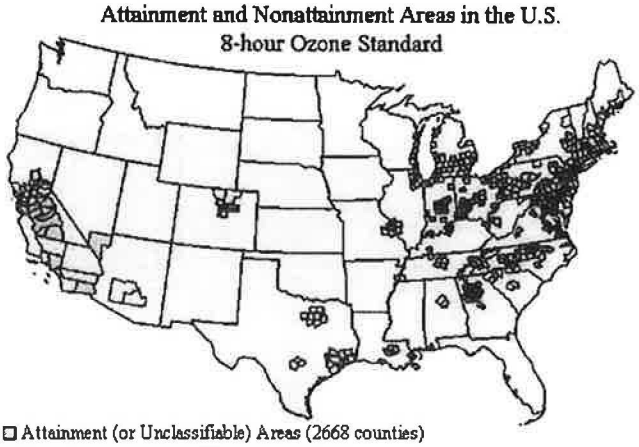


Figure 14: U.S. counties with ozone levels above EPA standards on April 15, 2004[1].

What is the magnitude of ozone pollution in Texas?

In Texas, 23 counties will exceed the EPA 8-hour ozone standard this year[1]. Of those, Brazoria, Galveston, and Harris counties are Texas' most polluted[110]. In 2003, Harris county (containing the city of Houston) ranked as the 6th most ozone-polluted county in the United States with 80 "Orange", 44 "Purple", and 11 "Red" ozone alert days. Tarrant County ranked #19 in the nation[111].

a. Primary prevention: Reducing Emissions

Ambient ozone pollution is a direct consequence of anthropogenic (human) production of nitrogen oxides (NOx) and volatile hydrocarbons that drive the generation of ozone through photochemical reactions. The majority of these primary pollutants are produced by vehicle emissions (53%) and power plants[1]. In the last 20 years, the total NOx emission has not changed despite attempts at reducing vehicle emissions per mile driven. The design of fuel-efficient cars and better fuel is offset by the fact that drivers are commuting farther each year[23].

Is there evidence that reducing human production of air pollutants could impact ozone levels or most importantly, lung health? The answer is Yes. During the 1996 Atlanta Olympics, a unique opportunity presented that proved the positive impact of improving air quality[5]. To accommodate visitors and athletes, traffic in downtown Atlanta was closed to private automobiles forcing residents and workers in the city to utilize public transportation through park-and-ride arrangements. During the Olympics, ozone levels were reduced an average of 11-19% in Atlanta and surrounding counties. Other index pollutants demonstrated similar reductions (Figure 15). After adjusting for temperature and climate changes, the reduction in ozone levels led to a 42% drop in asthma ED visits and hospitalizations[5].

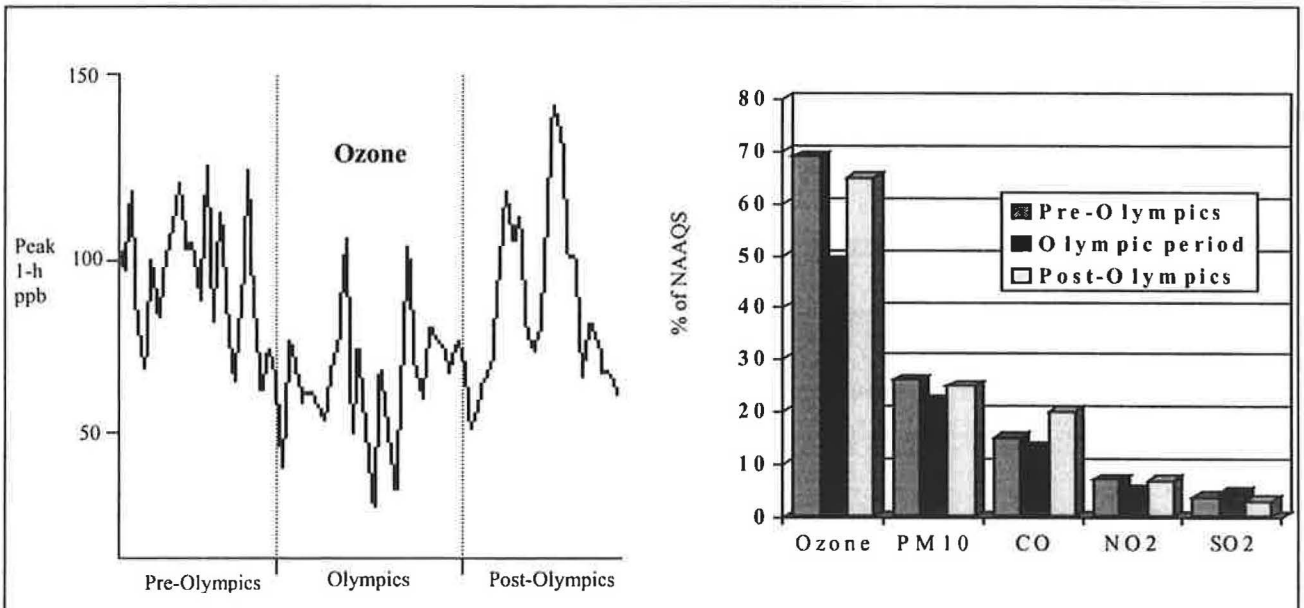


Figure 15: Reduction in the mean levels of ozone and four other major index pollutants during the 1996 Atlanta Olympics. From Friedman MS (2001)[5]

The Clear Skies Initiative 2003:

During the State of the Union Address on January 28, 2003, President Bush urged the U.S. Congress to pass the "Clear Skies Initiative" mandating a significant reduction in precursor pollutants from power plants (Table 5)[110]. This initiative would update the current Clean Air Act of 1990. This Bill proposes a reduction of power plant emissions by utilizing a "cap-and-trade" system that allows compliant power plants with pollutant

output below the cap to sell leftover points to plants that are not meeting standards. This incentive would drive healthy corporate competition focusing on developing technological advances that would reduce pollutants without the need for costly local and federal governmental oversight[110].

Table 5: The Clean Skies Initiative of 2003 proposes the following reduction in primary and secondary pollutants[110]

	Actual Emissions in 2000	First Phase 2010	Second Phase 2018	Reduction at Full Implementation
Nitrogen Oxides (NOx)	5.1 million tons	2.1 million tons	1.7 million tons	67%
SO2	11.2 million tons	4.5 million tons	3 million tons	73%
Mercury	48 tons	26 tons	15 tons	69%

The Clear Skies Initiative proposes that a reduction in nitrogen oxides (NOx) and volatile organic compounds (VOC's) would decrease ambient ozone levels allowing 263 counties (77 million people) to meet EPA NAAQS standards[110]. This reduction in ozone and particulate matter is expected to yield $113 billion dollars in annual health benefits by the year 2020 through prevention of:

- 12.5 million days with respiratory symptoms causing school absences, lost work days, or activity restriction
- 30,000 total hospitalizations (15,000 due to asthma)
- 23,000 non-fatal heart attacks (primarily due to particulate matter PM)
- 8,800 new cases of chronic bronchitis
- 14,100 premature deaths[110]

This proposed bill has met heavy criticism by environmental groups and bi-partisan Congress representatives (www.congress.org) and has yet to be approved by Congress. The main criticisms include the potential for unattainable deadlines and the failure to address global warming through the establishment of caps on carbon dioxide emissions[112,113].

What can we do to reduce emissions?

- **Technological advances:** Design and purchase low emission vehicles[114]. The average automobile sold in the United States has a fuel efficiency rating that is half of the 40-50 miles-per-gallon (mpg) seen in currently available fuel-efficient cars. This is due to the popularity of SUV's[114].

- **Community planning:** Enforce vehicle emission inspection for all motor vehicles; promote public transportation and carpooling; support bicycling and bike paths; utilize park-and-ride systems; limit city sprawling; and design affordable housing close to businesses and cities[114].

b. Secondary prevention: Behavior modification and antioxidant therapy

Most of the health policy pertaining to ozone pollution focuses on increasing public awareness and promoting behavioral modifications in susceptible populations. Most cities with high ozone pollution publish or report the current air quality and ozone alerts in newspapers, radio broadcasts, and television news. The following secondary prevention strategies have been suggested[114-116]:

- Implement air purification in schools
- Build schools away from major roads; limit traffic around schools.
- Keep children (especially age < 10 y/o or asthmatics) indoors during ozone alert days
- Limit outdoor exercise to the hours before 11AM and after 8PM

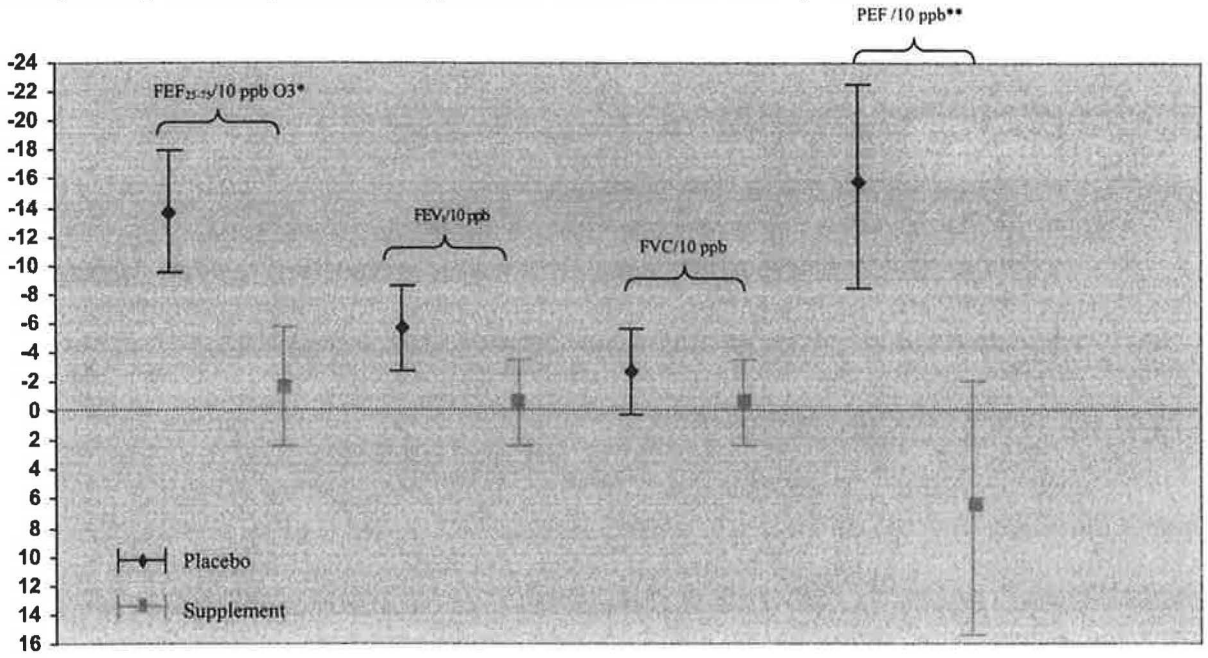
Role of corticosteroids and antioxidants:

Ozone exposure is characterized by increased mucosal permeability and neutrophil influx into the airways. In animal models, this is reduced by pre-treatment with corticosteroids[117]. In healthy humans, pre-treatment with inhaled corticosteroids has not had similar results[118]. In contrast, asthmatics given corticosteroids demonstrate inhibition of neutrophil influx but no effect on lung function decline[117]. This finding is not surprising since this decrement appears to be neurally-mediated and independent of inflammation[69,117].

Research has focused on the effect of dietary supplementation with antioxidants and vitamins in reducing ozone toxicity. For 2 weeks, Samet and colleagues supplemented the diet of 31 healthy adults with either placebo or a supplement containing 250 mg of Vitamin C, 50 IU of Vitamin E, and 12 ounces of a vegetable cocktail[32]. The subjects underwent BAL and spirometry before and after the exposure to 400 ppb O_3 for 2 hours. The antioxidant-supplemented group had elevated serum anti-oxidants and sustained a less significant drop in FEV_1 and FVC than the placebo group. Interestingly, no effect on BAL IL-6 or neutrophils was noted.

In Mexico City, the beneficial effect of anti-oxidant supplementation was demonstrated in a prospective study of 158 asthmatic children exposed to high ambient ozone pollution[12]. A placebo or a combination of Vitamin E 50 mg and Vitamin C 250 mg was administered daily for almost 2 years. Children whose diets were supplemented with anti-oxidant vitamins had less ozone-related decrements in FEF 25-75 and peak expiratory flows (PEF) (Figure 16)[12,119]. Based on this data, asthmatic children exposed to high ambient ozone pollution may benefit from antioxidant supplementation above the minimum daily requirement.

Figure: 16: Anti-oxidant supplementation protects against ozone-induced decrements in lung function in children with asthma. Change in lung function is plotted for a 10 ppb increase in ambient ozone levels. *p < 0.05



Adapted from Romieu I. (2002)[12]

XII. CONCLUSION:

This Protocol has reviewed the effects of ozone on lung health. Animal and human models of acute and chronic exposure have confirmed the inflammatory and cellular responses induced by breathing ozone at levels at or below EPA NAAQS standards. Extensive epidemiological data has confirmed that these inflammatory changes lead to measurable increases in respiratory symptoms, pulmonary dysfunction, school absenteeism, hospitalization, and mortality. Populations at risk include young children, asthmatics and COPD patients. Recent animal models suggest that chronic ozone exposure alters post-natal airway development with a decrease in distal airway branching and lung function and may predispose to chronic lung disease.

Air pollution is one of the few triggers of lung disease exacerbation for which we have some control and input. At an individual level, there are a variety of primary and secondary interventions that can be immediately applied to reduce pollutant emission and their effects on susceptible populations. The methods necessary to achieve a nationwide compliance with air pollution control have generated much political discourse and debate. Further studies should address the financial costs and long-term benefits of reducing air pollutants.

Finally, why should we be concerned about the quality of the air we breathe? The American Lung Association answers this question with its simple motto: "When you can't breathe, nothing else matters"[120].

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