OZONE HEALTH EFFECTS:
Repeated Exposure and Sensitive Subjects

Lawrence J. Folinsbee

National Center for Environmental Assessment
U.S. EPA
Research Triangle Park, NC 27711

Ozone has a broad range of health effects in humans. I intend to focus primarily upon two aspects of these health effects: (1) responses to repeated ozone exposures and the potential implication of these studies for understanding chronic effects of ozone and (2) the mechanisms of response to ozone in sensitive subjects and how these may be useful in understanding ozone-associated morbidity. In this regard, the focus will be mainly on human controlled exposure studies.

1. REPEATED EXPOSURES

Reported effects of long-term residence in communities with high ozone levels include a seasonal variation in ozone-induced pulmonary function responses, impediments to lung development in children, and acceleration of the age-related decline in lung function in adults. There is very limited evidence that long-term repeated ozone exposure may be related to an increased incidence of asthma in adults. Finally, several recent studies are suggestive of an association between acute ozone exposure and cardiorespiratory mortality.

1.1 Community Exposures

Studies of effects of ozone and lung function have been reported in the literature for decades. A lower sensitivity to ozone in people resident in communities with high ambient ozone levels is well known [1]. Moreover, studies indicated that this effect has a seasonal fluctuation that is consistent with a depression of response during repeated ozone exposure and the return of a "normal" response with a period of several months of limited ozone exposure [2]. A recent pilot study [3] of incoming freshman at the University of California (Berkeley) indicated that the level of performance on some tests of lung function (especially FEF75% and FEF25-75%), in relation to that expected for age, race and gender, were inversely related to estimated lifetime exposure to ozone (i.e., a higher lifetime ozone exposure resulted in a poorer performance on lung function tests). These observations suggest that chronic ozone exposure in children may be associated with interference with lung development.

1.2 Controlled Exposures

Over the past twenty years, many studies of repeated exposure to ozone in volunteer subjects have been performed. In general, these studies have shown that humans exposed to ozone on

successive days gradually develop an attenuated response to ozone which, over the course of 3-5 days, leads to the absence or near absence of pulmonary function or symptom responses to ozone. This has been referred to as "adaptation," although in the usual biological sense it is not an adaptation since, with no further exposure, the responses return to the pre-exposure levels within a week or so. If the initial exposure conditions are severe enough to cause marked pulmonary function and symptom responses, the individual often has an exaggerated response on the second day of exposure [4,5]. Repeated ozone exposure has also been associated with a temporary decline in baseline lung function [5,6]. Studies in which a seasonal attenuation of pulmonary function response is observed [2] show that the seasonal attenuation persists for several months whereas the attenuation observed with repeated laboratory exposures persists for no more than a couple of weeks [7]. The absence of pulmonary function or symptom responses could have suggested the erroneous conclusion that once one became "adapted" to ozone, that there were no further effects.

Acute exposure to ozone causes an inflammatory response characterized by release of various pro-inflammatory mediators (e.g., interleukins IL-6, IL-8, and prostaglandin PGE2), infiltration of neutrophils (PMN), activation of alveolar macrophages, and increased epithelial permeability. Only recently has there been any attempt to examine the inflammatory and cellular correlates of an attenuated function and symptom response in humans. Devlin et al. [9] showed that some

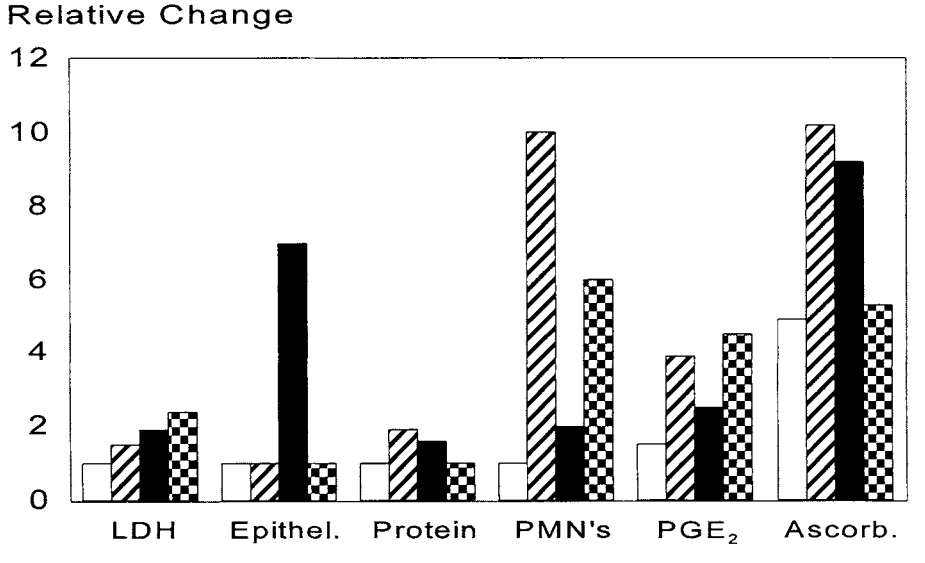

Figure 1. Lactate dehydrogenase (LDH), epithelial cell number, protein, neutrophils (PMN's), prostaglandin E_2 (PGE_2), and ascorbate content of BAL fluid after air (open bars) or ozone exposure (Day 1, hatched; Day 5, solid; return exposure, checkered) ([Devlin et al., [9]).

inflammatory and cellular responses associated with acute ozone exposure were also attenuated after five consecutive days of ozone exposure (Figure 1). Notably absent were increases in airway neutrophils and IL-6 hallmarks of the inflammatory response in healthy individuals acutely exposed to ozone. In addition, prostaglandin E_2 levels, usually markedly elevated after ozone exposure, showed only a slight increase after the fifth exposure. Epithelial cells, not normally

seen immediately after an acute exposure, were present in the BAL after the fifth exposure, possibly the result of already damaged cells physically knocked loose during the lavage process or from delayed sloughing of damaged cells. In addition, the bronchoalveolar lavage (BAL) fluid level of the enzyme lactate dehydrogenase (LDH), an indicator of cellular damage, was increased. This latter marker indicates that ozone continued to cause cell damage even in the absence of inflammation, symptoms, and lung function changes. When subjects were reexposed to ozone either 10 or 20 days later, the absence of an increase in BAL protein, epithelial cells, or the antioxidant ascorbate suggested a continued attenuation of these responses, although pulmonary function and symptom responses were similar to "pre-adaptation" levels. The changes in BAL protein suggest that the epithelium which replaces ozone-damaged epithelial cells is less "leaky." It is likely that cellular repair processes leading to a modified epithelium, that is more resistant to attack by ozone (such as observed in laboratory animals repeatedly exposed to ozone) [10], may also alter cellular/inflammatory responses. However, the more rapid return of pulmonary function and symptom responsiveness is consistent with the likely neural mediation of these responses.

Relationships between changes in spirometric lung function and symptoms and biochemical and cellular markers, in response to ozone exposure, have been of interest for some time. In this study [9], spirometry and symptom responses generally showed an initial decline, accentuated on the second day, and gradually diminishing by the fifth exposure. Despite the temporal alignment of the changes in spirometry and symptoms, there was no significant correlation between symptoms and function within the group of subjects at any specific time point. However, individuals exhibited consistent relationships between the magnitude of their individual changes in spirometry and their symptoms across the series of exposures. There was no evidence of a relationship between the biochemical and cellular markers and the changes in spirometry and symptoms. An increase in airway responsiveness to bronchoconstrictor agents has been shown, in other studies [4], to be increased during acute and repeated exposure to ozone (Fig 2). The changes in airway responsiveness were not correlated with the spirometric and symptom responses.

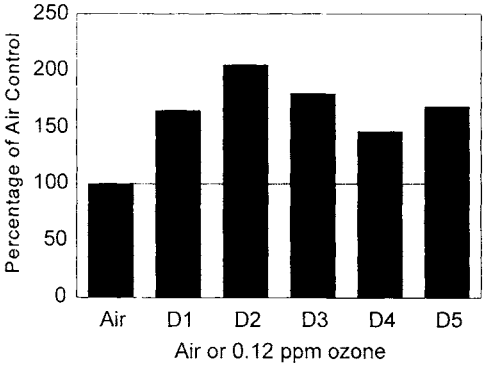

Figure 2. Airway responsiveness following exposure to air or 0.12 ppm ozone for 6.6 h on five consecutive days (Folinsbee et al., [4])

2.0 SENSITIVE SUBJECTS

There is a broad range of responsiveness to ozone within the population. Of particular concern from a public health standpoint is the identification of specific groups within the population who may be more sensitive as a result of specific characteristics, especially those characteristics associated with respiratory disease. Individuals with asthma, a chronic inflammatory airway disease, appear to be more responsive to ozone exposure.

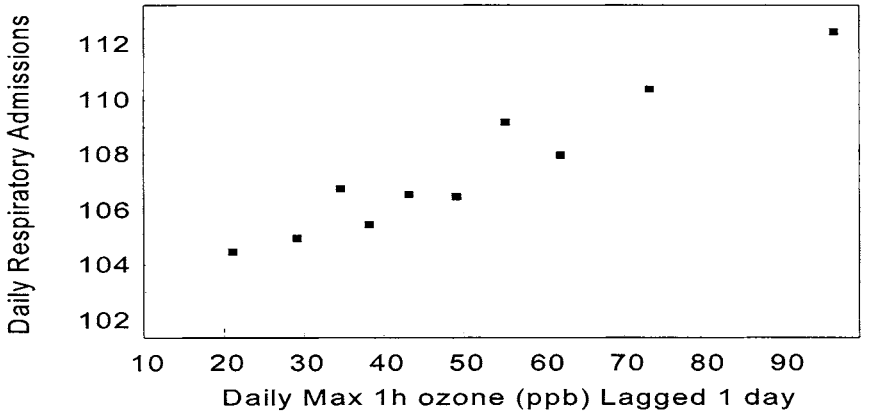

Figure 3. Respiratory admissions for 168 hospitals in Ontario, Canada in relation to daily 1 h maximum ozone concentration, lagged one day (Redrawn from Burnett et al., [11])

2.1 Community Exposures

Ambient exposure to ozone is associated with increased hospital admissions, increased visits to hospital emergency rooms, and increased numbers of asthma attacks and respiratory symptoms associated with asthma. Approximately 30 years ago, ambient ozone levels were reported to be associated with hospital admissions in Los Angeles [12]. More recent reports have detailed the association with respiratory hospital admissions in other cities such as London, New York, Toronto (Figure 3), and Rotterdam. Thurston et al., [13] have estimated that approximately 10-20% of summertime respiratory hospital admissions are related to ozone. This amounts to an estimated 1-3 ozone-related respiratory hospital admissions per day per 100 ppb ozone for each million in the population. In addition to increased asthma symptoms and asthma attacks, ozone accounts for increased numbers of asthmatics who present to emergency rooms (ER) in areas such as Atlanta [14], Los Angeles, Montreal, New Jersey, and Mexico City. It has been estimated that ozone accounts for as much as 15-20% of asthma ER visits during the summer and that asthma ER visits may increase as much as 30-40% on the highest ozone days [13,14]. These observations suggest that asthmatics may be more responsive to ozone than non-asthmatics.

2.2 Controlled Exposures

Exposure of asthmatics, usually with mild disease, to low concentrations of ozone in a controlled exposure facility has been conducted by a number of investigators. Although many of these studies showed little, if any, difference in spirometric responses between asthmatics and non-asthmatics, the interpretation of these studies may have been limited by the low concentration of ozone used [15]. More recent studies [16-21] conducted at higher ozone concentrations or for longer durations show somewhat greater changes in spirometry or airway resistance in asthmatics (Figure 4). The differences between healthy and asthmatic subjects exposed under the same conditions vary considerably among studies and range from no difference to a two-fold greater response in asthmatics. Nevertheless, these differences in response to ozone are considerably smaller than the differences in response to SO_2 [22] or other

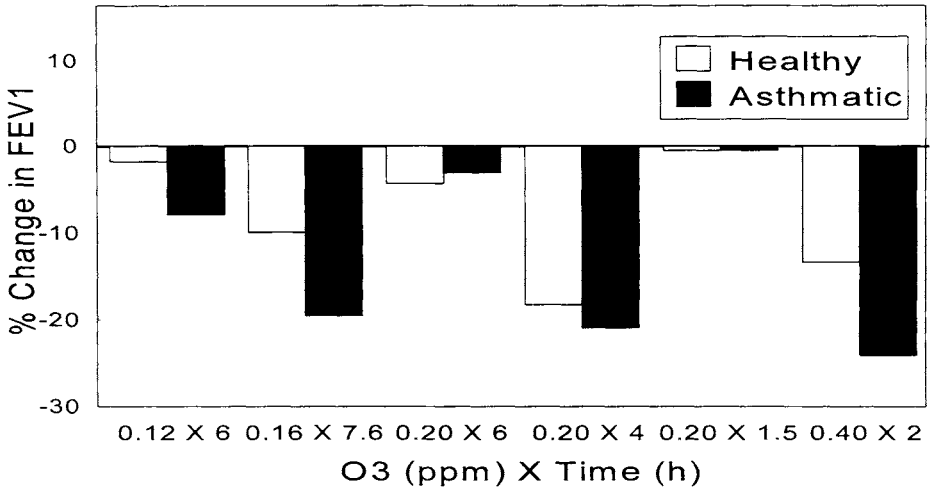

Figure 4. Change in FEV_1 (forced expired volume in one second) in response to ozone exposure of the specified concentration (ppm) and duration (h) from 6 different studies. Data from Linn et al., [17]; Horstman et al., [18]; Basha et al., [19]; Scannell et al., [20]; Linn et al., [21]; Kreit et al., [16]; respectively, left to right.

non-specific broncho-constrictor agents for which differences between asthmatics and non-asthmatics may be as much as 100-fold or more. The presence of inflammation in the airways of asthmatics led a number of investigators [19,20; RB Devlin, U.S. EPA, personal communication, 1997) to hypothesize that the inflammatory response would be increased in asthmatics exposed to ozone. In these studies, asthmatics had a greater number of neutrophils (a key cellular marker of ozone-induced inflammation in non-asthmatics) in their lavage fluid following ozone exposure than did non-asthmatics (Figure 5). In 3 studies conducted under comparable conditions, the mean percentage of neutrophils in BAL after ozone exposure in non-asthmatics ranged from 7-9% and in asthmatics ranged from 8-16%. The study with the smallest difference between asthmatics and non-asthmatics was one which employed only asthmatics with a known allergic response to house dust mite antigen. These individuals had elevated levels of eosinophils (the characteristic inflammatory cell in asthma) in their lavage fluid.

These above observations provide a mechanistic connection with the observation, from epidemiologic studies, that induction of asthma attacks is associated with ozone exposure. Horstman et al. [18] studied a group of moderate asthmatics exposed to 0.16 ppm ozone for 7.6 hours. The FEV_1 response of the asthmatics was about twice as large as that of the non-asthmatics and wheezing was experienced by more than half of the asthmatics. Treatment with an inhaled beta-adrenergic agonist partially reversed the decline in FEV_1 and alleviated symptoms, indicating that a substantial portion of these responses were due to bronchoconstriction (Figure 6). In non-asthmatics, in contrast, beta-agonists do not alleviate the decrease seen in FVC (Forced Vital Capacity) or FEV_1. In non-asthmatics, the FEV_1 response appears to be due primarily to a neurally mediated restriction in maximum inspired volume that can be promptly alleviated by a topical local anaesthetic [23] or by systemic opioid analgesics [24].

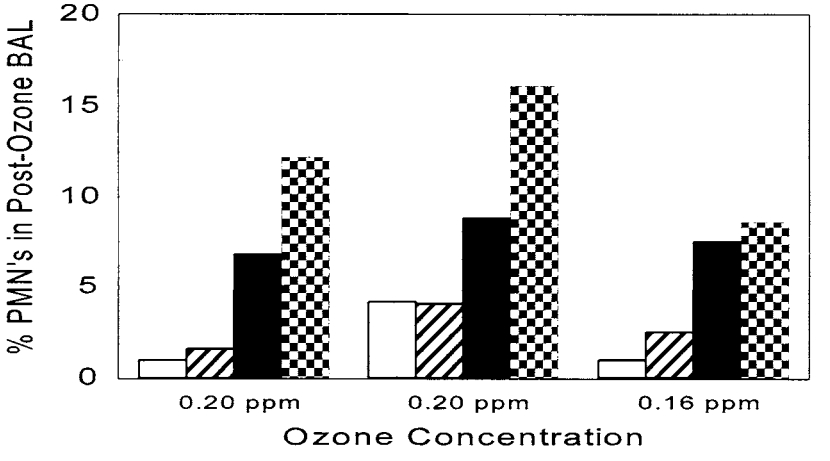

Figure 5. Percentage of neutrophils (PMN) in bronchoalveolar lavage (BAL) performed after air and ozone exposure (ppm) in both healthy and asthmatic individuals. (Clear bars: Healthy-Air; Hatched bars: Asthmatic-Air; Solid bars: Healthy-Ozone; Checkered bars: Asthmatic-Ozone). Data from Basha et al. [19], 6 h @ 25 L/min; Scannell et al. [20], 4 h @ 45 L/min; Unpublished data courtesy of R. Devlin, 7.6 h @ 25 L/min.

Since ozone is known to increase non-specific bronchial responsiveness and induce inflammatory processes, it was clear that ozone had the potential to increase bronchial responsiveness to inhaled specific antigens. An increased response to specific antigen after 3 h exposure 0.25 ppm ozone has been demonstrated by Jörres et al. [25] in both mild allergic asthmatics and allergic rhinitics, although the response was much less pronounced in the individuals with allergic rhinitis. In asthmatics, the dose of allergen which caused a 10% decline in FEV_1 after air exposure caused a 28% decline after ozone exposure. In contrast, the allergic rhinitics experienced no significant change after air exposure and an 8% change after ozone exposure. At lower ozone concentrations, the augmentation of allergen responses is less clear; one study has reported such an effect [26] and another has shown no significant effect under the same conditions [27]. Kehrl et al. [28] also reported an increase in response to house dust mite antigen in mild allergic asthmatics 16-18 h after completion of a 7.6 h exposure to 0.16 ppm ozone. These latter results not only support the findings of Jörres et al. [25] but more importantly indicate that an increased response to antigen can persist beyond the immediate post-exposure period. The association of hospital admissions or ER visits with ozone exposure is often strongest with a one-day lag, suggesting that the ozone-induced increase in reactivity to antigen may trigger a more severe asthma attack than would antigen exposure in the absence of ozone.

3. MORTALITY AND OZONE

A question that may be answered by the 21st Century is whether there is an association between ozone exposure and mortality. Although some studies have suggested such a relationship, there have been many questions raised about methodology and statistical approaches, precluding the possibility of coming to a satisfactory conclusion. In many of these

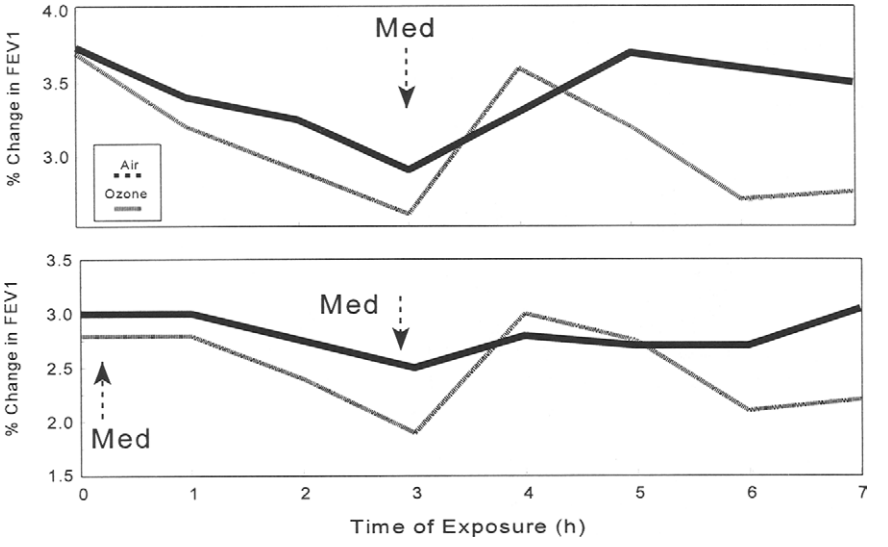

Figure 6. FEV_1 of two asthmatic subjects exposed to 0.16 ppm ozone or air for 7.6 h including mild exercise. Beta-adrenergic agonist medication was self-administered, as indicated by the arrows, in both air and ozone exposures. Note the marked improvement in FEV_1 after medication and the larger overall decline during the ozone exposure. (Redrawn from Horstman et al., [18])

analyses, the effect of particulate matter may have been a confounding factor [29]. A number of new studies have begun to look at this question again, using time-series analyses, and tend to show a positive relationship between ozone exposure and daily mortality. The association of ozone and mortality in these newer studies appears to be separate from the effects of other pollutants. [30-32].

What remains puzzling is that, in two cities with high ozone levels, Mexico City and Los Angeles, the association for ozone and mortality does not remain significant when particulate levels are taken into consideration. [29,33]. Asthma-related mortality cannot account for the apparent increase in mortality associated with ozone. Although an accelerated rate of decline of lung function [34] could contribute to mortality this also seems to be an unlikely explanation.

4. CONCLUSIONS

Clearly, human responses to ozone exposure are modified by repeated exposure. Ongoing lung cell damage was previously demonstrated in laboratory animals repeatedly exposed to ozone. Despite the attenuation of spirometric lung function and symptom responses in repeatedly exposed humans, we now know that lung cell damage continues, as does the repair process. The processes that lead to the changes in ozone responsiveness, either from a series of acute exposures in a controlled exposure chamber or from seasonal exposure in an oxidant polluted community, are not well understood from a human lung cell biology perspective. There is some suggestion that seasonal ambient exposures may have some implications for long term lung health. It is

likely that persons with impaired or hypersensitive respiratory systems may be impacted to the greatest extent. The association of asthma morbidity end-points with ozone exposure is clear. Although several ppm of ozone can induce life threatening effects in healthy humans, there is presently no mechanistic explanation of how very low (tens of parts per billion) concentrations of ozone could induce death.

This document has been peer-reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. The views expressed are those of the author and do not necessarily reflect the views of the agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Hackney JD, Linn WS, Buckley RD, and Hislop HJ. Studies on adaptation to ambient oxidant air pollution: effects of ozone exposures in Los Angeles residents vs. new arrivals. Environ Health Perspect 18:141-146 (1976).
2. Linn WS, Avol EL, Shamoo DA, Peng RC, Valencia LM, Little D, and Hackney JD. Repeated laboratory ozone exposures of volunteer Los Angeles residents: an apparent seasonal variation in response. Toxicol Indust Health 4:505-520 (1988).
3. Kunzli N, Lurmann F, Segal M, Ngo L, Balmes J, Tager IB. Association between lifetime ambient ozone exposure and pulmonary function in college freshmen — Results of a pilot study. Environ Res 72: 8-23, 1997.
4. Folinsbee LJ, Horstman DH, Kehrl HR, Harder S, Abdul-Salaam S, Ives PJ. Respiratory responses to repeated prolonged exposure to 0.12 ppm ozone. Am J Respir Crit Care Med 149:98-105, 1994.
5. Folinsbee LJ, Devlin RB, Koren HS. Time course of pulmonary response to repeated ozone exposure in man. Presented at the Annual Meeting of the American Thoracic Society, Boston, MA, May 1994. Am Rev Respir Dis 149 (#4, pt. 2): A151, 1994.
6. Horvath SM, Gliner JA, Folinsbee LJ. Adaptation to ozone: Duration of effect. Am. Rev. Respir. Dis. 123:496-499, 1981.
7. Linn WS, Medway DA, Anzar UT, Valencia LM, Spier CE, Tsao FS, Fischer DA, and Hackney JD. Persistence of adaptation to ozone in volunteers exposed repeatedly for six weeks. Am Rev Respir Dis 125:491-495 (1982).
8. Koren HS, Devlin RB, Graham DE, Mann R, Horstman DH, Kozumbo WJ, Becker S, McDonnell WF, and Bromberg PA. Ozone-induced inflammation in the lower airways of human subjects. Am Rev Respir Dis 139:407-415 (1989).
9. Devlin RB, Folinsbee LJ, Biscardi FH, Hatch G, Becker S, Madden M, Robbins MK, Koren HS. Inflammation and cell damage induced by repeated exposure of humans to ozone. Inhalation Toxicology 9:211-235, 1997.
10. Tepper JS, Costa DL, Lehmann JR, Weber MF, and Hatch GE. Unattenuated structural and biochemical alterations in the rat lung during functional adaptation to ozone. Am Rev Respir Dis 140: 493-501 (1989).
11. Burnett RT, Dales RE, Raizenne ME, Krewski D, Summers PW, Roberts GR, Raad-Young M, Dann T, Brook JR,. Effects of low levels of ambient ozone and sulfates on the frequency of respiratory admissions to Ontario hospitals. Environ Res 65:172-194, 1994.
12. Sterling TD, Pollack SV, Phair JJ. Urban hospital morbidity and air pollution. Arch Environ Health 15:362-374, 1967.
13. Thurston GD, Ito K, Kinney PL, Lippmann, M. A multi-year study of air pollution and respitatory hospital admissions in three New York State metrpolitan areas: results for 1988

and 1989 summers. J Exposure Anal Environ Epidemiol 2:429-450, 1992.
14. White MC, Etzel RA, Wilcox WD, Lloyd C. Exacerbations of childhood asthma and ozone pollution in Atlanta. Environ Res 1994; 65:56-68.
15. Linn WS, Buckley R, Speir C, Blessey R, Jones M, Fischer D, and Hackney JD. Health effects of ozone exposure in asthmatics. Am Rev Respir Dis 117:835-843 (1978).
16. Kreit JW, Gross KB, Moore TB, Lorenzen TJ, D'Arcy J, and Eschenbacher WL. Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol 66:217-222 (1989).
17. Linn WS, Shamoo DA, Anderson KR, Peng R-C, Avol EL, Hackney JD. Effects of prolonged repeated exposure to ozone, sulfuric acid, and their combination in healthy and asthmatic volunteers. Am J Respir Crit Care Med 1994; 150:431-440.
18. Horstman DH, Ball BA, Brown J, Gerrity TR, Folinsbee LJ. Comparison of pulmonary responses of asthmatic and nonasthmatic subjects performing light exercise while exposed to a low level of ozone. Toxicol Ind Health 11:369-385, 1995
19. Basha MA, Gross KB, Gwizdala CJ, Haidar AH, Popovich J. Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filtered purified air. Chest 1994; 106:1757-1765.
20. Scannell CH, Chen LL, Aris R, Tager I, Christian D, Ferrando R, Welch B, Kelly T, Balmes JR. Greater ozone-induced inflammatory responses in subjects with asthma. Am J Respir Crit Care Med 1996; 154:24-29.
21. Linn WS, Anderson KR, Shamoo DA, Edwards SA, Webb TL, Hackney JD, Gong H. Controlled exposures of young asthmatics to mixed oxidant gases and acid aerosol. Am J Respir Crit Care Med 1995; 152:885-891.
22. Folinsbee, LJ. Sulfur oxides: Controlled human exposure studies. In: Lee SD, Schneider T. (eds). Comparative Risk Analysis and Priority Setting for Air Pollution Issues, Proceedings of the 4th U.S.-Dutch International Symposium, June 1993, Keystone CO. Pittsburgh: Air & Waste Management Assoc., 1995. pp. 326-334.
23. Hazucha MJ, Bates DV, Bromberg PA. Mechanism of action of ozone on the human lung. J Appl Physiol 67:1535-1541 (1989).
24. Passanante A, Hazucha MJ, Seal E, Folinsbee L, Bromberg PA. The role of analgesia in modulating lung function response to ozone in man (abstr) . Anesth Analg 1995; 80:S371.
25. Jorres R, Nowak D, Magnussen H. Effects of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. Am J Respir Crit Care Med 1995; 153:56-64.
26. Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA, Szalai JP, Raizenne M, Slutsky AS, Zamel N. Effect of Low Concentrations of Ozone on Inhaled Allergen Responses in Asthmatic Subjects. The Lancet 338:199-203, 1991.
27. Ball BA, Folinsbee LJ, Peden DB, Kehrl HR. Allergen bronchoprovocation of patients with mild allergic asthma after ozone exposure. J Allergy Clin Immunol. 98:563-572, 1996.
28. Kehrl HR, Ball B, Folinsbee L, Peden D, Horstman D. Increased specific airway reactivity of mild allergic asthmatics following 7.6 hr exposures to 0.16 ppm ozone. Presented at the Annual Meeting of the American Thoracic Society, San Francisco, CA, May 1997. Am J Respir Crit Care Med 155 (#4, pt. 2): A731, 1997.
29. Kinney PL, Ito K, Thurston GD. A sensitivity analysis of mortality/PM10 associations in Los Angeles. Inhalation Toxicol 7:59-69, 1995.
30. Samet JM, Zeger SL, Kelsall JE, Xu J. Air Pollution and Mortality in Philadelphia, 1974-1988. Report to the Health Effects Institute. (March, 1997).
31. Sartor F, DeMuth C, Snacken R, Walckiers D. Mortality in the elderly and ambient ozone

concentration during the hot summer, 1994, in Belgium. Environ Res 72: 109-117, 1997.
32. Anderson HR, Ponce de Leon A, Bland JM, Bower JS, Strachan DP. Air pollution and daily mortality in London: 1987-92, Brit Med J 312:665-669, 1996.
33. Loomis DP, Borja-Aburto VH, Bangdiwala SI, Shy CM. Ozone Exposure and Daily Mortality in Mexico City: a Time-series Analysis. Research Report #75, Cambridge, MA: Health Effects Institute, 1996.
34. Detels, R., Tashkin, D.P., Sayre, J.W., Rokaw, S.N., Coulson, A.H., Massey, F.J., and Wegman, D.H. The UCLA population studies of chronic obstructive respiratory disease: 9. Lung function changes associated with chronic exposure to photochemical oxidants; a cohort study among never smokers. Chest 92:594-603 (1987).