

Mechanisms of pollution-induced airway disease: *in vivo* studies

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Allergy 1997; 52 (suppl 38): 37–44. © Munksgaard 1997.

Several studies have investigated the effects of ozone, sulphur dioxide (SO₂), and nitrogen dioxide (NO₂) on lung function in normal and asthmatic subjects. Decreased lung function has been observed with ozone levels as low as 0.15 ppm – this effect is concentration dependent and is exacerbated by exercise. A number of lines of evidence suggest that the effect on lung function is mediated, at least in part, by neural mechanisms. In both normals and asthmatics, ozone has been shown to induce neutrophilic inflammation, with increased levels of several inflammatory mediators, including prostaglandin E₂. However, in normal subjects, none of the markers of inflammation correlate with changes in lung function. The lung function changes in asthmatics may be associated with inflammatory effects; alternatively, ozone may prime the airways for an increased response to subsequently inhaled allergen. Indeed, an influx of both polymorphonucleocytes and eosinophils has been observed in asthmatic patients after ozone exposure. It has been suggested that the effect of ozone on classic allergen-induced bronchoconstriction may be more significant than any direct effect of this pollutant in asthmatics. SO₂ does not appear to affect lung function in normal subjects, but may induce bronchoconstriction in asthmatics. Nasal breathing, which is often impaired in asthmatics, reduces the pulmonary effects of SO₂, since this water-soluble gas is absorbed by the nasal mucosa. NO₂ may also influence lung function in asthmatics, but further research is warranted. SO₂ and NO₂ alone do not seem to have a priming effect in asthmatics, but a combination of these two gases has resulted in a heightened sensitivity to subsequently inhaled allergen.

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Key words: asthma; eosinophils; inflammation;
lung function; nitrogen dioxide; ozone; sulphur
dioxide.

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Introduction

Air pollution is a significant public health concern worldwide. A number of anthropogenic pollutants are present in the atmosphere, including ozone, sulphur dioxide (SO₂), nitrogen dioxide (NO₂), acid aerosols, and other particulate matter. Epidemiological studies suggest that each of these pollutants may be associated with increased respiratory morbidity and mortality. These studies have led to both animal and controlled exposure ('clinical') studies in which human volunteers were exposed to specific air pollutants to assess their effects on lung function.

Several clinical studies suggest that ambient pollutants exert an effect on lung function through neurally-mediated mechanisms. For example, a decreased forced vital capacity (FVC) is observed in subjects exposed to ozone, which is associated

with a subjective inability to perform a full inspiration. In asthmatics, SO₂ induces bronchospasm, which is reversible with either β -agonists or anticholinergic medications, again suggesting a neurally-mediated process.

Additionally, a number of pollutants, including ozone, SO₂ and NO₂, have proinflammatory actions. Each of these gases has been shown to induce inflammatory cell influx into the airway, and also cause a number of proinflammatory changes in epithelial cells. Indeed, initial signals from the epithelium, such as the release of various cytokines and lipids, may be fundamental in mediating the inflammatory action of pollutants.

Asthma is a disease characterized by increased airway sensitivity to inhaled irritants, increased airway inflammation, and injury to the epithelium. Given the effect of ambient air pollutants, such as ozone, SO₂ and NO₂, on lung mechanics, airway

inflammation, and proinflammatory changes in epithelial cells, it seems likely that asthmatics may be more sensitive than the general population to the effects of these gases. The proinflammatory effect of these agents suggests that they may not only exert direct effects in asthma, but that they may prime the airways, such that the response of extrinsic asthmatics to inhaled allergen is also enhanced.

This paper reviews the effect of ozone, SO₂ and NO₂ on lung function, with a special emphasis on the inflammatory actions of these pollutants and their subsequent effects in asthmatics.

Ozone

Normal subjects – lung mechanics

In normal subjects, ozone causes a reproducible decrease of FVC and forced expiratory volume in 1 s (FEV₁), increased non-specific bronchial responsiveness, and substernal discomfort when taking a deep breath (1, 2). These effects are dependent on ozone concentration, duration of exposure, and the level of exercise (with a corresponding increase in minute ventilation) (3). Indeed, exposure to ozone without exercise generally reveals no effect on lung function at levels below 0.50 ppm (1, 3). With exercise, ozone induces an increase in respiratory frequency, and the level of ozone required to cause decreases in FEV₁, FVC, airway resistance, and symptoms, can be much lower (4).

Dose-response studies employing an alternating rest and exercise protocol over 2.5 h, with a target ventilation rate of 67 L/min (normal resting ventilation being 15 L/min), at ozone concentrations of 0.12, 0.18, 0.24, 0.30 and 0.40 ppm, revealed that as little as 0.12 ppm ozone could cause a significant decrement in FVC, FEV₁, and forced expiratory flow at 25–75% of vital capacity (FEF_{25–75%}) (3). Furthermore, FVC, FEV₁, FEF_{25–75%}, airway resistance, respiratory frequency, and symptoms, were all influenced in a concentration-dependent manner at ozone levels above 0.24 ppm. Other studies using comparable exercise regimens (ventilation rates of 68 L/min and 70 L/min) confirm that ozone-induced changes in lung function occur at levels as low as 0.15 and 0.16 ppm (5, 6).

Studies examining the effect of a number of pharmacological agents on respiratory responses to ozone support the concept that the effect of ozone on lung mechanics is mediated, at least in part, by neural mechanisms. Beckett et al. (7) revealed that atropine inhibited ozone-induced decreases in airway resistance (although not spirometry), suggesting that vagally-mediated influences on bronchial smooth muscle may play a role.

In addition to cholinergically-mediated responses, ozone may exert effects via cyclooxygenase-dependent mechanisms. A positive correlation has been reported between levels of prostaglandin E₂ (PGE₂) (a cyclooxygenase pathway product) in bronchoalveolar lavage (BAL) fluid and decreased lung function in individuals exposed to ozone (8). Additionally, several investigators have shown that cyclooxygenase inhibitors, such as ibuprofen and indomethacin, can attenuate ozone-induced decreases in spirometry (9–12).

Lidocaine, when applied to the upper airway, also results in partial inhibition of ozone-induced decreases in spirometry. These results suggest that lidocaine-sensitive irritant receptors in the upper airway, when stimulated by ozone, exert a reflex-mediated decrease in inspiratory effort (13). Other neurally-mediated mechanisms, involving effects on opioid receptors, may also play a role in ozone-induced respiratory symptoms. This is supported by preliminary data in which sufentanyl (a short-acting narcotic) normalized lung function in subjects with reduced FVC and FEV₁ after ozone exposure (14). Thus, pain responses which are sensitive to opioids may partially account for the pulmonary response to ozone.

Neuropeptides may also be involved in the effect of ozone on lung function. Krishna et al. (15) exposed seven healthy subjects to 0.2 ppm ozone and clean air (on two separate occasions) for 2 h with intermittent exercise. Bronchial biopsies obtained 6 h after exposure were stained for substance P (SP) and calcitonin gene-related peptide (CGRP). The biopsies obtained after ozone exposure revealed submucosal neurons containing CGRP but not SP, indicating that SP had been released following ozone exposure, whereas those obtained after clean air exposure revealed neurons containing both CGRP and SP.

Normal subjects – inflammation

In normal volunteers, controlled exposure ranging from 0.10 to 0.4 ppm ozone has been shown to induce neutrophilic inflammation of airways as observed in both BAL fluid and bronchial mucosal biopsy. While the time course of ozone-induced inflammation has not been explored completely, neutrophil influx appears to be maximal between 1–6 h and persists for 18 h (16–20). Increased levels of interleukin-6 (IL-6), PGE₂, leukotriene B₄ (LTB₄), thromboxane B₂ (TXB₂), fibronectin, plasminogen activator, and elastase in BAL fluid have also been reported following ozone exposure (8). In studies where data are available 1 h and 18 h after ozone exposure, changes in IL-6 and PGE₂ are maximal 1 h after exposure, whereas fibronectin

tin and plasminogen activator are higher 18 h after exposure (8).

Studies examining the effect of ibuprofen on BAL inflammatory endpoints demonstrate that ozone-induced increases in polymorphonuclear neutrophils (PMN), fibronectin, total protein, lactate dehydrogenase, LTB_4 , and complement protein 3a were not inhibited, as were changes in IL-6, TXB_2 , and PGE_2 . It is notable that these subjects exhibited a blunted effect of ozone on lung function following ibuprofen treatment, consistent with studies employing indomethacin (12). Another preliminary study which examined the effect of pre-treatment with a combination of oral and inhaled glucocorticoids on ozone-induced changes in spirometry and lower airway inflammation revealed that corticosteroids have no significant effect on either PMN influx or lung function (21). It is intriguing that oral glucocorticoids (40 mg prednisone daily for three consecutive days prior to ozone exposure), which would logically cause decreased levels of arachidonic acid products via inhibition of phospholipase A_2 (PLA_2) had no effect on lung function. Taken together, these studies suggest that blockade of PLA_2 is not sufficient to inhibit ozone-induced changes in lung function. It is possible that with ozone stimulation, arachidonic acid is released into the cytoplasm in a non- PLA_2 dependent fashion, where it can be acted upon by the cyclooxygenase cascade.

In vitro studies of epithelial cells or epithelial cell lines have suggested possible mechanisms by which ozone may influence inflammation. Epithelial cell lines exposed to ozone generate the eicosanoids PGE_2 , TXB_2 , $PGF_{2\alpha}$, the proinflammatory cytokines IL-6 and IL-8, and the proinflammatory protein fibronectin. Nasal epithelial cells placed into culture respond to high level ozone (0.5 ppm) by generating intercellular adhesion molecule-1 (ICAM-1), the major adhesion molecule for neutrophils and eosinophils in the airway, as well as the cytokines IL-1, IL-6, and tumour necrosis factor- α (TNF- α). In addition, epithelial permeability in human subjects is increased after ozone exposure (22–24). Thus, epithelial cells most likely play a major role in the inflammatory response to ozone, while neural responses (C fibre, pain fibres, and possibly cholinergic fibres) exert the major effect on lung function changes.

With the exception of PGE_2 , none of the above-mentioned markers of inflammation correlate with changes in lung function in normal subjects. Furthermore, no association has been made between ozone-induced inflammation and airway responsiveness in normal subjects. Thus, the clinical significance of ozone-induced inflammation in normal subjects remains unclear. It has been sug-

gested that this proinflammatory effect of ozone would be of greater significance in asthma, a disorder characterized in part by eosinophilic inflammation of the lower airways.

Asthmatics – lung mechanics

A number of epidemiological studies suggest that increased ambient ozone levels are linked to exacerbation of asthma (25). Bates and Sizto (26) examined hospital admission rates for asthma over a summer season and found a significant correlation between asthma admissions and increased ambient ozone and sulphate levels on the previous day. The time-lag between ozone exposure and increased evidence of disease exacerbation suggests that inflammatory changes, rather than immediate decreases in FVC, account for lung function changes in both normal subjects and asthmatics. An alternative possibility is that ozone might prime the bronchial airways of asthmatics for increased responses to subsequently encountered allergens.

Initial controlled exposure studies in asthmatics with mild disease found little increased sensitivity to ozone compared with non-asthmatic subjects at concentrations of ≤ 0.25 ppm employing exposure protocols that involved little or no exercise (27). However, it is possible that experiments employing low levels of exercise underestimate the effect of ozone in asthma. Indeed, Kreit et al. (28) have demonstrated that, with more rigorous exercise (ventilation of 30 L/min/m²), exposure to 0.4 ppm ozone for 2 h causes greater changes in airway resistance in asthmatics than in normals. Non-specific airway hyperresponsiveness was also enhanced in asthmatics after ozone exposure. Silverman (27) also demonstrated that exposure to 0.25 ppm ozone for 2 h resulted in decreased pulmonary function in asthmatics. Other studies indicate that ozone not only causes the well described decrease in FVC, but also causes true bronchospasm in asthmatics (28–30). Thus, ozone has been demonstrated to directly worsen airflow in asthma.

However, while ozone exposure with exercise may result in true bronchospasm in asthmatics beyond the baseline ozone effect observed in normal subjects, recent studies (31, 32) have revealed that ozone exposure prior to exercise produces no effect on subsequent exercise-induced bronchospasm (EIB). Weymer et al. (32) exposed a cohort of mild asthmatics with EIB to ozone (0.0, 0.1, 0.25 and 0.40 ppm) for 1 h with mild exercise (enough to result in a ventilation rate of 20–30 L/min). Exercise challenge was performed 1 h later if the FEV_1 had returned to 90% of pre-exposure baseline. Even though there was a dose-

dependent effect of ozone exposure on both lung function and subjective symptom scores immediately after exposure, the resultant EIB was no greater after exposure to any ozone concentration than following a control exposure to clean air. A separate study examined the effect of exposure to 0.12 ppm ozone at rest for 1 h in asthmatics with EIB, and also revealed no exacerbation of EIB attributable to ozone (31).

Asthmatics – inflammation

As with non-asthmatics, exposure to ozone induces neutrophilic inflammation in both the nasal and bronchial airway. Bascom et al. (33) initially examined the effect of ozone on nasal inflammation in subjects with allergic rhinitis and found an increase in both PMNs as well as eosinophils after a 4-h exposure to 0.5 ppm ozone. These findings are similar to those obtained from our laboratory, in which nasal lavage fluid from extrinsic asthmatics exposed to 0.4 ppm ozone for 2 h was examined. Again, ozone appeared to induce increases in both eosinophils as well as the level of eosinophil cationic protein. Thus, in these two nasal studies, ozone was able to induce not only an influx of PMNs, but also eosinophil influx into the airways of atopic subjects.

Subsequent investigations have employed bronchoscopy to examine the effect of ozone on bronchial inflammation in asthmatics. Basha et al. (34) reported that a cohort of five asthmatics had a greater PMN response to ozone exposure than a cohort of five non-asthmatics, as reflected in BAL fluid. Furthermore, there was also an enhanced IL-8 response in the asthmatics. In a larger study, Scannell et al. (35) also compared the effect of exposure to 0.2 ppm ozone for 2 h on lower airway inflammation in 19 asthmatics and 20 non-asthmatic volunteers. Bronchoscopy was performed 18 h later with lavage fluid collected according to three methods – lavage from an isolated proximal airway, analysis of the first 10 mL of traditional BAL (labelled the bronchial fraction), as well as traditional BAL fluid. Asthmatics had increased levels of PMNs, lactate dehydrogenase, total protein, myeloperoxidase, fibronectin, and IL-8, with each of the three sampling techniques. Asthmatics also had a greater PMN response in the BAL fluid than non-asthmatics, and also had a trend for increased IL-8 levels. Thus, these two studies suggest that asthmatics may be more sensitive to the inflammatory effects of ozone than non-asthmatics. Of note was the absence of eosinophils in lavage fluid obtained from asthmatics.

In contrast to these studies, preliminary reports from our laboratory indicate that extrinsic asth-

matics exposed to 0.16 ppm ozone for 8 h have a substantial increase in both PMNs and eosinophils when compared with the response to clean filtered air. Bronchial samples included traditional BAL fluid as well as separate examination of the first 20 mL recovered (the bronchial fraction), the latter yielding more striking differences between clean air and ozone exposure in asthmatics. Indeed, the enhanced eosinophil level after ozone exposure was very apparent in the bronchial fraction; a total of seven subjects have completed the study to date (36). This observation is consistent with that of Frampton et al. (37) who observed a two-fold increase in eosinophils following ozone exposure.

Effect of ozone exposure on subsequent responses to inhaled allergen in asthmatics

The four studies described above all indicate that asthmatics have a substantial inflammatory response to ozone. While two of the studies failed to identify eosinophils in lavage fluid after ozone exposure, the presence of increased inflammation in asthmatics suggests that they may be more susceptible to the effects of subsequently inhaled allergen. This would be congruent with epidemiological observations showing that increased ambient ozone correlates with asthma exacerbation on the following day, a finding that suggests a priming effect of ozone on subsequent response to indoor allergens. As an initial test of this hypothesis, Molino et al. (38) examined seven seasonally allergic asthmatics after exposure to 0.12 ppm ozone at rest for 1 h who were subsequently challenged with inhaled allergen. Six of the seven subjects required less inhaled allergen to induce a 15% decrease in FEV₁ after ozone exposure than after clean air exposure. However, two recent studies examining the effect of low-dose ozone exposure (0.12 ppm for 1 h and 0.2 ppm for 2 h) did not demonstrate an effect on response to inhaled allergen (39).

Jorres et al. (40) reported increased sensitivity to inhaled allergen in 18 of 23 mild asthmatics after exposure to 0.25 ppm ozone for 3 h with intermittent exercise. Smaller changes were also noted in 11 subjects with allergic rhinitis. Both groups, as well as a cohort of normal subjects, had equivalent changes in methacholine responsiveness and lung function. Examination of allergen-induced nasal inflammation in asthmatics revealed that 0.4 ppm ozone also augments the late-phase eosinophilic response to allergen (41). Taken together, these findings suggest that the effect of ozone exposure on classic allergen-induced bronchoconstriction may be more significant than the direct effect of ozone in asthmatics. It is less clear what level of

ozone is required to exert deleterious effects on lung function in asthmatics.

Sulphur dioxide and nitrogen dioxide

SO₂ is released into the atmosphere primarily as a result of industrial combustion of high sulphur-containing coal and oil. Common sources of SO₂ include paper, steel, and cement manufacturing plants. Controlled exposure studies clearly reveal the ability of SO₂ to induce exacerbations of asthma. While these studies fail to demonstrate an effect of SO₂ on pulmonary function in normal subjects at levels as high as 0.6 ppm, several investigators have demonstrated bronchoconstriction in asthmatics at concentrations as low as 0.25 ppm (42–48).

Unlike other pollutants, SO₂ exerts a rapid effect on lung function in asthmatics; significant responses occur within 2 min and maximal response is noted within 5–10 min. Spontaneous recovery occurs as early as 30 min after challenge, with a refractory period of up to 4 h. Repeated exposure to low levels of SO₂ has been shown to result in tolerance to subsequent exposure. In one study, repeated exercise during SO₂ exposure resulted in diminished airway responsiveness to SO₂. However, initial responsiveness was restored within 6 h after the last exposure (49–52).

Responses to SO₂ include a decrease in FEV₁ (decreases as high as 60% have been observed) and an increase in airway resistance. Asthmatics have a modest variation in sensitivity to SO₂ – some experience lung function decrements at levels of 0.25 ppm, while others have no response at 2.0 ppm. However, levels above 0.50 ppm will usually induce bronchospasm in asthmatics. Exercise augments bronchial responses to SO₂, and combined exercise and SO₂ have additive effects in asthmatics. Symptoms associated with SO₂ inhalation include wheezing, chest discomfort, and dyspnoea.

Interestingly, nasal breathing protects against the pulmonary effects of SO₂ because this highly water soluble gas is absorbed very efficiently by the nasal mucosa. This may explain why exercise increases its effect, because most people shift from nasal breathing to oronasal respiration with exercise sufficient to cause a ventilation rate of 35 L/min. However, even with heavy exercise, up to 60% of inspired air passes over the nasal mucosa. This may be an important feature in asthmatics, who frequently suffer from allergic rhinitis or sinusitis, both of which cause decreased nasal airflow and increased oral breathing, thus decreasing the percentage of inspired air exposed to nasal tissue (53).

A number of pharmacological agents attenuate

the effect of SO₂ in asthmatics, including β -agonists, anti-muscarinic agents, cromolyn sodium, and nedocromil. Indeed, β -agonists have the most potent effect in mitigating SO₂-induced bronchospasm. Interestingly, chronic use of methylxanthines and inhaled corticosteroids, as well as acute use of antihistamines, has not been shown to affect the sensitivity to SO₂. Many investigators currently believe that SO₂ acts as a non-specific bronchoconstricting agent, analogous to the action of inhaled histamine and methacholine, to which asthmatics also have increased sensitivity compared with normal subjects (54, 55). These studies, combined with the increased sensitivity of asthmatics to SO₂, suggest a cholinergically-mediated neural mechanism of action. However, recent observations by Sandstrom et al. (56) demonstrate that very mild airway inflammation may also be induced by SO₂ exposure. Thus, the mechanisms by which SO₂ may influence asthma remain incompletely explained.

NO₂, a precursor to photochemical smog, is found in ambient outdoor air in urban and industrial regions and, in conjunction with sunlight and hydrocarbons, results in the production of ozone. Automobile exhaust is the most significant source of outdoor NO₂, although power-generating plants and other industrial sources that burn fossil fuels also release NO₂ into the environment. However, the most significant exposure to NO₂ occurs indoors, with the use of gas cooking stoves and kerosene heaters.

Controlled chamber studies reveal no consistent effect of NO₂ on airway function in normal subjects or asthmatics. Some investigators report that low levels of NO₂ induce increased airway reactivity to pharmacological or cold air challenge in asthmatics, while others found no such effect on methacholine reactivity in another cohort of asthmatics (42, 51, 57–59). Higher levels of NO₂ (4.0 ppm) have been reported to alter airway function in asthma. The mechanism of effect on airway function is also unclear. In studies of normal subjects, NO₂ was much less potent than ozone in inducing airway PMN influx (60), but it has been shown to increase proinflammatory cytokine production in epithelial cells after *in vitro* pollutant exposure (61). Overall, these data suggest that NO₂ could influence airway function in asthmatics, but there is a need for further research.

Priming effects of SO₂ and NO₂ in asthma

SO₂ exposure augments the response to other environmental agents that exacerbate bronchospasm. Exposure to ozone prior to SO₂ exposure increases bronchial sensitivity to SO₂ in asthmatics

(62). Exposure to cold dry air also exacerbates the bronchospastic effect of SO₂ in asthmatics (51). However, unlike ozone, SO₂ exposure alone has not been shown to exacerbate allergen-induced bronchospasm.

As with SO₂, NO₂ (0.4 ppm) has not been shown to enhance sensitivity to subsequently inhaled allergen in asthmatics, suggesting that it does not have a priming effect for allergen-induced inflammation. In a recent study of nasal inflammation in asthmatics (63), exposure to 0.4 ppm NO₂ for 6 h did not induce mast cell degranulation, eosinophil activation (as reflected by levels of eosinophil cationic protein in nasal lavage fluid), neutrophil activation (as shown by myeloperoxidase release), or cause an increase in IL-8. Likewise, such exposure did not enhance mast cell degranulation immediately following allergen challenge. However, there was an increase in eosinophil cationic protein in nasal lavage fluid following allergen challenge when this was preceded by NO₂ exposure. Thus, NO₂ appears to have a modest priming effect for the late-phase response to allergen.

Although SO₂ and NO₂ alone do not appear to have a priming effect in the airways of asthmatics, it has recently been demonstrated that exposure to a mixture of 0.2 ppm SO₂ and 0.4 ppm NO₂ for 6 h enhances sensitivity of mild asthmatics to inhaled allergen (64). Indeed, in this study, the combination of these two gases resulted in a 60.5% mean decrease in allergen required to induce a 20% fall in FEV₁ ($P=0.015$). This finding highlights the importance of examining the effect of pollutant mixtures, as well as individual pollutants, in assessing health risks, especially in special populations such as asthmatics.

Summary

Air pollution is a continuing problem worldwide, with certain pollutants, such as ozone and SO₂, clearly having marked effects on lung function. In addition, NO₂ probably also causes a decrease in lung function. Although the immediate effect of inhalation of these gases on lung function has been studied intensely, their role in modulating airway inflammation may be of greater significance, especially in assessing their effect in asthma and other pulmonary disorders. Asthma severity is strongly correlated to the degree of airway inflammation, particularly eosinophilic inflammation. Environmental conditions that contribute to development of airway inflammation are thought to be major risk factors in asthma pathogenesis. While allergen exposure is the traditional environmental stress considered by clinicians, pollutant exposure could also be important. Indeed, as outlined above, SO₂, ozone, and NO₂ have all

been reported to enhance non-specific airway reactivity in human volunteers and increase the response to inhaled allergens in allergic asthmatics.

While the mechanisms by which these pollutants alter airway function and inflammation are unclear, certain novel themes are developing. Non-adrenergic, non-cholinergic neurons have been suggested to play a role in the respiratory response to pollutants in studies of ozone-induced decreases in lung function, employing both pharmacologic effects and direct assessment of substance P depletion in the airway. Additionally, the proinflammatory action of epithelial cells, by generation of active mediators, loss of barrier function, and expression of adhesion molecules has been suggested in both *in vitro* and *in vivo* studies. Pursuing these themes may lead to better understanding of the cellular mechanisms by which pollutants alter lung function and inflammation, and in turn allow for more complete risk assessment and personal intervention strategies.

References

1. Bates DV, Hazucha MJ. The short-term effects of ozone on the human lung. US Government Printing Office 1973;93(15):507-40.
2. Hazucha MJ, Bates DV. Combined effect of ozone and sulphur dioxide on human pulmonary function. *Nature* 1975;257:50-1.
3. McDonnell WF, Horstman DH, Hazucha MJ et al. Pulmonary effects of ozone exposure during exercise: dose-response characteristics. *J Appl Physiol: Respirat Environ Exercise Physiol* 1983;54(5):1345-52.
4. McDonnell WF, Kehrl HR, Abdul Salaam S et al. Respiratory response of humans exposed to low levels of ozone for 6.6 hours. *Arch Environ Health* 1991;46:145-50.
5. Kulle TJ, Sauder LR, Hebel JR et al. Ozone response relationships in healthy nonsmokers. *Am Rev Respir Dis* 1985;132:36-41.
6. Linn WS, Avol EL, Shamoo DA et al. A dose-response study of healthy, heavily exercising men exposed to ozone at concentrations near the ambient air quality standard. *Toxicol Ind Health* 1986;2(1):99-112.
7. Beckett WS, McDonnell WF, Horstman DH et al. Role of the parasympathetic nervous system in acute lung response to ozone. *J Appl Physiol: Respirat Environ Exercise Physiol* 1985;59:1879-85.
8. Koren HS, Devlin RB, Becker S et al. Time-dependent changes of markers associated with inflammation in the lungs of humans exposed to ambient levels of ozone. *Toxicol Pathol* 1991;19(4):406-11.
9. Ying RL, Gross KB, Terzo TS et al. Indomethacin does not inhibit the ozone-induced increase in bronchial responsiveness in human subjects. *Am Rev Respir Dis* 1990; 142:817-21.
10. Schelegle ES, Adams WC, Siefkin AD. Indomethacin pretreatment reduces ozone-induced pulmonary function decrements in human subjects. *Am Rev Respir Dis* 1987; 136:1350-4.
11. Eschenbacher WL, Ying RL, Kreit JW et al. Ozone-induced lung function changes in normal and asthmatic subjects and the effect of indomethacin. *Atmos Ozone Res Policy Implic* 1989;493-9.

12. Hazucha MJ, Madden MC, Pape C et al. Effects of cyclooxygenase inhibition on ozone-induced respiratory inflammation and lung function changes. *Eur J Physiol Occup Med* 1995 (in press).
13. Folinsbee LJ. Human health effects of air pollution. *Environ Health Perspect* 1993;100:45-56.
14. Passannante A, Hazucha MJ, Seal E et al. Nociceptive mechanisms modulate ozone-induced human lung function decrements [Abstract]. *Anesth Analg* 1995;80:S371.
15. Krishna M, Springall D, Meng QH et al. Effects of 0.2 ppm ozone on the sensory nerves in the bronchial mucosa of healthy humans [Abstract]. *Am J Respir Crit Care Med* 1996;153(4):A700.
16. Aris RM, Christian D, Hearne PQ et al. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. *Am Rev Respir Dis* 1993;148:1363-72.
17. Koren HS, Devlin RB, Graham DE et al. Ozone-induced inflammation in the lower airways of human subjects. *Am Rev Respir Dis* 1989;139:407-15.
18. Schelegle ES, Siefkin AD, McDonald RJ. Time course of ozone-induced neutrophilia in normal humans. *Am Rev Respir Dis* 1991;143:1353-8.
19. Devlin RB, McDonnell WF, Mann R et al. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. *Am J Respir Cell Mol Biol* 1991;4:72-81.
20. Seltzer J, Bigby BG, Stulbarg M et al. O₃-induced change in bronchial reactivity to methacholine and airway inflammation in humans. *J Appl Physiol* 1986;60:1321-6.
21. Bottei GM, Devlin RB, Bromberg PA et al. The effect of corticosteroids on lower airway inflammation and pulmonary function after ozone exposure [Abstract]. *Am J Respir Crit Care Med* 1995;151:A29.
22. McKinnon KP, Noah T, Madden M et al. Cultured human bronchial epithelial cells release cytokines, fibronectin, and lipids in response to ozone exposure. *Chest* 1992;101:22S.
23. McKinnon KP, Madden MC, Noah TL et al. In vitro ozone exposure increases release of arachidonic acid products from a human bronchial epithelial cell line. *Toxicol Appl Pharmacol* 1993;118:215-23.
24. Beck NB, Koenig JQ, Lucht DL et al. Ozone can increase the expression of intercellular adhesion molecule-1 and the synthesis of cytokines by human nasal epithelial cells. *Inhal Toxicol* 1994;6:345-57.
25. Balmes JR. The role of ozone exposure in the epidemiology of asthma. *Environ Health Perspect* 1993;101:219-24.
26. Bates DV, Sizto R. Relationship between air pollutant levels and hospital admissions in Southern Ontario. *Can J Public Health* 1983;74:117-22.
27. Silverman F. Asthma and respiratory irritants (ozone). *Environ Health Perspect* 1979;29:131-6.
28. Kreit JW, Gross KB, Moore TB et al. Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. *J Appl Physiol: Respirat Environ Exercise Physiol* 1989;66:217-22.
29. Ball B, Horstman D, Folinsbee L et al. Pulmonary responses in asthmatics performing light exercise in clean air (air) and 0.16 ppm ozone (O₃) [Abstract]. *Am J Respir Crit Care Med* 1993;147:A640.
30. Horstman D, Roger LJ, McDonnell W et al. Increased specific airway resistance (S_{Raw}) in asthmatics exercising while exposed to 0.18 ppm ozone (O₃). *Physiologist* 1984;27:212.
31. Fernandes ALG, Molino NA, McClean PA et al. The effect of pre-exposure to 0.12 ppm of ozone on exercise-induced asthma. *Chest* 1994;106:1077-82.
32. Weymer AR, Gong H, Lyness A et al. Pre-exposure to ozone does not enhance or produce exercise-induced asthma. *Am J Respir Crit Care Med* 1994;149:1413-9.
33. Bascom R, Naclerio RM, Fitzgerald TK et al. Effect of ozone inhalation on the response to nasal challenge with antigen of allergic subjects. *Am Rev Respir Dis* 1990;142:594-601.
34. Basha MA, Gross KB, Gwizdala CJ et al. Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filtered purified air. *Chest* 1994;106:1757-65.
35. Scannell CH, Chen LL, Aris R et al. Greater ozone-induced inflammatory responses in subjects with asthma. *Am J Respir Crit Care Med* 1996;154:24-9.
36. Peden D, Boehlecke B, Horstman D, Devlin RB. Influx of bronchial neutrophils and eosinophils in asthmatics after exposure to 0.16 ppm ozone [Abstract]. *Am J Respir Crit Care Med* 1996;153:A700.
37. Frampton MW, Torres A, Voter KZ et al. Ozone exposure in subjects with asthma increases BAL lymphocyte number and activation [Abstract]. *Am J Respir Crit Care Med* 1996;153:A700.
38. Molino NA, Wright SC, Katz I et al. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. *Lancet* 1991;338:199-203.
39. Ball BA, Folinsbee LJ, Peden DB et al. Response to allergen bronchoprovocation of mild allergic asthmatics following low level ozone exposure [Abstract]. *Am J Respir Crit Care Med* 1995;151:A27.
40. Jörres R, Nowak D, Magnussen H. The effects of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. *Am J Respir Crit Care Med* 1996;153(1):56-64.
41. Peden DB, Setzer RW Jr, Devlin RB. Ozone exposure has both a priming effect on allergen-induced responses and an intrinsic inflammatory action in the airways of perennially-allergic asthmatics. *Am J Respir Crit Care Med* 1995;151:1336-45.
42. Molino NA. Effect of air pollution on atopic asthma. *Medicina* 1992;52:363-7.
43. Boushey HA. Bronchial hyperreactivity to sulphur dioxide: physiologic and political implications. *J Allergy Clin Immunol* 1982;69(4):335-8.
44. Witek TJ, Schachter EN. Airway responses to sulfur dioxide and methacholine in asthmatics. *J Occup Med* 1985;27(4):265-8.
45. Hackney JD, Linn WS, Bailey RM et al. Time course of exercise-induced bronchoconstriction in asthmatics exposed to sulfur dioxide. *Environ Res* 1984;34:321-7.
46. Horstman D, Roger LJ, Kehrl H et al. Airway sensitivity of asthmatics to sulfur dioxide. *Toxicol Ind Health* 1986;2(3):289-98.
47. Kehrl HR, Roger LJ, Hazucha MJ et al. Differing response of asthmatics to sulfur dioxide exposure with continuous and intermittent exercise. *Am Rev Respir Dis* 1987;135:350-5.
48. Folinsbee LJ, Bedi JF, Horvath SM. Pulmonary response to threshold levels of sulfur dioxide (1.0 ppm) and ozone (0.3 ppm). *J Appl Physiol: Respirat Environ Exercise Physiol* 1985;58:1783-7.
49. Roger LJ, Kehrl HR, Hazucha MJ et al. Bronchoconstriction in asthmatics exposed to sulfur dioxide during repeated exercise. *J Appl Physiol: Respirat Environ Exercise Physiol* 1985;59(3):784-91.
50. Horstman DH, Seal E Jr, Folinsbee LJ. The relationship between exposure duration and sulfur dioxide bronchoconstriction in asthmatic subjects. *Am Ind Hyg Assoc J* 1988;49(1):38-47.
51. Pierson WE, Koenig JQ. Respiratory effects of air pollution on allergic disease. *J Allergy Clin Immunol* 1992;90:557-66.
52. Gong H Jr. Health effects of air pollution. A review of clinical studies. *Clin Chest Med* 1992;13:201-14.

53. Linn WS, Shamoo DA, Spier CE et al. Respiratory effects of 0.75 ppm sulfur dioxide in exercising asthmatics: influence of upper-respiratory defenses. *Environ Res* 1983;30: 340–8.
54. Heath SK, Koenig JQ, Morgan MS et al. Effects of sulfur dioxide exposure on African-American and Caucasian asthmatics. *Environ Res* 1994;66:1–11.
55. Bigby B, Boushey H. Effects of nedocromil sodium on the bronchomotor response to sulfur dioxide in asthmatic patients. *J Allergy Clin Immunol* 1993;92:195–7.
56. Sandstrom T, Stjernberg N, Andersson M. Cell responses in bronchoalveolar fluid after exposure to sulfur dioxide: a time response study. *Am Rev Respir Dis* 1989;140:1828–31.
57. Bauer MA, Utell MJ, Morrow PE et al. Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. *Am Rev Respir Dis* 1986; 134:1203–8.
58. Linn WS, Shamoo DA, Spier CE et al. Controlled exposure of volunteers with chronic obstructive pulmonary disease to nitrogen dioxide. *Arch Environ Health* 1985;40(6):313–7.
59. Anto JM, Sunyer J. Nitrogen dioxide and allergic asthma: starting to clarify an obscure association. *Lancet* 1995; 345:402–3.
60. Sandstrom T, Stjernberg N, Eklund A et al. Inflammatory cell response in bronchoalveolar lavage fluid after nitrogen dioxide exposure of healthy subjects – a dose-response study. *Eur Respir J* 1991;4:332–3.
61. Devalia JL, Campbell AM, Sapsford RJ et al. Effect of nitrogen dioxide on synthesis of inflammatory cytokines expressed by human bronchial epithelial cells *in vitro*. *Am J Respir Cell Mol Biol* 1993;9:271–8.
62. Koenig JQ, Covert DS, Hanley QS et al. Prior exposure to ozone potentiates subsequent response to sulfur dioxide in adolescent asthmatic subjects. *Am Rev Respir Dis* 1990;141:377–80.
63. Wang JH, Devalia JL, Duddle JM, Hamilton SA, Davies RJ. Effect of six hour exposure to nitrogen dioxide on early phase nasal response to allergen challenge in patients with a history of seasonal allergic rhinitis. *J Allergy Clin Immunol* 1995;96:669–76.
64. Devalia JL, Rusznak C, Herdman MJ, Trigg CJ, Tarraf H, Davies RJ. Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. *Lancet* 1994;344:1668–71.