

**NITROGEN DIOXIDE:  
EVALUATION OF CURRENT CALIFORNIA AIR QUALITY  
STANDARDS WITH RESPECT TO PROTECTION OF CHILDREN**

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## 1. EXTENDED ABSTRACT

Nitrogen dioxide (NO<sub>2</sub>) is the most abundant and toxic of the nitrogen oxides formed from combustion of fossil fuels, and ambient concentrations are related to traffic density as well as point sources. Indoor NO<sub>2</sub> levels may exceed those found outdoors. When inhaled, NO<sub>2</sub> persists to the lung periphery because of its relatively low solubility. Greater than 60% of inhaled NO<sub>2</sub> is deposited, predominantly in the centri-acinar region, and the fraction deposited increases with exercise. Epidemiological studies have found relationships between both outdoor and indoor NO<sub>2</sub> levels and respiratory illness, decrements in lung function, and exacerbation of asthma, especially in children. Outdoor NO<sub>2</sub> was associated with increased infant mortality and intrauterine mortality in Sao Paulo, Brazil. However, these studies are subject to exposure misclassification, and generally fail to consider a possible role of indoor and outdoor particle exposure as a confounding factor. NO<sub>2</sub> may represent a marker for exposure to traffic- or combustion-related pollution in these epidemiological studies. Human clinical studies generally fail to show effects of exposure concentrations at or below the current California standard of 0.25 ppm, which supports the concept that NO<sub>2</sub> is a marker of pollution rather than a cause of direct effects at ambient levels. However, exposure to NO<sub>2</sub> at concentrations only slightly above 0.25 ppm appear to enhance responsiveness to allergen challenge in subjects with asthma.

## **2. BACKGROUND**

Combustion of fossil fuels results in the oxidation of nitrogen-containing compounds and the formation of nitrogen oxides. There are at least 7 species of nitrogen oxide compounds: nitric oxide (NO), nitrogen dioxide (NO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O), nitrogen trioxide (NO<sub>3</sub>), dinitrogen trioxide (N<sub>2</sub>O<sub>3</sub>), dinitrogen tetroxide (N<sub>2</sub>O<sub>4</sub>), and dinitrogen pentoxide (N<sub>2</sub>O<sub>5</sub>). These species are largely interconvertible, and therefore referred to collectively as NO<sub>x</sub>. Nitrogen dioxide is the most abundant in the atmosphere, and represents the greatest risk to human health. The U.S. Environmental Protection Agency has established a National Ambient Air Quality Standard (NAAQS) for NO<sub>2</sub> of 0.053 ppm (100 µg/m<sup>3</sup>), measured as an annual arithmetic mean. The State of California has established only a short-term (1 hour) standard for NO<sub>2</sub> of 0.25 ppm (470 µg/m<sup>3</sup>).

Nitrogen dioxide is considered an important outdoor pollutant not only because of potential health effects, but because it is an essential precursor in the formation of tropospheric ozone via photochemical reactions, and contributes to the formation of atmospheric acids and secondary particles. These issues will not be discussed in this review, which will focus on the health effects of exposure to NO<sub>2</sub> itself.

This review will not address the role of NO<sub>2</sub> in ozone or acid formation via photochemical reactions, and will only briefly discuss the chemistry, sources, and dosimetry of NO<sub>2</sub>. A number of reviews of these topics are available.

## **3. PRINCIPAL SOURCES AND EXPOSURE ASSESSMENT**

The primary sources for NO<sub>2</sub> are internal combustion engines, both gasoline and diesel powered, as well as point sources, especially power plants. U.S. emissions of NO<sub>x</sub> in 1996-1997 were approximately 23,000 short tons per year, with roughly 11,000 tons contributed by fuel combustion from non-transportation sources (Office of Air and Radiation, 1998). In 1991, 8.9 million people resided in counties that exceeded the NAAQS for NO<sub>2</sub>, with the highest annual concentrations occurring in Southern California (Bascom et al., 1996). National mean concentrations of NO<sub>2</sub> decreased 14% from 1988 to 1997, to about 20 ppb, although NO<sub>x</sub> emissions decreased little during that time period, and increased 1% in 1996-1997 (Office of Air and Radiation, 1998). Since 1970, total NO<sub>x</sub>

emissions have increased 11% and emissions from coal-fired power plants have increased 44%. During the past 5 years, all U.S. counties have been in compliance with the Federal NO<sub>2</sub> standard.

Compliance with the Federal NAAQS for NO<sub>2</sub> does not preclude substantial short-term peak concentrations, and the California standard of 0.25 ppm for 1 hour continues to be exceeded, although with less frequency. In 1999, maximum one-hour values for NO<sub>2</sub> were highest in the counties of Riverside (0.307 ppm) and Imperial (0.286), with annual mean concentrations of 0.022 and 0.035, respectively (Office of Air and Radiation, 1998).

Because NO<sub>2</sub> concentrations are related to traffic density, commuters in heavy traffic may be exposed to higher concentrations of NO<sub>2</sub> than those indicated by regional monitors. In one study of personal exposures by Los Angeles commuters (Baker et al., 1990), in-vehicle NO<sub>2</sub> concentrations, averaged over 1 week of travel, ranged from 0.028 to 0.170 ppm, with a mean of 0.078 ppm. This was 50% higher than ambient concentrations measured at local monitoring sites.

Indoor NO<sub>2</sub> levels, in the presence of an unvented combustion source, may exceed those found outdoors. Natural gas or propane cooking stoves release NO<sub>2</sub>, as do kerosene heaters. Peak levels exceeding 2.0 ppm have been measured in homes with gas stoves (Leaderer et al., 1984), and exposures during cooking have been measured as high as 0.6 ppm for up to 45 minutes (Goldstein et al., 1988). It is important to recognize that outdoor NO<sub>2</sub> levels provide a “background” for the higher peaks that may occur indoors; thus higher outdoor levels may drive higher peaks indoors, with outdoor levels contributing approximately 50% to indoor levels (Marbury et al., 1988).

Distance of residences from roadways appears to influence indoor NO<sub>2</sub> levels. In Tokyo, Japan, NO<sub>2</sub> exposure among adult women, age 40-60 years, was determined at varying distances from the roadside, using personal monitoring and monitoring inside and outside the home (Nakai et al., 1995). The highest mean personal exposure levels were found in women living closest to the roadway at 63.4 ppb, compared with 55.3 ppb farthest from the roadway. Personal monitoring in homes with unvented combustion sources were less clearly correlated to distance from the roadway than homes without combustion sources. In another study in the Netherlands (Roorda-Knape et al., 1999), NO<sub>2</sub> levels in

school classrooms were found to be significantly correlated with traffic density and distance of the school from the roadway.

Concentrations of NO<sub>2</sub> as high as 4 to 5 ppm have been measured inside ice hockey arenas, from operation of natural gas-fueled ice resurfacing machines in the presence of inadequate ventilation (Hedberg et al., 1989). These exposures have been associated with “epidemics” of acute respiratory illness in exposed players and fans.

### **3.1 Dosimetry**

Nitrogen dioxide is an oxidant gas that dissolves in water to form nitric acid, and also reacts with lipids and proteins in cells. It likely reacts either within the lung epithelial lining fluid or in the epithelial cell membrane, and probably does not penetrate beyond the epithelium as an intact molecule (Postlethwait et al., 1990). Toxic effects are presumably related to the effects of NO<sub>2</sub> and its reaction products on lung cells.

Nitrogen dioxide is less reactive than ozone, and is relatively insoluble; therefore, removal of inhaled NO<sub>2</sub> in the upper airway is limited. Dosimetric studies indicate that most inhaled NO<sub>2</sub> is retained in the lungs and deposited primarily in peripheral airways, particularly the terminal bronchiolar region. Miller et al. (Miller et al., 1982) developed a dosimetric model for NO<sub>2</sub> in the human which indicated that the NO<sub>2</sub> dose to the transitional airways increased three- to four-fold compared with the more proximal airways, and then decreased again in the alveolar region. Using this model, increases in tidal volume from 500 to 1500 mL would increase lung uptake from 60% to 90%, primarily attributable to increased alveolar uptake. Approximately 15 times more NO<sub>2</sub> would be delivered to pulmonary tissue at maximum tidal volume, as would occur during heavy exercise, than during rest. Data from a clinical study (Bauer et al., 1986) were supportive of these predictions. Fifteen asthmatic subjects were exposed to 0.3 ppm NO<sub>2</sub> via mouthpiece for 20 minutes at rest, followed by 10 minutes of exercise. Expired NO<sub>2</sub> concentrations were measured continuously. NO<sub>2</sub> deposition was 72±2% at rest, increasing to 87±1% with moderate exercise. These findings indicate that the NO<sub>2</sub> dose to the distal airways and alveolar space, and therefore toxic effects in this region, would be substantially increased by exercise.

## **4. DESCRIPTION OF KEY STUDIES**

The assessment of health risks of exposure to NO<sub>2</sub> and other ambient pollutants depends on three types of investigations: epidemiological studies, human clinical studies, and animal exposure and toxicology studies. In addition, in vitro exposure of cells and tissues assist with determining mechanisms of effects. Traditionally, epidemiological studies have focused on symptoms, doctor visits, hospitalizations, medication use, pulmonary function measures, and mortality as health outcomes. Clinical studies have focused on symptoms, changes in pulmonary function (principally spirometry), and occasionally assessment of non-specific airways responsiveness, in part because these measurements are relatively simple, safe, and reproducible. More recently, innovative approaches have been used to examine pollutant effects on respiratory host defense, airway inflammation, cardiac effects, and systemic effects. This review will first summarize findings from epidemiological studies, followed by human clinical studies. Although animal and in vitro exposure studies *per se* will not be addressed in detail in this review, particularly relevant data from these approaches will be addressed in the appropriate context. Emphasis will be placed on relevant studies within the past 5 years, particularly those dealing with the health of children.

### **4.1 Epidemiological Studies**

#### **4.1.1 Outdoor**

A number of epidemiological studies have sought evidence for health effects of exposure NO<sub>2</sub> outdoors, along with other pollutants, in both adults and children. A selection of studies published since 1995 are summarized in Table 1. Several studies show significant relationships between ambient NO<sub>2</sub> levels and health effects, including respiratory symptoms, episodes of respiratory illness, lung function, and even mortality. However, because NO<sub>2</sub> shares sources with other pollutants, especially fine particles, epidemiological studies are often unable to distinguish the relative importance of NO<sub>2</sub> in causing health effects. Particular caution is needed in interpreting the results of studies measuring ambient concentrations of NO<sub>2</sub>, but not particles. Indeed, many studies conducted over the past 10 years in a variety of locations around the world have observed a strong relationship between fine particle levels and both mortality and morbidity. That

NO<sub>2</sub> appears strongly correlated with health outcomes in a few of these studies is perhaps not surprising, given the close correlation between NO<sub>2</sub> and particles.

Beginning in the 1970s, epidemiological studies in Chattanooga, Tennessee examined the relation between respiratory illnesses and ambient levels of NO<sub>2</sub>. Shy and colleagues (Shy et al., 1970) tracked the respiratory symptoms of 871 families (4,043 individuals) selected from five schools situated near a munitions factory in Chattanooga. This factory emitted NO<sub>2</sub> into surrounding areas. The ambient 24-hr mean NO<sub>2</sub> levels were 0.083 ppm in the high exposure area, 0.063 ppm in the intermediate area, and 0.043 ppm in the low area. Total suspended particulate and sulfate concentrations were similar across the three areas. Biweekly questionnaires indicated that the rates of acute respiratory illness were higher among the families living in the relatively high exposure area, although the rates were not consistently associated with the exposure gradient among the three schools in the high exposure area. Differences in family size, income, or education did not explain the observed associations. Parental smoking habits did not appear to influence the illness rates among children.

A subsequent study in the same Chattanooga community (Pearlman et al., 1971) studied lower respiratory tract infections in 3,217 school children and infants. Physician's office records were used to validate the parental reports of illness. Episodes of bronchitis were reported more often for school children living two and three years in the high and intermediate ambient NO<sub>2</sub> areas. This pattern was not observed in the infants, and no significant difference in incidence was observed between the high and intermediate areas. The incidence of croup and pneumonia did not differ significantly among the three exposure areas. Control for socioeconomic status and for parental smoking was not mentioned.

In further collection and analyses of data and from the Chattanooga studies (Love et al., 1982), including improved estimates of environmental exposures data, there was an apparent increase in lower respiratory illness in children who resided in an area previously defined as having high exposure to NO<sub>2</sub>, although exposure levels at the time of the illnesses were comparable across the study region. The authors noted that the increased illnesses could not be attributed unequivocally to the atmospheric NO<sub>2</sub>.

In analyses of another EPA database from Chattanooga, Harrington and Krupnick (Harrington & Krupnick, 1985) found a statistically significant relationship between NO<sub>2</sub> and

reports of acute respiratory illness for children 12 years of age and younger. However, there was no clear exposure-response relationship.

Braun-Fahrlander and colleagues (Braun-Fahrlander et al., 1992) followed respiratory symptoms of 625 Swiss children in two cities using a daily symptom diary. Exposures to NO<sub>2</sub> were estimated using passive samplers placed outside the residence location and inside in the room where the child spent the most time. The concentrations of NO<sub>2</sub> indoors and outdoors were not associated with symptom incidence rates. The duration of symptom episodes was associated with outdoor but not indoor NO<sub>2</sub> concentration.

The Swiss Study on Air Pollution and Lung Disease in Adults (SAPALDIA) (Zemp et al., 1999; Schindler et al., 1998; Ackermann-Lieblich et al., 1997) examined the long-term effects of air pollution exposure in a cross-sectional and longitudinal study of 8 areas in Switzerland. Significant associations were observed between symptoms (chronic phlegm, chronic cough, breathlessness at rest, dyspnea on exertion) and both NO<sub>2</sub> and particles (Zemp et al., 1999). In the cross-sectional component of the study (Schindler et al., 1998), a significant negative correlation was observed between NO<sub>2</sub> and both FVC ( $\beta = -0.0123$ ,  $p < 0.001$ ) and FEV<sub>1</sub> ( $\beta = -0.0070$ ,  $p < 0.001$ ). NO<sub>2</sub> levels correlated strongly with PM<sub>10</sub> levels ( $r = 0.91$ ), making it impossible to determine the role of specific pollutants in the observed associations.

Frischer et al. (Frischer et al., 1993) studied 423 Austrian school children living in 4 small towns with varying levels of outdoor NO<sub>2</sub> levels. The children were assessed with spirometry and cold air inhalation challenge on two occasions 6 months apart. Lung function increased for the children coincident with an overall decrease in NO<sub>2</sub> levels, and NO<sub>2</sub> levels were found to be predictive for an increase in FVC. However, the study does not provide convincing evidence for causality in the relationship; the amount of lung function increase was consistent with that expected from increase in height during the interval. No measurements of ambient particles were reported.

A population-based study in the Netherlands (Boezen et al., 1998) suggested that, in adults, airways hyperresponsiveness at baseline increased risk for respiratory symptoms from air pollution. Relationships were observed between exposure to NO<sub>2</sub> and to PM<sub>10</sub> with respiratory symptoms, only in the group with some measure of airway lability.

Associations have also been observed between NO<sub>2</sub> levels and emergency visits for asthma in Valencia, Spain (Tenias et al., 1998), in Barcelona (Castellsague et al., 1995), in Israel (Garty et al., 1998), and in Santa Clara County, California (Lipsett et al., 1997). In these studies, NO<sub>2</sub> effects may have been reflective of the pollutant mix rather than NO<sub>2</sub> alone.

Children with asthma appear to be more susceptible to the health effects of air pollution in general, although the specific role of NO<sub>2</sub> exposure remains in question. McConnell et al (McConnell et al., 1999), reporting data from the Southern California Children's Health Study, found positive associations between indices of air pollution, including NO<sub>2</sub>, PM<sub>10</sub>, and PM<sub>2.5</sub>, and respiratory symptoms in children with asthma. The strongest association was with NO<sub>2</sub> (Figure 1). No association was seen for children without asthma. Particles, NO<sub>2</sub>, and acids were too highly correlated to allow estimation of individual pollutant effects. Krämer et al. (Krämer et al., 2000) examined the relationship between NO<sub>2</sub> exposure, as assessed by outdoor and personal monitors, and the prevalence of atopy and rhinitis in 9 year old children. Interestingly, a significant relationship for both endpoints was observed with outdoor NO<sub>2</sub> levels, but not with levels obtained from personal monitoring. This suggests that a factor associated with outdoor air pollution, other than NO<sub>2</sub>, may be playing a causative role.

A large study of visits to doctors' offices in London for respiratory complaints (Hajat et al., 1999) found different pollutant associations for children than for adults. Among children, positive associations were found between asthma visits and both NO<sub>2</sub> and CO. The strongest relationship was during the summer, when the percentage change in asthma visits for a 10 to 90<sup>th</sup> percentile increase in 24-hour NO<sub>2</sub>, lagged by one day, was 13.2% (CI: 5.6-21.3%). For adults, the only significant association was for PM<sub>10</sub>. This finding suggests that children and adults may differ in their susceptibilities to components of the ambient pollutant mix.

Associations between NO<sub>2</sub> levels and mortality have been observed. A very brief report (Garcia-Aymerich et al., 2000) examined mortality in a cohort of patients with chronic obstructive pulmonary disease in Barcelona. Significant relationships were found between mortality and increases in SO<sub>2</sub>, 1 hour maximum NO<sub>2</sub>, and 24 hour average NO<sub>2</sub>, but not black smoke. However, data for black smoke nearly missed significance, and it is likely that

NO<sub>2</sub> represents a surrogate for pollution in general in this study. Saldiva et al. (Saldiva et al., 1994) studied mortality among children under age 5 in Sao Paulo, Brazil, a city with dense traffic, high pollution levels, and high infant mortality. Mortality due to congenital malformations, neonatal events, or prematurity was excluded. Mean NO<sub>2</sub> (NO<sub>x</sub>) levels were 0.127 ppm. Only NO<sub>2</sub>, and not PM<sub>10</sub>, ozone, SO<sub>2</sub>, or CO, was associated with mortality in this study, with an estimated odds of 1.30, 95% CI: 1.17-1.43.

The Sao Paulo group subsequently examined the influence of pollutant exposure on intrauterine mortality (Pereira et al., 1998). Again, the strongest single-pollutant coefficient was for NO<sub>2</sub> (0.0013/μg/m<sup>3</sup>, p<0.01, Figure 2), with lesser coefficients for SO<sub>2</sub> and CO. No significant relationship was seen for PM<sub>10</sub> or ozone. An index combining the effects of NO<sub>2</sub>, SO<sub>2</sub>, and CO associated most strongly with fetal mortality. The authors postulated one mechanism may be formation of methemoglobin in the fetus; fetal hemoglobin is more easily oxidized than that in adults.

Exposure to ambient fine particulate matter has been associated with increases in cardiovascular mortality. Peters and colleagues (Peters et al., 2000) used a novel approach to determine whether ambient pollution levels were associated with cardiac arrhythmias. The investigators obtained data from patients with implantable cardiac defibrillators, determining the number of times the defibrillator was activated in response to an arrhythmia, and correlating this with ambient concentrations of particles and gases. The strongest association was with NO<sub>2</sub>, with a 1 to 2-day lag. For example, the odds ratio for having at least 10 defibrillator events in association with a 26 ppb increase in NO<sub>2</sub> was 2.79, with 95% confidence intervals of 1.53-5.10. The concentration-response relationship was steeper for NO<sub>2</sub> than for PM<sub>2.5</sub> or black carbon (Figure 3). This is a potentially instructive study because NO<sub>2</sub> levels were highest in the winter, and PM<sub>2.5</sub> levels were highest in the summer. Correlation between NO<sub>2</sub> and PM<sub>2.5</sub> measurements were lower (r=0.57) than in many epidemiological studies, allowing some ability to attribute effects. The authors hypothesized that NO<sub>2</sub> may be a marker for the more toxic emissions associated with local traffic-related pollution, rather than PM alone, which is a mixture of combustion and transported particles. The data are also consistent with toxicity related to NO<sub>2</sub> as a component of the ambient pollutant mixture.

### 4.1.2 Indoor

The indoor setting provides the potential for discrimination between NO<sub>2</sub> and particle effects, because stoves burning natural gas emit primarily NO<sub>x</sub>. Many studies have examined the potential for health effects of indoor NO<sub>2</sub> exposure, especially in children; many of these studies, and their methodological problems, have been reviewed (Samet & Utell, 1990; Samet & Spengler, 1991; Frampton et al., 1991b), and only selected studies will be mentioned here. Key studies published since 1995 are summarized in Table 2.

Reports during the 1970's, from the United Kingdom (Melia et al., 1977) and the U.S. (Speizer et al., 1980), suggested that residence in a home with a gas stove increased the frequency of respiratory symptoms and of respiratory illness among children less than 2 years of age. Small sample size, inadequate control of potential confounding factors (e.g., presence of other children in the household, day care attendance, exposure to environmental tobacco smoke, and socioeconomic status) and potential misclassification of exposure and outcome limit the validity of these investigations. In particular, misclassification of exposure by using gas stoves as a surrogate for NO<sub>2</sub> exposure, and small sample sizes, may tend to bias many of the studies toward no effect.

In a pilot study, Goldstein and associates (Goldstein et al., 1988) monitored NO<sub>2</sub> exposures for 5 days in asthmatic subjects with a portable continuous monitoring instrument held at breathing level before, during, and after they used a gas stove for cooking. The limited data suggested that at average NO<sub>2</sub> levels below 0.3 ppm there were no consistent effects on lung function, while at concentrations above 0.3 ppm most of the asthmatic subjects showed a drop in forced vital capacity (FVC).

To reduce the problem of small sample size, Hasselblad et al. (Hasselblad et al., 1992) reported a meta-analysis of 11 epidemiological studies of respiratory illness in children and residential NO<sub>2</sub> exposure. The authors found an estimated 20% increase in risk of respiratory illness in children per 15 ppb increment in indoor NO<sub>2</sub> exposure.

A number of additional studies have been published since 1990, with continued mixed results. A prospective cohort study of infants conducted in Albuquerque, New Mexico (Samet et al., 1993) attempted to address many of the issues of previous studies related to sample size and exposure misclassification. Exposures to NO<sub>2</sub> and respiratory illnesses were monitored prospectively from birth to 18 months of age in a cohort of 1,205 infants

living in homes with gas and electric cooking stoves, without smoking. NO<sub>2</sub> exposures were estimated from serial measurements of bedroom NO<sub>2</sub> concentrations. Respiratory illnesses were quantified from reports of symptoms and illnesses from mothers and validated by home visits. No consistent trends in incidence or duration of illness were observed by level of NO<sub>2</sub> exposure at the time of illness or during the prior month, or by type of stove. However, indoor NO<sub>2</sub> levels were very low in this study.

Neas et al. (Neas et al., 1991) reported that a composite measure of respiratory symptoms increased monotonically with measured annual average NO<sub>2</sub> concentrations within the home, among children in the Harvard Six Cities study. Symptoms included shortness of breath with wheeze, chronic wheezes, chronic cough, phlegm, or bronchitis. Residential NO<sub>2</sub> levels were not associated with pulmonary function. On the other hand, Dijkstra et al. (Dijkstra et al., 1990) found no associations between chronic cough, persistent wheeze, or shortness of breath with wheeze with indoor NO<sub>2</sub> measurements in the homes of children in the Netherlands.

More recent studies have utilized personal monitoring methods in an attempt to improve exposure classification. Mukala et al. (Mukala et al., 1999) prospectively studied personal exposure to NO<sub>2</sub> for periods of 13 weeks among 163 preschool children in Helsinki, using individual passive diffusion monitors. Daily diaries of symptoms were kept by the parents, and in a subset of 53 children, peak expiratory flow rates were measured in the morning and evening. Co-variables considered in the model included allergy, education, smoking, stove type, and outdoor pollutant concentrations (NO, NO<sub>2</sub>, O<sub>3</sub>, SO<sub>2</sub>, and total suspended particles). The median personal NO<sub>2</sub> exposure was 21.1 µg/m<sup>3</sup> (0.011 ppm), with a maximum of 99 µg/m<sup>3</sup> (0.05 ppm). An increased risk of cough was associated with increasing NO<sub>2</sub> exposure (risk ratio = 1.52; 95% confidence interval 1.00-2.31). There were no significant effects on other respiratory symptoms or peak flow.

In Australia, where unvented natural gas cooking and heating are common, Pilotto et al. (Pilotto et al., 1997) queried respiratory symptoms and school absences among 388 children from 6 to 11 years of age, and monitored indoor NO<sub>2</sub> levels at their schools, which were chosen for having either unvented gas heating or electric heating. Classroom monitoring of NO<sub>2</sub> levels was conducted intermittently over several months. A significant increase in sore throat, colds, and absences from school were found for children in

environments with hourly peak levels 80 ppb, compared with background levels of 20 ppb. Exposure-response relationships were evident for each outcome. However, no measurements of other pollutants, either indoor or outdoor, were provided. Caution must be used in interpreting the findings from cross-sectional studies, because many factors other than pollutant levels may influence differences between populations.

In the Latrobe Valley of Australia (Garrett et al., 1998), NO<sub>2</sub> levels were monitored in eighty homes, on 5 separate occasions for 4 days each, and health questionnaires administered to the 148 children residing in those homes. 58 of the children were asthmatic, although the diagnostic criteria were not provided. Children underwent allergy prick testing and monitored their peak flow rates for a 2-week period in the winter and spring. The indoor median NO<sub>2</sub> concentration was 6.0 ppb, with a maximum of 128 ppb. Respiratory symptoms were more common in children exposed to a gas stove (odds ratio 2.3, CI 1.00-5.2), even after adjusting for NO<sub>2</sub> levels (odds ratio 2.2, CI 1.0-4.8). Atopic children tended to have a greater risk than non-atopic children. NO<sub>2</sub> concentration was not a significant risk factor for symptoms. The authors conclude that gas stoves may pose a risk apart from NO<sub>2</sub>. However, the relative paucity of NO<sub>2</sub> monitoring data for each home may have provided insufficient statistical power to demonstrate an association. More important weaknesses in the study are the inclusion of homes with cigarette smokers, and the failure to monitor other pollutants, either inside or outside the home. These factors may have confounded the findings.

Jarvis et al. (Jarvis et al., 1996) studied symptoms, lung function, and atopy in 15,000 adults aged 20-44 years in Britain, as part of the European Community Respiratory Health Survey. Women, but not men, who reported cooking with gas had an increased risk for symptoms consistent with asthma, such as wheezing (odds ratio (OR) 2.07, CI 1.41-3.05), waking with shortness of breath (OR 2.32, CI 1.25-4.34), and "asthma attacks" (OR 2.60, CI 1.20-5.65). Lung function was measured in a subset of subjects, and FEV<sub>1</sub> was reduced 3.1% of predicted for women cooking with gas compared to those using other means, after adjusting for age, smoking, and town of residence. Total and specific IgE levels were not associated with gas stove use. There was no protective effect associated with use of an exhaust fan. The authors boldly concluded from their estimate of the population attributable risk fraction that "the prevalence of wheeze with breathlessness in

young women would be reduced by between 8% and 48% if cooking with gas were abandoned.” Although studies such as this are limited by the potential for exposure misclassification and the influence of other environmental and biological factors, the findings are consistent with women spending more time at cooking than men, and with reports of increased responsiveness to allergen challenge following NO<sub>2</sub> exposure (see below).

Taken together, studies of the health effects of exposure to NO<sub>2</sub> indoors fail to make a convincing case for association with respiratory illness in either children or adults. The findings of the Hasselblad meta-analysis (Hasselblad et al., 1992) must be interpreted with caution because the 11 studies used in the analysis employed varying methodologies and study populations. Small sample size, potential for misclassification, inclusion of smokers in many of the studies, and failure to consider potential effects of outdoor pollution, or other indoor pollutants, may bias many of the studies. For example, burning of natural gas in gas stoves emits ultrafine particles in addition to NO<sub>2</sub>, and the cooking process is also a source of particles. It is possible that observed health effects associated with gas stove use may represent health effects of particle exposure, or of particles combined with NO<sub>2</sub>. This may explain why Garrett et al. (Garrett et al., 1998), found a significant relationship between respiratory symptoms in children and gas stove use, but not indoor NO<sub>2</sub> levels. The Samet et al. study of infants in Albuquerque (Samet et al., 1993) provides convincing evidence that indoor NO<sub>2</sub>, at the very low concentrations found in that study, are not associated with respiratory illnesses in children under 18 months of age.

## **4.2 Clinical Studies**

### **4.2.1 Studies with Healthy Subjects**

*Effects on Pulmonary Function:* Studies examining responses of healthy volunteers to acute exposure to NO<sub>2</sub> have generally failed to show alterations in lung mechanics of healthy volunteers (Hackney et al., 1978; Kerr et al., 1979; Frampton et al., 1991a; Azadniv et al., 1998). Exposures ranging from 75 minutes to 3 hours at concentrations up to 4.0 ppm NO<sub>2</sub> (Linn et al., 1985b; Mohsenin, 1987b; Mohsenin, 1988) did not alter pulmonary function. Curiously, Bylin and associates (Bylin et al., 1985) found increased airway resistance after a 20-minute exposure to 0.25 ppm NO<sub>2</sub> and decreased airway resistance

after a 20-minute exposure to 0.5 ppm NO<sub>2</sub>, but no change in airway responsiveness to aerosolized histamine challenge in the same subjects. Overall, there is little convincing evidence that exposure of healthy volunteers to NO<sub>2</sub> at levels as high as 4.0 ppm alters airway mechanics, as measured by spirometry or flow resistance.

Several observations indicate that NO<sub>2</sub> exposures in the range of 1.5-2.0 ppm cause small but significant increases in airway responsiveness. Mohsenin (Mohsenin, 1988) found that a 1-hour exposure to 2 ppm NO<sub>2</sub> increased responsiveness to methacholine, as measured by changes in specific airway conductance, without directly affecting lung function. Furthermore, pretreatment with ascorbic acid prevented the NO<sub>2</sub>-induced increase in airway responsiveness (Mohsenin, 1987b). A mild increase in responsiveness to carbachol was observed following a 3-hour exposure to 1.5 ppm NO<sub>2</sub>, but not to intermittent peaks of 2.0 ppm (Frampton et al., 1991a).

Few human clinical studies of NO<sub>2</sub> have included elderly subjects. Morrow et al. (Morrow et al., 1992) studied the responses of 20 healthy volunteers, 13 smokers and 7 nonsmokers of mean age 61 years, following exposure to 0.3 ppm NO<sub>2</sub> for 4 hours with light exercise. There was no significant change in lung function related to NO<sub>2</sub> exposure for the group as a whole. However, the 13 smokers experienced a slight decrease in FEV<sub>1</sub> during exposure, and their responses were significantly different from the 7 nonsmokers (% change in FEV<sub>1</sub> at end of exposure: -2.25 vs. +1.25%, p = 0.01).

*Effects on Host Defense:* Clinical studies have attempted to address the question of whether NO<sub>2</sub> exposure increases susceptibility to infection. Goings et al. (Goings et al., 1989) exposed healthy volunteers to either 1-3 ppm NO<sub>2</sub> or to air for 2 hours per day for 3 consecutive days. A live, genetically engineered influenza A vaccine virus was administered intranasally to all subjects after exposure on day 2. Infection was determined by virus recovery from nasal washings, a 4-fold or greater increase in antibody titer, or both. The findings of this study were inconclusive, in part because of limitations in sample size. In addition, the attenuated, cold-adapted virus used in the study was incapable of infecting the lower respiratory tract, where NO<sub>2</sub> may have its greatest impact on host defense.

Another approach has been to obtain lavaged cells from NO<sub>2</sub>-exposed individuals and examine their handling of infectious virus *in vitro*. Several NO<sub>2</sub> exposure scenarios,

including continuous low-level exposure or intermittent peak exposures have been examined (Frampton et al., 1989). Alveolar macrophages obtained by BAL 3 1/2 hours after a 3-hour continuous exposure to 0.60 ppm NO<sub>2</sub> tended to inactivate influenza *in vitro* less effectively than cells collected after air exposure. The effect was observed in cells from 4 of the 9 subjects studied; alveolar macrophages from these 4 subjects increased release of interleukin-1 after exposure to NO<sub>2</sub>, whereas cells from the remaining 5 subjects decreased release of interleukin-1 following exposure. However, in a subsequent study (Azadniv et al., 1998) involving 2.0 ppm NO<sub>2</sub> exposures for 6 hours with intermittent exercise, no effect on alveolar macrophage function or inactivation of influenza virus was observed, either immediately or 18 hours after exposure.

*Airway Inflammation:* Unlike ozone exposure, NO<sub>2</sub> exposure at near-ambient levels (i.e., less than 2.0 ppm) does not cause a significant influx of polymorphonuclear leukocytes (PMN) into the airways and alveoli (Frampton et al., 1989). NO<sub>2</sub> appears to be much less potent than ozone in eliciting a neutrophilic inflammatory response.

However, prolonged exposure to NO<sub>2</sub> at concentrations only slightly above peak levels occurring indoors can cause mild airway inflammation. Healthy volunteers exposed to 2.0 ppm NO<sub>2</sub> for 6 hours with intermittent exercise (Azadniv et al., 1998) showed a slight increase in the percentage of PMN obtained in bronchoalveolar lavage fluid 18 hours after exposure (air: 2.2±0.3%; NO<sub>2</sub>: 3.1±0.4%). In a separate group of subjects, no effects of this exposure protocol were found on alveolar macrophage phenotype or expression of the adhesion molecule CD11b or receptors for IgG when assessed immediately after exposure (Gavras et al., 1994). Blomberg et al. (Blomberg et al., 1997) reported that 4-hour exposures to 2.0 ppm NO<sub>2</sub> resulted in an increase in interleukin-8 and PMN in the proximal airways of healthy subjects, although no changes were seen in bronchial biopsies. This group also studied the effects of repeated 4-hour exposures to 2 ppm NO<sub>2</sub> on 4 consecutive days, with BAL, bronchial biopsies, and BAL fluid antioxidant levels assessed 1.5 hours after the last exposure (Blomberg et al., 1999). The bronchial wash fraction of BAL fluid showed a two-fold increase in PMN and a 1.5-fold increase in myeloperoxidase, indicating persistent mild airway inflammation with repeated NO<sub>2</sub> exposure. Interestingly, small but

significant decrements in FVC and FEV<sub>1</sub> were observed after the first exposure, which returned to baseline following subsequent exposures.

There is evidence from both animal and human studies that exposure to NO<sub>2</sub> may alter lymphocyte subsets in the lung and possibly in the blood. Lymphocytes, particularly cytotoxic T cells and NK cells, play a key role in host defense against respiratory viruses by eliminating infected host cells. Richters and colleagues (Damji & Richters, 1989) (Richters & Damji, 1988; Richters & Richters, 1989; Kuraitis & Richters, 1989) showed that mice exposed to NO<sub>2</sub> at levels as low as 4 ppm for eight hours demonstrate reductions in populations of CD8<sup>+</sup> (cytotoxic/suppressor) lymphocytes in the spleen. In humans, Sandstrom et al. (Sandstrom et al., 1991) observed a significant, dose-related increase in lymphocytes and mast cells recovered by BAL 24 hours after a 20-minute exposure to NO<sub>2</sub> at 2.25 - 5.5 ppm. Rubinstein et al. (Rubinstein et al., 1991) found that a series of 4 daily 2-hour exposures to 0.60 ppm NO<sub>2</sub> resulted in a small increase in NK cells recovered by BAL. In contrast, repeated exposures to 1.5 or 4 ppm NO<sub>2</sub> for 20 minutes every 2<sup>nd</sup> day on six occasions resulted in decreased CD16<sup>+</sup>56<sup>+</sup> and CD19<sup>+</sup> cells in BAL fluid, 24 hours after the final exposure (Sandstrom et al., 1992b; Sandstrom et al., 1992a). No effects were seen on PMN or total lymphocytes. Finally, Azadniv et al. (Azadniv et al., 1998) observed a small but significant reduction in CD8<sup>+</sup> T lymphocytes in peripheral blood, but not BAL, 18 hr following single 6 hour exposures to 2.0 ppm NO<sub>2</sub>.

Differing exposure protocols and small numbers of subjects among these studies may explain the varying and conflicting findings. Furthermore, the clinical significance of transient, small changes in lymphocyte subsets is unclear. However, even small changes in susceptibility to respiratory viruses resulting from exposure to NO<sub>2</sub> may have a significant public health impact because of the large number of individuals exposed in the home, both to NO<sub>2</sub> and to respiratory viruses. However, clinical studies provide little evidence for effects on lung function, airway inflammation, or host defense impairment in healthy subjects at outdoor ambient exposure concentrations.

*Induction of Emphysema:* Clinical emphysema in humans has been linked with deficient proteinase inhibitor activity in the lung, presumably via inactivation by cigarette smoke. One mechanism by which chronic NO<sub>2</sub> exposure may result in structural lung injury is through inactivation of lung proteinase inhibitors. Animal models involving prolonged

exposure to relatively high levels of NO<sub>2</sub> have found pathological changes of emphysema (Evans et al., 1976; Lafuma et al., 1987). Mohsenin and Gee (Mohsenin and Gee, 1987) exposed healthy volunteers to 3 or 4 ppm NO<sub>2</sub> for 3 hours and observed a 45% decrease in the functional activity of  $\alpha_1$ -proteinase inhibitor in BAL fluid. Supplementation with vitamins C and E prior to exposure abrogated the effect of 4.0 ppm NO<sub>2</sub> on elastase inhibitory capacity of the alveolar lining fluid (Mohsenin, 1991). In contrast, Johnson et al. (Johnson et al., 1990) found no effect of exposure for 3 hours to continuous 1.5 ppm or intermittent peaks of 2.0 ppm NO<sub>2</sub> on either the concentration (immunoassay) or functional activity of  $\alpha_1$ -proteinase inhibitor in BAL fluid. The absence of an effect in the Johnson study may reflect the lower exposure levels used.

Frampton et al. (Frampton et al., 1989) observed a 47% increase in  $\alpha_2$ -macroglobulin, a metalloproteinase inhibitor released by alveolar macrophages, in BAL fluid 3 and 1/2 hours following 3-hour exposures to 0.60 ppm NO<sub>2</sub>. This protein may have local immunoregulatory effects as well as provide local protection against proteinases. Its increase following NO<sub>2</sub> exposure suggests a protective response. However, no change in BAL fluid levels of  $\alpha_2$ -macroglobulin were observed following similar exposures to 1.5 ppm NO<sub>2</sub> (Frampton et al., 1989).

#### **4.2.2 Studies of People with Asthma**

Orehek and colleagues (Orehek et al., 1976) were the first to report that relatively brief exposures of asthmatics to low-level NO<sub>2</sub> (0.1 ppm) might enhance subsequent responsiveness to challenge with a broncho-constricting drug. Although NO<sub>2</sub> alone caused an increase in airway resistance in only 3 of 20 asthmatics, bronchial responsiveness to carbachol increased in 13 of these 20 subjects. However, this report was challenged because of the retrospective separation of responding from non-responding subjects. Hazucha and colleagues (Hazucha et al., 1983) failed to confirm these results in a study of 15 asthmatic subjects. Although there were some differences in techniques and patient selection between the Orehek and Hazucha studies, it seems likely that the findings of Orehek and coworkers reflect a retrospective stratification of subjects into “responder” and “non-responder” groups that was not justified *a priori*. Other investigators have also been unable to confirm effects of 0.1-0.2 ppm NO<sub>2</sub> on lung function in either asthmatic

adolescents (Koenig et al., 1985; Koenig et al., 1988) or in mildly asthmatic adults (Koenig et al., 1985; Bauer et al., 1986; Orehek et al., 1976; Bylin et al., 1985; Hazucha et al., 1983; Koenig et al., 1988; Kleinman et al., 1983; Linn et al., 1986; Mohsenin & Gee, 1987; Morrow & Utell, 1989; Roger et al., 1990).

Kleinman and colleagues (Kleinman et al., 1983) evaluated the response of lightly exercising asthmatic subjects to inhalation of 0.2 ppm NO<sub>2</sub> for 2 hours, during which resting minute ventilation was doubled. Although NO<sub>2</sub> did not cause alterations in flow rates or airways resistance, approximately two-thirds of the subjects experienced increased responsiveness to methacholine after inhalation of NO<sub>2</sub> compared with clean air, as assessed by specific airway resistance.

In view of the inconclusive findings at 0.1 and 0.2 ppm NO<sub>2</sub>, Bauer and colleagues (Bauer et al., 1986) studied the effects of mouthpiece exposure to 0.3 ppm NO<sub>2</sub> for 30 minutes (20 minutes at rest followed by 10 minutes of exercise at approximately 40 L/min) in 15 asthmatics. At this level, NO<sub>2</sub> inhalation produced significant decrements in forced expiratory flow rates after exercise, but not at rest. Furthermore, after airway function was allowed to return to baseline during a 1-hour recovery period, isocapnic cold-air hyperventilation elicited increased airway responsiveness in the asthmatics who had earlier been exposed to NO<sub>2</sub>.

Roger and coworkers (Roger et al., 1990), in a comprehensive, concentration-response experiment, were unable to confirm the results of a previous pilot study suggesting airway responses in asthmatic subjects. Twenty-one male asthmatics exposed to NO<sub>2</sub> at 0.15, 0.30, and 0.60 ppm for 75 minutes did not experience significant effects on lung function or airway responsiveness compared with air exposure. Bylin and coworkers (Bylin et al., 1985) found significantly increased bronchial responsiveness to histamine challenge compared with sham exposure in 8 atopic asthmatics exposed to 0.30 ppm NO<sub>2</sub> for 20 minutes. Five of 8 asthmatics demonstrated increased reactivity, while 3 subjects showed no change, as assessed by specific airway resistance. Mohsenin (Mohsenin, 1987a) reported enhanced responsiveness to methacholine in eight asthmatic subjects exposed to 0.50 ppm NO<sub>2</sub> at rest for 1 hour; airway responsiveness was measured by partial expiratory flow rates at 40% vital capacity, which may have increased the sensitivity for detecting small changes in airway responsiveness. Strand et al. (Strand et al., 1996)

found increased responsiveness to histamine among 19 asthmatic subjects 5 hours after a 30 minute exposure to 0.26 ppm NO<sub>2</sub>, with intermittent exercise.

The inconsistent results of these studies have not been satisfactorily explained. It is evident that a wide range of responses occur among asthmatics exposed to NO<sub>2</sub>. This variation may in part reflect differences in subjects and exposure protocols: mouthpiece vs. chamber, obstructed vs. non-obstructed asthmatics, sedentary vs. exercise, and requirements for medication. Identification of factors that predispose to NO<sub>2</sub> responsiveness requires further investigation. These studies have typically involved volunteers with mild asthma; data are needed from more severely affected asthmatics who may be more susceptible. Overall, there is little convincing evidence that short-term exposures to NO<sub>2</sub> at outdoor ambient concentrations significantly alter lung function or non-specific airway responsiveness in most people with mild asthma. However, outdoor levels influence indoor concentrations, which may reach peak levels that are clinically important for some adults and children with asthma.

*Effects on Allergen Responsiveness:* The potential for NO<sub>2</sub> exposure to enhance responsiveness to allergen challenge in asthmatics deserves special mention. Several recent studies, summarized in Table 3, have reported that low-level exposures to NO<sub>2</sub>, both at rest and with exercise, enhance the response to specific allergen challenge in mild asthmatics. Tunnicliffe et al. (Tunnicliffe et al., 1994) reported exposures of 8 subjects with asthma to 400 ppb NO<sub>2</sub> for only 1 hour at rest, and found increased responsiveness to a fixed dose of allergen, both during the early and late phases of the response. No significant effect was seen at 100 ppb, but the data suggested an exposure-response relationship. Davies' group from the U.K., in two reports (Devalia et al., 1994; Rusznak et al., 1996), described an effect of exposure to the combination of 400 ppb NO<sub>2</sub> and 200 ppb SO<sub>2</sub>, but not either pollutant alone, on subsequent allergen challenge in mild asthmatics. Strand and colleagues (Strand et al., 1998) from Sweden demonstrated increases in both the early and late phase responses to allergen following 4 daily repeated exposures to 260 ppb NO<sub>2</sub> for 30 minutes, at rest. Finally, Jenkins et al. (Jenkins et al., 1999) exposed asthmatic subjects to NO<sub>2</sub>, ozone, and their combination using two different protocols that varied time of exposure and gas concentration, but kept the total exposure constant. All three exposures of the high concentration regimen (200 ppb ozone, 400 ppb NO<sub>2</sub>, and the

combination for 3 hours), but not the low concentration regimen, enhanced subsequent responsiveness to allergen.

Additional data from both animal exposure and in vitro exposure studies provide support for enhancement of allergen responsiveness by NO<sub>2</sub> exposure. Gilmour (Gilmour, 1995) has reviewed the evidence in animal models. Of particular interest is a rat model of house-dust-mite sensitivity in which a 3-hour exposure to 5 ppm NO<sub>2</sub>, after a priming injection and pulmonary challenge with antigen, increased the specific immune response and immune-mediated pulmonary inflammation. NO<sub>2</sub> exposure also enhanced lymphocyte proliferation responses to allergen in both the spleen and mediastinal lymph nodes. Schierhorn et al. (Schierhorn et al., 1999) observed increased histamine release by cultured human nasal mucosa from surgical resections in response to exposure to NO<sub>2</sub> at 200 and 800 µg/m<sup>3</sup> (106 and 424 ppb) for 24 hours. The magnitude of the effect was more pronounced than for ozone.

These recent studies involving allergen challenge appear relatively consistent in demonstrating effects at concentrations that occur indoors, and suggest that NO<sub>2</sub> may enhance both allergen sensitization and its associated inflammatory response. Confirmation of these findings is needed from other centers. However, the rising incidence, prevalence, and mortality from asthma makes these observations particularly important and timely. Additional work is needed in understand more completely the exposure-response characteristics, effects of exercise, relationship to severity of asthma, role of asthma medications, and other clinical factors. Animal and in vitro studies are needed to establish the precise mechanisms involved.

#### **4.2.3 Chronic Obstructive Pulmonary Disease**

Few studies have examined responses to NO<sub>2</sub> in subjects with chronic obstructive pulmonary disease (COPD). In a group of 22 subjects with moderate COPD, Linn and associates (Linn et al., 1985a) found no pulmonary effects of 1-hour exposures to 0.5, 1.0, and 2.0 ppm NO<sub>2</sub>. In a study by Morrow and colleagues (Morrow et al., 1992), 20 subjects with COPD were exposed for 4 hours to 0.3 ppm NO<sub>2</sub> in an environmental chamber, with intermittent exercise. Although progressive decrements in lung function occurred during the exposure, significant decreases were not found for FVC until the end of the exposure. The decrement in lung volume occurred without changes in flow rates. The difference in

results between the Linn and Morrow studies may reflect the difference in duration of exposure. It is worth noting that changes in lung function were typical of the “restrictive” pattern seen with ozone rather than the obstructive changes described by some with NO<sub>2</sub> exposure in asthmatics.

## **5. INTERACTIONS**

Environmental exposures to NO<sub>2</sub> do not occur singly, but rather as a complex mixture of pollutants, and failure to consider the presence of other pollutants may confuse interpretation of the observed effects. Recent data suggest exposure to low concentrations of NO<sub>2</sub> at rest may enhance the response to allergen inhalation in subjects with asthma (see section 4.2.2). When considering mixtures of anthropogenic pollutants, it may be impossible to separate the effects of one component from those of other components, particularly with the possibilities of synergistic or antagonistic interactions. In considering the health effects of mixtures, potential causal pathways should be carefully delineated. For example, some reports have suggested that HONO may contribute to the health effects attributed to indoor NO<sub>2</sub> (Spengler et al., 1990).

Efforts have been made to study effects of NO<sub>2</sub>-ozone mixtures on pulmonary function. These studies have generally revealed no interactive effects; the observed pulmonary function decrements appear to reflect the ozone component of the mixtures. Hazucha et al., (Hazucha et al., 1994) found that pre-exposure of healthy women to 0.6 ppm NO<sub>2</sub> for 2 hours enhanced the development of nonspecific airway responsiveness induced by a subsequent 2-hour exposure to 0.3 ppm ozone, with intermittent exercise.

Relatively high-level, prolonged (6 hours/day, up to 90 days) exposure to NO<sub>2</sub> (14.4 ppm) and ozone (0.8 ppm) results in a syndrome of progressive pulmonary fibrosis in rats (Rajini et al., 1993), associated with a sustained increase in procollagen gene expression in the central acini (Farman et al., 1999). This does not occur with either gas alone, indicating a true synergistic effect. The relevance of this observation for human ambient exposures is not clear, given the high exposure concentrations used in the study, and absence of evidence for alveolar fibrosis or restrictive lung disease in epidemiological studies.

Bermudez et al. (Bermudez et al., 1999) examined DNA strand breaks in BAL cells from rats exposed to ozone (0.3 ppm), to NO<sub>2</sub> (1.2 ppm), and the combination. Ozone and the combination exposure increased DNA strand breaks to a similar degree compared with air exposure, but NO<sub>2</sub> alone had no effect.

The effects of NO<sub>2</sub> exposure on SO<sub>2</sub>-induced bronchoconstriction have been examined, but with inconsistent results. Jorres and Magnussen (Jorres & Magnussen, 1991) found an increase in airways responsiveness to SO<sub>2</sub> in asthmatic subjects following exposure to 0.25 ppm NO<sub>2</sub> for 30 minutes, yet Rubinstein et al. (Rubinstein et al., 1990) found no change in responsiveness to SO<sub>2</sub> inhalation following exposure of asthmatics to 0.30 ppm NO<sub>2</sub> for 30 minutes.

Overall, there are little definitive data suggesting that NO<sub>2</sub> interacts with other pollutants in causing human health effects. However, human clinical studies have not systematically addressed the effects of pollutant combinations containing NO<sub>2</sub>, in part because of the complexity of the experimental design and the difficulty in studying the most susceptible subjects.

## **6. CONCLUSIONS**

Evidence for human health effects of exposure to ambient NO<sub>2</sub> derives from epidemiological, human clinical, and animal exposure studies. This review has focused primarily on epidemiological and human exposure studies; those studies published since 1995 that appear most relevant to the current re-evaluation of the California air quality standard for NO<sub>2</sub> are indicated with an asterisk in the first column of Tables 1-3.

Many studies have found an increased incidence of respiratory illness in children associated with indoor NO<sub>2</sub> exposure, and a meta-analysis indicates that a long-term increase in exposure to NO<sub>2</sub> of 15 ppb is associated with an increase in illness odds of approximately 20% in children but not in adults. However, these studies are subject to exposure misclassification, and generally fail to consider a possible role of indoor and outdoor particle exposure as a confounder.

Several recent epidemiological studies examining health outcomes related to outdoor pollutant exposure have found the strongest indicator of health effects to be NO<sub>2</sub>. Because outdoor NO<sub>2</sub> concentrations correlate strongly with fine particles, and because a

substantial body of evidence now exists associating exposure to fine particles with increased morbidity and mortality, NO<sub>2</sub> is presumed to be a marker for traffic-related pollution, rather than a direct cause of the observed effects. Epidemics of respiratory illness described in ice hockey arenas with a poorly functioning Zamboni, which emit NO<sub>2</sub>, would suggest that exposure to NO<sub>2</sub> at levels of 4 to 5 ppm, with exercise, can cause significant acute respiratory illness in some people. However, natural gas combustion also emits ultrafine particles (less than 100 nm in diameter), and it is possible that these particles were present and contributed in causing the observed episodes of respiratory illness. Taken together, the epidemiological evidence would indicate that traffic- and combustion-related pollutant exposure has adverse health effects, and that NO<sub>2</sub> is an important atmospheric marker of exposure. We cannot exclude the possibility that NO<sub>2</sub>, as part of that ambient mixture, plays an important role in causing the observed health effects.

Responses to NO<sub>2</sub> exposure in clinical studies are characterized by marked variability, which directs attention toward identifying determinants of susceptibility, including the pattern of exposure, age of subjects, underlying diseases, antioxidants in the diet, and presence of other pollutants in the atmosphere. Most human clinical studies do not show effects with concentrations at or below the current California standard of 0.25 ppm. Recent studies from the UK and Sweden suggest that exposure to NO<sub>2</sub> at concentrations as low as 0.26 to 0.4 ppm, at rest, enhances responsiveness to allergen challenge in subjects with asthma. Animal models of allergic asthma support the observation, and in vitro studies using human nasal epithelium suggest the mechanism may involve enhanced mast cell degranulation and histamine release.

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## 8. FIGURE LEGENDS

**Figure 1.** Association between ambient NO<sub>2</sub> concentrations in (ppb) and production of phlegm in the Children's Health Study. A concentration-response relationship was seen for children with asthma. Adapted from Figure 2 in McConnell et al., 1999.

**Figure 2.** Relative risk of intrauterine mortality for increasing concentrations of NO<sub>2</sub> (quintiles of the 5-day moving average, in  $\mu\text{m}^3$ ). Adapted from Figure 4 in Pereira et al., 1998.

**Figure 3.** Associations between defibrillator discharges and quintiles of 2-day lagged values of PM<sub>2.5</sub>, black carbon, and NO<sub>2</sub>, adjusted for season, minimum temperature, humidity, trend, and day of the week. Adapted from Figure 2 in Peters et al., 2000.

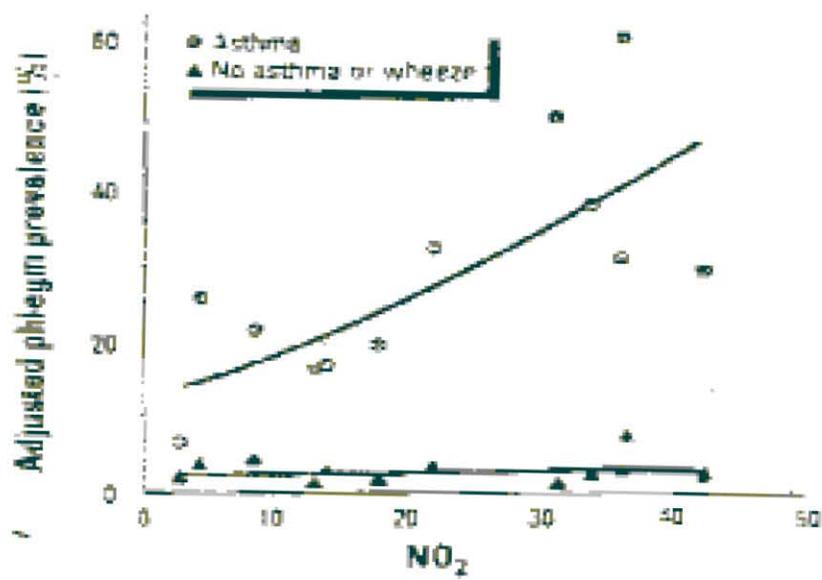


Figure 1

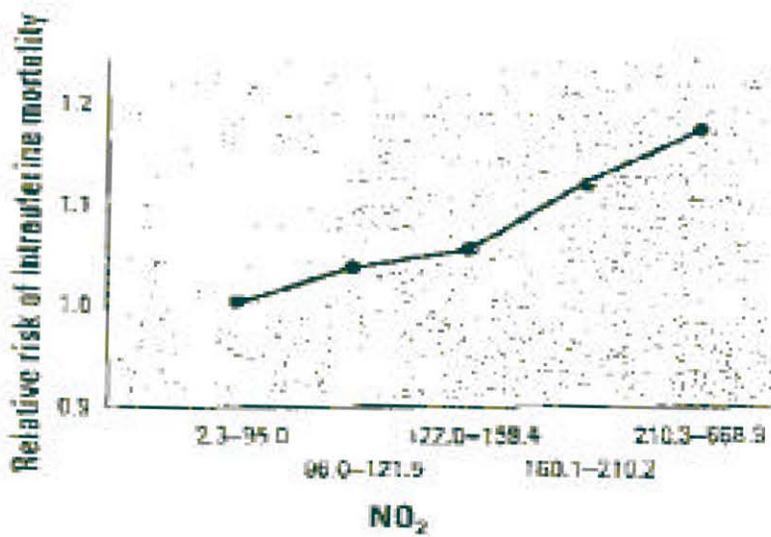


Figure 2

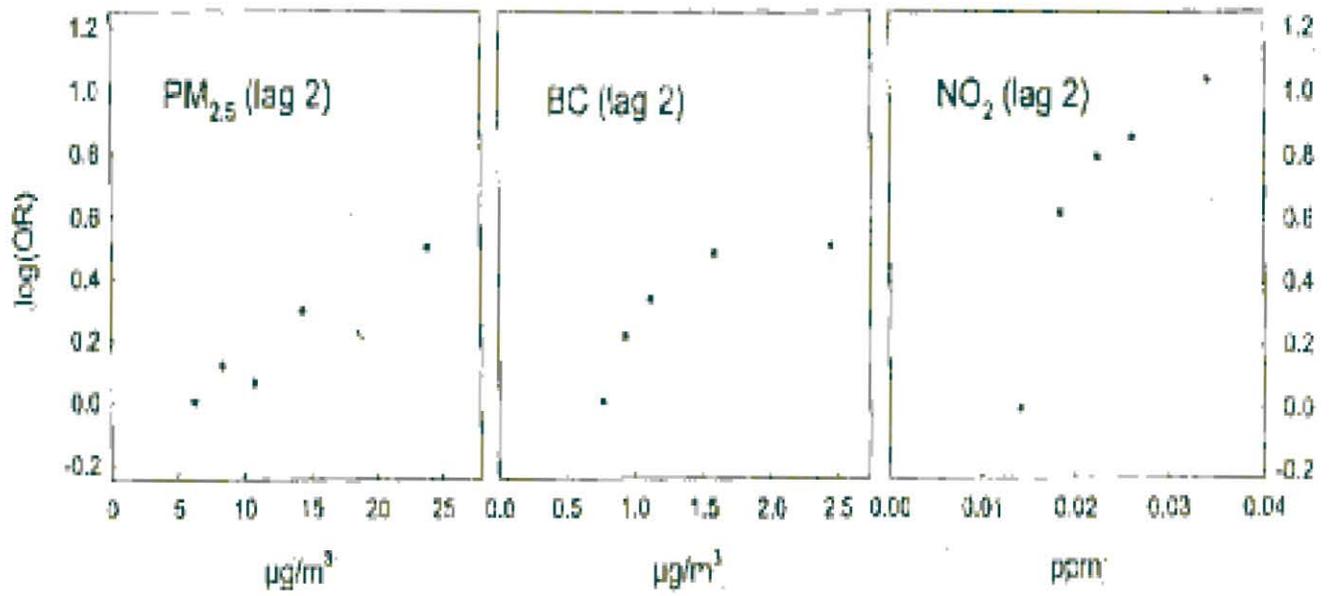


Figure 3

**Table 1.** Epidemiological studies of outdoor NO<sub>2</sub> exposure (since 1995). \* Indicates particular relevance to Standard.

Reference	Location	Participants	Approach & Methods	Exposure Levels (ppb)	Findings
(Boezen et al., 1998)	Netherlands	288 adults age 48-73 yrs, with and without respiratory symptoms.	Time series, respiratory symptoms	24-h means: Urban, 46; Rural, 27	Group with airways hyperresponsiveness or peak flow lability experienced increased symptoms with exposure to NO <sub>2</sub> or PM <sub>10</sub>
(Castellsague et al., 1995)	Barcelona	Asthmatics age >14 visiting emergency departments	Time series	24-h means: Summer, 55; Winter, 53	Relative risk for asthma visits associated with both black smoke and NO <sub>2</sub> .
(Garcia-Aymerich et al., 2000)	Barcelona	Patients with COPD	Time series mortality	Not given	Associations between mortality and NO <sub>2</sub> , but black smoke not significant.
(Garty et al., 1998)	Israel	1076 Children age 1-18 yrs presenting to ED with asthma attack	Time series	Weekly means ~50-250	Emergency department visits correlated with concentrations of NO <sub>2</sub> and SO <sub>2</sub> .
*(Hajat et al., 1999)	London, UK	Patients with respiratory complaints visiting physicians' offices	Time series analysis of Dr visits for asthma and lower respiratory diseases	Annual Mean 33.6, SD 10.5	Significant associations between asthma consultations and NO <sub>2</sub> for children, PM <sub>10</sub> for adults
(Krämer et al., 2000)	Germany	317 children age 9 yrs	Time series, with both outdoor and personal monitoring	Weekly means: Outdoors, 84-116; Personal, 43-50	Atopy related to outdoor NO <sub>2</sub> levels (OR=1.81) but not personal NO <sub>2</sub> exposure. No measurements of other pollutants.
*(Lipsett et al., 1997)	Santa Clara County, California	Asthmatics making visits to ED.	Time series, ED visits for asthma at 3 hospitals. ED visits for gastroenteritis were control population.	Mean 1-h peak, 69, SD 28	Significant PM <sub>10</sub> risk, dependent on temperature. NO <sub>2</sub> also significant but not when PM <sub>10</sub> factored in.
*(McConnell et al., 1999)	Southern California	4 <sup>th</sup> , 7 <sup>th</sup> , and 10 <sup>th</sup> grade children with or without asthma in 12 suburban communities	Cross-sectional	24 h means: 21.9, range 2.7-42.6	"Bronchitis" symptoms in children with asthma associated with NO <sub>2</sub> and PM <sub>10</sub> levels. Effects of PM <sub>10</sub> , NO <sub>2</sub> , and acid inseparable because all were closely correlated.

**Table 1 (continued)**

Reference	Location	Participants	Approach & Methods	Exposure Levels (ppb)	Findings
*(Moolgavkar et al., 1997)	Minneapolis-St. Paul & Birmingham	Elderly adults hospitalized for pneumonia and chronic obstructive pulmonary disease	Time series	24 h means: 16.3; 10-90 <sup>th</sup> percentile, 7.9-25.3	NO <sub>2</sub> associated with hospital admissions in Minneapolis-St. Paul, but ozone gave strongest association, and other pollutants were inseparable.
*(Pereira et al., 1998)	Sao Paulo, Brazil	Unborn children	Time series, intrauterine mortality	24-h means 296, SD 153	NO <sub>2</sub> showed strongest association with fetal mortality
*(Peters et al., 2000)	Boston, MA	Patients with implantable cardiac defibrillators	Time series	24 h means 23; 5-95 <sup>th</sup> percentile, 11-37	Increased risk of defibrillator discharge associated with 1-2 day lagged NO <sub>2</sub> levels
(Pershagen et al., 1995)	Stockholm, Sweden	204 infants age 4-48 mos. hospitalized for "wheezing bronchitis" (cases), 409 controls.	Case-control study; model estimates of outdoor NO <sub>2</sub> concentrations at home address	1-h values, mean ~100, range 38-660	Increased risk of hospitalization related to NO <sub>2</sub> exposure in girls, RR = 2.7 (p=0.02), but not boys. NO <sub>2</sub> levels considered a surrogate for air pollution in general.
(Schindler et al., 1998)	Switzerland (SAPALDIA)	Children	Cross-sectional, pulmonary function	Annual means 17-109. Estimated average personal exposure: 24-93	Negative correlation between NO <sub>2</sub> and both FVC and FEV <sub>1</sub> . NO <sub>2</sub> levels correlated strongly with PM <sub>10</sub> levels (r=0.91),
(Studnicka et al., 1997)	Austria	Children age 7 yrs	Cross-sectional, 8 non-urban communities with varying pollution from traffic.	Overall means: 6-17	Prevalence of asthma significantly associated with long-term NO <sub>2</sub> exposure. No particle measurements
(Tenias et al., 1998)	Valencia, Spain	Asthmatics age >14 identified from emergency department (ED) visits	Ecological time series, ED visits	1 h means 189, 5-95 <sup>th</sup> percentiles 134-288	Relative risk for ED visit significant for NO <sub>2</sub> 24 hour mean, NO <sub>2</sub> 1 hour maximum, and ozone 1 hour maximum. Not significant for SO <sub>2</sub> or black smoke.
(Zemp et al., 1999)	Switzerland	9,651 adults age 18-60 yrs (SAPALDIA study)	Time series, respiratory symptoms	Annual mean 67, range 17-109	Significant associations between symptoms (chronic phlegm, chronic cough, breathlessness at rest, dyspnea on exertion) and both NO <sub>2</sub> and particles. Effects of NO <sub>2</sub> and particles could not be distinguished.

**Table 2.** Epidemiological studies of indoor NO<sub>2</sub> exposure (since 1995). \* Indicates particular relevance to Standard.

Reference	Location	Participants	Approach & Methods	Findings
(Garrett et al., 1998)	Australia	Healthy and asthmatic children 7-14 yr.	Prospective. Intermittent monitoring in homes with and without gas stoves. Respiratory symptoms, peak flow, skin prick testing. Smoking homes included, no measurements of other pollutants.	Relationship between gas stove, but not NO <sub>2</sub> levels, and respiratory symptoms.
* (Jarvis et al., 1996)	East Anglia, UK	Adults age 20-44 yrs	Cross-sectional, questionnaire, lung function and IgE levels on a subset	Gas stove use associated with increased symptoms and decreased lung function in women, but not men
(Magnus et al., 1998)	Oslo, Norway	Oslo birth cohort: Children age <2 yrs who developed 2 episodes of bronchial obstruction or 1 episode lasting >4 months.	Case-control study; personal and home monitoring	No effect of NO <sub>2</sub>
(Moran et al., 1999)	U.K.	National Child Development Study, cohort born in 1958 (age 34-35 yrs at time of study). 1449 examined, 1119 with "chest disease" and 330 controls.	Retrospective cohort study. Gas or electric cooking, health status, lung function, skin tests.	No association between gas cooking in childhood or adulthood and incidence of asthma, respiratory symptoms, or allergic sensitization. Slightly lower FEV <sub>1</sub> associated with gas cooking in men only.
* (Mukala et al., 1999)	Helsinki	Pre-school children in day care, 3-6 yr. No information on baseline health status.	Prospective. Personal monitoring of NO <sub>2</sub> exposure and respiratory symptoms, peak flow in a subset of subjects.	Relationship between NO <sub>2</sub> exposure and cough
* (Pilotto et al., 1997)	Australia	School children 6-11 yr. No information on baseline health status.	Prospective. Fixed monitoring in homes and schools with electric versus gas heating. Respiratory symptoms and school absences. No measurements of other pollutants.	Hourly peak levels 80 ppb associated with increased sore throat, colds, and absences

**Table 3.** Effects of NO<sub>2</sub> exposure on response to inhaled allergen. \* Indicates particular relevance to Standard.

Reference	Location	Participants	Approach & Methods	Findings
*(Devalia et al., 1994; Rusznak et al., 1996)	United Kingdom	Mild asthmatics	6 h exposures to combination of 400 ppb NO <sub>2</sub> and 200 ppb SO <sub>2</sub> .	Increased allergen responsiveness to combination of NO <sub>2</sub> and SO <sub>2</sub> , but not to individual gases. Effect persists 48 h, maximal at 24 h.
*(Jenkins et al., 1999)	United Kingdom	11 patients with mild asthma	1) 6-h exposures to air, 100 ppb ozone, 200 ppb NO <sub>2</sub> , and combination followed by allergen challenge; 2) 3-h exposures to air, 200 ppb ozone, 400 ppb NO <sub>2</sub> , and combination; all with intermittent exercise.	All of the second exposure scenarios (ozone, NO <sub>2</sub> , and combination), but none of the first exposure scenarios, resulted in enhanced responsiveness to allergen. Authors conclude that response may have a concentration threshold.
*(Strand et al., 1997)	Sweden	18 patients with mild asthma, age 18-50 yrs	Exposure to 490 µg/m <sup>3</sup> NO <sub>2</sub> (260 ppb) for 30 min at rest	Late phase, but not early phase, response to allergen enhanced by NO <sub>2</sub> .
*(Strand et al., 1998)	Sweden	16 patients with mild to moderate asthma, age 21-52 yrs	4 daily repeated exposures to 260 ppb NO <sub>2</sub> for 30 min at rest	Significant increases in both early and late phase response to allergen after 4 <sup>th</sup> day of exposure.
*(Tunnicliffe et al., 1994)	United Kingdom	10 nonsmoking mild asthmatics age 16-60 yrs. 8 subjects completed.	Exposure to air, 100 ppb, and 400 ppb NO <sub>2</sub> for 1 hr at rest, separated by at least 1 week, followed by allergen challenge.	Post-challenge reduction in FEV <sub>1</sub> after 400 ppb NO <sub>2</sub> was greater than after air, for both the early (p<0.009) and late (p<0.02) responses. No difference in nonspecific airway responsiveness.
(Wang et al., 1995b; Wang et al., 1995a)	United Kingdom	2 groups of 8 subjects with allergic rhinitis	Exposure to 400 ppb NO <sub>2</sub> (at rest?) for 6 h followed by nasal allergen challenge and nasal lavage	Increase in myeloperoxidase and eosinophil cationic protein in nasal lavage fluid following allergen challenge.