Air Quality Criteria for Oxides of Nitrogen

Volume III of III
DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
The U.S. Environmental Protection Agency (EPA) promulgates the National Ambient Air Quality Standards (NAAQS) on the basis of scientific information contained in criteria documents. In 1971, the first air quality criteria document for nitrogen oxides (NO\textsubscript{x}) was issued by the National Air Pollution Control Administration, a predecessor of EPA. On the basis of scientific information contained in that document, NAAQS were promulgated for nitrogen dioxide (NO\textsubscript{2}) at levels of 0.053 ppm (100 \(\mu\text{g/m}\text{\textsuperscript{3}}\)), averaged over 1 year. The last full-scale NO\textsubscript{x} criteria document revision was completed by EPA in 1982, leading to an Agency decision in 1985 to reaffirm the annual average NO\textsubscript{2} NAAQS of 0.053 ppm. The present, revised criteria document, Air Quality Criteria for Oxides of Nitrogen, assesses the current scientific basis for periodic reevaluation of the NO\textsubscript{2} NAAQS in accordance with the provisions identified in Sections 108 and 109 of the Clean Air Act.

Key chapters in this document evaluate the latest scientific data on (a) health effects of NO\textsubscript{x} measured in laboratory animals and exposed human populations and (b) effects of NO\textsubscript{x} on agricultural crops, forests, and ecosystems, as well as (c) NO\textsubscript{x} effects on visibility and nonbiological materials. Other chapters describe the nature, sources, distribution, measurement, and concentrations of NO\textsubscript{x} in the environment. These chapters were prepared and peer reviewed by experts from various state and Federal government offices, academia, and private industry for use by EPA to support decision making regarding potential risks to public health and the environment. Although the document is not intended to be an exhaustive literature review, it is intended to cover all the pertinent literature through early 1993.

The Environmental Criteria and Assessment Office of EPA’s Office of Health and Environmental Assessment acknowledges with appreciation the contributions provided by the authors and reviewers and the diligence of its staff and contractors in the preparation of this document at the request of EPA’s Office of Air Quality Planning and Standards.
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III-xxx
13. STUDIES OF THE EFFECTS OF NITROGEN COMPOUNDS ON ANIMALS

13.1 INTRODUCTION

This chapter discusses the effects of the oxides of nitrogen (NO\textsubscript{x}) in experimental animals. Previous reviews of the literature have appeared in a criteria document (U.S. Environmental Protection Agency, 1982) A World Health Organization summary has also been published (World Health Organization, 1977)

Most of the data presented in this chapter relate to the effects of nitrogen dioxide (NO\textsubscript{2}) on experimental animals because the vast majority of the NO\textsubscript{x} literature is on NO\textsubscript{2}. The results of the few comparative NO\textsubscript{x} studies show that NO\textsubscript{2} appears to be the most toxic of the NO\textsubscript{x} species, but definitive conclusions on relative quantitative potency must await new information on major NO\textsubscript{x} species. The majority of the literature describes the effects of NO\textsubscript{2} on the respiratory tract, however, extrapulmonary effects also have been observed and are included here. A broad range of NO\textsubscript{2} concentrations have been evaluated, but only studies conducted at less than 18,800 \( \mu \)g/m\textsuperscript{3} (10 ppm) NO\textsubscript{2} are discussed, with emphasis on those studies at exposure concentrations of 9,400 \( \mu \)g/m\textsuperscript{3} (5.0 ppm) or less, with the exception of studies on dosimetry and emphysema.

Discussions of the available literature on the effects of other NO\textsubscript{x} compounds and mixtures containing NO\textsubscript{2} are also included in this chapter. These sections are short because of the general lack of information in these areas.

13.2 NITROGEN DIOXIDE

13.2.1 Respiratory Tract Transport and Absorption

13.2.1.1 Introduction

Dosimetry refers to measurement or estimation of the quantity of a chemical absorbed by target sites such as the pulmonary region tissue or, more locally, the tissue of the centriacinar region. Dosimetry allows exposure-response data into be transformed to dose-response relationships. However, to quantitatively extrapolate animal data to humans,
knowledge of dosimetry and species sensitivity must both be considered. Even when two species receive an identical local tissue/cellular dose, cellular sensitivity and repair/defense mechanisms determine the extent and type of injury produced. These mechanisms are likely to vary among humans and animals because of dissimilarities in pharmacokinetics, genetic makeup, metabolic rates, detoxification systems, and/or other factors. At present, knowledge of dosimetry is more advanced than knowledge of species sensitivity, inhibiting quantitative animal-to-human extrapolation of effective NO₂ concentrations. Nevertheless, knowledge of interspecies differences and similarities in dosimetry alone is crucial to the process of risk assessment, as will be discussed.

The compound most directly responsible for toxic effects may be the inhaled gas, here NO₂, or chemical reaction products or metabolites. Complete identification of the actual toxic agents and their integration into dosimetry is a complex issue that has not been fully resolved. Thus, most dosimetry investigations, which are difficult enough, are concerned with the dose of the primary inhaled chemical. In the context of interspecies dosimetric extrapolation, a further confounding factor can be the units of dose (e.g., mass retained per breath, mass retained per breath per body weight, mass retained per breath per respiratory tract surface area). That is, when comparing dose between species, what is the relevant measure of dose? This question, like the previous issue, has not been answered, units are often dictated by the type of experiment and/or by the choice of the investigators.

Theoretical (modeling) and experimental studies are used to obtain information on dose. Experiments have been conducted to obtain direct measurements of absorbed NO₂ in the total respiratory tract, the upper respiratory tract (region proximal to the tracheal entrance), and in the lower respiratory tract (region distal to tracheal entrance). However, obtaining experimental absorption data for smaller regions or locations, such as specific airways or in the centriacinar region where lesions due to NO₂ occur (see Section 13.2.2.4), is extremely difficult, and may not be possible in the near future because of technical limitations. Nevertheless, experimentation is important for determining dose, assessing hypotheses and concepts, and validating mathematical models that may be of use in predicting dose to specific sites.

Theoretical studies are based on the use of mathematical models developed for the purposes of simulating the uptake and distribution of gases that are absorbed in the tissues.
and fluids of the respiratory tract, usually at the generational level. Because the factors affecting the transport and absorption of gases are general to all mammals, a model that uses appropriate species and/or disease-specific anatomical and ventilatory parameters can be used to describe absorption by different-sized, aged, or diseased members of the species. Models may also be used to identify areas needing additional research, to make inter- and intraspecies dose comparisons, to compare and reconcile data from different experiments, to predict dose in conditions not possible or feasible experimentally, to better understand the processes involved in toxic effects, and to design experiments.

The amount of NO$_2$ acting at a given site is dependent on the nearby airway luminal or airspace concentration and the transport of the gas to the site. In the movement of the gas from the airway lumen or airspace to the cellular components of the respiratory tract, NO$_2$ first comes in contact with the liquid or fluid that lines the cells or tissues of the respiratory tract (mainly consisting of mucus and periciliary fluid in the upper respiratory tract and tracheobronchial region and of surfactant film and serous fluid in the pulmonary region). Nitrogen dioxide reacts chemically with the constituents of fluids and tissues. The reactions with the chemical components of the liquid lining are likely to reduce the quantity of the inhaled gas reaching the tissue. However, reactions in the lining may also produce products that, in turn, may increase toxicity above that produced by the direct action of NO$_2$ alone.

### 13.2.1.2 Principles of Gas Uptake and Dosimetry Models

To further our understanding of the absorption of gases, mathematical models have been developed to simulate the processes involved and to predict absorption by various regions and sites within the respiratory tract.

Animal species-specific information that characterizes respiratory tract morphology and anatomy and transport processes, including chemical reactions, is needed for mathematical modeling. Anatomical information needed includes data about the physical dimensions and geometry of the structural elements of the respiratory tract (e.g., airways, alveoli), the liquid linings, the underlying tissues, and the capillary blood system. In the air phase or air compartment (airway lumens and airspaces), the processes of convection, molecular diffusion, turbulence, dispersion, and the loss or gain of gaseous species to and from the respiratory tract walls must be taken into account. Factors to be considered in lung fluids
and tissues include the biochemical constituents, chemical reactions, solubility, molecular diffusion, convection due to mucociliary action, and capillary blood flow. A detailed discussion of these factors can be found in Overton (1984) and Ultman (1988).

Although variations in physiological processes and structures occur between species, laboratory animals and humans are very similar, enabling dosimetry models to be developed based on the valid concept of a general mammalian respiratory tract structure and physiological processes that apply to both humans and laboratory animals. As a result, only one dosimetry model is needed for the simulation of uptake in several species or subjects. However, species- or subject-dependent structural and physiological data in a form useable by such a model is required to simulate the absorption of NO_2 by a specific animal or human.

There is only one reported theoretical investigation of NO_2 dosimetry, it was discussed originally in Miller et al. (1982) and later in Overton (1984). The dosimetry model used for this investigation was one developed for modeling of ozone (O_3) uptake, however, because NO_2, like O_3, is highly reactive in the respiratory tract tissues and fluids and is not very soluble, this model is considered to be valid for NO_2 (Miller et al., 1982). Because there are very few NO_2 dosimetry modeling results and the principles of O_3 uptake are general and apply to NO_2, the following is a discussion of the general aspects and the factors that have been considered for dosimetry models of O_3.

For dosimetry models, lung dimensions and form usually are accounted for by using airway or anatomical models based on upper respiratory tract, tracheobronchial, and pulmonary region data. The upper respiratory tract can be modeled as a series of segments along the path through the upper respiratory tract airways, segment dimensions (length, diameter, surface areas, etc.) are often based on cast data (e.g., Schreider and Raabe [1981] for the rat and Patra et al. [1986] for a human child). In the lower respiratory tract, the complex and numerous branching airways are represented by a sequence of sets of right circular cylinders (e.g., Weibel [1963] for humans and Yeh et al. [1979] for rats). Each set corresponds to a generation and each cylinder represents an airway or alveolar duct. This type of lower respiratory tract model simplifies the development of dosimetry models in that all paths from the trachea to the last generation airway or duct are assumed identical. Thus,
a description of transport and absorption in one airway in a given generation is the same as any other airway in that generation (Overton, 1984)

Although gas transport in the airways is a three-dimensional problem, the use of the stylized anatomical models have lead to the development of methods that allow the description of transport in terms of a one-dimensional, time-dependent partial differential equation (Overton, 1984) The change with respect to time of the average cross-sectional concentration of the inhaled gas in an airway is related to axial dispersion, convection, and wall loses due to physical absorption and chemical reactions Transfer to the liquid lining is often described in terms of mass transfer coefficients and the liquid-phase and gas-phase interfacial concentrations, related by Henry’s law constant In respiratory tract fluids and tissues, only chemical reactions and transport perpendicular to the interfaces between the air, liquid, and tissue compartments have been taken into account Chemical reactions in the tissue and fluids have been modeled in several ways, depending on assumptions about the type and nature of the reactions that effectively describe the reaction processes Reactions have been modeled as instantaneous or first-order and time-dependent or steady state, depending on the investigators and the state of understanding Dosimetry models also account for ventilation, which plays an important role in delivered dose, some models include the effects of expansion and contraction to account for the fact that dimensions vary during the breathing cycle (Overton et al., 1987)

Postlethwait and Bidani (1990) exposed ventilated, isolated rat lungs to 18,800 to 118,000 μg/m³ (10 to 63 ppm) NO₂ for up to 1 h and found evidence for both linear and nonlinear chemical reaction processes NO₂ uptake (mass of NO₂ retained) was proportional to inhalation rates from 2 to 14 μg NO₂/min, but saturated with greater NO₂ inhalation rates For the lower rates, reactions can be modeled as first order kinetics Evidence for linear reactions was also observed in an in vitro experiment in which Postlethwait et al. (1990) exposed rat bronchoalveolar lavage fluid to NO₂ (≤19,700 μg/m³, 10 5 ppm) How this relates to in vivo conditions is not clear The authors suggested that the renewal of biochemical compounds plays a role in NO₂ uptake and that it is the rate of this renewal that is limiting the uptake A further understanding of the nonlinearity aspects is needed to judge if linearity is a reasonable assumption for environmental conditions or if nonlinearity must be taken into account (e.g., renewal)
In another in vitro experiment by Postlethwait and coworkers (Postlethwait et al., 1991), rat bronchoalveolar lavage fluid was used to explore whether or not unreacted NO$_2$ can penetrate the epithelial liquid lining to underlying sites. The investigators concluded that inhaled NO$_2$ (≤18,800 µg/m$^3$, 10 ppm) does not penetrate the epithelial liquid lining to underlying sites. In addition to implying a very high chemical reactivity in this layer, the investigators suggested that cytotoxicity is the result of chemical reaction products formed in the liquid lining.

In light of the work of Postlethwait and coworkers, it is of interest to note that for the theoretical NO$_2$ dosimetry investigation of Miller et al. (1982), NO$_2$ chemical reactions were modeled as instantaneous and only as occurring in the liquid lining. Further, a renewal process was assumed. On the other hand, the tissue was assumed to be very reactive and NO$_2$ was predicted to reach and react with the tissue throughout most of the lower respiratory tract. Uptake saturation would not have been predicted by the model and, in contrast to Postlethwait and Bidani (1990) and Postlethwait et al. (1990), the model predicted nonlinear uptakes at low NO$_2$ concentrations and linear uptakes at high NO$_2$ concentrations (Miller et al., 1978).

The word "uptake" is often used in conjunction with gas dosimetry. Generally, the meaning of this word depends on context and should be defined to reduce ambiguity, if the meaning is not obvious. Uptake can denote a measure of the quantity of gas absorbed (e.g., 100 g) in a region or at a specific site. Unless otherwise stated, the terms "fractional uptake" and "percent uptake" refer, respectively, to the fraction or the percent of the inhaled NO$_2$ retained by the specified respiratory tract region.

13.2.1.3 Dosimetry of Nitrogen Dioxide

Based on the findings of NO$_2$-induced lesions in the respiratory tract of experimental animals (Section 13.2.2.4), it appears that NO$_2$ is absorbed along the entire respiratory tract.

*Upper Respiratory Tract Absorption*

The upper respiratory tract uptake of NO$_2$ has been experimentally estimated in dogs, rats, and rabbits. Using unidirectional flow, Yokoyama (1968) measured NO$_2$ uptake in the isolated upper airways of dogs and rabbits exposed to 7,520 to 77,100 µg/m$^3$ (4 to 41 ppm).
The upper respiratory tract of the two species was observed to remove 42.1% (standard deviation = 14.9%) of the NO₂ drawn through the noses. The authors did not discuss the relative humidity of the air. If it were not sufficiently high, the continuous airflow would dehydrate the mucous membrane, possibly affecting the uptake properties of the upper respiratory tract. Cavanagh and Morris (1987) exposed the isolated upper respiratory tract of naive and previously exposed rats (76,000 μg/m³, 40 ppm NO₂) under unidirectional flow and found the uptakes to be 28 and 25%, respectively. The relative humidity of the "inhaled" air was maintained so as to be equivalent to 92% at 37°C. The reported uptake difference between the naive and previously exposed rats may not be significant because a multivariable analysis of variance, and not an analysis of variance, test should have been used to analyze the data.

Kleinman and Mautz (1991) exposed six tracheostomized dogs to 1,880 or 9,400 μg/m³ (1.0 or 5.0 ppm) NO₂ and measured uptake during inhalation. Ventilation rates were varied from approximately 2 to 14 L/min by adding carbon dioxide (CO₂) to the inspired air. During mouth breathing, fractional uptake was not dependent on concentration and the mean fractional uptake decreased from 65% to 30% as the ventilation rate increased for 2 to 8 L/min. However, for nasal breathing, exposure to 1,880 μg/m³ NO₂ resulted in significantly greater fractional uptake than did exposure to 9,400 μg/m³. For the lower exposure concentration, the mean nasal fractional uptake decreased from 55% to 40% over the same ventilation range. Although the mean fractional nasal uptake was larger than oral uptake, at ventilation levels corresponding to moderate exercise, there was no statistically significant difference.

**Lower Respiratory Tract Absorption**

Com et al. (1976) estimated the average mass transfer coefficient (K) of NO₂ in the lower respiratory tract of cats. The values of K measured were associated with the region between the trachea and a portion of the lung associated with the "right lateral thoracic region between the fourth and fifth rib." No dependence of K on NO₂ concentration changes or ventilatory rates was observed. According to Postlethwait and Bidani (1990), the estimated value of K is unreasonably large. Also, a comparison of the Com et al. (1976) value of K to theoretical values inferred from Overton et al. (1987) for O₃ indicate that the
experimentally determined $K$ for NO$_2$ is over 240 times as large as those used for modeling ozone uptake. Further work is needed to determine mass transfer coefficients of NO$_2$.

Postlethwait and Mustafa (1981) measured the uptake of NO$_2$ by an isolated ventilated perfused rat lung. The isolated lungs were exposed for 90 min to 9,400 µg/m$^3$ (5.0 ppm) NO$_2$ at a ventilation rate of 45 mL/min. Thirty-six percent of the ventilated NO$_2$ was retained. In a later similar experiment with the exposure concentration ranging from 7,520 to 37,600 µg/m$^3$ (4 to 20 ppm) and the minute volume ranging from 45 to 130 mL/min for different groups of lungs, Postlethwait and Mustafa (1989) found that the quantity of NO$_2$ retained was related linearly to the inhaled quantity of NO$_2$, the percent uptake ranged from 60 to 72% with an average of 65%. These results differ considerably from the first experiment and no reasons for the differences in results were given. Although the tidal volumes in these experiments are realistic, the breathing frequencies are generally much lower than for rats breathing normally. Similar experiments should be performed using more realistic ventilatory parameters.

In addition to measuring upper respiratory tract uptakes in dogs, Kleinman and Mautz (1991) also measured lower respiratory tract uptake. They found that the fractional uptake of NO$_2$ by dog lungs ventilated through a tracheostomy was about 90% of the NO$_2$ inspired by the lungs. This fractional uptake was basically independent of ventilation rates from 3 to 16 L/min, but was somewhat higher at lower ventilation rates. From 1 to 4 L/min, some of their fractional uptakes were greater than 100%, which is not possible. The investigators hypothesized that the greater than 100% values were related to an experimental error in the dead space correction equation used for estimation of fractional uptake. How this type of error affects other measurements is not clear, but the effect of instrument dead space on estimates of fractional uptake should decrease as tidal volume increases.

Results from simulations of NO$_2$ uptake in the lower respiratory tract were described by Miller et al. (1982) and Overton (1984). The model used for this investigation was the same as the dosimetry model described in Miller et al. (1978) for O$_3$, but with the diffusion coefficient and Henry’s law constant appropriate to NO$_2$, however, values of the latter constant and the chemical reactions were considered uncertain. The investigation was mainly a sensitivity study of the effects of Henry’s law constant and reaction rates for which the upper limit of the latter was assumed to be the reaction rate of O$_3$. For humans, the results
indicate that NO₂ is absorbed throughout the lower respiratory tract, but the major dose to
tissue would be delivered in the centracinar region, that is, the junction between the
conducting and respiratory airways. Simulations also predicted that peak tissue levels would
occur in this same anatomical region of the rat, guinea pig, and rabbit. These findings are
consistent with the site of morphological effects (Section 13.2.4). Beyond this region there
is a rapid fall in the NO₂ dose delivered to tissue. Depending on the tracheal concentration
and tidal volume, the model predicted that 75 to 95\% of the NO₂ entering the trachea could
be retained in the lower respiratory tract tissues and fluids. However, these predictions are
dependent on the investigator's choices of values for the uncertain parameters. The results
also predict that exercise will increase the amount of NO₂ delivered to and absorbed in the
pulmonary region over that at rest, and will reduce percent uptake in the tracheobronchial
region (Miller et al., 1982, Overton, 1984).

**Total Respiratory Tract Absorption**

Total respiratory tract uptake has been measured in healthy and diseased humans.
Healthy humans were exposed by Wagner (1970) to a nitric oxide (NO)/NO₂ mixture
containing 550 to 13,500 μg/m³ (0.29 to 7.2 ppm) NO₂ for brief (but unspecified) periods.
Of the inhaled NO₂, 81 to 90\% was absorbed during normal respiration, this increased to
91 to 92\% with maximal ventilation. Bauer et al (1986) exposed adult asthmatics to
560 μg/m³ (0.3 ppm) NO₂ for 30 min. The exposed subjects inhaled NO₂ by mouthpiece for
20 min at rest, then exercised for 10 min on a bicycle ergometer (30 L/min). The inspired
and expired NO₂ concentrations were measured, showing that the average uptake was 72\% at
rest, whereas the average uptake was 87\% during exercise, a statistically significant increase.
The effects of NO₂ exposure on pulmonary function in humans following this exposure
regime are reported in Chapter 15 of this document.

Russell et al. (1991) exposed rats to NO₂ labeled with oxygen-15 ([¹⁵O]-NO₂) and
determined the distribution of this compound in the upper and lower respiratory tract. They
found that the combined nasopharynx and larynx contained 94\% of the radiolabeled NO₂
retained by the respiratory tract. In the lower respiratory tract, the trachea and the five lung
lobes each contained from 0.6 to 1.5\% of the retained [¹⁵O]-NO₂.
Kleinman and Mautz (1991) also measured the fractional uptake of the entire respiratory tract of female beagle dogs exposed to 9,400 μg/m³ (50 ppm) while standing at rest or exercising on a treadmill. The dogs were not tracheostomized, but breathed through a small respiratory mask. During rest at the end of exercise and during continuous rest, the fractional uptake of the entire respiratory tract was measured to be about 78%. The fractional uptake was 94% during the exercise exposures.

**Effect of Exercise on Respiratory Tract Dosimetry**

Experiments indicate that increased ventilation decreases percent uptake in the upper respiratory tract, and increases the percent uptake in the lower and total respiratory tract. In all cases, the effect of increased ventilation is an increase in the quantity of NO₂ absorbed by the individual regions. The reduction of percent uptake in the upper respiratory tract due to increased ventilation results in a greater proportion of inhaled NO₂ being delivered to the lower respiratory tract. Also, the switch from oronasal to oral breathing at high exercise levels is expected to increase the delivery of NO₂ to the lower respiratory tract because the percent of NO₂ removed by the mouth is less than that removed by the nasal cavity.

Aharonson et al. (1974) predicted a negative correlation between percent upper respiratory tract uptake and ventilation using a model. The model analyzed data from experiments on the uptake of vapors by the nose. The model was based on assumptions of quasi-steady-state flow, mass balance, that the flux of a trace gas at the air-mucus interface is proportional to the gas-phase concentration of the trace gas and a local mass transfer coefficient. Miller et al. (1985) and Overton et al. (1987) illustrated the effects of ventilation on the lower respiratory tract uptake of O₃. The theoretical work of Miller et al. (1985) on uptake in humans shows that exercise has a minimal effect in the tracheobronchial region on tissue dose (i.e., quantity of O₃ absorbed by unit area of tissue per unit time) and a pronounced increase in the dose to the pulmonary region tissues. As the exercise level was increased, the maximum tissue dose (at the first respiratory bronchile for the resting state) shifted distally several generations and increased by a factor of 19 (over that of the resting state and at the generation of the shifted maximum) for the highest simulated exercise level. Furthermore, this dose increase was more than twice the ratio of the exercise to rest minute volumes. On the whole, the pulmonary region absorbed 13 times as much at
the highest ventilation rate than at the lowest ventilation rate. In contrast, the tracheobronchial region dose only increased by a factor of 1.4 for the same ventilation rate increase. The simulation results for rats (Overton et al., 1987) are in agreement with these predictions. Because the upper respiratory tract delivers a greater proportion of inhaled NO\textsubscript{2} to the lower respiratory tract during exercise than at rest, the factors quoted above are expected to be conservative. Thus, the modeling results predict that exercise (with respect to the resting state) delivers a disproportionately greater quantity of the inhaled mass to the pulmonary region and an even greater disproportionate quantity to the more distal pulmonary region surfaces. Qualitatively, similar conclusions for NO\textsubscript{2} are reasonable.

**Systemic Dosimetry**

Once deposited, NO\textsubscript{2} dissolves in lung fluids, and various chemical reactions occur, giving rise to products that are found in the blood and other body fluids. Svorcova and Kaut (1971) suggested that inhaled NO\textsubscript{2} entered the bloodstream based upon estimates of radiolabelled nitrate and nitrite levels in the blood and urine of rabbits following exposure to 45,120 \(\mu\text{g/m}^3\) (24 ppm) NO\textsubscript{2} for 4 h. The initial transformation products following NO\textsubscript{2} absorption have been the subject of some speculation, however.

The distribution of NO\textsubscript{2} radiolabeled with nitrogen-13 (560 to 1,710 \(\mu\text{g/m}^3\) [0.3 to 0.91 ppm] inhaled for 7 to 9 min) in rhesus monkeys was investigated by Goldstein et al. (1977b). They concluded that inhaled NO\textsubscript{2} was distributed throughout the lungs, and that it probably reacts with the water molecules in the fluids of the respiratory tract to form nitrous acid (HONO) and nitric acid (HNO\textsubscript{3}), the authors suggested that the acids were responsible for subsequent toxic effects. Based upon the absorption of NO\textsubscript{2} by isolated ventilated perfused rat lungs, Postlethwait and Mustafa (1981) proposed that the main reaction of NO\textsubscript{2} was not with lung water, but with readily oxidizable tissue components (e.g., proteins and lipids) to produce nitrite. These investigators found that 70% of absorbed NO\textsubscript{2} appeared as nitrite in perfusate and lung tissue, and that the concentration of nitrite produced increased with time during exposure. They also hypothesized that nitrite in the blood may then be oxidized to nitrate by interaction with hemoglobin in red blood cells. In a similar experiment, Postlethwait and Mustafa (1989) exposed isolated perfused rat lungs, to various concentrations (7,520 to 37,600 \(\mu\text{g/m}^3\), 4 to 20 ppm) and minute volumes (45 to 130 L/min).
and found that the amount of nitrite detected in the perfusate was proportional (56%) to the amount of NO₂ retained by the lung. Saul and Archer (1983) provided support for this pathway using an in vivo system in which rats were exposed for 24 h to NO₂ concentrations of 2,260 to 16,540 μg/m³ (1.2 to 8.8 ppm). They also concluded that the main reaction pathway of absorption in the lungs was the reaction of NO₂ with oxidizable tissue components to produce nitrite. This product may then serve as a precursor for other chemical reactions at extrarespiratory sites.

The current data base indicates that once NO₂ is absorbed in lung fluids, the subsequent reaction products are rapidly taken up and then translocated via the bloodstream. For example, intratracheally instilled nitrite has been shown to be rapidly absorbed into blood from the lungs (Parks et al., 1981). Oda et al. (1979, 1980b), after exposing rats to 20,900 to 113,000 μg/m³ (11.1 to 59.9 ppm) NO₂ labeled with nitrogen-15 for 0.5 to 53.9 h, found increased levels of labeled nitrogen in the lungs, kidneys, plasma, and urine, as well as an increased level of nitrite in plasma. In a later study, Oda et al. (1981) noted a concentration-dependent increase in both nitrite and nitrate levels in the blood of mice during 1-h exposures to 9,400 to 75,200 μg/m³ (5 to 40 ppm) NO₂. The blood levels of nitrite and nitrate declined rapidly after exposures ended, with the decay half-times of a few minutes for nitrite and about 1 h for nitrate. The shorter time for the former was ascribed to its rapid reaction (oxidation) with hemoglobin, producing nitrate and methemoglobin (Oda et al., 1981; Kosaka et al., 1979, Case et al., 1979), although such measurements were not made.

Free nitrate in blood is generally excreted in urine (Greene and Hiatt, 1954, Hawksworth and Hill, 1971).

**Summary**

The important physical, chemical, and biological factors involved in the uptake of NO₂ by the respiratory tract were reviewed. These factors must be taken into account in order to interpret and understand experimental dosimetry results and to develop models that simulate NO₂ uptake for interspecies extrapolation purposes. With respect to dosimetry, the following has been observed. Total respiratory tract uptake in humans ranged from 72 to 92% depending on the investigation and the breathing state (Wagner, 1970, Bauer et al., 1986). The percent total uptake was found to increase with increasing exercise level.
respiratory tract uptakes have been measured in dogs, rabbits, and rats (Yokoyama, 1968, Cavanagh and Morris, 1987, Kleinman and Mautz, 1991, Russell et al., 1991). Uptake values ranged from as low as 25% to as high as 94% depending on the study, species, airflow rate, and mode of breathing (nasal or oral). Percent upper respiratory tract uptakes were found to decrease with increasing ventilation, uptakes via nasal breathing were determined to be significantly greater than oral breathing uptakes. For the lower respiratory tract, Kleinman and Mautz (1991) found that the fractional uptake of NO$_2$ by dog lungs ventilated through a tracheostomy was about 90% of the inspired dose. Uptake values of 36 and 72% have been reported for isolated, ventilated, perfused rat lungs (Postlethwait and Mustafa, 1981, Postlethwait and Mustafa, 1989). Experimental evidence indicated that NO$_2$ chemically reacted with lung tissue, but did not penetrate directly to blood. However, the reaction products, labeled nitrogen compounds, have been found throughout the body of experimental animals (Oda et al., 1979, 1980b).

There has been very little modeling of the uptake of NO$_2$. The results of the only simulation study predicted that the maximum NO$_2$ tissue dose in humans, rats, guinea pigs, and rabbits occurred in the vicinity of the centriacinar region where morphological lesions are commonly observed (Miller et al., 1982, Overton, 1984). Modeling has been used to estimate the effect of increasing ventilation on the distribution of absorbed O$_3$, which is similar to NO$_2$, in humans (Miller et al., 1985, Overton et al., 1987). These simulations, the qualitative results of which were expected to apply to NO$_2$, predicted that increasing ventilation had little effect on uptake in the tracheobronchial region, but greatly enhanced pulmonary region uptake.

### 13.2.2 Respiratory Effects

#### 13.2.2.1 Host Defense Mechanisms

The respiratory tract defenses encompass many interrelated responses, however, for simplicity, they can be divided into two major components: the physical defense mechanisms and the cellular defense mechanisms. The physical defense mechanisms in the upper and lower airways (mucociliary system) begin with aerodynamics (Newhouse et al., 1976). There is a large amount of turbulence experienced by the incoming airstream, causing the nasopharyngeal removal of many of the large particles ($\geq 10$ $\mu$m). Smaller particles can also
be deposited on the mucociliary escalator of the tracheobronchial region. Particles deposited on the mucus layer are removed by ciliary action that directs overlying mucus, particles, and absorbed gases toward the pharynx where they are swallowed or expectorated (Breeze and Wheeldon, 1977; Green, 1970; Newhouse et al., 1976; Proctor, 1977). This mucociliary clearance is the first line of defense of the conducting airways.

The second component of the pulmonary defense system is the cellular defense mechanisms (phagocytic and immunologic reactions), which operate mostly in the pulmonary region of the lung. Large mononuclear cells, alveolar macrophages (AMs), are the first line of cellular defense (Hocking and Golde, 1979). The role of AMs in host defense is diverse and varied, including such important activities as detoxifying and/or removing inhaled particles, maintaining sterility against inhaled microorganisms, interacting with lymphoid cells in a variety immunologic reactions, and removing damaged or dying cells from the alveoli through phagocytosis (Fels and Cohn, 1986). Alveolar macrophages migrate throughout the pulmonary region and it is this mechanism that allows contact with foreign material entering the lungs. Once phagocytosis has occurred, the particle is encased in a phagocytic vesicle and fuses with lysosomes to form phagolysosomes, the prime subcellular compartment for the killing of engulfed bacteria (Nathan et al., 1980; Silverstein et al., 1977). During the phagocytosis process, AMs release oxygen radicals and enzymes either into the phagosome or into the external milieu (Johnston et al., 1978). Oxygen radicals and enzymes are believed to be essential for bacterial killing by phagocytes. Both oxidation and decarboxylation of bacterial membranes appear to be the major mechanisms by which oxygen radicals induce bacterial killing (Klebanoff, 1968, Strauss et al., 1970).

Polymorphonuclear leukocytes (PMNs), another phagocytic cell, are present in a small percentage in normal lungs. In response to a variety of insults, there can be an influx of PMNs from blood into the lung by chemotaxis. Once recruited to the lung, PMNs then ingest and kill opsonized microbes and other foreign substances by mechanisms that are similar to those described for AMs (Sibille and Reynolds, 1990). In contrast to AMs, PMNs contain substantial amounts of myeloperoxidase stored in the primary granules and, therefore, are an important source of hydroxide radicals, considered to be major bactericidal agents (Klebanoff, 1982).
In most cases, optimal phagocytosis of microorganisms by PMNs and AMs requires the presence of opsonins. Opsonins are immunoglobulins that have the capacity to enhance phagocytosis of microorganisms. When the phagocytic response is not sufficient to effectively remove particles from the respiratory tract, immunologic (humoral and cell-mediated immunity) responses are provided by the lymphocytes. The humoral part of this system primarily involves the B cells that function in the synthesis and secretion of antibodies (immunoglobulin A [IgA], immunoglobulin G [IgG], immunoglobulin M [IgM]) into the blood and body fluids. Secretory IgA is found in the upper respiratory tract and tracheobronchial region and its primary role is to inhibit microbial attachment to the conducting airway surfaces. Immunoglobulin G is the predominant class of immunoglobulins of the lower respiratory tract. These immunoglobulins act to enhance macrophage functioning. Immunoglobulin M is also present in the lower respiratory tract, but in low concentrations. The interactions of these defense mechanisms are complicated and not completely understood.

The cell-mediated component primarily involves T lymphocytes, which are the effectors of cellular defense. These cells are responsible for delayed hypersensitivity and defense against viral, fungal, bacterial, and neoplastic disease. Alveolar macrophages may be responsible for the initiation of humoral and cellular immune responses by the ingestion of particles or soluble antigens and the "processing" and "presenting" of these antigens for specific antigen-reactive B and T cells. This process stimulates the B and T cells to proliferate and differentiate into effectors of humoral (antibody production) and cell-mediated (cytotoxic lymphocytes) immunity. It is these responses that are important in acute and chronic infections and form the basis for antimicrobial immunity (Harada and Repine, 1985).

Alveolar macrophages also secrete alpha, beta, and gamma interferon and platelet activating factor. The interferons, protein substances produced by virus-invaded cells that prevent the replication of the virus (Lefkowitz et al., 1984, Lefkowitz et al., 1983), have potentially important implications in modulating the antiviral and immune activities of AMs. Recently, AMs were found to release a factor first thought to be related to the interferon family and therefore initially named interferon beta 2. It was later determined to be a cytokine (Wong and Clark, 1988). Cytokines are believed to play an immunoregulatory role in the respiratory tract defenses, affecting inflammatory responses and tumoricidal activities.
(Dinarello et al., 1986) Platelet activating factors may play an important role in the inflammatory response and are capable of PMN aggregation and of the release of arachidonic acid metabolites from PMNs (Braquet and Rola-Pleszczynski, 1987).

Although antiviral pulmonary immune functions have not been adequately evaluated after NO₂ exposure, their proper functioning is essential in humoral and cell-mediated immunity against viral disease. These functions include (1) interferon production in bronchoalveolar lavage (BAL) fluid, (2) AM function, (3) natural killer (NK) activity, (4) cytotoxic T lymphocyte activity, and (5) antibody production (Burleson, 1987). A host infected with virus manifests a cascade of immune responses, both specific and nonspecific. Local and circulating interferons (alpha, beta, and gamma) are produced in the first 24 h after viral exposure. Interferons have antiviral, antitumor, and immunoregulatory activities. Interferons serve as an immunoregulator to stimulate the immunological activity of both AMs and lymphocytes exhibiting NK activity. Interferon, AMs, and NK cells all contribute to the nonspecific antiviral immunity of the host. Cytotoxic T lymphocytes are the first specific immunological response against viruses. These cells can be activated by interferons and therefore, are most relevant in defense against viral infections and tumors.

**Mucociliary Clearance**

The effectiveness of the mucociliary system is dependent upon the integrity of the cilia and respiratory epithelia, the chemophysical properties of the mucus, and the rate of mucus transport. Viral and bacterial infections and various chemicals can lead to over or undersecretion of mucus, alterations in mucous flow characteristics, and loss or paralysis of the cilia. The cilia can respond to insults in a number of ways, changes in beat frequency, cessation of ciliary beating, and/or development of abnormal forms of cilia. Substances that produce such disruption or impairment of this defense system can result in an excess accumulation of cellular secretions, increased acute bacterial and viral infections, chronic bronchitis, and prolonged pulmonary complications possibly associated with the pathogenesis of chronic obstructive pulmonary disease or bronchial cancer through the accumulation of inhaled carcinogens (Schlesinger et al., 1987a).

There are numerous reports of significant loss of cilia and ciliated cells in the bronchiolar epithelium. A description of these histopathological changes can be found in
Section 13 2 2 4, addressing the morphological effects of NO\textsubscript{2} exposure. This section and Table 13-1 only summarize NO\textsubscript{2} effects on mucociliary activity.

Clearance of marker substances deposited in the airways has been used to assess the effects of NO\textsubscript{2} on mucociliary clearance. This method has merit as an index of overall efficiency of mucociliary clearance. The mucociliary clearance of inhaled tracer particles deposited into the tracheobronchial tree of rabbits exposed 2 h/day for 2, 7, or 14 days to 0, 560, or 1,880 $\mu$g/m\textsuperscript{3} (0, 0.3, or 1.0 ppm) NO\textsubscript{2} did not alter the rate of removal (Schlesinger et al., 1987a). Using a different approach discussed later in this section, short-term physical clearance of bacteria was not affected at concentrations up to 27,800 $\mu$g/m\textsuperscript{3} (14.8 ppm) (4 h) (Goldstein et al., 1973, Parkei et al., 1989).

Giordano and Morrow (1972) demonstrated significant impairment of tracheobronchial clearance rates in rats following exposure to 11,280 $\mu$g/m\textsuperscript{3} (6.0 ppm) NO\textsubscript{2} for 6 weeks. This decrease in ciliary clearance was not accompanied by any observable abnormality of the airways. A 2-h exposure to 14,100 $\mu$g/m\textsuperscript{3} (7.5 ppm) failed to alter the tracheal mucus velocity in sheep, but exposure to twice that concentration for 2 h produced a significant slowing of mucus movement (Abraham et al., 1980). Nitrogen dioxide-induced effects on airway clearance appear to be both concentration and duration-dependent, however, it would take prolonged exposure to high concentrations to induce alterations that would have detrimental health effects. Data would indicate that even a severely damaged airway epithelium has the ability to maintain mucus transport (Abraham, 1984).

A few in vitro experiments have examined the effect of NO\textsubscript{2} on isolated ciliated epithelium cells and tracheal rings. Kita and Omichi (1974) reported that exposure to NO\textsubscript{2} at concentrations greater than 9,400 $\mu$g/m\textsuperscript{3} (5.0 ppm) resulted in a decreased rate of ciliary beating. Schiff (1977) isolated and exposed hamster tracheal ring cultures to 3,760 $\mu$g/m\textsuperscript{3} (2.0 ppm) NO\textsubscript{2} for 1.5 h/day, 5 days/week for 1 to 4 weeks. After 14 days of exposure, the NO\textsubscript{2} produced decreased ciliary beating.

**Alveolar Macrophages**

The effectiveness of AMs depends on the type, number, and viability of the cells. The cells must maintain an intact membrane, mobility, and phagocytic activity, and have functioning enzyme systems. Evidence from both in vivo and in vitro studies indicate that
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>560 g/m³</td>
<td>0.3</td>
<td>2 h/day, 14 days</td>
<td>M</td>
<td>NS</td>
<td>Rabbit (New Zealand)</td>
<td>No effect on tracheobronchial clearance</td>
<td>Schlesinger et al (1987a)</td>
</tr>
<tr>
<td>1,880 g/m³</td>
<td>1.0</td>
<td>In vitro, 15 h/day, 5 days/week, 1-4 weeks</td>
<td>NS</td>
<td>2 weeks</td>
<td>Hamster (Syrian Golden)</td>
<td>After 2-week exposure, ciliary beating activity was decreased and morphological changes were observed</td>
<td>Schiff (1977)</td>
</tr>
<tr>
<td>3,760 g/m³</td>
<td>2.0</td>
<td>In vitro</td>
<td>NS</td>
<td>2 weeks</td>
<td>Rat (Golden)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400 g/m³</td>
<td>5.0</td>
<td>In vitro</td>
<td>N/A</td>
<td>Decrease in ciliary beating activity</td>
<td>Kita and Omichu (1974)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11,280 g/m³</td>
<td>6.0</td>
<td>7 days/week, 6 weeks</td>
<td>F</td>
<td>NS</td>
<td>Rat (Long Evans)</td>
<td>Decrease in mucociliary velocity, functional impairment reversed by 1 week postexposure</td>
<td>Giordano and Morrow (1972)</td>
</tr>
<tr>
<td>14,100 g/m³</td>
<td>7.5</td>
<td>2 h</td>
<td>NS</td>
<td>NS</td>
<td>Sheep (NS)</td>
<td>Slowing of mucus velocity at highest concentration</td>
<td>Abraham et al (1980)</td>
</tr>
<tr>
<td>28,200 g/m³</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,000 g/m³</td>
<td>10</td>
<td>2 h</td>
<td>M</td>
<td>NS</td>
<td>Rabbit (New Zealand)</td>
<td>No effect on bronchial clearance rate</td>
<td>Schlesinger et al (1987b)</td>
</tr>
</tbody>
</table>

*M = Male
NS = Not Stated
F = Female
NO₂ exposure alters these functions, thereby increasing the risk of the host to disease.

Human clinical studies of AMs are discussed more fully in Section 15.6

**In Vivo Exposure.** Alveolar macrophages isolated from mice that had been continuously exposed to 3,760 µg/m³ (2 0 ppm) NO₂ for 33 weeks or exposed to 940 µg/m³ (0.5 ppm) NO₂ with a 1-h peak to 3,760 µg/m³ (2 0 ppm) for 5 days/week for 21 weeks showed distinctive morphological changes as compared to controls (Aranyi et al., 1976). Examples of structural changes observed included the loss of surface processes, appearance of fenestrae, bleb formation, and denuded surface areas. Exposure to a lower level (i.e., 940 µg/m³ [0.5 ppm] continuous or 188 µg/m³ [0.1 ppm] continuous with a 3-h peak to 1,880 µg/m³ [1.0 ppm]) for periods of up to 24 weeks did not result in any significant identifiable morphological or biochemical changes. Such morphological changes would be expected to interfere with a number of cellular functions, such as chemotaxis and phagocytosis.

Studies have shown that exposure to NO₂ at concentrations greater than 9,400 µg/m³ (5.0 ppm) causes concentration-related increases in the number of AMs (Suzuki et al., 1986, Rombout et al., 1986, Sherwin et al., 1968). A 12-week exposure to 2,256 or 7,520 µg/m³ (1.2 or 4.0 ppm) NO₂ increased the AM population by 30% over that of controls (Mochitake et al., 1992). Alveolar macrophage accumulation has also been reported after 15 weeks of exposure at lower concentrations, such as to either 9,400 µg/m³ (5.0 ppm) NO₂ or to 1,880 µg/m³ (1.0 ppm) with two daily 1-h peaks to 9,400 µg/m³ (Gregory et al., 1983). After a 1-day exposure to 5,000 µg/m³ (2 7 ppm) NO₂, Rombout et al. (1986) reported an increase in the number of AMs in the terminal bronchioles and proximal alveoli of rats. The increase in AMs was associated with morphological changes in the respiratory tract, as discussed in Section 13.2.2.4 addressing NO₂-induced changes in lung morphology. No effect was seen at 1,000 or 2,500 µg/m³ (0.53 or 1.33 ppm).

Chang et al. (1986) and Crapo et al. (1984) studied the response of 1-day-old and 6-week-old rats following exposure to 0 5 ppm (940 µg/m³) NO₂, 23 h/day for 6 weeks. Another group of 6-week-old rats was exposed to 2 0 ppm (3,760 µg/m³) for the same duration. Two daily 1-h peaks of three times the baseline level (i.e., 2,820 and 11,280 µg/m³, 1 5 and 6 0 ppm) were applied Monday-Friday. In these studies, the older
rats were more responsive to NO\textsubscript{2} than younger animals, showing a significant increase in both number and volume of AMs. The studies conducted by Azoulay-Dupuis et al. (1983) also demonstrated that in both the rat and the guinea pig, newborns were less affected by a 3-day exposure to 3,760 and 18,800 \( \mu \)g/m\(^3\) (2.0 ppm and 10 ppm) NO\textsubscript{2} than older animals based on changes in lung structure and superoxide dismutase activity in isolated AMs.

Mochitate et al. (1986) reported a significant increase in the total number of AMs isolated from rats during 10 days of exposure to 7,520 \( \mu \)g/m\(^3\) (4.0 ppm) NO\textsubscript{2}. At this exposure concentration, AMs accounted for 97% of the total lavaged cells. The number of PMNs did not show any significant increase over this exposure period. Alveolar macrophages from exposed animals also exhibited increased metabolic activity as measured by the activities of glucose-6-phosphate dehydrogenase, glutathione peroxidase, and pyruvate kinase, which were significantly increased by 1.29-fold, 1.17-fold, and 1.2-fold, respectively. Simultaneously with these effects, the exposed AMs exhibited a significant increase in the rate of synthesis of protein and DNA. All responses peaked on Day 4 and all values had returned to control levels by the tenth day.

Suzuki et al. (1986) found that NO\textsubscript{2} exposure significantly increased the number of AMs in BAL fluid from rats exposed to either 7,520 or 15,000 \( \mu \)g/m\(^3\) (4.0 or 8.0 ppm) NO\textsubscript{2}. In this study, the exposure continued for 10 days, but the effect became most significant at the fifth day of exposure. The viability of these isolated cells was decreased on Day 1 and remained depressed for the remainder of the exposure period. There was no evidence that either exposure caused an increase of PMNs. However, Schlesinger (1987b) failed to find any significant changes in the number or the viability of AMs in lung lavage from rabbits exposed to 1,880 \( \mu \)g/m\(^3\) (1.0 ppm) NO\textsubscript{2}, 2 h/day for 13 days.

Rose et al. (1989) reported a concentration-dependent decrease in AM phagocytosis in the lower respiratory tract of mice exposed to 1,880 or 9,400 \( \mu \)g/m\(^3\) (1.0 or 5.0 ppm) NO\textsubscript{2} for 6 h on 2 consecutive days, but the decrease was only significant at 9,400 \( \mu \)g/m\(^3\). Mice were intratracheally inoculated with gold-198 colloid following NO\textsubscript{2} exposure. When the exposure was increased to 28,200 \( \mu \)g/m\(^3\) (15 ppm) NO\textsubscript{2}, further effects on AM phagocytosis were not noted. The authors suggested that the lack of additional effects on AM phagocytosis with increasing NO\textsubscript{2} concentrations may be due to an influx of phagocytic cells in the lower respiratory tract due to an inflammatory response to the NO\textsubscript{2}.

Additional
studies at 9,400 μg/m³ did not show an effect on the infectivity of murine cytomegalovirus for AMs.

The phagocytic activity of rat AMs was significantly depressed after 5 days of exposure to 15,000 μg/m³ (8 0 ppm) (Suzuki et al., 1986) A similar suppression was noted following exposure to 7,520 μg/m³ (4 0 ppm), but only after 7 days of exposure. In all cases, the phagocytic activity of these affected cells recovered to the control values at Day 10 of exposure. Suzuki et al. (1986) proposed that the suppression of phagocytosis might be due to the ability of NO₂ to cause membrane lipid peroxidation. The study by Dowell et al. (1971) adds support to this hypothesis. As reported in Section 13.2.4 on lung morphology, evidence of mitochondrial damage was noted in AMs from dogs exposed to 5,640 μg/m³ (3 0 ppm), and became most evident in dogs exposed to 13,160 μg/m³ (7 0 ppm) NO₂. The authors described this effect as a manifestation of damage to the membrane function. The apparent recovery of the AMs after 10 days of exposure is thought to be due to the influx of new AMs into the alveoli. The morphological changes were also associated with changes in lung biochemistry.

Schlesinger (1987b) did not find any significant changes in the number or the viability of AMs in BAL fluid from rabbits exposed to 560 or 1,880 μg/m³ (0 3 or 1 0 ppm) NO₂, 2 h/day for 2, 6, or 13 days. Although there were no effects on the numbers of AMs that phagocytized latex spheres, 2 days of exposure to 560 μg/m³ decreased the phagocytic capacity (i.e., number of spheres phagocytized per cell), the higher level of NO₂ increased this parameter. After 6 or 13 days of exposure, phagocytosis was normal. Lefkowitz et al. (1986) failed to find any depression in phagocytosis after mice were exposed for 7 days to 9,400 μg/m³ (5 0 ppm) NO₂.

Alveolar macrophages isolated from humans exposed for 3 h to 1,130 μg/m³ (0 6 ppm) NO₂ had a tendency (p < 0 07) to be less able to inactivate influenza virus than controls cells (Frampton et al., 1989). However, when the concentration was reduced to 94 μg/m³ (0 05 ppm) NO₂ with three 15 min peaks to 3,760 μg/m³ (2 0 ppm) (same product of concentration and time [C × T] as the 1,120 μg/m³ regimen), the rate of viral inactivation was unchanged. Alveolar macrophages from humans exposed for 4 h to 3,760 μg/m³ (2 0 ppm) exhibited a decrease in phagocytosis of Candida albicans (Devlin et al., 1992). See Section 15.6 for a fuller discussion of these human studies.
Alveolar macrophages obtained by lavage from baboons exposed to 3,760 μg/m³ (2.0 ppm) NO₂ for 8 h/day, 5 days/week for 6 mo had significantly impaired responsiveness to migration inhibitory factor produced by sensitized lymphocytes (Greene and Schneider, 1978). This substance affects the behavior of AMs by inhibiting free migration, which in turn interferes with the functional capacity of these defense cells. The random mobility of AMs was significantly depressed in rabbits following a 2 h/day exposure for 13 days to 560 μg/m³ (0.3 ppm) but not at 1,880 μg/m³ (1.0 ppm) (Schlesinger, 1987b). Such effects are important in the mediation of local immunologic responses in the lung and would be expected to prolong the residence time of the multitude of inhaled deposited particles in the deep lung.

Vollmuth et al. (1986) studied the clearance of strontium-85 tagged polystyrene latex spheres from the lungs of rabbits following a single 2-h exposure to 560, 1,880, 5,600, or 18,800 μg/m³ (0.3, 1.0, 3.0, or 10 ppm) NO₂. An acceleration in clearance (decreased daily retention) was evident immediately after exposure to the two lowest NO₂ concentrations. A similar effect was observed by Schlesinger and Gearhart (1987). At the higher levels of NO₂, an acceleration in clearance was not evident until midway through the 14-day postexposure period. Repeated exposure to 1,880 or 18,800 μg/m³ NO₂, 2 h/day for 14 days produced a response similar to a single exposure at the same concentration, indicating that, with repeat exposures, some attenuation may be produced after the initial exposure (Vollmuth et al., 1986). However, in rats exposed chronically (7 h/day, 5 days/week, 18 to 22 mo) to 17,900 μg/m³ (9.5 ppm) NO₂, there was no effect on long-term clearance of radiolabeled fused aluminosilicate particles (Mauderly et al., 1990).

Several studies have reported that NO₂ exposure significantly decreases the ability of AMs to produce superoxide anion radical, which may limit the antibacterial activity of these cells. Amoruso et al. (1981) presented evidence of such an effect in rats exposed to NO₂ concentrations ranging from 2,444 to 31,960 μg/m³ (1.3 to 17 ppm). The duration of the NO₂ exposure, other than a statement that it was an acute exposure, was not given. In this study, the author's objective was to compare the measured NO₂ response with O₃ effects, and the data were expressed in terms of ppm-h, making it impossible to determine the specific concentration and duration of exposure that elicited the effect. Superoxide anion radical reduction began after exposure to 18.3 ppm-h NO₂. Production was reduced by 50%
after 29 ppm-h, and at 51 ppm-h, the highest exposure tested, the production was decreased by 85%. Devlin et al. (1992) also observed a decrease in the release of superoxide anion from AMs of humans exposed (with exercise) for 4 h to 3,760 μg/m³ (2 ppm). Suzuki et al. (1986) reported a marked decrease in the ability of rat AMs to produce superoxide anion radical following a 10-day exposure to either 7,520 or 15,040 μg/m³ (4 or 8 ppm). At the highest concentration, the depression was significant only on exposure Days 3, 5, and 10.

A number of animal studies have been performed to induce various structural, functional, and biochemical changes in AMs by exposing the test animals to exceedingly high concentrations (i.e., greater than 9,400 μg/m³, 50 ppm NO₂). Some of these studies, along with the studies conducted at lower exposure concentrations, are listed in Table 13-2 to familiarize the reader with the broad spectrum of biological responses that AMs can exhibit following NO₂ exposure.

**In Vitro Exposure.** Voisin and co-workers (Voisin et al., 1977, Voisin and Aerts, 1984) have shown concentration-related effects after exposure for 30 min to NO₂ concentrations as low as 188 μg/m³ (0.1 ppm). In this system, the AMs, attached to cellulose acetate fibers, were floated on top of a nutrient medium that diffused through the filter, maintaining cell viability without submerging the cells. These floating cells were then exposed to NO₂. In these studies, guinea pig AMs seemed to be sensitive to NO₂, exhibiting a reduction in phagocytic and normal bactericidal activity, reduced adenosine triphosphate (ATP) content, and major morphological changes. The severity of these changes was related to the NO₂ concentration (Voisin et al., 1977). Increasing either the exposure concentration to 9,400 μg/m³ (5 ppm) or the exposure duration to 24 h resulted in complete destruction of the cell (Voisin and Aerts, 1984). As described more fully in Section 15.6, normal human AMs exposed in vitro to high levels of NO₂ (9,400, 18,800, or 28,200 μg/m³, 5, 10, or 15 ppm) for 3 h did not exhibit any change in cell viability or the release of either neutrophil chemotactic factor or interleukin-1 (Pinkston et al., 1988).

Robison et al. (1990) exposed AMs from rats to 188, 1,880, 9,400, or 37,600 μg/m³ (0.1, 1, 0, 5, 0, or 20 ppm) NO₂ for 1 h in vitro to determine if NO₂-induced infiltration of PMNs in vivo was in response to the synthesis of leukotriene B₄ (LTB₄) by AMs. Alveolar
### TABLE 13-2. EFFECTS OF NITROGEN DIOXIDE ON ALVEOLAR MACROPHAGES

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>94 base + 3,760 peaks</td>
<td>0 05 base + 3 h base + three 15-min peaks</td>
<td>NS</td>
<td>NS</td>
<td>Human</td>
<td>No effects at 0.05 ppm NO₂ with peaks, trend (p &lt; 0.07) towards AMs losing ability to inactivate influenza virus at 0.6 ppm</td>
<td>Frampton et al (1989)</td>
<td></td>
</tr>
<tr>
<td>1,130</td>
<td>0.6</td>
<td>3 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>188</td>
<td>0.1</td>
<td>1 h</td>
<td>NS</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>At 5.0 ppm increase in LTB₄, concentration-related decrease in SOD production in AMs at ≥1.0 ppm, increase in LDH in AMs at 5.0 and 20 ppm</td>
<td>Robison et al (1990)</td>
</tr>
<tr>
<td>1,880</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37,600</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>Continuous, 24 weeks</td>
<td>NS</td>
<td>NS</td>
<td>Mouse</td>
<td>No effects on AM morphology at 0.5 ppm continuous or 0.1 ppm base + peak exposures</td>
<td>Aranyi et al (1976)</td>
</tr>
<tr>
<td>188 base + 1,880 peak</td>
<td>0.1 base + Continuous base + 3-h peak, 5 days/week, 24 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,760</td>
<td>2.0</td>
<td>Continuous, 33 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>940 base + 3,760 peak</td>
<td>0.5 base + Continuous base + 1-h peak, 5 days/week, 33 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
TABLE 13-2 (cont’d). EFFECTS OF NITROGEN DIOXIDE ON ALVEOLAR MACROPHAGEsa

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>560 μg/m³</td>
<td>0.3</td>
<td>2 h/day, M</td>
<td>NS</td>
<td>Rabbit (New Zealand)</td>
<td>Decreased phagocytic ability of AMs at 0.3 ppm after 2 days of exposure, increased at 1.0 ppm after 2 days of exposure, no effect on cell number or viability, random mobility reduced at 0.3 ppm only, no effects after 6 days of exposure</td>
<td>Schlesinger (1987b)</td>
</tr>
<tr>
<td>1,880 μg/m³</td>
<td>1.0</td>
<td>2, 6, 13 days, 14 days</td>
<td>M</td>
<td>NS</td>
<td>Increase in alveolar clearance</td>
<td>Schlesinger and Gearhart (1987)</td>
</tr>
<tr>
<td>560 μg/m³</td>
<td>0.3</td>
<td>2 h/day up to M</td>
<td>NS</td>
<td>Rabbit (New Zealand)</td>
<td>Concentration-related acceleration in clearance of particles from lung with the greatest increase at two lowest concentrations, effects from repeated exposures similar to those seen after acute exposures to same concentrations</td>
<td>Vollmuth et al. (1986)</td>
</tr>
<tr>
<td>940 base + 0.5 base + 2,820 peak</td>
<td>1.5 peak</td>
<td>Base</td>
<td>M</td>
<td>1 day and 6 weeks</td>
<td>Trend towards increase in number of AMs and cell volume in younger animals, increase in number of AMs and cell volume in older rats</td>
<td>Crapo et al. (1984)</td>
</tr>
<tr>
<td>3,760 base + 2.0 base + 11,300 peak</td>
<td>6.0 peak</td>
<td>22 h/day, 7 days/week, + two 1-h peaks, 5 days/week, 6 weeks</td>
<td>Rat (Fischer 344)</td>
<td>Increase in AMs in highest exposed group, no effects noted in 2 lowest exposure groups</td>
<td>Rambout et al. (1986)</td>
<td></td>
</tr>
<tr>
<td>1,000 μg/m³</td>
<td>0.5</td>
<td>Continuous, M</td>
<td>6 weeks</td>
<td>Rat (Wistar)</td>
<td>Exposure-related decrease in AM phagocytosis from 1.0-5.0 ppm, decrease was not further affected by 15 ppm</td>
<td>Rose et al. (1989)</td>
</tr>
</tbody>
</table>
TABLE 13-2 (cont’d). EFFECTS OF NITROGEN DIOXIDE ON ALVEOLAR MACROPHAGES*

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,880 28,200 45,120</td>
<td>10+</td>
<td>7 h/day, 5 days/week</td>
<td>NS</td>
<td>NS</td>
<td>Rat (Long Evans)</td>
<td>Stimulated clearance of particles from lung at lowest concentration, but decreased clearance rate at two highest concentrations</td>
<td>Fern and Leach (1977)</td>
</tr>
<tr>
<td>1,880 9,400</td>
<td>10</td>
<td>7 h/day, 5 days/week</td>
<td>M/F</td>
<td>14-16 weeks</td>
<td>Rat (Fischer 344)</td>
<td>Accumulation of AMs Superimposed peak exposures produced changes that may persist with continued exposures</td>
<td>Gregory et al (1983)</td>
</tr>
<tr>
<td>1,880 base + 9,400 peaks</td>
<td>10</td>
<td>Base 7 h/day, 5 days/week, two 15-h peaks/day, 15 weeks</td>
<td>M/F</td>
<td>14-16 weeks</td>
<td>Rat (Fischer 344)</td>
<td>Increased clearance rate at two highest concentrations</td>
<td></td>
</tr>
<tr>
<td>2,440-32,000</td>
<td>13-17</td>
<td>NS (&quot;acute&quot;)</td>
<td>F</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>Decreased production of superoxide anion radical</td>
<td>Amoroso et al (1981)</td>
</tr>
<tr>
<td>3,760 19,000</td>
<td>20</td>
<td>3 days</td>
<td>M/F</td>
<td>5, 10, 21</td>
<td>Guinea pig (Dunkin Hartley)</td>
<td>Newborns were less affected than adults when AMs were tested for SOD levels</td>
<td>Azoulay-Dupuis et al (1983)</td>
</tr>
<tr>
<td>3,760</td>
<td>20</td>
<td>8 h/day, 5 days/week, 6 mo</td>
<td>M/F</td>
<td>3-4 years</td>
<td>Baboon</td>
<td>Impaired AM responsiveness to migration inhibitory factor</td>
<td>Greene and Schneider (1978)</td>
</tr>
<tr>
<td>3,760</td>
<td>20</td>
<td>4 h</td>
<td>NS</td>
<td>NS</td>
<td>Human</td>
<td>Decreased phagocytosis and superoxide anion release</td>
<td>Devlin et al (1992)</td>
</tr>
<tr>
<td>5,000</td>
<td>27</td>
<td>24 h</td>
<td>M</td>
<td>6 weeks</td>
<td>Rat (Wistar)</td>
<td>Increase in number of AMs</td>
<td>Rombout et al (1986)</td>
</tr>
<tr>
<td>5,640-30,100</td>
<td>3-16</td>
<td>3 h</td>
<td>NS</td>
<td>NS</td>
<td>Dog (Beagle)</td>
<td>Enhanced swelling of AMs</td>
<td>Dowell et al (1971)</td>
</tr>
</tbody>
</table>
### TABLE 13-2 (cont’d). EFFECTS OF NITROGEN DIOXIDE ON ALVEOLAR MACROPHAGES

<table>
<thead>
<tr>
<th>NO(_2) Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,770 36</td>
<td>1 h</td>
<td>F NS</td>
<td>Rat</td>
<td>Enhanced macrophage agglutination with concanavalin A at both concentrations tested</td>
<td>Goldstein et al (1977a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22,700 121</td>
<td>2 h</td>
<td>(Sprague-Dawley)</td>
<td>(in vitro)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,520 4</td>
<td>6 h/day, 7, 14, or 21 days</td>
<td>M NS</td>
<td>Rat (Wistar)</td>
<td>Changes in morphology at all concentrations, increase in number of AMs at ≥ 10 ppm, phagocytic capacity reduced after 14 and 21 days of exposure to 25 ppm</td>
<td>Hooftman et al (1988)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19,000 10</td>
<td>7, 14, or 21 days</td>
<td>M WIStar</td>
<td>Increase in number of AMs, no increase in PMNs, increased metabolic activity, protein, and DNA synthesis, all responses peaked on Day 4 and returned to normal on Day 10</td>
<td>Mochitate et al (1986)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47,000 25</td>
<td>21 days</td>
<td>AMs at ~10 ppm, phagocytic capacity reduced after 14 and 21 days of exposure to 25 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,520 40</td>
<td>Up to 10 days</td>
<td>NS</td>
<td>Rat (Wistar)</td>
<td>Increase in number of AMs at both concentrations, reaching a peak on Day 3 and 5, no increase in number of PMNs, decrease in AM viability throughout exposure period</td>
<td>Suzuki et al (1986)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15,000 80</td>
<td>10 days</td>
<td>(Fischer 344)</td>
<td>Increase in superoxide radical production, but at 4 ppm, the effect became significant on Days 3, 5, and 10, at 8 ppm, the effect was significant at all time periods tested</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400 50</td>
<td>7 days</td>
<td>F NS</td>
<td>Mouse (CD-1)</td>
<td>No effect on phagocytic activity</td>
<td>Lefkowitz et al (1986)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>µg/m³</td>
<td>ppm</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age (Strain)</td>
<td>Species (Strain)</td>
<td>Effects</td>
</tr>
<tr>
<td>-------------------</td>
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<td>---------</td>
</tr>
<tr>
<td>9,400</td>
<td>5</td>
<td>3 h after infection with paramfluenza 3 virus</td>
<td>NS</td>
<td>NS</td>
<td>Rabbit (New Zealand)</td>
<td>AMs lost resistance to challenge with rabbit pox virus after exposure to 15 ppm</td>
<td>Acton and Myrvik (1972)</td>
</tr>
<tr>
<td>28,200</td>
<td>15</td>
<td>3 h</td>
<td>M</td>
<td>F</td>
<td>Humans (In vitro exposure)</td>
<td>No change in cell viability, release of neutrophil chemotactic factor, or interleukin-1</td>
<td>Pinkston et al (1988)</td>
</tr>
<tr>
<td>9,400</td>
<td>5</td>
<td>24 h</td>
<td>NS</td>
<td>NS</td>
<td>Rabbit (New Zealand)</td>
<td>Inhibition of phagocytic activity</td>
<td>Gardner et al (1969) Acton and Myrvik (1972)</td>
</tr>
<tr>
<td>13,200</td>
<td>70</td>
<td>5 days/week, 18-22 mo</td>
<td>M</td>
<td>18 weeks</td>
<td>Rat (Fischer 344)</td>
<td>No effect on long-term clearance of radiolabeled tracer particles</td>
<td>Mauderly et al (1990)</td>
</tr>
<tr>
<td>19,000</td>
<td>10</td>
<td>47,000</td>
<td>24 h</td>
<td>M</td>
<td>Guinea pig</td>
<td>63% increase in epithelial cells positive for macrophage congregation</td>
<td>Sherwin et al (1968)</td>
</tr>
<tr>
<td>47,000</td>
<td>25</td>
<td>24 h</td>
<td>F</td>
<td>NS</td>
<td>Mouse (Swiss)</td>
<td>Increase in total pulmonary cells in animals infected with some species of bacteria</td>
<td>Jakab (1988)</td>
</tr>
</tbody>
</table>

a NS = Not stated
b AMs = Alveolar macrophages
LTP₄ = Leukotriene B₄
LDH = Lactate dehydrogenase
M = Male
F = Female
SOD = Superoxide dismutase
PMNs = Polymorphonuclear leukocytes

Only one female used in study
macrophage production of LTB4 was not affected by exposure to 188, 1,880, or 37,600 μg/m3, however, levels of LTB4 were elevated following exposure to 9,400 μg/m3 NO2. Products from AMs exposed to 9,400 or 37,600 μg/m3 enhanced PMN chemotaxis. Superoxide production was decreased in a concentration-related manner, starting with AMs exposed to 1,880 μg/m3 NO2. It was suggested that the decrease in superoxide production may be the result of NO2-induced lipid peroxidation processes at the plasma membrane. An NO2-induced decrease in AM viability was also suggested in the 9,400- and 37,600-μg/m3-exposed AMs by changes in cellular lactate dehydrogenase (LDH) content.

**Humoral and Cell-Mediated Immunity**

It is most relevant to assess the effects of inhaled compounds on cell-mediated and antibody responses in the lung itself, because this is the primary site of defense against respiratory infections. However, because of technical obstacles, which in some cases have only recently been overcome, many studies have assessed the responses to inhaled pollutants or NO2 in the spleen or peripheral blood. These studies are more difficult to interpret in terms of enhanced risk of respiratory infection. However, the immune system is one of the many potential targets of inhaled pollutants, such as NO2. In some cases, the immune system can be affected immediately, whereas under different conditions, the changes may be secondary to the injury of other organs or to a general deterioration of the host’s health. In either case, these changes can often seriously compromise the health and well-being of the exposed host. Such changes can result in a suppression in immune function, thereby decreasing defenses against infectious and neoplastic disease. Enhanced immune activity may result in an exaggerated response to an antigenic stimulus causing hypersensitivity. There are many examples of such undesirable immunological changes that have been associated with chemical exposure both in laboratory animals and humans. These changes can, under certain circumstances, trigger a specific response in the immune system, possibly leading to a subsequent pathological state that is more serious than the original lesions.

Because the immune system is a multicomponent, highly regulated system, it offers numerous target sites for NO2 action and there are some studies that indicate that high concentrations of NO2 (> 9,400 μg/m3, 50 ppm) can significantly depress the immune response, as determined by one or more tests available to assess the functional integrity of
the specific components Unfortunately, there are only a very few studies conducted at near ambient concentrations, and, independent of the concentrations tested, only a few of the numerous immune parameters have been evaluated

Exposing sheep to 9,400 \( \mu g/m^3 \) (5 0 ppm) \( \text{NO}_2 \), 1 5 h/day for 10 to 11 days showed that such intermittent, short-term exposure may temporarily alter their pulmonary immune responsiveness (Joel et al , 1982) One technique commonly used in determining the production of antibody forming cells is to measure the number of plaque-forming cells (PFCs) in the spleen or blood of immunized animals In this study, the authors assessed immunological response by monitoring the daily output of PFCs in the efferent lymph of caudal mediastinal lymph nodes Although the number of sheep used was small and the data were not analyzed statistically, it would appear that in the animals that were immunized with horse red blood cells (a T-cell dependent antigen) 2 days, but not 4 days, after initiation of \( \text{NO}_2 \) exposure, the output of PFCs was below controls Blastogenic responses of T cells from the efferent pulmonary lymph and blood also appeared depressed

Hillam et al (1983) examined the effects of a 24-h exposure to 9,400, 18,800, and 48,900 \( \mu g/m^3 \) (5, 10, and 26 ppm) \( \text{NO}_2 \) on cellular immunity in rats after intratracheal immunization of the lung with sheep red blood cells (SRBCs) Cellular immunity was evaluated by antigen-specific lymphocyte stimulation assays of pooled lymphoid cell suspensions from either the thoracic lymph nodes or the spleen A concentration-response effect with elevated cellular immunity was observed

Studies conducted by Fenters et al (1971, 1973) and Ehrlich and Fenters (1973) using squirrel monkeys showed the impact of \( \text{NO}_2 \) on the humoral immune response to intratracheally delivered influenza vaccine In monkeys exposed for 493 days (16 mo) to 1,880 \( \mu g/m^3 \) (1 0 ppm) \( \text{NO}_2 \) and immunized with monkey-adapted virus (A/PR/8/34), the serum neutralizing antibody titers were significantly increased earlier, and to a greater degree, than in controls (Fenters et al , 1973, Ehrlich and Fenters, 1973) In monkeys exposed to 9,400 \( \mu g/m^3 \) (5 0 ppm) \( \text{NO}_2 \) for a total of 169 days and immunized with mouse-adapted influenza virus (A/PR/8), serum neutralization titers were initially lower than controls; no significant difference was observed by 133 days of exposure (Fenters et al , 1971; Ehrlich and Fenters, 1973) In all of these studies, the hemagglutination inhibition antibody titers were not affected The authors discussed these differences, suggesting that
the difference in the virus used for immunization played a role, along with exposure differences. The authors also hypothesized that exposure to 1,880 \( \mu g/m^3 \) NO\(_2\) improved the establishment and survival of the monkey-adapted virus within the respiratory tract, resulting in an increase in antibody production.

The results of Holt et al. (1979) suggest that both the nature of and rate of change in immunological function from NO\(_2\) exposure can be both concentration- and time-dependent. Tests were conducted at 7-week intervals to assess the functioning ability of the immune system of mice exposed to 18,800 \( \mu g/m^3 \) (10 ppm) NO\(_2\), 2 h/day for periods of up to 30 weeks (Holt et al., 1979). Chronic exposure exhibited a general suppression in antibody titers and ability of the T cells to function in a graft versus host reaction. However, the more acute exposures resulted in an enhancement of immunological responsiveness.

A series of immunological studies designed to examine the effects of NO\(_2\) on the humoral antibody response to SRBCs was reported by Fujimaki and Shimizu (1981) and Fujimaki (1989). They exposed mice for 12 h to 9,400, 37,600, and 75,200 \( \mu g/m^3 \) (5, 20, and 40 ppm) NO\(_2\) and reported a significant suppression in PFCs (primary antibody response) in response to SRBCs at the two highest levels of exposure. When mice were exposed to 7,520 \( \mu g/m^3 \) (4.0 ppm) NO\(_2\) for 3, 7, 14, or 56 days, no suppression in antibody response was observed.

Using this same model, Fujimaki et al. (1982) reported a similar effect in mice (i.e., suppression of primary antibody PFC response in the spleen) after 4 weeks of continuous exposure to 752 and 3,000 \( \mu g/m^3 \) (0.4 and 1.6 ppm) NO\(_2\). At the higher concentration, there were no significant differences observed in the activities of the T and B lymphocytes. Secondary antibody response was not affected at 752 \( \mu g/m^3 \), but was slightly enhanced at 3,000 \( \mu g/m^3 \) exposure level. However, Mairget et al. (1978) found that the normal transformation response of mouse splenic T and B cells to phytohemagglutinin (PHA) and bacterial lipopolysaccharide (LPS), respectively, was suppressed following NO\(_2\) exposure. Mice were exposed to 940 \( \mu g/m^3 \) (0.5 ppm) NO\(_2\), 24 h/day, 7 days/week for up to 1 year or to a baseline concentration of NO\(_2\) (188 \( \mu g/m^3 \), 0.1 ppm) for 24 h/day, 7 days/week with 3-h peaks 5 days/week to either 470, 940, or 1,880 \( \mu g/m^3 \) (0.25, 0.5, or 1.0 ppm) NO\(_2\). The decrease in mitogenic responses of splenic lymphocytes to PHA and LPS was not concentration- or duration-dependent in the base + peak exposure groups. The decrease in
T-cell mitogenesis was linearly related to the increased duration of continuous exposure to 940 \( \mu g/m^3 \) NO\(_2\).

Lefkowitz et al (1986) employed several methods to measure immunooactivity of mice exposed to 9,400 \( \mu g/m^3 \) (5 0 ppm) NO\(_2\), 24 h/day for 6 days and injected with SRBCs after the first day of exposure. Nitrogen dioxide did not affect hemagglutination antibody titers or cell-mediated immunity (blastogenesis of splenic T cells), but did significantly reduce the number of splenic PFCs to SRBCs. The authors stated (data were not shown) that mice exposed to 2,820 \( \mu g/m^3 \) (1.5 ppm) NO\(_2\) for 14 or 21 days also showed a 33 and 50\% decrease, respectively, in the number of PFCs.

Kosmider et al (1973b) exposed guinea pigs to 1,880 \( \mu g/m^3 \) (1.0 ppm) NO\(_2\) for 6 mo and reported a significant reduction in all serum immunoglobulin fractions and complement. Decreased levels of these substances may lead to an increase in the frequency, duration, and severity of an infectious disease. Mice exposed to a baseline of 940 \( \mu g/m^3 \) (0.5 ppm) plus peak of 3,760 \( \mu g/m^3 \) (2.0 ppm) NO\(_2\) for 3 mo had decreased serum levels of IgA and exhibited nonspecific increases in serum IgM, IgG, and IgG\(_2\) (Ehrlich et al., 1975).

Effects on lymphocyte populations were tested in mice by Richters and Damji (1988). The percentage of the total T-lymphocyte population was lower in the spleens of mice exposed for 7 weeks (7 h/day, 5 days/week) to 470 \( \mu g/m^3 \) (0.25 ppm) NO\(_2\). The percentages of mature helper/inducer T lymphocytes and T-cytotoxic/suppressor lymphocytes were also lower in the spleen of exposed animals. There were no statistically significant changes in the percentages of NK cells or mature T cells. Mice exposed to 670 \( \mu g/m^3 \) (0.35 ppm) for 7 h/day, 5 days/week for 12 weeks also showed a suppression in the percentage of total matured T lymphocytes, but no statistically significant effect on any specific subpopulation. However, when the exposure was increased to 7,520 \( \mu g/m^3 \) (4.0 ppm) for 8 h, the percentage of total T lymphocytes, T-helper/inducer lymphocytes, and T-cytotoxic/suppressor lymphocytes was significantly lower in mice (Damji and Richters, 1989). The most susceptible subpopulation was the large T-cytotoxic/suppressor lymphocytes.

Richters and Damji (1990) reported similar findings in mice exposed to 470 \( \mu g/m^3 \) (0.25 ppm) NO\(_2\), 7 h/day, 5 days/week when the exposure time was increased to up to 181 days. The splenic T-helper/inducer (CD\(_4^+\)) lymphocytes were reduced, no effects were

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observed on T-cytotoxic/suppressor cells. Spontaneously developing lymphomas in NO₂-exposed animals had progressed more slowly than those in control animals. This was attributed to the NO₂-induced reduction in the T-helper/inducer lymphocytes.

Conversely, Selgrade et al. (1991) reported that exposure to an NO₂ peak slowly rising to 2,820 μg/m³ (1.5 ppm) for 6 h/day, 5 days/week, superimposed on a baseline concentration of 940 μg/m³ (0.5 ppm) for 22 h/day, 7 days/week for up to 78 weeks did not affect the splenic or circulating T-cell response to mitogens in rats. There were also no NO₂-related effects on the B-cell population. There was a transient decrease in splenic NK cell activity. This effect was, however, only noted after 3 weeks of exposure.

Mice that were vaccinated with influenza virus (A-2/Taiwan/1/64) after 3 mo of continuous exposure to 3,760 μg/m³ (2.0 ppm) or to 940 μg/m³ (0.5 ppm) NO₂ with a 1-h daily, 5 day/week peak exposure to 3,760 μg/m³ (2.0 ppm) had mean serum neutralizing antibody titers that were fourfold lower than clean air controls (Ehrlich et al., 1975). The hemagglutination inhibition titers in these animals were unchanged. This agrees with the Fenters et al. (1971) findings in squirrel monkeys exposed to 9,400 μg/m³ (5.0 ppm) for 1 year and inoculated with A/PR/8/34 influenza virus.

Immune response to murine cytomegalovirus was not substantially affected by NO₂, even though NO₂ increased susceptibility to infection (Rose et al., 1989). Mice were exposed to 9,400 μg/m³ (5.0 ppm) NO₂ for 6 h/day on 2 days prior to viral intratracheal inoculation and 4 days after inoculation. Splenic lymphocyte response to PHA or circulating specific antibody titers were not changed. However, lymphocyte secondary proliferative response to the viral antigen was decreased.

Few studies have been undertaken to assess the effects of NO₂ on interferon production. Mice exposed to either 9,400 or 47,000 μg/m³ (5 or 25 ppm) NO₂ for 3 to 7 days had serum levels of interferon similar to controls (Lefkowitz et al., 1984, Lefkowitz et al., 1983).

An increase in certain immunological functions may also be detrimental to the host’s health by stimulating the immune system to react against the host’s own tissue. Balchum et al. (1965) identified such an effect when guinea pigs were exposed to 9,400 μg/m³ (5.0 ppm) or 28,200 μg/m³ (15 ppm) NO₂. There was a noticeable increase in the titer of serum antibodies against lung tissue in all test animals exposed, starting after 160 h of
exposure These antibody titers continued to increase with the increases in NO₂ concentration and exposure duration.

The effects of NO₂ exposure on the immune system appear to be concentration- and time-dependent. Some studies suggest little effect, whereas others suggest suppression or activation, depending not only on concentration, but also on length of exposure, species tested, and specific end points measured. Table 13-3 summarizes the reported immunological effects following exposure to NO₂.

*Interaction with Infectious Agents*

Different experimental approaches using laboratory animals have been employed in an effort to determine the functional efficiency of the host’s pulmonary defenses following NO₂ exposure. In the most commonly used infectivity model, animals are randomly selected for exposure to either a pollutant, in this case NO₂, or filtered air. After exposure, the treatment groups are combined and exposed for approximately 15 min to an aerosol of a viable agent, such as *Streptococcus* sp, *Klebsiella pneumoniae*, *Diplococcus pneumoniae*, influenza A2/Taiwan virus, or A/PR/8 influenza virus. The animals are then returned to clean air for a holding period (usually 15 days) and the mortality rates in the NO₂-exposed and the air-exposed groups are compared. If the normal pulmonary defenses are functioning properly, the deposited viable microorganisms will be quickly killed and the lungs will remain sterile, and only a small percentage (typically between 5 to 15%) of the control animals will succumb to the laboratory infection. However, if host defenses are compromised by the chemical exposure, mortality rates will be higher (Ehrlich, 1963, 1966, 1980; Gardner et al., 1982, Coffin and Gardner, 1972, Henry et al., 1970).

A wide variety of mammalian species, including humans, share an array of defensive mechanisms that are anatomically and physiologically integrated in the respiratory tract to prevent and control most invading infectious organisms. The infectivity model is an excellent indicator of a weakened host defense system. The effects seen in laboratory animals represent alterations in host defenses. Studies have shown that these responses are valid across species, sensitive to a variety of chemicals, supported by mechanistic studies, and capable of epidemiological confirmation. Similar alterations in these basic defense
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>470 μg/m³</td>
<td>7 h/day, 5 days/week, 7 weeks</td>
<td>F</td>
<td>6 weeks</td>
<td>Mouse (AKR/cum)</td>
<td>Reduced percentage of total T-cell population and trend towards reduced percentage of certain T-cell subpopulations and NK cells</td>
<td>Richters and Damji (1988)</td>
</tr>
<tr>
<td>470 μg/m³</td>
<td>7 h/day, 5 days/week, 181 days</td>
<td>F</td>
<td>5 weeks</td>
<td>Mouse (AKR/cum)</td>
<td>Reduced percentage of total T-cell population and percentages of T-helper/inducer cells on Days 37 and 181</td>
<td>Richters and Damji (1990)</td>
</tr>
<tr>
<td>670 μg/m³</td>
<td>7 h/day, 5 days/week, 12 weeks</td>
<td>M</td>
<td>6 weeks</td>
<td>Mouse (C57BL/6J)</td>
<td>Trend towards suppression in total percentage of T cells Percentages of other T-cell subpopulations lower, but not significantly</td>
<td>Richters and Damji (1988)</td>
</tr>
<tr>
<td>752 μg/m³</td>
<td>Continuous, 4 weeks</td>
<td>M</td>
<td>7 weeks</td>
<td>Mouse (BALB/c)</td>
<td>Decreased primary splenic PFC response at both concentrations, increased secondary antibody response at 1.6 ppm</td>
<td>Fujimaki et al (1982)</td>
</tr>
<tr>
<td>3,000 μg/m³</td>
<td>Continuous, up to 1 year</td>
<td>M</td>
<td>10-11 weeks</td>
<td>Mouse (CD-1)</td>
<td>Linear decrease in PHA-induced mitogenesis with NO₂ duration</td>
<td>Maigetter et al (1978)</td>
</tr>
<tr>
<td>188 base +, 470, 940, or 1,880 peak</td>
<td>24 h/day, 7 days/week base +, 3 h/day, 5 days/week peak for up to 1 year</td>
<td>M</td>
<td>10 weeks</td>
<td>Rat (Fischer 344)</td>
<td>No effect on splenic or circulating B- and T-cell response to mitogens, decrease in splene NK cell activity only after 3 weeks exposure No histological changes in lymphoid tissue</td>
<td>Selgrade et al (1991)</td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age (Strain)</td>
<td>Effects</td>
<td>Reference</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>940 base + 3,760 peak</td>
<td>24 h/day, 5 days/week base + 1 h daily peaks for 3 mo</td>
<td>M</td>
<td>6 weeks (CD-1)</td>
<td>Vaccination with influenza A2/Taiwan virus followed exposure Decrease in serum neutralizing antibody, hemagglutination inhibition titer unchanged, before virus challenge, NO₂ exposure decreased serum IgA and increased IgG₁, IgM, and IgG₂, after virus, serum IgA unchanged and IgM increased</td>
<td>Ehrlich et al (1975)</td>
<td></td>
</tr>
<tr>
<td>1,880</td>
<td>Continuous, 16 mo</td>
<td>M</td>
<td>NS</td>
<td>Monkey (Squirrel)</td>
<td>Monkeys challenged five times with monkey-adapted influenza virus during NO₂ exposure Hemagglutination inhibition antibody titer not altered Compared to controls, NO₂ caused an earlier and greater increase in serum neutralization antibody titers to the virus</td>
<td>Fenters et al (1973)</td>
</tr>
<tr>
<td>1,880</td>
<td>6 mo</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Intranasal challenge with K. pneumoniae after exposure Decreased hemolytic activity of complement, decrease in all immunoelectrophoretic fractions</td>
<td>Kosmider et al (1973b)</td>
</tr>
<tr>
<td>2,820</td>
<td>24 h/day, 7, 14 or 21 days</td>
<td>F</td>
<td>NS</td>
<td>Mouse (CD-1 and C57BL)</td>
<td>Splenic PFCs reduced on Day 14 and 21 by 33 and 50%, respectively, no effect on cell-mediated immune system or hemagglutinating titers</td>
<td>Lefkowitz et al (1986)</td>
</tr>
<tr>
<td>7,520</td>
<td>Continuous, up to 56 days</td>
<td>M</td>
<td>8-10 weeks (BALB/c)</td>
<td>No suppression of antibody response to SRBCs</td>
<td>Fujimaki (1989)</td>
<td></td>
</tr>
<tr>
<td>7,520</td>
<td>8 h</td>
<td>F</td>
<td>6 weeks (C57BL/6c)</td>
<td>Alteration in T lymphocyte subpopulation</td>
<td>Damj and Richters (1989)</td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>1.5 h/day, 10-11 days</td>
<td>M/F</td>
<td>NS</td>
<td>Sheep (Dorset)</td>
<td>Reduction in PFCs in lymph, depressed blastogenic response of T cell from lymph and blood</td>
<td>Joel et al (1982)</td>
</tr>
<tr>
<td>9,400</td>
<td>4 or 7 h/day, 5 days/week, 5 5 mo</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (New England)</td>
<td>Serum antibodies against lung tissue increased with concentration and duration of exposure</td>
<td>Balchum et al (1965)</td>
</tr>
<tr>
<td>9,400</td>
<td>Continuous, 169 days</td>
<td>M</td>
<td>NS</td>
<td>Monkey (Squirrel)</td>
<td>Monkeys challenged 4× with mouse-adapted influenza virus Initial depression in serum neutralization titers with return to normal by Day 133, no effect on hemagglutin inhibition titer</td>
<td>Fenters et al (1971)</td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age (Strain)</td>
<td>Effects</td>
<td>Reference</td>
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<td>-------------------</td>
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</tr>
<tr>
<td>9,400 47,000</td>
<td>5 0</td>
<td>7 days</td>
<td>F</td>
<td>8-12 Mouse (C57BL/6)</td>
<td>No effect on serum interferon levels</td>
<td>Lefkowitz et al (1983, 1984)</td>
</tr>
<tr>
<td></td>
<td>25 0</td>
<td>3 days</td>
<td>F</td>
<td>NS Mouse (CD-1, C57BL)</td>
<td>No effect on serum antibody titers in mice exposed for 6 days (only exposure tested), decrease in splenic PFCs was noted in some groups exposed for 7 or 15 days</td>
<td>Lefkowitz et al (1986)</td>
</tr>
<tr>
<td>9,400 18,800 48,900</td>
<td>5 0</td>
<td>24 h/day, 6, 7, or 15 days</td>
<td>F</td>
<td>NS Mouse (Fischer 344)</td>
<td>Concentration-related elevation of cellular immunity in thoracic lymph nodes and spleen after immunizing the lung with SRBCs</td>
<td>Hillam et al (1983)</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>24 h</td>
<td>M</td>
<td>24-28 weeks Rat (Fischer 344)</td>
<td>Decrease in secondary proliferative response of splenic lymphocytes to viral antigen</td>
<td>Ehrlich and Fenters (1973)</td>
</tr>
<tr>
<td>9,400</td>
<td>5 0</td>
<td>Continuous, 6 mo</td>
<td>NS</td>
<td>NS Monkey (Squirrel)</td>
<td>Depressed postvaccination serum neutralizing antibody formation</td>
<td>Rose et al (1989)</td>
</tr>
<tr>
<td></td>
<td>6 0</td>
<td>6 h/day, 6 days</td>
<td>NS</td>
<td>4-6 weeks Mouse (CD-1)</td>
<td>Mice infected with murine cytomegalovirus after second day of exposure. No effect on circulating specific antibody titer to virus or splenic lymphocyte response to PHA. Decrease in secondary proliferative response of splenic lymphocytes to viral antigen</td>
<td>Fujimaki and Shimizu (1981)</td>
</tr>
<tr>
<td>9,400 37,600 75,200</td>
<td>5 0</td>
<td>12 h</td>
<td>M/F</td>
<td>6-9 weeks Mouse (BALB/c)</td>
<td>No effect on primary and secondary splenic PFC response at 5 ppm, at higher levels, suppression in primary antibody response</td>
<td>Fujimaki et al (1981)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Continuous, 19, 26, or 33 days</td>
<td>NS</td>
<td>Guinea pig (BFA-ZH-Kisselg)</td>
<td>No effect on antibody production</td>
<td>Hidekazu and Fuji (1980)</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10,000</td>
<td>5 3</td>
<td>Continuous, 19, 26, or 33 days</td>
<td>F</td>
<td>NS Guinea pig (BFA-ZH-Kisselg)</td>
<td>No effect on antibody production</td>
<td>Antweiller et al (1975)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>2 h/day, 30 weeks</td>
<td>F</td>
<td>6-8 weeks Mouse (BALB/c)</td>
<td>Marked depression in ability of T cells to respond to nonspecific stimuli, reduced ability to reject tumors</td>
<td>Holt et al (1979)</td>
</tr>
</tbody>
</table>

*F = Female  
*NK = Natural killer cells  
*M = Male  
*IgM = Immunoglobulin M  
*NS = Not stated  
*PFCs = Plaque forming cells  
*IgA = Immunoglobulin A  
*IgG = Immunoglobulin G  
*SRBCs = Sheep red blood cells
mechanisms that occur in animals could also occur in humans because they have equivalent pulmonary defenses. In the animal model frequently used, mortality is the sensitive response indicator for alterations in host defense functioning. However, with today's medical care, few people die of bacterial pneumonia, so a better comparison in humans would be the prevalence of respiratory illness in the community, as discussed in Chapter 14 of this document (epidemiological studies). Such a comparison is proper because both mortality (animals) and morbidity (humans) result from a loss of pulmonary defenses. However, different exposure levels, patterns, and durations may be required to produce alterations to human host defenses. Table 13-4 summarizes the effects of exposure to NO₂ and infectious agents.

An enhancement in mortality following exposure to NO₂ and a pathogenic microorganism could be due to several factors. Studies by Goldstein et al. (1973) showed decreases in pulmonary bactericidal activity following NO₂ exposure. In their first experiments, mice breathed aerosols of *Staphylococcus aureus* (*S. aureus*) labeled with radioactive phosphorus and then were exposed to NO₂ for 4 h. Physical removal of the bacteria was not affected by any of the NO₂ concentrations used up to 27,800 μg/m³ (14.8 ppm). Concentrations of 13,200, 17,300, and 27,800 μg/m³ (7.0, 9.2, and 14.8 ppm) NO₂ lowered bactericidal activity by 7, 14, and 50%, respectively, when compared to controls. Lower NO₂ concentrations (3,570 and 7,140 μg/m³, 1.9 and 3.8 ppm) had no significant effect. The cause of the alteration was an impairment of the AM's bactericidal activity and not an impairment of the host's mechanical clearance system. In another experiment (Goldstein et al., 1973), mice breathed 1,880, 4,320, and 12,400 (1.0, 2.3, and 6.6 ppm) NO₂ for 17 h and then were exposed to an aerosol of radiolabeled *S. aureus*. Four hours later, the animals were examined. No difference in the number of bacteria inhaled was found in the NO₂-exposed animals. Concentrations of 4,320 and 12,400 μg/m³ NO₂ decreased pulmonary bactericidal activity by 6 and 35%, respectively, compared to controls. Exposure to 1,880 μg/m³ NO₂ had no significant effect. Goldstein et al (1973) hypothesized that the decreased bactericidal activity was due to defects in AM function. Jakab (1987, 1988) confirmed these findings and reported that the concentration of NO₂ required to suppress normal pulmonary bactericidal activity in mice depends on the specific
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Infective Agent</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 base + 0 1</td>
<td>Continuous, base + twice/day 1-h peaks, 5 days/week for 15 days</td>
<td>F NS</td>
<td>Mouse (CD-1)</td>
<td>Streptococcus</td>
<td>No effect</td>
<td>sp</td>
<td>Gardner (1980)</td>
<td></td>
</tr>
<tr>
<td>2,256 base + 4,700</td>
<td>7 days/week weeks (CD-I) sp</td>
<td>Increased mortality</td>
<td>Graham et al (1987)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>376 base + 1,504</td>
<td>23 h/day, 7 days/week base + twice daily 1-h peaks, 5 days/week for 1 year</td>
<td>F 6-8 weeks</td>
<td>Mouse (CD-1)</td>
<td>Streptococcus</td>
<td>Peak plus baseline caused significantly greater mortality than baseline</td>
<td>Miller et al (1987)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>564-940 base + 0 5</td>
<td>Continuous, 4 weeks</td>
<td>Mouse (ICR JCL)</td>
<td>A/PR/8 virus</td>
<td>High incidence of adenomatous proliferation of peripheral and bronchial epithelial cells, NO₂ alone and virus alone caused less severe alterations</td>
<td>Motomya et al (1973)</td>
<td>No enhancement of effect of NO₂ and virus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 13-4 (cont'd). INTERACTION OF NITROGEN DIOXIDE WITH INFECTIOUS AGENTS

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Infective Agent</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>940 ppm</td>
<td>Intermittent, 6 or 18 h/day, up to 12 mo</td>
<td>F</td>
<td>NS</td>
<td>Mouse (Swiss)</td>
<td>K. pneumoniae</td>
<td>Increased mortality after 6 mo intermittent exposure or after 3, 6, 9, or 12 mo continuous exposure, following 12 mo exposure, increased mortality was significant only in continuously exposed mice</td>
<td>Ehrlich and Henry (1968)</td>
</tr>
<tr>
<td>18,800 ppm</td>
<td>Continuous, 39 days</td>
<td>F</td>
<td>NS</td>
<td>Mouse (ICR, dd)</td>
<td>A/PR/8 virus</td>
<td>Increased susceptibility to infection</td>
<td>Ito (1971)</td>
</tr>
<tr>
<td>940-52,700 ppm</td>
<td>Varied</td>
<td>F</td>
<td>NS</td>
<td>Mouse (CD-1) sp</td>
<td>Streptococcus sp</td>
<td>Increased mortality with increased time and concentration, concentration is more important than time</td>
<td>Gardner et al (1977a,b)</td>
</tr>
<tr>
<td>940 ppm</td>
<td>3 h/day, 3 mo</td>
<td>F</td>
<td>6-8 weeks</td>
<td>Mouse (CD₂F₁, CD-1)</td>
<td>Streptococcus sp</td>
<td>Increase in mortality with reduction in mean survival time</td>
<td>Ehrlich et al (1979)</td>
</tr>
<tr>
<td>940 ppm</td>
<td>24 h/day, 7 days/week, 3 mo</td>
<td>F</td>
<td>NS</td>
<td>Mouse (CF-1)</td>
<td>K pneumoniae</td>
<td>Significant increase in mortality after 3-day exposure to 50 ppm, no effect at other concentrations, but control mortality very high</td>
<td>McGrath and Oyervides (1985)</td>
</tr>
<tr>
<td>940 ppm</td>
<td>4 h</td>
<td>M/F</td>
<td>8-10 weeks</td>
<td>Mouse (C57BL/6N)</td>
<td>Mycoplasma pulmonis</td>
<td>Decrease in intrapulmonary killing only at 50 ppm</td>
<td>Davis et al (1992)</td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Infective Agent</td>
<td>Effects</td>
</tr>
<tr>
<td>-------------------</td>
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<td>---------</td>
</tr>
<tr>
<td>1,880</td>
<td>10</td>
<td>17 h</td>
<td>M</td>
<td>NS</td>
<td>Mouse (Swiss)</td>
<td>S aureus</td>
<td>No difference in number of bacteria deposited, but at the two highest concentrations, there was a decrease in pulmonary bactericidal activity of 6 and 35%, respectively, no effect at 10 ppm</td>
</tr>
<tr>
<td>4,324</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12,408</td>
<td>66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880</td>
<td>10</td>
<td>4 h</td>
<td>F</td>
<td>NS</td>
<td>Mouse (Swiss)</td>
<td>S aureus</td>
<td>Injection with corticosteroids increased NO₂-induced impairment of bactericidal activity at ≥2.5 ppm</td>
</tr>
<tr>
<td>4,700</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880</td>
<td>10</td>
<td>48 h</td>
<td>M</td>
<td>NS</td>
<td>Mouse (Swiss Webster)</td>
<td>Streptococcus sp</td>
<td>Increased proliferation of Streptococcus in lung of exposed mice but no effect with Staphylococcus</td>
</tr>
<tr>
<td>4,700</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880</td>
<td>10</td>
<td>3 h</td>
<td>F</td>
<td>5-6 weeks</td>
<td>Mouse (CD-1)</td>
<td>Streptococcus sp</td>
<td>Exercise on continuously moving wheels during exposure increased mortality at 30 ppm</td>
</tr>
<tr>
<td>5,640</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880</td>
<td>10</td>
<td>6 h/day, 6 days</td>
<td>NS</td>
<td>4-6 weeks</td>
<td>Mouse (CD-1)</td>
<td>Cytomegalovirus</td>
<td>Increase in virus susceptibility at 50 ppm only</td>
</tr>
<tr>
<td>4,700</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,820-94,000</td>
<td>15-50</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>Mouse (NS) Hamster (NS) Monkey (Squirrel)</td>
<td>K pneumoniae</td>
<td>Significant increased mortality in mice, hamsters, and monkeys at NO₂ concentrations of ≥35, ≥35, and 50 ppm, respectively</td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Infective Agent</td>
<td>Effects</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------</td>
<td>--------</td>
<td>-----</td>
<td>------------------</td>
<td>-----------------</td>
<td>------------------------------------------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>2,820 ppm</td>
<td>Continuous or intermittent, 7 h/day, 7 days/week, up to 15 days</td>
<td>F</td>
<td>NS</td>
<td>Mouse (CD-1)</td>
<td>Streptococcus sp</td>
<td>After 1 week, mortality with continuous exposure was greater than that for intermittent, after 2 weeks, no significant difference between continuous and intermittent exposure</td>
<td>Gardner et al (1979) Coffin et al (1977)</td>
</tr>
<tr>
<td>6,580 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased mortality with increased duration of exposure, no significant difference between continuous and intermittent exposure, with data adjusted for total difference in C X T, mortality essentially the same</td>
<td></td>
</tr>
<tr>
<td>2,820 base + 8,100 + peak</td>
<td>Continuous 64 h, then peak for 1, 3, 5, or 7 h, then continuous 18 h base</td>
<td>F</td>
<td>NS</td>
<td>Mouse (CD-1)</td>
<td>Streptococcus sp</td>
<td>Mortality increased with 3 5- and 7-h single peak when bacterial challenge was after an 18-h baseline exposure</td>
<td>Gardner (1980) Gardner et al (1982) Graham et al (1987)</td>
</tr>
<tr>
<td>8,100 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mortality proportional to duration when bacterial challenge was immediate, but not 18 h postexposure</td>
<td></td>
</tr>
<tr>
<td>2,820 ppm</td>
<td>7 h/day, 4, 5, and 7 days</td>
<td>NS</td>
<td>NS</td>
<td>Mouse (NS)</td>
<td>Streptococcus sp</td>
<td>Elevated temperature (32 °C) increased mortality after 7 days</td>
<td>Gardner et al (1982)</td>
</tr>
<tr>
<td>3,570 ppm</td>
<td>4 h</td>
<td>M</td>
<td>NS</td>
<td>Mouse (NS)</td>
<td>S aureus</td>
<td>Physical removal of bacteria unchanged by exposure Bactericidal activity decreased by 7, 14, and 50%, respectively, in three highest NO₂-exposed groups</td>
<td>Goldstein et al (1973)</td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age (Strain)</td>
<td>Species (Strain)</td>
<td>Infective Agent</td>
<td>Effects</td>
</tr>
<tr>
<td>-------------------</td>
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<td>--------</td>
<td>--------------</td>
<td>------------------</td>
<td>----------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2,820</td>
<td>1.5</td>
<td>3 h</td>
<td>F</td>
<td>6-10</td>
<td>Mouse</td>
<td>Streptococcus</td>
<td>Increased mortality in mice exposed to ≥2.0 ppm</td>
</tr>
<tr>
<td>9,400</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,820</td>
<td>1.5</td>
<td>2 h</td>
<td>NS</td>
<td>6-8</td>
<td>Mouse</td>
<td>K_pneumoniae</td>
<td>No effect at 1.5 or 2.5 ppm, increased mortality at 3.5 ppm and above</td>
</tr>
<tr>
<td>4,700</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increase in mortality when K_pneumoniae challenge 1 and 6 h after 5 or 10 ppm NO₂ exposure, effect at 15 ppm</td>
</tr>
<tr>
<td>6,580</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>9,400</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28,200</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,760</td>
<td>2.0</td>
<td>1.5 h/day,</td>
<td>NS</td>
<td>2 weeks</td>
<td>Hamster</td>
<td>A/PR/8/34</td>
<td>Peak virus production in tracheal explants occurred earlier</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 days/week</td>
<td></td>
<td></td>
<td>(Golden Syrian)</td>
<td>influenza virus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>for 1, 2, and 3 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,700</td>
<td>2.5</td>
<td>4 h</td>
<td>F</td>
<td>NS</td>
<td>Mouse</td>
<td>S_aureus, Proteus</td>
<td>Concentration-related decrease in bactericidal activity at ≥4 0 ppm with S_aureus when NO₂ exposure after bacterial challenge, when NO₂ exposure was before challenge, effect at 10 ppm, NO₂ concentrations &gt; 5 0 ppm required to affect bactericidal activity for other tested microorganisms</td>
</tr>
<tr>
<td>7,500</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>5.0</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td></td>
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<tr>
<td>28,200</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>5.0</td>
<td>Continuous, 2 mo</td>
<td>M</td>
<td>NS</td>
<td>Monkey</td>
<td>K_pneumoniae or A/PR/8 influenza virus</td>
<td>Increased viral-induced mortality (1/3) Increase in Klebsiella-induced mortality (2/7), no control deaths</td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>Continuous, 1 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased virus-induced mortality (6/6) within 2-3 days after infection, no control deaths Increase in Klebsiella-induced mortality (1/4), no control deaths</td>
</tr>
</tbody>
</table>
**TABLE 13-4 (cont’d). INTERACTION OF NITROGEN DIOXIDE WITH INFECTIOUS AGENTS**

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Infective Agent</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>9,400</td>
<td>50</td>
<td>4 h</td>
<td>M/F</td>
<td>6-10 weeks</td>
<td><em>Mycoplasma pulmonis</em></td>
<td>NO₂ increased incidence and severity of pneumonia lesions and decreased the number of organisms needed to induce pneumonia, no effect on physical clearance, decreased mycoplasmal killing and increased growth, no effect on specific IgM in serum, C57Bl/6N mice generally more sensitive than C3H/HeN mice</td>
<td>Parker et al (1989)</td>
</tr>
<tr>
<td>18,880</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>2 h</td>
<td>M/F</td>
<td>NS</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>Clearance of bacteria from lungs of 10-, 15-, and 35-ppm groups delayed or prevented</td>
<td>Henry et al (1969)</td>
</tr>
<tr>
<td>28,200</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65,800</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94,000</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**

- F = Female
- NS = Not stated
- *K pneumonae* = *Klebsiella pneumoniae*
- M = Male
- *S aureus* = *Staphylococcus aureus*
- C × T = The product of concentration and time
invading organism. For example, exposure to \(\geq 7,520 \, \mu g/m^3\) (4.0 ppm) NO\(_2\) for 4 h after bacterial challenge depressed lung bactericidal activity in mice against deposited \(S. \) aureus, but it required a concentration of 18,800 to 37,600 \(\mu g/m^3\) (10 to 20 ppm) before the lung’s ability to kill \(P. \) Pasteurella and \(P. \) Proteus was impaired. These effects became more significant with increasing concentrations.

The combination of corticosteroid (subcutaneous injection) and NO\(_2\) exposure (4 h) significantly impaired the intrapulmonary killing of staphylococci at concentrations of \(\geq 4,700 \, \mu g/m^3\) (\(\geq 2.5\) ppm) (Jakab, 1988). Without corticosteroids, bactericidal activity was only decreased at \(\geq 9,400 \, \mu g/m^3\). No NO\(_2\)- and corticosteroid-related effects on intrapulmonary killing were noted in mice exposed to 1,880 \(\mu g/m^3\) (1.0 ppm) NO\(_2\). These results would demonstrate that such a pretreated host is more susceptible to the effects of NO\(_2\). The implication of this finding is the probable existence of a high-risk population (because of immunosuppression, chronic lung disease, or old age) whose altered host status makes them more susceptible to infection following NO\(_2\) exposure (Green, 1970).

Parker et al. (1989) made similar observations in mice exposed for 4 h to 9,400 or 18,800 \(\mu g/m^3\) (5 or 10 ppm) NO\(_2\) and infected with \(M. \) pulmonis. The higher concentration of NO\(_2\) increased mortality. Both concentrations reduced lung bactericidal activity and increased the number of bacteria within the lung, producing an increase in the incidence and severity of murine respiratory mycoplasmosis lesions. There was no effect on physical clearance. When NO\(_2\) exposure was decreased (940, 1,880, or 3,760 \(\mu g/m^3\), 0.5, 1.0, or 2.0 ppm), using the same exposure model, the bactericidal activity of the lungs was not affected, although 9,400 \(\mu g/m^3\) (5.0 ppm) decreased bactericidal activity (Davis et al., 1992).

Differences in species susceptibility to NO\(_2\) or to a pathogen may play a role in the enhancement in mortality seen in experimental animals. An enhancement in mortality was noted in mice, hamsters, and monkeys exposed to NO\(_2\) for 2 h followed by a challenge of \(K. \) pneumoniae (Ehrlich, 1975). However, differences in susceptibility were noted between the species. Squirrel monkeys exposed continuously to NO\(_2\) levels of 9,400 and 18,800 \(\mu g/m^3\) (5.0 and 10 ppm) for 2 and 1 mo, respectively, showed increased mortality following a challenge with \(K. \) pneumoniae and reduced lung clearance of viable bacteria (Henry et al., 1970). Two of seven monkeys exposed to 9,400 \(\mu g/m^3\) for 2 mo died, and the
rest had bacteria in the lungs on autopsy. Ehrlich (1975) found that hamsters exhibited enhanced mortality after exposure for 2 h to NO₂ concentrations of ≥65,800 μg/m³ (∼35 ppm), but not at 9,400 to 47,000 μg/m³ (5 to 25 ppm). The mouse was the most sensitive to NO₂ exposure, as evidenced by enhanced mortality following a 2-h exposure to 6,580 μg/m³ (3.5 ppm), but not to 2,820 to 4,700 μg/m³ (1.5 to 2.5 ppm) (Ehrlich, 1975).

Purvis and Ehrlich (1963) also reported no effect on mortality in mice exposed for 2 h to 2,820 or 4,700 μg/m³ (1.5 or 2.5 ppm), increase mortality occurred at 6,580 μg/m³ (3.5 ppm) and higher. However, when Streptococcus sp was the infectious agent, a 3-h exposure to 3,760 μg/m³ (2.0 ppm) NO₂ caused an increased in mortality in mice (Ehrlich et al., 1977).

Squirrel monkeys exposed to 9,400 or 18,800 μg/m³ (5 or 10 ppm) NO₂ for 2 or 1 mo, respectively, also showed increased susceptibility to a laboratory induced viral influenza infection (Henry et al., 1970). All six animals exposed to the highest concentration died within 2 to 3 days of infection with the influenza virus. At the lower concentration, one of three monkeys died. Susceptibility to viral infection was enhanced when the NO₂ exposure occurred 24 h after the infectious challenge.

McGrath and Smith (1984) investigated whether changes in susceptibility to bacterial infections in mice were related causally to the effects of NO₂ on respiration. Because no spontaneous changes in respiratory patterns were produced following a 3-day exposure to 9,400 μg/m³ (5.0 ppm) NO₂, the authors concluded that any such relationship was unlikely. Refer to Section 13.2.2.3 on pulmonary function for details of the study.

The importance of the test microorganism used with the infectivity models was also demonstrated by Sherwood et al. (1981). These researchers illustrated that exposure to 1,880 μg/m³ (1.0 ppm) NO₂ for 48 h increased the propensity of virulent group C streptococci, but not S. aureus, to proliferate within the lungs of mice and cause earlier mortality.

The relationships of concentration and time to susceptibility to respiratory infection and to subsequent mortality in infections with Streptococcus sp were examined by Gardner et al. (1977a,b). When NO₂ concentrations were varied from 2,820 to 52,600 μg/m³ (1.5 to 28 ppm) and the duration of exposure varied from 0.25 to 4.7 h so that C × T equaled a value of 7 ppm-h, exposure to high concentrations of NO₂ for brief periods of time resulted in increased susceptibility and mortality.
in greater mortality than did prolonged exposures to lower concentrations (Gardner et al., 1977b). This indicated that susceptibility to infection was influenced more by the concentration of NO₂ than by the duration of the exposure.

In the same study (Gardner et al., 1977a,b), a linear exposure-duration response was observed in mice exposed to from 940 to 52,640 μg/m³ (0.5 to 28 ppm), indicating that mortality increases with increasing length of exposure to a given concentration of NO₂ (Figure 13-1). Mortality also increased with increasing concentration of NO₂, as indicated by the steeper slopes with higher concentrations. When C × T was held constant, the relationship between concentration and time produced significantly different mortality responses. At a constant C × T of approximately 21 ppm·h, a 14-h exposure to 2,820 μg/m³ (1.5 ppm) NO₂ increased mortality by 12.5%, whereas a 1.5-h exposure to 26,300 μg/m³ (14 ppm) NO₂ enhanced mortality by 58.5%. These results confirmed that concentration is more important than time in determining the degree of injury induced by NO₂ in this model. According to Larsen et al. (1979), NO₂ modeling studies have shown that the concentration (c) of NO₂ expected to cause a certain mortality level (z) as a function of the hours of exposure (t) can be expressed as:

\[ c = 9.55 \times (2.42)^{t^{0.33}} \]

Gardner et al. (1979) and Coffin et al. (1977) also compared the effect of continuous versus intermittent exposure to NO₂ followed by bacterial challenge with *Streptococcus* sp. Mice were exposed either continuously or intermittently (7 h/day, 7 days/week) to 2,820 or 6,580 μg/m³ (1.5 or 3.5 ppm) NO₂. The results of continuous and intermittent exposure to 6,580 μg/m³ (3.5 ppm) for periods up to 15 days indicated that there was a significant increase in mortality for each of the experimental groups with increasing duration of exposure. When the data were adjusted for the difference in C × T, the mortality was essentially the same for the continuous and intermittent groups. The continuous exposure of mice to 2,820 μg/m³ NO₂ increased mortality after 24 h of exposure. During the first week of exposure, the mortality was significantly higher in mice exposed continuously to NO₂ than in those exposed intermittently. By the 14th day of exposure, the difference between intermittent and continuous exposure became indistinguishable. This suggests that fluctuating levels of NO₂ may ultimately be as toxic as sustained high levels (Gardner et al., 1979).

When mice were exposed continuously or intermittently (6 or 18 h/day) to 940 μg/m³ (0.5 ppm) NO₂ for up to 12 mo, murine resistance to *K. pneumoniae* infection was not
Figure 13-1. Mortality enhancement for mice exposed to nitrogen dioxide at various concentrations and for various durations prior to challenge with streptococci. At all concentrations, prolonged exposure results in enhanced mortality, but the severity of resistance reduction is more directly related to concentration.

Source Gardner et al (1977b)

...
intermittent exposure regimen to produce a level of effect equivalent to that of a continuous exposure.

A significant increase in percent mortality and a decrease in relative mean survival time was noted in mice exposed continuously to 9,400 \( \mu g/m^3 \) (50 ppm) for 3 days (McGrath and Oyervides, 1985). However, similar continuous exposure for 24 h/day, 7 days/week to 940, 1,880, and 2,820 \( \mu g/m^3 \) (0.5, 1.0, and 1.5 ppm) \( NO_2 \) for 3 mo did not produce a difference in either of these parameters. These subchronic exposure results do not agree with Ehrlich and Henry (1968), who found excess mortality in mice after continuous exposure to 940 \( \mu g/m^3 \) (0.5 ppm) for 3 mo, but the short-term exposure results do agree with those of Gardner et al. (1979) and Ehrlich (1980). The inconsistency may also be attributed to the fact that the McGrath and Oyervides (1985) study had 95% mortality in the control groups, making it virtually impossible to detect a \( NO_2 \)-induced enhancement in mortality.

Purvis and Ehrlich (1963) investigated the persistence of the effect of \( NO_2 \) using the infectivity model. They found a significant increase in excess mortality in mice exposed for 2 h to 9,400 \( \mu g/m^3 \) (50 ppm) \( NO_2 \), followed by a challenge with \( K. pneumonieae \). They reported that the increased mortality occurred when the infectious challenge was given 1 or 6 h postexposure, but was no longer present if the infectious challenge was given 27 h after the animals were removed from the inhalation chambers.

Gardner (1980), Gardner et al. (1982), and Graham et al. (1987) reported further investigations on the response of mice to airborne infections during or following intermittent exposure to \( NO_2 \). These studies investigated the toxicity of \( NO_2 \) peak exposures superimposed on a lower continuous background level of \( NO_2 \). Such a regimen approximates the pattern of exposure that humans receive in the urban environment. Mice were exposed to \( NO_2 \) peaks of 8,460 \( \mu g/m^3 \) (4.5 ppm) for 1, 3, 5, or 7 h and then were challenged with \( Streptococcus \) sp either immediately or 18 h postexposure. Mortality was proportional to the duration of the peak when the mice were exposed to bacteria immediately following \( NO_2 \) exposure, but mice had recovered from the exposure by 18 h. When a peak exposure of 8,460 \( \mu g/m^3 \) was superimposed on a continuous background of 2,820 \( \mu g/m^3 \) (1.5 ppm) for 64 h preceding and 18 h following the peak, mortality was significantly enhanced by a peak exposure lasting 3.5 or 7 h when the infectious agent was administered 18 h after the peak exposure. Possible explanations for these differences due to the presence
or absence of a background exposure are that mice continuously exposed are not capable of recovery or that new AMs or PMNs recruited to the site of infection are impaired by the continuous exposure to NO₂. The effect of multiple peaks was examined by exposing mice for 2 weeks to two daily 1-h peaks (morning and afternoon) (5 days/week) of 8,460 μg/m³ superimposed on a continuous background (7 days/week) of 2,820 μg/m³ NO₂. Mice were exposed to the infectious agent either immediately before or after the morning peak exposure. When the infectious agent was given before the morning peak exposure, the increase in mortality did not closely approach that of a continuous exposure to 2,820 μg/m³ NO₂. However, in mice exposed after the morning peak, by the second week of exposure, the increased mortality over controls approached that equivalent to continuous exposure to 2,820 μg/m³ NO₂. These findings demonstrate that the pattern of exposure determines the response and that the response is not predictable based on a simple C × T relationship.

Further investigations into the effects of NO₂ on murine antibacterial lung defenses have been conducted using a peak to baseline ratio of 4:1, which is not uncommon in the urban environment (Miller et al., 1987). For 1 year, mice were continuously exposed 23 h/day, 7 days/week to a baseline of 376 μg/m³ (0.2 ppm) or to this baseline level on which a 1-h peak of 1,500 μg/m³ (0.8 ppm) NO₂ was superimposed two times a day, 5 days/week. The animals exposed to the baseline level did not reveal any significant treatment-related effects, however, the infectivity mortality of the mice exposed to the baseline plus peak regimen was significantly greater than that of either the NO₂ background exposed mice or the control mice. This chronic study indicates that short-term peaks of NO₂ can cause detectable effects on antibacterial lung defenses. This baseline and peak exposure also affected pulmonary function (see Section 13.2.2.3) (Miller et al., 1987).

Mice exposed continuously for 3 mo to 564 to 940 μg/m³ (0.3 to 0.5 ppm) NO₂ followed by a challenge with A/PR/8 influenza virus showed significant pulmonary pathological responses (Motomiya et al., 1973). A greater incidence of adenomatous proliferation of bronchial epithelial cells resulted from the combined exposures of virus plus NO₂ than with either the viral or NO₂ exposures alone. Continuous NO₂ exposure for an additional 3 mo did not enhance the effect of NO₂ or the subsequent virus challenge.

Ito (1971) challenged mice with influenza A/PR/8 virus after continuous exposure to 940 to 1,880 μg/m³ (0.5 to 1.0 ppm) NO₂ for 39 days and to 18,800 μg/m³ (10 ppm) NO₂,
2 h daily for 1, 3, and 5 days. Acute and intermittent exposure to 18,880 \( \mu g/m^3 \) NO\(_2\), as well as continuous exposure to 940 to 1,880 \( \mu g/m^3 \) NO\(_2\), increased the susceptibility of mice to influenza virus, as demonstrated by increased mortality. Further, when isolated hamster tracheal organ explants were exposed in vitro for 1, 2, and 3 weeks to 3,760 \( \mu g/m^3 \) (2 0 ppm), 1.5 h/day for 5 days/week and then immediately infected with influenza virus (A/PR/8/34), the maximum virus titer reached was the same for both the exposed and unexposed explants. However, NO\(_2\) exposure caused the peak virus production to occur earlier (Schiff, 1977).

The lower respiratory tract of mice became significantly more susceptible to murine cytomegalovirus infection after 6-h exposures for 6 days to 9,400 \( \mu g/m^3 \) (5 0 ppm), but not \( \leq 4,700 \mu g/m^3 \) (\( \leq 2.5 \) ppm) NO\(_2\) (Rose et al., 1988, 1989). Viral replication was routinely increased in the lungs of mice exposed to 9,400 \( \mu g/m^3 \) NO\(_2\) over that of controls. There was also an increase in the incidence of lung lesions in the NO\(_2\)-exposed mice. The NO\(_2\)-exposed animals could be infected with an inoculum 100 times less than that required to infect air-exposed animals. Further, the NO\(_2\)-exposed animals were more susceptible to viral reinfection, whereas the air-exposed controls appeared to resistant to reinfection. However, AM antiviral capacity did not appear to be altered. Humoral virus-specific antibody titers were not affected by NO\(_2\), nor were splenic lymphocyte proliferative responses to viral antigens.

Exposure to a NO\(_2\) concentration of 9,400 \( \mu g/m^3 \) (5 0 ppm) did not significantly alter the course of a paramyxovirus (murine sendai virus) infection in mice as measured by the infectious pulmonary virus titers in the lungs. However, this concentration of NO\(_2\), when combined with the virus exposure, did increase the severity of the pulmonary disease process (viral pneumonitis) (Jakab, 1988).

Only one human clinical study has examined the effect of NO\(_2\) on infectivity rate (Goings et al., 1989). Subjects were exposed to 1,880, 3,760, or 5,640 \( \mu g/m^3 \) (1.0, 2.0, or 3 0 ppm) NO\(_2\) for 3 consecutive days (2 h/day) and inoculated intranasally with attenuated influenza A/Korea/reassortment virus on the second of these days. No statistically significant effects were observed, although there was a trend towards an increase in infectivity rates. However, this study had a low statistical power to detect small changes. See Section 15.6 for an expanded discussion of this study.
Stress, in addition to influencing the lethality of a particular exposure concentration, has been shown to enhance the toxic effect of NO₂. Mice placed on continuously moving exercise wheels during exposure to 5,640 μg/m³ (30 ppm) NO₂, but not 1,880 μg/m³ (10 ppm), for 3 h showed enhanced mortality over nonexercised NO₂-exposed mice using the streptococcal infectivity model (Il!ng et al., 1980). The presence of other environmental factors, such as O₃ (Ehrlich et al., 1977, Gardner, 1980, Gardner et al., 1982, Graham et al., 1987), elevated temperatures (Gardner et al., 1982), or tobacco smoke (Henry et al., 1971), also augments the effect of NO₂ on host resistance to infection. When mice were stressed by elevated temperature (32 °C) and exposed to 2,820 μg/m³ (1.5 ppm) NO₂, there was a significant enhancement in mortality rate after 7 days of exposure (Gardner et al., 1982).

**Summary**

The host defense system is one of the many potential targets whose function has been shown to be altered significantly by exposure to NO₂. Evidence would indicate that any breach in these defenses should be considered as a possible indicator of an increased risk of infectious pulmonary and/or systemic disease.

As discussed in the section on lung morphology, NO₂ causes structural alterations in the ciliated cells of the airways, however, significant impairment of tracheobronchial clearance rates generally were not seen at levels ≤18,800 μg/m³ (10 ppm) NO₂ (Schlesinger et al., 1987a,b). This would indicate that even a severely damaged airway epithelium (i.e., loss of cilia) still has the ability to maintain mucus transport at a normal rate, and that the exposure of experimental animals to NO₂ would have to be to concentrations >18,800 μg/m³ to induce any significant alterations that would have detrimental health effects.

Within the pulmonary region of the lung, the primary cellular defense affected by both acute and long-term exposure to NO₂ is the AM. Nitrogen dioxide causes a depression of phagocytic activity, reduces cell viability, disrupts membrane integrity, reduces the total number of available cells, produces morphological changes, and decreases bactericidal activity. Although a few of these effects were seen following exposure to concentrations <1,880 μg/m³ (1.0 ppm) (Schlesinger, 1987b, Rose et al., 1989), most of the studies...
showed effects at concentrations between 1,880 and 9,400 µg/m³ (1 0 and 5 0 ppm) NO₂
(Goldstein et al., 1973, Greene and Schneider, 1978, Hooftman et al., 1988, Dowell et al.,
1971, Suzuki et al., 1986, Acton and Myrvik, 1972) Evidence indicates that these cells are
no longer capable of isolating, transporting, detoxifying, or clearing inhaled substances.

The systemic cell-mediated and humoral immune system is also a target for NO₂, as
evidenced by experimental animal studies. The immunological effects reported seem to be
variable. Some studies show effects and others do not. It has been suggested that long-term
exposure may result in a suppression of the various humoral and cell-mediated functions,
whereas shorter exposures may cause an enhancement of immunological activity. The
response seems to be dependent not only on concentration and duration of exposure, but also
on animal species and the specific immunological end point measured. Research on the
systemic immune system in mice, guinea pigs, and monkeys indicates that subchronic and
chronic exposure at or below 1,880 µg/m³ (1 0 ppm) can suppress T- and B-cell
responsiveness to mitogens and can decrease the number of T cells (Richters and Damj,
1988, 1990, Maigetter et al., 1978). Nitrogen dioxide influences the production of serum-
neutralizing antibodies to viruses and humoral primary antibody response to SRBCs
(Fujimaki et al., 1982, Ehrlich et al., 1975, Fenters et al., 1973). Other immunological
effects attributed to NO₂ include an increase in IgM and IgG, and a decrease in IgA serum
levels (Ehrlich et al., 1975). The significance of many of these changes is uncertain, and
studies conducted at lower levels of exposure and for longer periods of time are needed to
improve our understanding of these immunological responses. In the absence of adequate
data, one can only speculate that if NO₂ affects the systemic immune system, it is likely that
it also would affect the pulmonary immune system.

The consequence of suppression of the various host defense mechanisms would
ultimately lead to increased microbial proliferation within the lung, resulting in increased
incidence and severity of pulmonary infections. Experimental animal studies have
demonstrated that both acute and chronic exposures to NO₂ can significantly increase
susceptibility to viral and bacterial infections. The exact exposures producing such effects
are dependent upon the animal species, the microbial species/strain, and the model used.
The infectivity model, in which air- and NO₂-exposed mice are challenged with a viable
microbial aerosol and mortality is measured, is the most sensitive. For example, a 39-day
exposure to 940 μg/m³ (0.5 ppm) NO₂ increased influenza-induced mortality (Ito, 1971), and a 6-mo exposure to 940 μg/m³ (0.5 ppm) NO₂ increased bacterial-induced mortality (Ehrlich and Henry, 1968). After an acute exposure, 3,760 μg/m³ (2.0 ppm) is the lowest level tested to produce significant mortality for the bacterial model (Ehrlich et al., 1977, Ehrlich, 1980).

The mouse streptococcal infectivity model has been applied extensively to elucidate C × T relationships (Gardner et al., 1977a,b). Exposure concentration has a predominant influence over that of exposure duration. In the urban air, the typical pattern of NO₂ is a low-level baseline exposure on which peaks are superimposed corresponding to peaks of NOₓ mobile source emissions. When the relationship of the peak to baseline exposure and of enhanced susceptibility to bacterial infection was investigated, the results indicated that no simplistic C × T relationship was present, and that peaks had a major influence on the outcome (Gardner, 1980, Gardner et al., 1982, Graham et al., 1987). When one compares the effect of a subchronic continuous one-level exposure to an exposure consisting of baseline and peaks having a lower C × T, the effect was roughly equivalent. In a 1-year chronic study with the infectivity model, the effect of a 376 μg/m³ (0.2 ppm) NO₂ baseline exposure (21 h/day, 7 days/week) was compared to baseline plus two daily 1-h peaks of 1,504 μg/m³ (0.8 ppm) NO₂ for 5 days/week. Only the baseline-plus-peak group exhibited significant increased susceptibility to bacterial infection (Miller et al., 1987).

The effects associated with NO₂ exposure on the host defense system are dependent on the concentration of the gas, the duration of the exposure, the animal species tested, and the specific end point of toxicity measured. As stated earlier, basic defense mechanisms are common across mammalian species. Thus, the summation of the effects on a number of host defense systems may make the mammalian host more vulnerable to infectious disease. Although the outcome measured in animals is mortality, morbidity would be expected to occur first or occur at exposures too low to induce mortality. In humans, especially those under medical treatment, such a loss in pulmonary defenses would be expected to result in an increased incidence of morbidity, especially in that segment of the population that may be more susceptible, such as young children or the elderly. In assessing or predicting such human risk from experimental animal data, it is understood that in humans, different levels of exposure to NO₂ may be required to produce effects similar to those seen in animals.
13.2.2.2 Lung Biochemistry

Studies of lung biochemistry in animals exposed to NO₂ have focused on either the putative mechanisms of toxic action of NO₂ or on detection of indicators of NO₂-induced tissue and cell damage. One theory to explain NO₂ toxicity is that NO₂ initiates lipid peroxidation in unsaturated fatty acids in membranes of target cells (Menzel, 1976). These changes are thought to cause cell injury or death, and the symptoms associated with NO₂ inhalation. An alternate theory is that NO₂ oxidizes water-soluble, low molecular weight reducing substances and proteins, resulting in a metabolic dysfunction that evidences itself as the toxic symptom (Freeman and Mudd, 1981). Nitrin dioxide may, in fact, act by both means and, as a consequence, may affect the intermediary metabolism of animals and their growth and maturation. Further, given that NO₂ dissolves in water to produce HONO and HNO₃, the possibility of an acid or a pH effect as a primary or secondary mechanism of injury should also be considered.

Lipid Metabolism

Table 13-5 summarizes the effects of NO₂ on lipids.

Various investigators have performed in vitro experiments on isolated lung cells or subcellular components to examine the effects of NO₂ on oxidation of unsaturated fatty acids. In a series of studies, Patel and Block (1986a,b) and Patel et al (1988) examined the effects of NO₂ exposure on cultured endothelial cells from either pulmonary arteries or aortae of pigs. After exposing the cells to 9,400 μg/m³ (5 ppm) NO₂ for 24 h, they observed changes in membrane fluidity, lipid peroxide formation, 5-hydroxytryptamine uptake, release of LDH (a marker for cell membrane abnormalities) and increases in GSH reductase and G-6-P dehydrogenase activities. The authors concluded that injury of endothelial cells by NO₂ can be ascribed to decreased membrane fluidity secondary to lipid peroxidation, and that the altered physical state of the cell membrane causes impaired functionality of the membrane, leading to cellular abnormalities of metabolism and biochemistry.

Sekharam et al (1991) found that oxidative damage in endothelial cells (from the pulmonary artery of pigs) exposed in vitro to 9,400 μg/m³ (5 ppm) for 48 h, phospholipase A₁ activity was increased in the cell membrane, but not in the mitochondrial.
### TABLE 13-5. EFFECTS OF NITROGEN DIOXIDE ON LIPID METABOLISM

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0 04</td>
<td>Continuous,</td>
<td>M 8 (Wistar)</td>
<td>Increased lipid peroxidation (TBA method) at 4 0 ppm after 9 mo and at 0 4 and 4 0 ppm after 18 mo, increased ethane exhalation at all levels at 9 and 18 mo, ethane exhalation returned to normal levels in highest group by 27 mo, no changes in total lipid, phospholipid, total cholesterol, or triglyceride contents, TBA reactants increased at 4 ppm (9 mo) and ≤0 4 ppm (18 mo)</td>
<td>Sagar et al (1984)</td>
</tr>
<tr>
<td>752</td>
<td>0 4</td>
<td>9, 18, or 27 mo</td>
<td>M 13 (Wistar)</td>
<td>Increased ethane exhalation after 9 and 18 mo</td>
<td>Ichnose et al (1983)</td>
</tr>
<tr>
<td>7,520</td>
<td>4 0</td>
<td>Continuous,</td>
<td>M 13 (Wistar)</td>
<td>Increased ethane exhalation and TBA-reactive substances during first week of exposure, returned to normal levels by fourth week, tendency towards increase thereafter</td>
<td>Ichnose and Sagar (1982)</td>
</tr>
<tr>
<td>75</td>
<td>0 04</td>
<td>Continuous,</td>
<td>M NS (Hartley)</td>
<td>No effect at 0 4 ppm, increase in lung lipid content in vitamin C-depleted, but not normal, animals at 1 0 ppm and above</td>
<td>Ichnose et al (1983)</td>
</tr>
<tr>
<td>225</td>
<td>0 12</td>
<td>6, 9, and 18 mo</td>
<td>M NS (Hartley)</td>
<td>Increased lung lipid content in vitamin C-depleted guinea pigs</td>
<td>Selgrade et al (1981)</td>
</tr>
<tr>
<td>752</td>
<td>0 4</td>
<td>Continuous,</td>
<td>M 13 (Wistar)</td>
<td>No effect on lung lavage fluid composition in normal or vitamin C-depleted animals</td>
<td>Ichnose et al (1983)</td>
</tr>
<tr>
<td>1,880</td>
<td>0 4</td>
<td>Continuous,</td>
<td>M NS (Hartley)</td>
<td>Increase in lipid peroxidation products</td>
<td>Balabaeva and Tabakova (1985)</td>
</tr>
<tr>
<td>1,880</td>
<td>1 0</td>
<td>Continuous,</td>
<td>M NS (Hartley)</td>
<td>Decrease in lecithin synthesis after 1 week, less marked depression after 2 weeks</td>
<td>Seto et al (1975)</td>
</tr>
<tr>
<td>1,880</td>
<td>1 0</td>
<td>Continuous,</td>
<td>M NS (Hartley)</td>
<td>1 ppm elevated thromboxane B₂, 3 ppm depressed thromboxane B₂, 10 ppm depressed 6-keto-PGF₁α, and thromboxane B₂, no changes noted in PGE₂, PGE₂α, or LTB₄</td>
<td>Schlesinger et al (1990)</td>
</tr>
<tr>
<td>5,640</td>
<td>1 0</td>
<td>Continuous,</td>
<td>M NS (Hartley)</td>
<td>1 ppm elevated thromboxane B₂, 3 ppm depressed thromboxane B₂, 10 ppm depressed 6-keto-PGF₁α, and thromboxane B₂, no changes noted in PGE₂, PGE₂α, or LTB₄</td>
<td>Schlesinger et al (1990)</td>
</tr>
<tr>
<td>18,800</td>
<td>1 0</td>
<td>Continuous,</td>
<td>M NS (Hartley)</td>
<td>1 ppm elevated thromboxane B₂, 3 ppm depressed thromboxane B₂, 10 ppm depressed 6-keto-PGF₁α, and thromboxane B₂, no changes noted in PGE₂, PGE₂α, or LTB₄</td>
<td>Schlesinger et al (1990)</td>
</tr>
</tbody>
</table>
## TABLE 13-5 (cont’d). EFFECTS OF NITROGEN DIOXIDE ON LIPID METABOLISM

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>Exposure</th>
<th>Gender Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/m³ ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,450 29</td>
<td>Continuous, 5 days/week, 9 mo</td>
<td>M NS</td>
<td>Rat (Long Evans)</td>
<td>Increase in lung wet weight (12.7%) and decrease in total lipids (8.7%), decrease in saturated fatty acid content of BAL fluid and tissue, increase in surface tension of BAL fluid</td>
<td>Arner and Rhoades (1973)</td>
</tr>
<tr>
<td>5,640 30</td>
<td>Continuous, 17 days</td>
<td>M NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>Decrease in linoleic and linolenic acid content of BAL fluid</td>
<td>Menzel et al (1972)</td>
</tr>
<tr>
<td>18,800 10</td>
<td>Continuous, 4 weeks</td>
<td></td>
<td></td>
<td>Decrease in polyunsaturated fatty acids in BAL and lung tissue</td>
<td></td>
</tr>
<tr>
<td>5,640-30,080 16</td>
<td>1 h</td>
<td>NS NS</td>
<td>Dog (Beagle)</td>
<td>Decrease in phospholipid content of BAL fluid from animals with NO₂-induced intraalveolar edema, increase in unsaturated fatty content of BAL lecithin at ≥5 0 ppm</td>
<td>Dowell et al (1971)</td>
</tr>
<tr>
<td>7,520 4 0</td>
<td>3 h</td>
<td>M/F 21-33 years</td>
<td>Human Pgf (in vitro)</td>
<td>Increased lipid peroxidation products in BAL fluid</td>
<td>Mohsenin (1991)</td>
</tr>
<tr>
<td>9,400 5 0</td>
<td>24 h</td>
<td>NS 6-7 mo</td>
<td>Pig (in vitro)</td>
<td>In endothelial cells of pulmonary artery and aorta, changes in membrane fluidity, lipid peroxide formation, release of LDH, and 5-hydroxytryptamine uptake</td>
<td>Patel and Block (1986a,b) Patel et al (1988)</td>
</tr>
<tr>
<td>9,400 5 0</td>
<td>48 h</td>
<td>NS 6-7 mo</td>
<td>Pig (in vitro)</td>
<td>Lipid alterations in endothelial cells from pulmonary artery</td>
<td>Sekharam et al (1991)</td>
</tr>
<tr>
<td>10,300 5 5</td>
<td>3 h/day, 7 and 14 days</td>
<td>M 8 weeks</td>
<td>Rat (Wistar)</td>
<td>Decrease in lyssolecithin acyltransferase in lung homogenate microsomes</td>
<td>Yokoyama et al (1980)</td>
</tr>
<tr>
<td>18,800 10</td>
<td>12 h</td>
<td>M NS</td>
<td>Rat (Wistar)</td>
<td>Changes in fatty acids of BAL phospholipids</td>
<td>Kobayashi et al (1984)</td>
</tr>
<tr>
<td>18,800 10</td>
<td>1, 3, 5, 7, 14 days</td>
<td>M NS</td>
<td>Rat (NS)</td>
<td>Changes in prostaglandins and thromboxane B₂ in BAL fluid</td>
<td>Kobayashi (1986)</td>
</tr>
</tbody>
</table>

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*M = Male
TBA = Thiorbarbamic acid
NS = Not stated
F = Female
6-keto-PGF₁α = 6-keto-prostaglandin F₁α
PGF₂α = Prostaglandin E₂α
PGE₂ = Prostaglandin E₂
LTP₄ = Leukotriene B₄
LDH = Lactate dehydrogenase
BAL = Bronchoalveolar lavage
or microsomal membranes. There was a significant increase in lyso-phosphatidylethanolamine and a significant decrease in phosphatidylethanolamine as a result of the increase in phospholipase A₁ activity. Phosphatidylserine content was also increased. However, the total phospholipid content of the plasma membrane was decreased compared to that of controls. The authors suggested a possible association between the increased activity of phospholipase A₁ and the increase in phosphatidylserine seen in the cell membrane of the NO₂-exposed endothelial cells after treating endothelial cells with exogenous phosphatidylserine and observing an 87% increase in phospholipase A₁ activity in the cell membrane.

Rietjens and co-workers (1986, 1987) exposed cultured rat AMs to NO₂ or O₃ by gas diffusion through a Teflon® film. Based on various experiments using different radical scavengers, the authors concluded that NO₂ and O₃ acted by different mechanisms, whereby NO₂ exerted its toxicity via a free radical-mediated peroxidative pathway and O₃ via a pathway involving the formation of lipid ozonides. Both NO₂ and O₃ appear to act at the level of lipid oxidation in causing AM toxicity. However, it should be noted that the chemical reactions of NO₂ or O₃ with organic compounds in aqueous solutions can be very complex (Glaze, 1986), and prediction of which pathway(s) may predominate in complex biological systems is by no means straightforward. For example, NO₂ or O₃ may react with unsaturated fatty acids, a component of lung phospholipids, but also with water-soluble reducing substances of low molecular weight or reducible groups on proteins. A discussion of the functional and structural effects of NO₂ on AMs appears in Sections 13.2.2.1 and 13.2.2.4 on host defense mechanisms and morphological effects, respectively.

Roehm et al. (1971) studied the in vitro oxidation of unsaturated fatty acids by NO₂ and O₃. Both NO₂ and O₃ initiated the oxidation of unsaturated fatty acids through free radicals. Typically, an induction period was noted with either anhydrous thin films or aqueous emulsions of linolenic acid exposed to 2,820 μg/m³ (1.5 ppm) NO₂. The addition of free radical-scavenging agents such as vitamin E, butylated hydroxytoluene, or butylated hydroxyanisole delayed the onset of oxidation in vitro. The rate of oxidation of linolenic acid in thin films was proportional to concentrations of NO₂ from 940 to 10,152 μg/m³ (0.5 to 5.4 ppm). Thin-layer chromatography of the oxidation products of linolenic acid showed a conversion to polar nitrogen-containing compounds and to peroxides. A suggested
mechanism of formation of these products (Menzel, 1976) involves addition of NO₂ across a double bond between two carbon atoms in an unsaturated fatty acid to form a nitro compound and a carbon-centered free radical. Such a radical can extract an electron from various potential electron donors, thereby initiating the chain reaction. Nitrohydroperoxides and fatty acid hydroperoxides are produced in vitro from the oxidation of unsaturated fatty acids by NO₂. Phenolic antioxidants can prevent the autooxidation of unsaturated fatty acids by NO₂ by reacting with both fatty acid hydroperoxyl free radicals and nitrohydroperoxyl free radicals generated by the addition of NO₂ to unsaturated fatty acids. It is not known whether this sequence of reactions is important in the lung in vivo.

Sagai et al. (1984) and Ichinose et al. (1983) reported an increase in thiobarbituric acid (TBA) reactants (an indication of lipid peroxides) in lung homogenates of rats exposed to 7,520 μg/m³ (4 ppm) NO₂ continuously for 9 mo. When exposure was increased to 18 mo, a concentration-related increase in TBA reactants occurred in rats exposed to 75, 752, and 7,520 μg/m³ (0.04, 0.4, and 4.0 ppm) NO₂, but the increase was only significant in animals exposed to the two highest concentrations.

Mohsenin (1991) reported the effects of NO₂ exposure on healthy human subjects. Subjects received either a placebo or vitamins C (1,500 mg/day) and E (1,200 IU/day) for 4 weeks and were then exposed to 7,520 μg/m³ (4 ppm) for 3 h. In BAL fluid, NO₂ exposure decreased the elastase inhibitory capacity of α₁-protease inhibitor, the major plasma and lung protease inhibitor of elastase. There was also an NO₂-induced increase in lipid peroxidation products (primarily conjugated dienes, but some malondialdehydes) in the BAL fluid. However, when subjects were supplemented with dietary vitamin C and E, these effects were prevented.

The exhalation of ethane in the breath was measured in assays of in vivo lipid peroxidation (Sagai et al., 1984, Ichinose et al., 1983). In the first series of studies, excess mortality in chamber control rats forced the use of room control rats in the statistical analyses, however, there was no major difference between room and chamber control values. At 75, 752, and 7,520 μg/m³ (0.04, 0.4, and 4.0 ppm) NO₂, 9 and 18 mo of exposure increased the exhalation of ethane. The two lower NO₂ concentrations also increased ethane exhalation after 27 mo of exposure, but ethane was within the control range in the 7,520 μg/m³-exposed group. Pentane exhalation was measured to determine if the lipid...
Peroxidation was bacterial in origin. Pentane was only increased after 18 mo of exposure to 75 and 752 \( \mu g/m^3 \) NO\(_2\), supporting the interpretation of the ethane results. In the second series of experiments, chamber control rats were used and rats were exposed to 75, 225, and 752 \( \mu g/m^3 \) (0.04, 0.12, and 0.4 ppm) NO\(_2\) for 6, 9, and 18 mo. After 6 mo, ethane exhalation was only increased in the 752 \( \mu g/m^3 \) group. All NO\(_2\) concentrations increased ethane exhalation after 9 and 18 mo of exposure. These studies showed that NO\(_2\) increased lipid peroxidation in a concentration- and exposure duration-related manner. An inverse relationship with lung antioxidant metabolism was also found (see later subsection on antioxidant metabolism). Shorter duration exposures also influence lipid peroxidation in rats, as measured by ethane exhalation. For example, exposure to \( \geq 2,256 \mu g/m^3 \) (\( \geq 1.2 \) ppm) NO\(_2\) increases ethane exhalation after 1 week of exposure. Levels of ethane had returned to control values after 4 weeks of NO\(_2\) exposure, at which time ethane levels began a slow rise again over the remainder of the 16-week exposure period (Ichnose and Sagai, 1982, Ichnose et al., 1983). Based on their body of work and other related studies, Sagai et al. (1984) suggested that the increased lipid peroxidation may be related to NO\(_2\)-induced thickening of alveolar walls, as reported in some lung morphology studies, and decreased O\(_2\) tension in arterial blood.

An increase in lung lipid peroxidation products has also been reported in pregnant rats exposed to 1,000 \( \mu g/m^3 \) or 10,000 \( \mu g/m^3 \) (0.53 or 5.3 ppm) NO\(_2\), 5 h/day for 21 days (Balabaeva and Tabakova, 1985). When the pregnant progeny of this group were exposed to the same NO\(_2\) exposure regimen, there was an exposure-related increase in the lung lipid peroxides. Nitrogen dioxide exposure was also reported to have an effect on lipid peroxidation in the liver in both pregnant and nonpregnant rats, and in the placenta. The findings are discussed later in Section 13.2.3.7.

Arner and Rhoades (1973) exposed rats for 9 mo to 5,450 \( \mu g/m^3 \) (2.9 ppm) NO\(_2\) for 24 h/day, 5 days/week. The lung wet weight increased by 13% compared to that of controls. The lipid content of the lung was significantly depressed by about 9%. The total saturated fatty acid content of the lungs was decreased. The largest decrease was seen in the phosphatidylethanolamine. Smaller decreases were seen in lecithin (phosphatidylcholine), phosphatidylinositol, and phosphatidylserine. Values for specific unsaturated fatty acids of biological importance were not reported. The lung surface tension extracts were reported as

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13-60
increased. The authors suggested that the increased surface tension corresponded to a decrease in the lung surfactant concentration.

Total lecithin was reduced in lung lavage fluid from beagle dogs with NO₂-induced intraalveolar edema (Dowell et al., 1971). Individual dogs were exposed for 1 h to 13,160 to 30,080 μg/m³ (7 to 16 ppm) NO₂. There was a decrease in total phospholipids, as compared to neutral lipids, in animals with intraalveolar edema. In animals without intraalveolar edema, exposed to 5,640 to 22,560 μg/m³ (3 to 12 ppm) NO₂ for 1 h, the phospholipid content was slightly greater than in control animals. There was also an increase in the amount of unsaturated fatty acids in the phospholipids from the lungs of animals exposed to 9,400 to 30,080 μg/m³ (5 to 16 ppm) NO₂ whether or not intraalveolar edema was present. These changes were not noted in the animals exposed to 5,640 μg/m³ NO₂. The physiological effects of NO₂ exposure in these animals is discussed in Section 13.2.2.4 on lung morphology.

Lecithin synthesis has also been reported depressed in the lungs of rabbits exposed to 1,880 μg/m³ (1.0 ppm) NO₂ for 2 weeks (Seto et al., 1975). The most marked effect was observed after 1 week of exposure and appeared to decline after the second week of exposure. Yokoyama et al. (1980) found few changes in lipid metabolism of rats exposed for 3 h/day for 7 and 14 days to 10,300 μg/m³ (5.5 ppm) NO₂. Lysolecithin acyltransferase activity in the microsomal fraction decreased when an unsaturated acid (linoleic) was used, but not when a saturated acid (palmitic) was the substrate. The supernatant fraction of this enzyme was unchanged, phospholipase A₁ and A₂ activities were not affected either.

Products of arachidonic acid metabolism are also affected by NO₂. The concentration of thromboxane B₂ was elevated in the BAL fluid from rabbits exposed to 1,880 μg/m³ (1.0 ppm) NO₂ for 2 h (Schlesinger et al., 1990). When exposure was increased to 5,640 μg/m³ (3.0 ppm), the concentration of thromboxane B₂ was depressed. Thromboxane B₂ levels were significantly below those of controls 24 h postexposure in rabbits exposed to 18,880 μg/m³ (10 ppm) NO₂. 6-Keto-prostaglandin F₁α was also depressed in rabbits exposed to 18,880 μg/m³ NO₂. Prostaglandins E₂ and F₂ and LTB₄ were not affected.

No effects on BAL lipid and protein content were observed in guinea pigs exposed to 752, 1,880, 5,460, or 9,400 μg/m³ (0.4, 1.0, 3.0, or 5.0 ppm) NO₂ for 72 h (Selgrade et al., 1981). However, vitamin C-depleted guinea pigs, having an average of 25% of the
normal blood vitamin C content, had greater BAL protein and lipid content, except for those guinea pigs exposed to 752 μg/m³ NO₂. In animals exposed to 9,400 μg/m³ NO₂, the changes in BAL fluid composition were correlated with mortality (50%) and alveolar edema as determined by conventional light microscopy.

Effects on Lung Amino Acids, Proteins, and Enzymes

Table 13-6 summarizes the effects of NO₂ on proteins and selected enzymes. Nitrogen dioxide can oxidize various reducible amino acids or side chains of proteins in aqueous solution (Freeman and Mudd, 1981). Suzuki et al. (1988) reported increased amounts of tryptophan metabolites in the urine of rats exposed for 2 weeks to 9,400 μg/m³ (50 ppm) NO₂. Concentrations of NO₂ above 9,400 μg/m³ produce lung edema with concomitant infiltration of serum protein and enzymes. Also, an influx of inflammatory cells (predominantly leukocytes) from blood and alterations in the epithelial cell types of the lung may occur. Thus, some reports of changes in lung enzyme activity and protein content may reflect either edema, altered inflammatory cell populations, and/or changes in cell types, rather than direct effects of NO₂ on lung cell enzymes.

As indicated earlier in this section, Saga! et al. (1984) reported a concentration-related increase in TBA reactants in the lungs of rats exposed to 75, 752, and 7,520 μg/m³ (0.04, 0.4, and 4.0 ppm) NO₂ continuously for 18 mo. However, total lung protein content was not affected by the NO₂ exposure. Gelzleichter et al. (1992a) investigated the effect of C × T on total BAL protein, PMNs, and epithelial cells in rats. Experimental animals were exposed to 6,770, 13,500, 20,300, or 27,100 μg/m³ (3.6, 7.2, 10.8, or 14.4 ppm) NO₂ for 24, 12, 8, or 6 h, respectively, for 3 consecutive days. The cumulative C × T was 259 2 ppm-h. Concentrations ≥13,500 μg/m³ increased BAL protein to a roughly equivalent extent. The 24-h exposure to 6,770 μg/m³ caused no effects. Epithelial cell increases followed a pattern similar to that of protein, but PMNs were only increased at the highest concentration.

Nitrogen dioxide has also been reported to increase the protein content of lung lavage in vitamin C-depleted guinea pigs (Selgrade et al., 1981, Sherwin and Carlson, 1973, Hatch et al., 1986, Slade et al., 1989). Selgrade et al. (1981) found effects as low as 1,880 μg/m³.
**TABLE 13-6. EFFECTS OF NITROGEN DIOXIDE ON LUNG AMINO ACIDS, PROTEINS, AND ENZYMES**

<table>
<thead>
<tr>
<th>NO$_2$ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu g/m^3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>72 h</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig</td>
<td>No effect at 0.4 ppm, increase in BAL protein in vitamin C-depleted, but not normal, animals at 1.0 ppm and above</td>
<td>Selgrade et al (1981)</td>
</tr>
<tr>
<td>1,880</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td>Increased BAL protein in vitamin C-depleted guinea pigs 15 h postexposure</td>
<td></td>
</tr>
<tr>
<td>5,640</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td>No effect on BAL protein</td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>5.0</td>
<td>3 h</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>Continuous, 1 week</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Increased lung protein content of guinea pigs with an unquantified vitamin C deficiency</td>
<td>Sherwin and Carlson (1973)</td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>Continuous, 1 week</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig</td>
<td>Complex concentration and duration dependence of effects Example at 0.4 ppm, cytochrome P-450 levels decreased at 2 weeks, returned to control level by 5 weeks At 1.2 ppm, cytochrome P-450 levels decreased initially, increased at 5 weeks, and decreased at 10 weeks Effects on succinate-cytochrome c reductase also</td>
<td>Takahashi et al (1986)</td>
</tr>
<tr>
<td>2,256</td>
<td>1.2</td>
<td>1 to 14 weeks</td>
<td>M</td>
<td>22-24 weeks</td>
<td>Rat (Wistar)</td>
<td>Decrease in cytochrome P-450 levels at 1.2 ppm</td>
<td>Mochitate et al (1984)</td>
</tr>
<tr>
<td>7,520</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td>Mouse (Swiss Webster)</td>
<td>No changes in lung serotonin levels, increase in brain serotonin and 5-hydroxyindoleacetic acid content</td>
<td>Sherwin et al (1986)</td>
</tr>
<tr>
<td>845</td>
<td>0.45</td>
<td>7 h/day, 4 weeks</td>
<td>M</td>
<td>NS</td>
<td>Mouse (Swiss Webster)</td>
<td>Increased content of serum proteins in homogenized whole lung tissue</td>
<td>Sherwin and Layfield (1976)</td>
</tr>
<tr>
<td>935</td>
<td>0.47</td>
<td>Continuous, 10, 12, 14 days</td>
<td>M</td>
<td>NS</td>
<td>Mouse (NS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Effects</td>
<td>Reference</td>
<td></td>
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</tr>
<tr>
<td>940 μg/m³, 0.5 ppm</td>
<td>6 h/day, 5 days/week, 4 weeks</td>
<td>M NS</td>
<td>Rat (Fischer 344)</td>
<td>0.5 ppm increase in urinary hydroxylysine output starting during Week 1, BAL hydroxylysine level, angiotensin-converting enzyme level, and BAL protein content unchanged</td>
<td>Evans et al (1989)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880 10</td>
<td>6 h/day</td>
<td>M/F 14-16 weeks</td>
<td>Rat (Fischer 344)</td>
<td>Changes in BAL and tissue levels of enzymes early in exposure, resolved by 15 weeks</td>
<td>Gregory et al (1983)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400 base</td>
<td>5 days/week, up to 15 weeks</td>
<td>M/F 50 base</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880 peak</td>
<td>5 days/week, 21 5-h peaks/day, up to 15 weeks</td>
<td>M/F 50 peak</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3,760 20</td>
<td>Continuous, 1-3 weeks</td>
<td>M NS</td>
<td>Guinea pig (NS)</td>
<td>Increase in number of LDH-positive cells with time of exposure Suggests Type 1 cells decrease as Type 2 cells increase</td>
<td>Sherwin et al (1973)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,760 20</td>
<td>14 days</td>
<td>M 12-24 weeks</td>
<td>Rat (Wistar)</td>
<td>Increased activity of lung glycolytic enzymes</td>
<td>Mochitate et al (1985)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,500 40</td>
<td>10 days</td>
<td>M 7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5,640 30</td>
<td>7 days</td>
<td>M/F 8 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>Various changes in lung homogenate protein and DNA content and enzyme activities, changes more severe in vitamin E-deficient rats</td>
<td>Elsayed and Mustafa (1982)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age (Strain)</td>
<td>Species</td>
<td>Effects</td>
<td>Reference</td>
</tr>
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</tr>
<tr>
<td>6,770 3 6 24 h M 10-12 Rat</td>
<td>24 h</td>
<td>Increased BAL protein at ≥ 7 2 ppm</td>
<td>Gelzleichter et al (1992a)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>13,500 7 2 12 h M 10-12 Rat</td>
<td>7 days</td>
<td>Initial decrease in lung protein content followed by an increase, changes in microsomal enzyme activities</td>
<td>Mochtate et al (1984)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20,300 10 8 8 h M 21-24 Rat</td>
<td>10 days</td>
<td>Increased gamma-glutamyl transferase on Days 14 and 21, no consistent effect on alkaline phosphatase, LDH, or total protein</td>
<td>Hoofman et al (1988)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>27,100 14 4 6 h M 21-24 Rat</td>
<td>45 days</td>
<td>No significant changes in lung homogenate parameters</td>
<td>Mustafa et al (1984)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,520 4 0 10 days M 21-24 Rat</td>
<td>16 h</td>
<td>Increased lung wet weight, alterations in lung antioxidant levels in vitamin C-deficient animals</td>
<td>Hatch et al (1986)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800 10 7 days M 21-24 Rat</td>
<td>3 h</td>
<td>Increased lung lavage fluid protein content in vitamin C-deficient animals</td>
<td>Csallany (1975)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400 5 0 14-72 h F NS Mouse</td>
<td>2 weeks</td>
<td>Increased amounts of the tryptophan metabolites and xanthurenic and kynurenic acids excreted in urine during Week 2 of exposure, but had returned to normal levels by Week 4</td>
<td>Suzuki et al (1988)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>9,400 5 0 6 h/day, 6 days NS 4-6 weeks Mouse</td>
<td>5 weeks</td>
<td>Modest increase in albumin in BAL no effect on LDH or lysosomal enzyme peroxidase</td>
<td>Rose et al (1989)</td>
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</tr>
</tbody>
</table>
TABLE 13-6 (cont'd). EFFECTS OF NITROGEN DIOXIDE ON LUNG AMINO ACIDS, PROTEINS, AND ENZYMES

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/m³</td>
<td>ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400-47,000</td>
<td>5 0-25</td>
<td>Continuous, 7 days</td>
<td>M</td>
<td>10-11 weeks</td>
<td>Rat (Sprague-Dawley)</td>
</tr>
<tr>
<td>9,400</td>
<td>5 0</td>
<td>3 h</td>
<td>NS</td>
<td>Rabbit (New Zealand)</td>
<td>Benzo[a]pyrene hydroxylase activity of tracheal mucosa not affected</td>
</tr>
<tr>
<td>37,600 94,000</td>
<td>20 50</td>
<td>Continuous, 1, 3, or 7 days</td>
<td>M</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
</tr>
<tr>
<td>15,000</td>
<td>8 0</td>
<td>Continuous, 14 days</td>
<td>F (NS)</td>
<td>Mouse (NS)</td>
<td>Increase in lung protein</td>
</tr>
<tr>
<td>17,900 18,800</td>
<td>9 5 10</td>
<td>7 h/day, 5 days/week, 6 mo</td>
<td>M</td>
<td>in utero and 6 mo</td>
<td>Rat (Fischer 344)</td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>Continuous, 14 days</td>
<td>M</td>
<td>8 weeks</td>
<td>Rat (Wistar)</td>
</tr>
<tr>
<td>18,800 37,600 56,400 75,200</td>
<td>10 20 30 40</td>
<td>4 h</td>
<td>M</td>
<td>NS</td>
<td>Rat (Long Evans)</td>
</tr>
</tbody>
</table>

NS = Not stated
LTB₄ = Leukotriene B₄
LDH = Lactate dehydrogenase
M = Male
BAL = Bronchialveolar lavage
F = Female
(1.0 ppm) after a 72-h exposure, but not after a 1-week exposure to 752 \( \mu g/m^3 \) (0.4 ppm). The results of the 1-week exposure apparently conflict with those of Sherwin and Carlson (1973), who found increased protein content of lavage fluid from vitamin C-deficient guinea pigs exposed to 752 \( \mu g/m^3 \) NO\(_2\) for 1 week. Differences in exposure techniques, protein measurement methods, and/or degree of vitamin C deficiency may explain the difference between the two studies. However, Sherwin and Carlson (1973) also reported increases in lavage fluid protein from normal guinea pigs exposed to 752 \( \mu g/m^3 \) (0.4 ppm) NO\(_2\) continuously over a 1-week period.

Hatch et al. (1986) found that the NO\(_2\)-induced increase in protein in lung lavage fluid in vitamin C-deficient guinea pigs was accompanied by an increase in lung content of nonprotein sulfhydryls and vitamin C and a decrease in vitamin E content. The increased susceptibility to NO\(_2\) was observed when lung vitamin C was reduced (by diet) to levels below 50% of normal values. A depletion of nonprotein sulfhydryls also enhances susceptibility to a high level of NO\(_2\) exposure (18,800 \( \mu g/m^3 \), 10 ppm) (Slade et al., 1989). Selgrade et al. (1981) expanded earlier studies of Sherwin and Carlson (1973) on the effects of vitamin C deficiency on NO\(_2\) toxicity. Taken together, these investigations support a role for dietary vitamin C in influencing the susceptibility of NO\(_2\)-exposed animals to increased protein and lipids in lung lavage. Because vitamin C is readily oxidized and reduced, it could serve to detoxify oxidative products formed by NO\(_2\) or to maintain the intracellular redox potential.

Utell et al. (1991) and Frampton et al. (1989) reported no significant changes in the content of total protein, albumin, or \( \alpha_2 \)-macroglobulin, a glycoprotein that may play a role in the local control of lung protease activity, of BAL fluid from healthy, nonsmoking volunteers exposed to 94 \( \mu g/m^3 \) (0.05 ppm) NO\(_2\) with three 15-min peak exposures to 3,760 \( \mu g/m^3 \) (2.0 ppm), to 1,128 \( \mu g/m^3 \) (0.6 ppm) continuously, or to 2,820 \( \mu g/m^3 \) (1.5 ppm) continuously. All NO\(_2\) exposures were for 3 h, and BAL fluid was obtained at 3.5 h (94-\( \mu g/m^3 \) + 3,760 \( \mu g/m^3 \) peaks and 2,820-\( \mu g/m^3 \) groups) or 18 h (1,128 \( \mu g/m^3 \)-group) postexposure. When BAL was performed 3.5 h after exposure to 1,128 \( \mu g/m^3 \) NO\(_2\), there was an increase in \( \alpha_2 \)-macroglobulin that was not seen under the other exposure regimes. Whether this increase in \( \alpha_2 \)-macroglobulin was indicative of an NO\(_2\)-induced change in the
protease-antiprotease balance or was a chance observation is not known (Frampton et al., 1989). (See Section 15.6 on clinical studies for details)

A major concern has been the effect of NO2 on the structural proteins of the lungs because elastic recoil is lost after exposure. Kosmider et al. (1973a) reported that the urinary hydroxyproline and acid mucopolysaccharide content of guinea pigs exposed to 1,880 μg/m3 (1.0 ppm) NO2 for 6 mo were significantly increased. Because the remodeling of bone is the major source (>90%) of urinary hydroxyproline in normal animals and dietary ascorbate status would affect hydroxyproline homeostasis, the significance of these observations to lung structure and function remains to be shown.

Because hydroxylysine, a modified amino acid, is unique to collagen and proteins containing collagen-like sequences, Evans et al. (1989) selected this compound for study as a biomarker of lung injury. During a series of experiments, rats were exposed to 1,880 to 56,400 μg/m3 (1 to 30 ppm) NO2, 6 h/day for 2 days. A concentration-dependent relationship was noted in the amount of hydroxylysine in the BAL fluid and the urinary hydroxylysine output. The increases in hydroxylysine were, however, only significant at NO2 exposure levels of ≥14,100 μg/m3 (7.5 ppm) for BAL fluid and ≥28,200 μg/m3 (15 ppm) for urinary output. The angiotensin-converting enzyme level and the total protein concentration of BAL fluid were significantly increased in the highest NO2-concentration groups.

When rats were exposed to 940 or 1,880 μg/m3 (0.5 or 1.0 ppm) NO2, 6 h/day, 5 days/week for 4 weeks, there was a gradual increase in urinary hydroxylysine output that became significant the week after exposure ended in the 1,880 μg/m3 exposed group, but was significant in the 940 μg/m3 exposed group starting during the first week of exposure (Evans et al., 1989). The amount of hydroxylysine in the BAL fluid of rats in the 1,880 μg/m3 group was significantly lower than that of controls immediately following exposure and remained significantly lower after a 4-week recovery period, whereas the angiotensin-converting enzyme level was significantly increased, returning to normal values 4 weeks after exposure ended. The hydroxylysine content of lavage fluid and the angiotensin-converting enzyme level were unchanged in the 940-μg/m3 exposed group. The total protein content of the BAL fluid was not significantly altered at either exposure level.

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Last and co-workers have examined the effects of NO₂ on collagen synthesis rates in lung minces from animals exposed in vivo for 7 days. In one study (Last et al., 1983), rats were continuously exposed to 9,400 to 47,000 μg/m³ (5 to 25 ppm) NO₂ for 7 days. The authors found a linear concentration-response curve for plots of collagen synthesis rate, with a correlation coefficient (least squares analysis) for fit of the data to a straight line of 0.92. Linear extrapolation of the line to an estimated no-observable-effect level gave a value of about 1,880 to 5,640 μg/m³ (1.0 to 3.0 ppm) NO₂. Last and Warren (1987) confirmed that exposure to 9,400 μg/m³ NO₂ increased collagen synthesis. It was assumed by these workers, although not proven, that the increases in lung collagen synthesis rate observed after acute exposure regimens are predictive of increases in total lung collagen (pulmonary fibrosis) after longer periods of exposure.

Only a modest increase in albumin, indicating a mild degree of injury to the pulmonary capillary membrane, was noted in mice exposed to 9,400 μg/m³ (5.0 ppm) NO₂, 6 h/day for 6 days or 9,400 μg/m³, 6 h/day for 2 days prior to viral inoculation and 6 h/day for 4 days immediately following inoculation (Rose et al., 1989). However, only minimal histopathological changes were noted in the NO₂-exposed, viral-inoculated animals. Lysosomal enzyme peroxidase and lactate dehydrogenase (LDH) activities were not affected. See also Section 13221 on host defense mechanisms.

Sherwin et al. (1972) exposed guinea pigs to 3,760 μg/m³ (2.0 ppm) NO₂ for 1, 2, or 3 weeks. They examined lung sections histochemically for LDH. With this technique, LDH is thought to be primarily an indicator of Type 2 cells rather than Type 1 cells. The number of Type 2 cells per alveolus was determined. In control lung sections, a mean value of 1.9 Type 2 cells per alveolus was found, with a range of 1.5 to 3.4. Exposure to NO₂ significantly increased the LDH content of the lower lobes of the lung by increasing the number of Type 2 cells per alveolus. The increase was progressive over the 3-week exposure period. The authors suggested that the increase in lung LDH content was due to the replacement of Type 1 cells by Type 2 cells, as shown in some of the morphological studies.

An increase in LDH in BAL fluid was reported in rats exposed to 1,880 to 9,400 μg/m³ (1.0 to 5.0 ppm) NO₂, 7 h/day, 5 days/week for 27 weeks (Gregory et al., 1983). By 15 weeks of exposure, LDH had returned to control values, even though
histological changes persisted. A baseline (1,880 µg/m³ [1.0 ppm]) plus peak (two 1-h peaks to 9,400 µg/m³ [5.0 ppm]) exposure had no effects. Alkaline phosphatase and LDH activities, as well as collagenous peptides were increased in BAL fluid of chronically exposed rats (17,900 µg/m³, 9.5 ppm, 7 h/day, 5 days/week, 24 mo) (Mauderly et al., 1990).

Glycolytic pathways are also increased by NO₂ exposure, apparently due to an increase in Type 2 cells (Mochitate et al., 1985). The most sensitive enzyme was pyruvate kinase. After a 14-day exposure to 3,760 µg/m³ (2.0 ppm) NO₂, the activity of this enzyme was increased. When the exposure concentration was increased to 7,520 and 18,800 µg/m³ (4 and 10 ppm), the pyruvate kinase activity was increased by Day 4 and 7, respectively.

Alterations in lung xenobiotic metabolism follow a complex pattern based on exposure duration and concentration in rats exposed to 752, 2,260, or 7,520 µg/m³ (0.4, 1.2, or 4.0 ppm) NO₂ (Takahashi et al., 1986). At 752 µg/m³, cytochrome P-450 levels had decreased by the second week of exposure, but returned to normal levels by the fifth week of exposure, where they remained at Week 10. An initial decrease in cytochrome P-450 was also seen in animals exposed to 2,260 µg/m³ NO₂, cytochrome P-450 levels returned to control level by Week 5 and decreased below control levels by Week 10. A similar pattern of response occurred in the highest concentration tested. Only 7,520 µg/m³ affected other microsomal electron-transport systems. The activity of succinate-cytochrome c reductase was decreased by the fourteenth week of exposure to 752 µg/m³ and even sooner at higher levels of exposure. Mochitate et al. (1984) also found a decrease in cytochrome P-450 levels after a 7-day exposure of rats to ≥2,260 µg/m³ NO₂.

The activity of benzo[a]pyrene hydroxylase in the tracheobronchial region of the lungs of rabbits exposed to 9,400, 37,600, or 94,000 µg/m³ (5, 20, or 50 ppm) NO₂ for 3 h was studied by Palmer et al. (1972). No effect was observed. Law et al. (1975) studied the effect of NO₂ on benzo[a]pyrene hydroxylase, microsomal O-methyl transferase, catechol O-methyl transferase, and supernatant catechol O-methyl transferase activity in rat lungs. Exposure to 75,200 or 132,000 µg/m³ (40 or 70 ppm) NO₂ for 2 h had no effects. Thus, the studies of Palmer et al. (1972) and Law et al. (1975) agree that NO₂ exposure does not affect total benzo[a]pyrene hydroxylase activity of the lung. The O-methyl transferase activity studied by Law et al. (1975) relates to the ability of the lung to metabolize catecholamine hormones.
Effects on Antioxidant Metabolism and Influence of Antioxidants

Table 13-7 summarizes the effects of NO₂ on antioxidant metabolism and antioxidants.

Menzel (1970) proposed that antioxidants might protect the lung from NO₂ damage by inhibiting lipid peroxidation. Data related to this hypothesis have been reported by Ayaz and Csallany (1978), Csallany (1975), Fletcher and Tappel (1973), Menzel et al. (1972), Mohsenin (1991), Slade et al. (1989), and Thomas et al. (1968). Many laboratories have observed changes in the activity of enzymes in the lungs of NO₂-exposed animals that regulate levels of glutathione (GSH), the major water-soluble reductant in the lung’s armamentarium (Tyson et al., 1982), or in lung content of GSH in rodents exposed to NO₂.

Buthionine sulfoxime, an inhibitor of GSH synthesis, has also been shown to cause increased lung damage in mice exposed to 1,960 μg/m³ (1 0 ppm) O₃, suggesting a role for GSH as a protective agent against oxidant gases in vivo (Sun et al., 1988). Chow and Tappel (1972) proposed an enzymatic mechanism for the protection of the lung against lipid peroxidation damage by O₃, involving coupled reactions of glucose-6-phosphate dehydrogenase (G-6-P dehydrogenase) (to produce reduced nicotinamide-adenine dinucleotide phosphate [NADPH]), GSH reductase (to regenerate nicotinamide-adenine dinucleotide phosphate [NADP]), and GSH peroxidase (to regenerate GSH). Chow et al. (1974) exposed rats to 1,880, 4,330, or 11,600 μg/m³ (1 0, 2.3, or 6 2 ppm) NO₂ continuously for 4 days to examine the effect on the GSH peroxidase system by measuring the activities of GSH reductase, G-6-P dehydrogenase, and GSH peroxidase in the soluble fraction of exposed rat lungs. Linear regression analysis of the correlation between NO₂ concentrations and enzymatic activities showed a significant positive correlation coefficient of 0.63 for GSH reductase and of 0.84 for G-6-P dehydrogenase. No correlation was found between the GSH peroxidase activity and the NO₂ exposure concentration. The activities of GSH reductase and G-6-P dehydrogenase were significantly increased during exposure to 11,600 μg/m³ NO₂. The possible role of edema and cellular inflammation in these findings was not examined. These researchers concluded that because exposure of rats to NO₂ had an insignificant effect on lung GSH peroxidase activity, but did significantly increase the activities of GSH reductase and G-6-P dehydrogenase, it appears that this oxidant attacks mainly GSH and NADPH.
<table>
<thead>
<tr>
<th>(\text{NO}_2) Concentration</th>
<th>(\mu g/m^3)</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0.04</td>
<td>Continuous, M</td>
<td>8 weeks (Wistar)</td>
<td>NPSHs increased at (\geq 0.4) ppm after 9 or 18 mo, GSH peroxidase activity decreased at (0.4) ppm after 18 mo and at (4.0) ppm after 9 and 18 mo, GSH reductase activity increased after a 9 mo exposure to (4.0) ppm, no effects on 6-P-G dehydrogenase, SOD, or disulfide reductase, some GSH S-transferase had decreased activities after 18-mo exposure to (\geq 0.4) ppm</td>
<td>Sagai et al (1984)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>9 and 18 mo</td>
<td>(Wistar)</td>
<td>Duration-dependent pattern for increase in activities of antioxidant enzymes, increase, peaking at Week 4 and then decreasing Concentration-dependent effects</td>
<td>Ichnose and Sagai (1982)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880</td>
<td>8.2</td>
<td>4 days (Sprague-Dawley)</td>
<td>VitamIn E-supplement reduced lipid peroxidation</td>
<td>Thomas et al (1967)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880</td>
<td>0.5</td>
<td>4 weeks (C57Bl/6J)</td>
<td>At 1 ppm, GSH-peroxidase activity decreased in vitamin E-deficient mice, and increased in vitamin E-supplemented mice</td>
<td>Ayaz and Csallany (1978)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>4 weeks (C57Bl/6J)</td>
<td>At 1 ppm, GSH-peroxidase activity decreased in vitamin E-deficient mice, and increased in vitamin E-supplemented mice</td>
<td>Ayaz and Csallany (1978)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>752-940</td>
<td>0.4-0.5</td>
<td>Continuous, F</td>
<td>NS Mouse (NS)</td>
<td>Growth reduced, vitamin E (30 or 300 mg/kg diet) improved growth</td>
<td>Csallany (1975)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>Continuous, M</td>
<td>8 weeks (Sprague-Dawley)</td>
<td>Activities of GSH reductase and G-6-P dehydrogenase increased at 6.2 ppm proportional to duration of exposure, plasma lysozyme and GSH peroxidase not affected at 6.2 ppm, no effects at 1.0 or 2.3 ppm</td>
<td>Chow et al (1974)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>ppm Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Effects</td>
<td>Reference</td>
<td></td>
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<td>-------------------</td>
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<td></td>
</tr>
<tr>
<td>2,260</td>
<td>1 2 Continuous, M</td>
<td>12 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>Increases in G-6-P dehydrogenase, isocitrate dehydrogenase, disulfide reductase, and NADPH cytochrome c reductase activities at 1 8 ppm only</td>
<td>Lee et al (1989, 1990)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,380</td>
<td>1 8 3 days</td>
<td>M/F</td>
<td>5-&gt;60 days</td>
<td>Rat (Wistar)</td>
<td>Decreased SOD activity in 21-day-old animals</td>
<td>Azoulay-Dupuis et al (1983)</td>
<td></td>
</tr>
<tr>
<td>3,760</td>
<td>2 0 3 days</td>
<td>M/F</td>
<td>5-&gt;60 days</td>
<td>Rat (Wistar)</td>
<td>G-6-P dehydrogenase increased at ≥2 ppm, at 2 ppm, 14 days of exposure needed</td>
<td>Mochltake et al (1985)</td>
<td></td>
</tr>
<tr>
<td>7,500</td>
<td>4 0 10 days</td>
<td>M</td>
<td>12-24 weeks</td>
<td>Rat (Wistar)</td>
<td>Increased lipid peroxidation (TBA-reactive substances) with vitamin E deficiency</td>
<td>Sevanian et al (1982)</td>
<td></td>
</tr>
<tr>
<td>13,200</td>
<td>7 0 4 days</td>
<td>M</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>No effects on parameters tested</td>
<td>Mustafa et al (1979)</td>
<td></td>
</tr>
<tr>
<td>18,800</td>
<td>10 4 days</td>
<td></td>
<td></td>
<td></td>
<td>Increase in lung weight, G-6-P dehydrogenase, GSH reductase, and GSH peroxidase activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28,200</td>
<td>15 1-7 days</td>
<td></td>
<td></td>
<td></td>
<td>Increase in lung weight, DNA content, G-6-P dehydrogenase, 6-P-G dehydrogenase, and GSH reductase activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increase in lung weight, DNA content, G-6-P dehydrogenase, 6-P-G dehydrogenase, and GSH reductase activities, no effect on lung protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age (Strain)</td>
<td>Effects</td>
<td>Reference</td>
<td></td>
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<tr>
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</tr>
<tr>
<td>7,520 ppm</td>
<td>3 h</td>
<td>M/F</td>
<td>21-33 years</td>
<td>Decreased elastase inhibitory capacity and increased lipid peroxidation products in BAL of subjects not administered supplement of vitamin C and E prior to NO₂ exposure</td>
<td>Mohsenin (1991)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11,000 ppm</td>
<td>4 h/day,</td>
<td>F</td>
<td>NS</td>
<td>Increase in GSH reductase and G-6-P dehydrogenase activities</td>
<td>Csallany (1975)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28,000 ppm</td>
<td>7 days</td>
<td></td>
<td></td>
<td>Increase in GSH levels, and GSH reductase, G-6-P dehydrogenase, and GSH peroxidase activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53,000 ppm</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17,900 ppm</td>
<td>7 h/day, 5 days/week, 6 mo</td>
<td>M</td>
<td>in utero and 6 mo</td>
<td>Increase in GSH reductase activity in younger rats and GSH peroxidase activity in older rats</td>
<td>Mauderly et al (1987)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17,900 ppm</td>
<td>7 h/day, 5 days/week, 24 mo</td>
<td>M</td>
<td>18 weeks</td>
<td>Increase in GSH reductase activity in BAL</td>
<td>Mauderly et al (1990)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[^]M = Male

NPSHS = Nonprotein sulfhydryls

GSH = Glutathione

G-6-P dehydrogenase = Glucose-6-phosphate dehydrogenase

6-P-G dehydrogenase = 6-phosphogluconate dehydrogenase

SOD = Superoxide dismutase

F = Female

NS = Not stated

NADPH = Nicotinamide-adenine dinucleotide phosphate

ICD = Isocitrate dehydrogenase

NADPH = Nicotinamide-adenine dinucleotide phosphate (reduced form)

TBA = Thiobarbituric acid
whereas $O_3$ not only initiates lipid peroxidation, but also directly attacks these reducing substances.

Nitrogen dioxide effects on antioxidant metabolism appears to follow a concentration-and exposure-duration response. Lee et al. (1990) reported that G-6-P dehydrogenase, 6-phosphogluconate dehydrogenase (6-P-G dehydrogenase), and NADP-specific isocitrate dehydrogenase were not affected after rats were exposed to 2,260 $\mu$g/m$^3$ (1.2 ppm) NO$_2$ continuously for 3 days. When NO$_2$ exposure was increased from 3 days to 16 weeks, there was an increase in GSH peroxidase, GSH reductase, G-6-P dehydrogenase, 6-P-G dehydrogenase, SOD, and disulfide reductase activities in rats from the first week of exposure that reached a maximum by the fourth week of exposure and thereafter gradually declined over the 16-week exposure period (Ichnose et al., 1983). The increase in antioxidant activity was exposure-dependent over the exposure range of 75 to 7,520 $\mu$g/m$^3$ (0.04 to 4.0 ppm) NO$_2$.

Sagai et al. (1984) and Ichnose et al. (1983) studied the effects of prolonged (9 and 18 mo) exposures to 75, 752, and 7,520 $\mu$g/m$^3$ (0.04, 0.4, and 4.0 ppm) NO$_2$ on rats. Nonprotein sulfhydryl levels were increased in the 752- and 7,520-$\mu$g/m$^3$-exposed groups after both exposure durations. Nine- and 18-mo exposures to 7,520 $\mu$g/m$^3$ caused a decrease in the activity of GSH peroxidase and an increase in G-6-P dehydrogenase activity. Glutathione peroxidase activity was also decreased in rats exposed to 752 $\mu$g/m$^3$ NO$_2$ for 18 mo. Three GSH S-transferases were also studied, two of which (aryl S-transferase and aralkyl S-transferase) exhibited decreased activities after 18 mo of exposure to 752 and 7,520 $\mu$g/m$^3$ NO$_2$. No effects were observed on the activities of 6-P-G dehydrogenase, SOD, or disulfide reductase. The decreases in antioxidant metabolism were inversely related to the formation of lipid peroxides (see previous subsection on lipid metabolism). Shorter exposures (4 mo) to NO$_2$ between 752 and 7,520 $\mu$g/m$^3$ also cause concentration- and duration-dependent effects on antioxidant enzyme activities (Ichnose and Sagai, 1982). For example, G-6-P dehydrogenase activity increased, reaching a peak at 1 mo, and then decreased towards control. Brefer (2-week) exposures to 752 $\mu$g/m$^3$ NO$_2$ caused no such effects in rats or guinea pigs (Ichnose and Sagai, 1989).

Age susceptibility to the effects of NO$_2$ (17,900 $\mu$g/m$^3$, 9.5 ppm, 7 h/day, 5 days/week) was examined by Mauderly et al. (1987). Rats were exposed for 6 mo,
beginning in utero or at 6 mo of age. In the older rats, only GSH peroxidase was increased, whereas in the younger rats only GSH reductase was increased.

Malnutrition of animals can drastically affect their response to toxicants, including NO₂. Experimental interest in this area has mainly focused on dietary lipids, vitamin E and other lipid-soluble antioxidants, and vitamin C and other water-soluble antioxidants. For example, Sevaman et al. (1982) reported an increase in the amount of TBA reactants in lung homogenate of vitamin E-deficient rats after 7 days of exposure to 5,640 μg/m³ (3.0 ppm) NO₂. Ayaz and Csallany (1978) exposed weanling mice continuously for 17 mo to 940 or 1,880 μg/m³ (0.5 or 1.0 ppm) NO₂ and fed the animals a basal diet that was either deficient in vitamin E or supplemented with 30 or 300 mg/kg of diet. Blood, lung, and liver tissues were assayed for GSH peroxidase activity. Exposure to 1,880 μg/m³ NO₂ suppressed GSH peroxidase activity in the blood and lungs. A combination of vitamin E deficiency and 1,880 μg/m³ NO₂ exposure resulted in the lowest GSH peroxidase activity in blood and lung. Liver GSH peroxidase activity was unaffected by either vitamin E deficiency or NO₂ exposure. Other studies (Hatch et al., 1986, Selgrade et al., 1981, Slade et al., 1989, Sherwin and Carlson, 1973) have also shown that vitamin C deficiency increases susceptibility to NO₂-induced increases in BAL protein.

Summary

Studies on the biochemical effects of NO₂ on the lung have focused on the mechanisms of the toxic action or indicators of tissue and cell damage. One theory describing the toxic action of NO₂ is that of lipid peroxidation of unsaturated fatty acids in target cell membranes (Menzel, 1976). An alternate theory is that NO₂ oxidizes water-soluble low molecular weight reducing substances and proteins (Freeman and Mudd, 1981). Studies show that regardless of the toxic action, many of the effects are concentration- or exposure duration-dependent.

Exposure to 75 μg/m³ (0.04 ppm) NO₂ increased lipid peroxidation (as indicated by increased ethane exhalation) in the lungs of rats exposed for 9 mo or longer. Ethane exhalation was also increased in rats exposed to 752 μg/m³ for 6 mo, but not in rats exposed to 75 or 225 μg/m³ (0.04 or 0.12 ppm) over the same time period (Sagai et al., 1984, Ichinose et al., 1983). Increases in lipid peroxidation products have also been reported in
healthy, nonsmoking humans exposed to 7,520 μg/m³ (4.0 ppm) NO₂ for 3 h (Mohsenin, 1991)

Increases in lavage fluid and urinary levels of hydroxyllysine were found in rats exposed to 1,880 to 56,400 μg/m³ (1 to 30 ppm) NO₂, 6 h/day for 2 days. The increases were, however, only significant at NO₂ levels ≥14,100 μg/m³ (7.5 ppm) for lavage fluid and ≥28,200 μg/m³ (15 ppm) for urinary output (Evans et al., 1989). Urinary secretion of hydroxyproline and acid mucopolysaccharides have been reported in guinea pigs exposed to 1,880 μg/m³ (10 ppm) NO₂ for 6 mo (Kosmider et al., 1973a). The significance of these observations to lung structure and function is unknown.

No changes in blood and lung GSH peroxidase activity were reported in mice exposed to 940 μg/m³ (0.5 ppm) NO₂ continuously for up to 17 mo, however, when the exposure concentration was increased to 1,880 μg/m³ (1.0 ppm), a suppression of GSH peroxidase activity was noted (Ayaz and Csallany, 1978). This enzyme activity was not affected in rats exposed continuously to up to 11,600 μg/m³ (6.2 ppm) NO₂ for 3 or 4 days (Chow et al., 1974, Lee et al., 1990), but was significantly decreased in rats exposed to 752 μg/m³ for 18 mo (Sagai et al., 1984, Ichinose et al., 1983).

Nitrogen dioxide effects on antioxidant metabolism appears to be both concentration- and exposure duration-dependent. No effect on G-6-P dehydrogenase, 6-P-G dehydrogenase, and NADP-specific isocitrate dehydrogenase activities was noted in rats exposed to 2,256 μg/m³ (1.2 ppm) NO₂ continuously for 3 days (Lee et al., 1990). However, an increase in GSH peroxidase, GSH reductase, G-6-P dehydrogenase, 6-P-G dehydrogenase, SOD, and disulfide reductase activities has been reported in rats exposed to 75 to 7,520 μg/m³ (0.04 to 4.0 ppm) NO₂ from the first week of exposure that reached a maximum by the fourth week of exposure and thereafter gradually declined over the 16 week exposure period (Ichinose et al., 1983). When exposure was increased to 9 to 18 mo, there was an increase in G-6-P dehydrogenase activity, but only in rats exposed to 7,520 μg/m³. No effects were observed on the activities of 6-P-G dehydrogenase, SOD, or disulfide reductase (Sagai et al., 1984, Ichinose et al., 1983).
13.2.2.3 Pulmonary Function

The key issues addressed by investigators evaluating the effects of NO₂ on pulmonary function in experimental animals were (1) the effects of low-level, long-term exposures to an urban pattern of NO₂, the lowest concentrations that stimulated respiratory reflexes and impaired gas exchange in the lung, and (2) differences in responses between very young and mature animals. Compared with humans, rats and hamsters used in experimental studies of NO₂ have very immature lungs at birth. Humans have approximately 50 million alveoli at birth, which multiply rapidly until age 3 years and slowly until about age 8 years, when alveolar development is complete. Growth continues until maturity, 16 to 18 years, through alveolar enlargement. Rats and hamsters are born with no true alveoli. Alveolar proliferation is most rapid between 4 and 30 days of age and is essentially complete by 40 days of age. Although hamster lungs have reached adult volumes and elasticity at 40 days of age, lung growth through alveolar enlargement continues in rats to 5 mo of age (Mauderly, 1989). Changes in pulmonary function parameters in the experimental animals from exposure NO₂ are shown in Table 13-8.

Nitrogen dioxide concentrations in urban areas are not constant, but consist of peak exposures superimposed on a relatively constant background level. Miller et al. (1987) evaluated this urban pattern of NO₂ exposure in mice using continuous 7 day/week, 23 h/day exposures to 376 μg/m³ (0.2 ppm) NO₂ with two daily (5 days/week) 1-h peak exposures to 1,500 μg/m³ (0.8 ppm) NO₂ for 32 and 52 weeks. Mice exposed to clean air and to the constant background concentration of 376 μg/m³ served as controls. Data from animals examined immediately and 30 days following both exposure regimens were combined for analysis because there was no statistical difference between the groups (i.e., immediately and 30 days postexposure). Most of the differences in pulmonary function were measured between groups exposed to background concentrations with diurnal peaks and those exposed to constant background NO₂ levels, although the same pattern of effects was found when comparing peak- and air-exposed animals. Both end-expiratory volume and vital capacity, the difference in lung volume between maximum inflation and deflation, were significantly lower in mice exposed to NO₂ with diurnal peaks than in mice exposed to the constant level of NO₂. Lung distensibility, measured as respiratory system compliance, also tended to be lower (p = 0.072) in mice exposed to diurnal peak exposures of NO₂ compared with
<table>
<thead>
<tr>
<th>NO$_2$ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>376</td>
<td>0.2</td>
<td>23 h/day, F</td>
<td>6-8 weeks</td>
<td>Mouse (CD-1)</td>
<td>Decreased vital capacity and respiratory system compliance following exposure to 0.2 ppm + 0 8-ppm peak compared with air-exposed and 0.2-ppm exposed rats</td>
<td>Miller et al (1987)</td>
<td></td>
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<tr>
<td>376 base + 0.2 base +</td>
<td></td>
<td>7 days/week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1,504 peak</td>
<td>0.8 peak</td>
<td>two 1-h peaks/day, 5 days/week, 32 and 52 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>750</td>
<td>0.4</td>
<td>1, 2, and 3 mo</td>
<td></td>
<td>Rat</td>
<td>Decreased heart rate following 1-mo exposure to 1.2 and 4.0 ppm, decreased body weight and PaO$_2$ following 3-mo exposure to 4.0 ppm NO$_2$</td>
<td>Suzuki et al (1981)</td>
<td></td>
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<tr>
<td>2,250</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,520</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>940 base + 0.5 base +</td>
<td></td>
<td>Continuous, M daily 1-h peaks for 1, 3, or 6 weeks</td>
<td>1 day and 7 weeks</td>
<td>Rat (Fischer 344)</td>
<td>Increased lung volume (at Weeks 3 and 6) and compliance (at Week 3) in neonates exposed to the two highest exposure levels. Decreased body weight and lung compliance in older rats following 6 weeks exposure to the highest concentration, older rats recovered by 3 weeks postexposure (younger rats not tested)</td>
<td>Stevens et al (1988)</td>
<td></td>
</tr>
<tr>
<td>2,820 peak</td>
<td>1.5 peak</td>
<td>with two daily 1-h peaks</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>1,880 base + 1.0 base +</td>
<td></td>
<td>60 peak</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>5,640 peak</td>
<td>3.0 peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,760 base + 2.0 base +</td>
<td></td>
<td>60 peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11,280 peak</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>6 h/day, M NS</td>
<td>5 days/week, 4 weeks</td>
<td>Rat (Fischer 344)</td>
<td>No effects 1 0 ppm, increase in vital capacity immediately following exposure and an increase in compliance 4 weeks postexposure at 0.5 ppm Functional changes associated with changes in mean linear intercept</td>
<td>Evans et al (1989)</td>
<td></td>
</tr>
<tr>
<td>1,880</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NO$_2$ Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age (Strain)</td>
<td>Species (Strain)</td>
<td>Effects</td>
<td>Reference</td>
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<tr>
<td>940 (base) + 2,820 (peak)</td>
<td>22 h/day, 7 days/week base + 6-h peak/day, 5 days/week, 1, 3, 12, 52, and 78 weeks</td>
<td>M</td>
<td>60 days Rat (Fischer 344)</td>
<td>Decreased AF$FE_{25}$ following 78 weeks of NO$_2$ exposure, frequency of breathing decreased throughout, with greatest decrease observed at 78 weeks</td>
<td>Tepper et al (1992)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880</td>
<td>Continuous, 493 days</td>
<td>M</td>
<td>NS Monkey (Squirrel)</td>
<td>Monkeys challenged with monkey-adapted influenza virus Minor NO$_2$-induced changes in tidal volume, minute volume, and respiration rate</td>
<td>Fenters et al (1973)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,760</td>
<td>8 h/day, 5 days/week, 8 weeks</td>
<td>M</td>
<td>8 weeks Hamster (Golden Syrian)</td>
<td>Increase in fixed lung volume, but no change in vital capacity or lung compliance following NO$_2$ exposures in both normal and elastase-treated animals</td>
<td>Lafuma et al (1987)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>30 min/day, twice/week for 7 weeks</td>
<td>M</td>
<td>NS Guinea pig (NS)</td>
<td>Last 5 weeks of NO$_2$ exposure followed by 10-min exposure to aerosolized albumin Increased dyspneic breathing during fourth through seventh week of NO$_2$-albumin exposure</td>
<td>Yoshida et al (1980b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>2 h, resting or exercising</td>
<td>F</td>
<td>2-9 years Dog (Beagle)</td>
<td>Statistically significant decrease in the ventilation equivalent for O$_2$ in exercising dogs</td>
<td>Klemman and Mautz (1991)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>Continuous, 2 mo</td>
<td>M</td>
<td>NS Monkey (Squirrel)</td>
<td>Monkeys challenged with monkey-adapted influenza virus Decreased tidal volume and increased respiratory rate</td>
<td>Henry et al (1970)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400</td>
<td>24 h</td>
<td>M</td>
<td>15-16 weeks Mouse (JCL ICR)</td>
<td>Concentration-related decrease in forced swimming and blood lactic acid immediately and 24 h following 4-min forced swim at 5 0 ppm</td>
<td>Suzuki et al (1982a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Effects</td>
<td>Reference</td>
<td></td>
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</tr>
<tr>
<td>9,400 µg/m³ 50 ppm</td>
<td>24 h</td>
<td>M</td>
<td>15-16 weeks</td>
<td>Mouse (JCL ICR)</td>
<td>Concentration-related increase in respiration rate, decrease in PaCO₂ at 5 ppm and in PaO₂ at ≥10 ppm</td>
<td>Suzuki et al (1982b)</td>
<td></td>
</tr>
<tr>
<td>18,800 µg/m³ 100 ppm</td>
<td>Continuous, 3-day to 50 ppm followed by 8 min to 100 ppm</td>
<td>F</td>
<td>NS</td>
<td>Mouse (CF-1)</td>
<td>The irritant response to 8-mm, 100-ppm NO₂ exposure, typified by increased respiratory rate and decreased tidal volume and minute volume, was lessened by preexposure to 50 ppm NO₂ for 3 days</td>
<td>McGrath and Smith (1984)</td>
<td></td>
</tr>
<tr>
<td>10,200 µg/m³ 54 ppm</td>
<td>3 h/day for 7, 14, or 30 days</td>
<td>M</td>
<td>7-9 weeks</td>
<td>Rat (Wistar)</td>
<td>Nonsignificant tendency toward increased lung volume at low inflation pressures at 30 days</td>
<td>Yokoyama et al (1980)</td>
<td></td>
</tr>
<tr>
<td>13,200-275,000 µg/m³ 70-146 ppm</td>
<td>1 h</td>
<td>M/F</td>
<td>5-10 weeks</td>
<td>Guinea pig (CRL COBS)</td>
<td>Concentration-related increase in sensitivity to inhaled histamine aerosols 10 min following NO₂ exposure, but not 2 and 19 h following exposure, concentration-related increase in respiratory rate 10 min following exposure and decrease in tidal volume 10 min and 2 and 19 h following exposure</td>
<td>Silbaugh et al (1981)</td>
<td></td>
</tr>
<tr>
<td>14,000 µg/m³ 75 ppm</td>
<td>2 h</td>
<td>NS</td>
<td>NS</td>
<td>Sheep (NS)</td>
<td>Increased pulmonary resistance immediately following 4-h exposure to 15 ppm NO₂, no consistent effects of exposure on airway reactivity to inhaled carbachol, arterial blood gases, and pulmonary and systematic hemodynamics</td>
<td>Abraham et al (1980)</td>
<td></td>
</tr>
<tr>
<td>28,000 µg/m³ 150 ppm</td>
<td>2 and 4 h</td>
<td>NS</td>
<td>NS</td>
<td>Sheep (NS)</td>
<td>Increased pulmonary resistance immediately following 4-h exposure to 15 ppm NO₂, no consistent effects of exposure on airway reactivity to inhaled carbachol, arterial blood gases, and pulmonary and systematic hemodynamics</td>
<td>Abraham et al (1980)</td>
<td></td>
</tr>
<tr>
<td>17,900 µg/m³ 95 ppm</td>
<td>7 h/day, 5 days/week, 24 mo</td>
<td>M</td>
<td>18 weeks</td>
<td>Rat (Fischer 344)</td>
<td>Increased lung volumes and lung compliance and decreased rate of forced exhalation in NO₂-exposed rats, no physiologically significant interaction between NO₂ and elastase-treatment</td>
<td>Mauderly et al (1990)</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 13-8 (cont’d). EFFECTS OF NITROGEN DIOXIDE ON PULMONARY FUNCTIONa

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>17,900 µg/m³</td>
<td>95</td>
<td>7 h/day, 5 days/week, 6 mo</td>
<td>M, in utero</td>
<td>Rat (Fischer 344)</td>
<td>No substantive effects of NO₂</td>
<td>Mauderly et al (1987)</td>
</tr>
<tr>
<td>18,800 µg/m³</td>
<td>10</td>
<td>2 h</td>
<td>M/F, NS</td>
<td>Monkey (Squirrel)</td>
<td>Monkeys challenged with Klebsiella pneumonia Decreased tidal volume that returned to normal or elevated levels within 24 h in most animals</td>
<td>Henry et al (1969)</td>
</tr>
<tr>
<td>28,200 µg/m³</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

aF = Female
PaO₂ = Arterial oxygen tension
M = Male
NS = Not stated
ΔFEF₂₅ = Change in forced expiratory flow at 25% of forced vital capacity
O₂ = Oxygen
PaCO₂ = Arterial carbon dioxide tension
constant NO₂ exposure. These changes suggest that up to 52 weeks of low-level NO₂ exposure with diurnal peaks produces some decrease in lung distensibility, resulting in decreased respiratory system compliance and vital capacity. Vital capacity was still decreased 30 days postexposure. Lung morphology (by light microscopy) in these mice showed no exposure-related lesions, however, the absence of observable morphologic lesions does not preclude the presence of subtle morphologic changes as discussed in Section 13.2.2.4. The decrease in lung distensibility measured in this study is consistent with the increase in vitro lung collagen synthesis rates measured in lung minces from rats exposed in vivo for 7 days to 9,400 to 47,000 µg/m³ (5 to 25 ppm) NO₂ (Last et al., 1983).

Tepper et al. (1992) exposed rats to 940 µg/m³ (0.5 ppm) NO₂, 22 h/day, 7 days/week, with a 6-h peak slowly rising to 2,820 µg/m³ (1.5 ppm) NO₂, 5 days/week for up to 78 weeks. Evaluations of pulmonary function conducted at 1, 3, 13, 52, and 78 weeks revealed a small, but statistically significant decrease in the frequency of breathing that was paralleled by a trend toward increased tidal volume, expiratory resistance, inspiratory and expiratory time. In general, these variables were most affected following 78 weeks of exposure. Also, at the 78-week evaluation, delta flow at 25% forced vital capacity was decreased. Taken together, these results might indicate airway obstruction, however, a more prudent conclusion would be that few, if any, significant functional effects were observed that might suggest degenerative lung disease.

Effects of exposure to diurnal peaks of NO₂ were also studied by Stevens et al. (1988). These investigators exposed 1-day- and 7-week-old rats to 940, 1,880, or 3,760 µg/m³ (0.5, 1.0, or 2.0 ppm) NO₂ with two daily 1-h peak exposures at three times the baseline concentration for 1, 3, and 7 weeks. The effects on rats beginning exposure at 1 day and 7 weeks of age were substantially different. In rats beginning exposure at 1 day of age, respiratory system compliance was increased following 3 weeks, but not 6 weeks of exposure to the 1,880 to 3,760 µg/m³ NO₂ baselines with peak exposures. In rats beginning exposure at 7 weeks of age, respiratory system compliance was decreased following 6 weeks of exposure to the two highest levels, and body weight was decreased following 3 and 6 weeks of exposure to the 3,760 µg/m³ baseline with peak exposures. The decreased compliance in the older rats similarly exposed to NO₂ was associated with morphological changes, particularly in the centriacinar alveolar region (Chang et al., 1986, Section 13.2.2.4). In the
older rats (younger rats not tested), pulmonary function changes returned to normal values by 3 weeks after exposure ceased

Mauderly et al. (1987) did not measure substantive differences in effects on pulmonary function parameters between rats exposed to 17,900 μg/m³ (9.5 ppm) NO₂, 7 h/day, 5 days/week either beginning exposure at 6 mo of age for 6 mo or beginning from the time of conception until 6 mo after birth. There was, however, a statistically significant increase in minute volume in the NO₂-exposed younger animals compared to air-exposed animals. The increase in minute volume was attributed to an increase in tidal volume. Because there were no changes in lung volumes, mechanics, or gas exchange, the increased minute volume apparently does not reflect a compensation for impaired lung function. No morphologic or morphometric differences were observed either. Differences in the observed effects of NO₂ between this study and that of Stevens et al. (1988) may possibly be attributed to differences in exposure schedules (7 h/day, 5 days/week versus 23 h/day with diurnal peaks), to differences in the stage of lung development at the time of sacrifice (alveolar expansion essentially complete at 6 mo, but still continuing at 13 weeks), or to some other factor.

In adult rats receiving a longer (24-mo) intermittent exposure to 17,900 μg/m³ (9.5 ppm) NO₂, lung volumes and compliance were increased (Mauderly et al., 1990). The percentage of forced vital capacity exhaled in 1 s was decreased. There were no structural correlates (light microscopy) to these changes.

Lung volumes and capacities were evaluated in anesthetized rats after exposure to 940 or 1,880 μg/m³ (0.5 or 1.0 ppm) NO₂, 6 h/day, 5 days/week for 4 weeks (Evans et al., 1989). No pulmonary function effects were noted in rats exposed to 1,880 μg/m³ NO₂ immediately after exposure ended or 4 weeks postexposure. There was, however, a significant increase in vital capacity at the end of the 4-week exposure period, and an increase in compliance following the 4-week recovery period in the 940-μg/m³-exposed group. The changes in the pulmonary function parameters were associated with changes in the mean linear intercept in the NO₂-exposed animals. Except for a nonsignificant trend for an increased lung volume at low inflation pressure, Yokoyama et al. (1980) observed no changes in ventilatory mechanics of rats exposed for 14 or 30 days (3 h/day) to 10,200 μg/m³ (5.4 ppm) NO₂. Histological changes were minor. A discussion of the...
biochemical changes and morphological findings in the lungs of these exposed animals appears in Sections 13.2.2.2 and 13.2.2.4.

Only minor changes in tidal volume, minute volume, and respiration rate were reported in squirrel monkeys exposed to 1,880 μg/m³ (1.0 ppm) NO₂ continuously for 493 days (16 mo) following viral challenge with monkey adapted influenza A/PR/8/34 virus (Fenters et al., 1973). These changes were not affected by the viral challenge, although the authors stated that slight emphysema and thickening bronchial and bronchiolar epithelium were noted in monkeys exposed to NO₂ and the influenza virus. When influenza challenge followed exposure to 9,400 μg/m³ (5.0 ppm) for 2 mo, there was a decrease in tidal volume and an increase in respiratory rate that had returned to normal values after 4 weeks (Henry et al., 1970).

Klemman and Mautz (1991) conducted pulmonary function studies on a group of beagle dogs exposed to 9,400 μg/m³ (5.0 ppm) NO₂ for 2 h while standing at rest or exercising. Resting dogs developed a shorter breathing time and a trend towards an increase in Vₑ and VO₂ compared to air-exposed animals, however, neither of these responses was significant. The only statistically significant effect in NO₂- and air-exposed exercising dogs was a decrease in the ventilation equivalent for oxygen (O₂). Other pulmonary function parameters examined included tidal volume, minute ventilation, ventilation equivalent for CO₂, pulmonary resistance, and dynamic compliance.

Lafuma et al. (1987) exposed 12-week-old hamsters to 3,760 μg/m³ (2.0 ppm) NO₂, 8 h/day, 5 days/week for 8 weeks. Half the animals had been pretreated intratracheally with elastase to produce a condition of experimental emphysema. Fixed lung volumes (20 cm water with 2.5% glutaraldehyde) were significantly higher in NO₂-exposed animals than in air-exposed controls, independent of elastase treatment. Vital capacity and pulmonary compliance were not affected by NO₂ exposure, however, morphometrically, the emphysematous lesion produced by elastase appeared to be aggravated by the NO₂ exposure. Mauderly et al. (1990) exposed rats to higher concentrations of NO₂ (17,900 μg/m³, 9.5 ppm) for longer times (7 h/day, 5 days/week for 24 mo) and also found no increased susceptibility, related to pulmonary function or morphological changes, to NO₂ in elastase-treated animals. Although there was an interaction of NO₂ and elastase treatment on forced expiratory flow at 10% of forced vital capacity, the authors attributed this response to either
a statistical fluke or a change of little physiological significance. See Section 13 2 2 4 for a further discussion of structural changes.

Suzuki and Tsubone, along with their colleagues, have conducted extensive studies on the effects of NO2 on respiratory and cardiac function in mice and rats. Because many cardiovascular effects observed following exposure to NO2 are most likely secondary to pulmonary edema and/or stimulation of sensory receptors in the respiratory tract, these changes will be discussed together.

Suzuki et al. (1984) reported that the heart rate in unanesthetized mice was lower following 1-mo exposure to 2,260 and 7,520 \( \mu g/m^3 \) (1.2 and 4.0 ppm) NO2, but not following 2- and 3-mo exposures. Arterial O2 tension (\( PaO_2 \)) was decreased following a 3-mo exposure to 7,520 \( \mu g/m^3 \) NO2. Respiratory rate was not affected by NO2 exposures. Suzuki et al. (1981) also exposed rats for up to 3 mo to between 752 and 7,520 \( \mu g/m^3 \) (0.4 and 4.0 ppm) NO2. After 3 mo of exposure to 7,520 \( \mu g/m^3 \) NO2, anesthetized rats, artificially ventilated at high frequencies, had a significant reduction in \( PaO_2 \).

Effects of 24-h exposures to 9,400, 18,800, 37,600, and 75,200 \( \mu g/m^3 \) (5, 10, 20, and 40 ppm) NO2 on swimming performance in mice were evaluated by Suzuki et al. (1982a). Blood lactic acid levels measured in mice after a 4-min forced swim were approximately 50% higher following exposure to 9,400 \( \mu g/m^3 \) NO2 compared with controls. Exposures to higher concentrations of NO2 (\( \geq 18,800 \mu g/m^3 \)) resulted in a concentration-related decrease in maximum forced swimming time. Changes in blood lactic acid levels in the Suzuki et al. (1982a) study indicate that although mice exposed to 9,400 \( \mu g/m^3 \) NO2 were able to swim as long as control mice, the cardiorespiratory system was not able to supply sufficient O2 to meet the metabolic demands of swimming, and anaerobic pathways were activated producing lactic acid.

Suzuki et al. (1982b) evaluated breathing pattern and gas exchange in mice following exposure to 9,400, 18,800, or 37,600 \( \mu g/m^3 \) (5, 10, or 20 ppm) NO2 for 24 h. The irritant effect of exposure to 9,400 \( \mu g/m^3 \) NO2 resulted in increased respiratory rate and an associated decrease in arterial CO2 tension (\( PaCO_2 \)), but no effect on \( PaO_2 \). Respiratory rates were increased at the two highest NO2 exposure concentrations, but because of impaired gas exchange associated with increased lung wet weight and lung water content, \( PaCO_2 \) was unchanged and \( PaO_2 \) was decreased following exposure. The studies of Suzuki...
and Tsubone together have shown that 30-min to 3-mo exposures of mice and rats to NO₂
congentrations of 9,400 μg/m³ NO₂ and greater stimulated respiratory reflexes that slow the
heart rate and produce concentration-related pulmonary edema, decreasing blood oxygenation
and impairing maximum exercise performance.

McGrath and Smith (1984) found that the irritant response in mice to an 8-min
exposure to 188,000 μg/m³ (100 ppm) NO₂ was lessened by a 3-day continuous preexposure
to 9,400 μg/m³ (50 ppm) NO₂. Considering both 188,000 μg/m³ NO₂ exposures and
phenyl diguanide injections as irritant challenges, 3 to 7 days of exposure to 7,520 to
9,400 μg/m³ (4.0 to 5.0 ppm) NO₂ lessened the response to 188,000 μg/m³ NO₂ exposure,
suggesting the development of a tolerance or attenuated response to NO₂ (McGrath and
Smith, 1984), but heightening of the response to phenyl diguanide injections (Tsubone and
Suzuki, 1984).

Yoshida et al. (1980b) exposed guinea pigs to 9,400 μg/m³ NO₂ (5.0 ppm). After four
preliminary 30-min NO₂ exposures, spread over 2 weeks, animals were exposed twice a
week for 10 weeks to 30 min of NO₂, followed 20 min later by a 10-min exposure to
aerosolized albumin. After 5 weeks of NO₂ plus albumin exposures (two 30-min
exposures/week), animals were exposed to aerosolized acetylcholine for 10 min. Results
were evaluated by grading the animals breathing pattern on a scale of 1 to 7, with
1 representing normal breathing and 7 almost total apnea with only rare respiratory efforts.
Using this relatively subjective measure, the authors state that dyspneic breathing patterns
were more severe in animals exposed to NO₂ followed by exposure to aerosolized albumin
compared to animals exposed to albumin alone, with the greatest differences occurring
between the fourth and seventh week of exposure to albumin. Animals previously exposed to
NO₂ plus albumin were also more affected by the final exposure to acetylcholine. Although
the authors state that the effects they observed are statistically significant, quantitative
measures of pulmonary function would allow for much more thorough statistical evaluation
and better definition of the functional changes occurring in the lungs.

Summary

Changes in pulmonary function parameters following NO₂ exposure in experimental
animals have shown consistent patterns among different treatment conditions and animals.
A significant increase in vital capacity has been reported immediately following the exposure of rats to 940 μg/m³ (0.5 ppm) NO₂, 6 h/day, 5 days/week for 4 weeks (Evans et al., 1989). When the animals were examined 4 weeks later, there was an increase in compliance in the NO₂-exposed animals. The changes in these pulmonary function parameters were associated with changes in the mean linear intercept. Exposures to diurnal peaks of NO₂ superimposed on a constant background level, simulating NO₂ patterns in the urban environment, produced a decrease in lung distensibility in both mice and rats (Miller et al., 1987, Stevens et al., 1988). These changes were very subtle, and in mice occurred at concentrations of 376 μg/m³ (0.2 ppm) NO₂ with peak exposures of 1,504 μg/m³ (0.8 ppm) after 52 weeks of continuous exposure (Miller et al., 1987). Impaired gas exchange was a predominant feature in mice following several months of exposure 7,520 μg/m³ (4.0 ppm) NO₂ and was reflected in decreased PaO₂ (Suzuki et al., 1984). When NO₂ exposure was increased to 9,400 μg/m³ (5.0 ppm) for 24 h, increased anaerobic metabolism, manifested by increased production of lactic acid, occurred (Suzuki et al., 1982).

Newborn and older animals are affected differently by NO₂ exposures, particularly rats exposed subchronically to continuous background concentrations with diurnal peaks (1,880 and 3,760 μg/m³ [1.0 and 2.0 ppm] with two daily 1-h peaks at three times the baseline concentration [Stevens et al., 1988]). Lung distensibility was increased transiently in 1-day-old rats exposed to NO₂ for 3 weeks, but was decreased in rats that were 7 weeks old at the beginning of exposure. When pulmonary function parameters were compared between 6 mo old rats exposed to NO₂ concentrations of 17,900 μg/m³ (9.5 ppm) for 6 mo and rats exposed to the same NO₂ regimen beginning at conception and continuing until 6 mo of age, there were no substantive differences noted between the two exposed groups (Mauderly et al., 1987).

All these studies taken together demonstrate that NO₂ produces subtle to major changes in pulmonary function, depending on the concentration and duration of exposure. Lung distensibility and gas exchange are the parameters most consistently affected by NO₂ exposure.
13.2.2.4 Morphologic Studies

Inhalation of NO₂ produces morphological alterations in the respiratory tract. Tables 13-9, 13-10, and 13-11 provide an overview of results of studies of respiratory system morphology following acute, subchronic, and chronic exposures to a wide range of NO₂ concentrations. Examination of the tables shows variability in responses at similar exposure levels in different studies. There are several possible explanations for these differences in response. Species, strain, and age of the experimental animals and the diet they were fed appear to be major factors. Although in most recent studies, the authors specify that specific pathogen-free animals were ordered from the supplier, the possibility of intercurrent disease acquired after they were shipped or during the experimental protocol can only be excluded by serology, microbial culture, and a complete necropsy at the end of the experiment.

Other major factors that influence the results reported are the methods and instruments used for morphological evaluation. Because NO₂ does not affect the cells and tissues of the lungs in a uniform manner (Stephens et al., 1972; Crapo et al., 1984), sampling procedures are important to the detection of lesions. Sampling procedures can also influence the validity of the morphometric data derived using stereological procedures. The type of microscopy used for morphological observations is also critical. Light microscopy (LM) permits detection of many NO₂ lesions and the examination of much larger areas of lungs than does transmission electron microscopy (TEM). However, LM provides neither the magnification nor the resolution required to detect some very significant morphological lesions that follow acute or low-concentration exposures. Conversely, inspection of small areas using TEM can easily miss the limited sites affected by NO₂. Scanning electron microscopy (SEM) is especially useful for examining the many surfaces of lungs at both low and high magnification. Scanning electron microscopy is the method of choice for inspecting airways for some subtle NO₂-induced changes in cell surfaces. Although qualitative judgments of the extent and severity of lesions can be made using all of the above instruments, quantitative evaluation requires morphometric methods employing appropriate sampling and stereological procedures. Morphometric techniques, especially those using TEM (Chang et al., 1986, 1988, Kubota et al., 1987), are essential for evaluating subtle changes resulting from exposure to low concentrations or evaluating the extent of recovery of either the epithelium or the underlying connective tissue interstitium.

13-89
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Procedures</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>940 µg/m³</td>
<td>0.5</td>
<td>4 h</td>
<td>LM</td>
<td>M</td>
<td>NS</td>
<td>Loss of cytoplasmic granules in and rupture of mast cells at 0.5 ppm; degranulation and decreased number of mast cells at 1 ppm</td>
<td>Thomas et al (1967)</td>
</tr>
<tr>
<td>940 µg/m³</td>
<td>0.5</td>
<td>Continuous, up to 6 days</td>
<td>LM</td>
<td>M</td>
<td>NS</td>
<td>Increased number of mast cells in trachea as exposure duration increased</td>
<td>Hayashi et al (1987)</td>
</tr>
<tr>
<td>3,760 µg/m³</td>
<td>2.0</td>
<td>3 days</td>
<td>LM</td>
<td>M/F</td>
<td>5, 10, 21, 45, 55, ≥60 days</td>
<td>At 2 ppm. no lesions observed at 10 ppm fibrin deposition in alveoli, some broncholar cilia loss</td>
<td>Azoulay-Dupuis et al (1983)</td>
</tr>
<tr>
<td>18,800 µg/m³</td>
<td>10</td>
<td>3 days</td>
<td>LM</td>
<td>TEM</td>
<td></td>
<td>At 2 ppm thickening of alveolar walls, edema, increase in macrophage number, loss of broncholar cilia, inflammation</td>
<td>Dunkin Hartley (1971)</td>
</tr>
<tr>
<td>3,760 µg/m³</td>
<td>2.0</td>
<td>3 days</td>
<td>LM</td>
<td>TEM</td>
<td></td>
<td>At 10 ppm severe loss of cilia in trachea and bronchioles, edema, hemorrhage, inflammation, focal emphysema (enlarged airspaces), increased number of Type 2 cells</td>
<td></td>
</tr>
<tr>
<td>5,640-30,100 µg/m³</td>
<td>3-16</td>
<td>1 h</td>
<td>LM</td>
<td>NS</td>
<td>NS</td>
<td>Edema at ≥7.0 ppm, some damage to alveolar cell mitochondria and cell membrane at ≥3.0 ppm</td>
<td>Dowell et al (1971)</td>
</tr>
<tr>
<td>9,400 µg/m³</td>
<td>5.0</td>
<td>24 h/day, 3 days</td>
<td>LM</td>
<td>M</td>
<td>60-75 days</td>
<td>No major morphological changes observed</td>
<td>Messiha et al (1983)</td>
</tr>
<tr>
<td>20,000 µg/m³</td>
<td>10</td>
<td>6 h</td>
<td>LM</td>
<td>TEM</td>
<td>M</td>
<td>6 weeks</td>
<td>Cilia loss, shortening of cilia, focal hypertrophy of broncholar epithelium</td>
</tr>
</tbody>
</table>

*M = Male  
F = Female  
LM = Light microscopy  
TEM = Transmission electron microscopy  
SEM = Scanning electron microscopy
TABLE 13-10. EFFECTS OF SUBCHRONIC EXPOSURE TO NITROGEN DIOXIDE ON LUNG MORPHOLOGY \(^a\)

<table>
<thead>
<tr>
<th>NO(_2) Concentration</th>
<th>ppm</th>
<th>Exposure Procedures</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu g/m^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>207</td>
<td>0.11</td>
<td>Continuous, LM</td>
<td>F</td>
<td>1, 3, 12, 21 mo (JCL-SD)</td>
<td>Various morphometric changes, depending on age and exposure level. Multifascic pattern (e.g., decrease in air-blood barrier thickness from 1-12 mo of age, and increase at 21 mo old)</td>
<td>Kyono and Kawai (1982)</td>
<td></td>
</tr>
<tr>
<td>865</td>
<td>0.46</td>
<td>TEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5,260</td>
<td>2.8</td>
<td>1 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16,500</td>
<td>8.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>0.34</td>
<td>6 h/day, LM</td>
<td>M</td>
<td>NS</td>
<td>Mouse</td>
<td>Type 2 cell hypertrophy and hyperplasia, increase in mean linear intercept and amount of alveolar wall area</td>
<td>Sherwin and Richters (1982)</td>
</tr>
<tr>
<td>940 base + 2,820 peak</td>
<td>0.5</td>
<td>23 h/day, 5 days/week</td>
<td>M</td>
<td>1 day and 6 weeks</td>
<td>Rat (Fischer 344)</td>
<td>In proximal alveolar region 0.5 ppm base + peak caused Type 2 cells to become spread over more surface area in neonates and adults, Type 2 cell hypertrophy and increase in number of AMs in adults, Type 2 cells thinner in neonates, 2.0 ppm base + peak (only adults studied) caused similar changes plus an increase in number of Type 1 cells, which were smaller than normal Type 1 cells. 0.5 ppm base + peak had increased interstitial matrix and fibroblast volume. In terminal bronchial region 0.5 ppm base + peak caused no effects on percentage distribution of ciliated cells and Clara cells in neonates or adults, but neonates (only) had an increase in ciliated cell surface area and mean lumenal surface area of Clara cells. 2.0 ppm base + peak (only adults studied) resulted in fewer ciliated cells with a reduced surface area and alterations in the shape of Clara cells.</td>
<td>Crapo et al (1984)</td>
</tr>
<tr>
<td>3,760 base + 11,300 peak</td>
<td>2.0</td>
<td>3 × base, 1-h peaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Various abbreviations and symbols are used throughout the table.
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure Procedures</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000 µg/m³</td>
<td>0.53</td>
<td>Continuous, 90 days</td>
<td>LM NS</td>
<td>NS</td>
<td>No lesions were visible by the technique used</td>
<td>Steadman et al (1966)</td>
</tr>
<tr>
<td>2,500 µg/m³</td>
<td>1.33</td>
<td>Continuous, up to 28 days</td>
<td>LM TEM</td>
<td>M 6 weeks</td>
<td>Rat (Wistar)</td>
<td>At 0.53 and 1.33 ppm no pathology</td>
</tr>
<tr>
<td>5,000 µg/m³</td>
<td>2.7</td>
<td></td>
<td>LM SEM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20,000 µg/m³</td>
<td>10.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20,000 µg/m³</td>
<td>10.6</td>
<td>6 h/day, up to 28 days</td>
<td>LM TEM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Procedures</td>
<td>Gender</td>
<td>Age (Strain)</td>
<td>Effects</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----</td>
<td>----------</td>
<td>------------</td>
<td>--------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>1,320-1,500</td>
<td>0-7-8</td>
<td>Continuous, 1 mo</td>
<td>LM</td>
<td>F</td>
<td>3 weeks Mouse (JCL ICR)</td>
<td>At exposure end mucous hypersecretion, focal degeneration and desquamation of mucous membrane, terminal broncholar epithelial hyperplasia, some alveolar enlargement, shortening of cilia, Type 1 cell edema After 1 mo postexposure minimal lesions persisted in some bronchioles, lymphocytic infiltration of tracheal and bronchial mucosa</td>
</tr>
<tr>
<td>1,880-2,820</td>
<td>10-15</td>
<td>8 h/day, 5 days/week, 8 weeks</td>
<td>LM</td>
<td>M</td>
<td>8 weeks Hamster (Golden Syrian)</td>
<td>Moderate alveolar enlargement, primarily at broncholar-alveolar duct junction, increase in mean linear intercept, decrease internal surface area of lung, no lesions in bronchial, broncholar, alveolar duct, or alveolar epithelium, no change in macrophage number</td>
</tr>
<tr>
<td>3,760</td>
<td>20</td>
<td>Continuous, 7-21 days</td>
<td>LM</td>
<td>M</td>
<td>NS Guinea pig (NS)</td>
<td>Type 2 cell hypertrophy at 7 or 21 days</td>
</tr>
<tr>
<td>3,760</td>
<td>20</td>
<td>Continuous, 6 weeks</td>
<td>LM</td>
<td>M</td>
<td>8 weeks Rat (Wistar)</td>
<td>Evidence of intercurrent disease, which may mask changes due to NO₂ exposure Some cilia loss in terminal bronchioles, some distended or disrupted alveolar walls</td>
</tr>
<tr>
<td>7,520</td>
<td>40</td>
<td>6 h/day, 5 days/week, up to 21 days</td>
<td>LM</td>
<td>M</td>
<td>NS Rat (Wistar)</td>
<td>At 4 ppm no lesions in nasal cavity or lungs At 10 ppm no lesions in nasal cavity, increased cellularity of walls of bronchioles, alveolar duct, and adjacent alveoli by 21 days, hypertrophy or hyperplasia of small bronchi and broncholar epithelium by 7 days At 25 ppm no lesions in nasal cavity, hypertrophy or hyperplasia of small bronchi or broncholar epithelium by 7 days, increase in cellularity of walls of respiratory bronchioles, alveolar ducts and adjacent alveoli by 7 days, some mononuclear infiltration of peribronchial areas</td>
</tr>
</tbody>
</table>
### TABLE 13-10 (cont'd). EFFECTS OF SUBCHRONIC EXPOSURE TO NITROGEN DIOXIDE ON LUNG MORPHOLOGY

<table>
<thead>
<tr>
<th>NO₂ Concentration (μg/m³)</th>
<th>ppm</th>
<th>Exposure</th>
<th>Procedures</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>18,880</td>
<td>10</td>
<td>Continuous, 14 days</td>
<td>LM TEM</td>
<td>M</td>
<td>NS</td>
<td>Rat (Wistar)</td>
<td>3 days swelling and vacuolar degeneration of Type 1 cells, swelling of Type 2 cells, desquamation of alveolar cells, interstitial edema, goblet cell hyperplasia in trachea and bronchi 7-14 days desquamation of Type 1 cells, hypertrophy and hyperplasia of Type 2 cells, slight thickening of alveolar wall, interstitial edema, desquamation of endothelial cells, swelling and vacuolar degeneration of nonciliated broncholar cells, loss of cilia and desquamation of bronchial epithelium</td>
<td>Hayashi et al (1987)</td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>Continuous, 1 mo</td>
<td>LM TEM</td>
<td>F</td>
<td>1, 3, 12, 21 mo</td>
<td>Rat (JCL SD)</td>
<td>Increased arithmetic mean thickness of air-blood barrier, increased thickness of both interstitial matrix and of cells, hyperplastic foci in middle and terminal bronchi</td>
<td>Kyono and Kawai (1982)</td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>Continuous, 6 weeks</td>
<td>TEM</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Type 2 cell hyperplasia, increase in lipid bodies and lamellae in cells</td>
<td>Yuen and Sherwin (1971)</td>
</tr>
</tbody>
</table>

---

*LM = Light microscopy
TEM = Transmission electron microscopy
F = Female
M = Male
NS = Not stated
AMs = Alveolar macrophages
SEM = Scanning electron microscopy*
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Procedures</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>0.04</td>
<td>Continuous, 9-27 mo</td>
<td>LM</td>
<td>M</td>
<td>8 weeks</td>
<td>Rat (JCL, Wistar)</td>
<td>At 0.04 ppm no significant change, but some tendency towards increase in arithmetic mean thickness of air-blood barrier</td>
<td>Kubota et al (1987)</td>
</tr>
<tr>
<td>750</td>
<td>0.4</td>
<td>Continuous, 9-27 mo</td>
<td>TEM</td>
<td>M</td>
<td>8 weeks</td>
<td>Rat (JCL, Wistar)</td>
<td>At 0.4 ppm slight increase in arithmetic mean thickness of air-blood barrier by 18 mo, becoming significant by 27 mo, some interstitial edema and slight change in bronchiolar and alveolar epithelium by 27 mo</td>
<td></td>
</tr>
<tr>
<td>7,520</td>
<td>4.0</td>
<td>Continuous, 9-27 mo</td>
<td>TEM</td>
<td>M</td>
<td>8 weeks</td>
<td>Rat (JCL-SD)</td>
<td>At 4 ppm hypertrophy and hyperplasia of bronchiolar epithelium and increase in arithmetic mean thickness of air-blood barrier at 9 mo, Clara cell hyperplasia, interstitial fibrosis, hypertrophy of Type 1 and Type 2 cells, and some decline in arithmetic mean thickness of air-blood barrier at 27 mo</td>
<td></td>
</tr>
<tr>
<td>380 base</td>
<td>0.2</td>
<td>23 h/day, 7 days/week</td>
<td>LM</td>
<td>F</td>
<td>6-8 weeks</td>
<td>Mouse (CD-1)</td>
<td>Slight to moderate interstitial pneumonia considered to be due to intercurrent disease rather than NO₂ exposure. Intercurrent disease may have masked effects of NO₂</td>
<td>Miller et al (1987)</td>
</tr>
<tr>
<td>1,470 peak</td>
<td>0.78</td>
<td>base + 2 1-h peaks, 5 days/week, 52 weeks.</td>
<td>LM</td>
<td>F</td>
<td>6-8 weeks</td>
<td>Mouse (CD-1)</td>
<td>At 1 ppm cell loss in terminal bronchioles, hyperplasia of Type 2 cells, interstitial edema, At 4 ppm cell loss in terminal bronchioles, hyperplasia of Type 2 cells, interstitial edema.</td>
<td></td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>Continuous, 7 mo</td>
<td>LM</td>
<td>M/F</td>
<td>4 weeks</td>
<td>Rat (JCL-SD)</td>
<td>At 0.5 ppm swelling of terminal bronchiolar cilia, hyperplasia of Type 2 cells At 1 ppm cilia loss in terminal bronchioles, hyperplasia of Type 2 cells, interstitial edema At 4 ppm cilia loss in terminal bronchioles, hyperplasia of Type 2 cells, interstitial edema, decrease in number lamellar bodies in Type 2 cells, lysosomes with osmiophilic lamellar structure in ciliated cells of terminal bronchioles</td>
<td>Yamamoto and Takahashi (1984)</td>
</tr>
</tbody>
</table>
### TABLE 13-11 (cont'd). EFFECTS OF CHRONIC EXPOSURE TO NO₂ ON LUNG MORPHOLOGY

<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>Experiments</th>
<th>Procedures</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>940 µg/m³ 0.5 ppm</td>
<td>6-24 h/day, 3-12 mo</td>
<td>LM NS NS Mouse (NS)</td>
<td>3 mo pneumonitis and alveolar size increase, loss of cilia in respiratory bronchioles and bronchial inflammation with 24 h/day, 6-12 mo pneumonitis, cilia loss, bronchial and bronchiolar inflammation, alveolar size increase</td>
<td>Blair et al (1969)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>940 µg/m³ 0.5 ppm</td>
<td>Continuous, up to 19 mo</td>
<td>LM TEM M NS Rat (Wistar)</td>
<td>Type 2 cell hypertrophy and interstitial edema by 4 mo, increased thickness of alveolar septa by 6 mo, fibrous pleural thickening by 19 mo</td>
<td>Hayashi et al (1987)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,500 µg/m³ 0.8 ppm</td>
<td>Continuous, lifetime (up to 33 mo)</td>
<td>LM NS 4 weeks Rat (Sprague-Dawley)</td>
<td>Minimal changes slight enlargement of alveoli and alveolar ducts, some rounding of bronchial and bronchiolar epithelial cells, increase in elastic fibers around alveolar ducts</td>
<td>Freeman et al (1966)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1,880 µg/m³ 1.0 ppm</td>
<td>Continuous, 16 mo</td>
<td>LM TEM M NS Monkey (Squirrel)</td>
<td>Slight to moderate interstitial pneumonia considered to be result of intercurrent disease rather than NO₂ exposure Intercurrent disease may have masked effects of NO₂</td>
<td>Fenters et al (1973)</td>
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<tr>
<td>1,880 µg/m³ 1.0 ppm</td>
<td>6 h/day, 5 days/week, up to 18 mo</td>
<td>LM M NS Dog (Mongrel)</td>
<td>At 1 ppm 6 mo no lesions observed, no pathology, 12 mo dilated alveoli and alveolar ducts, 18 mo dilated alveoli, edema, thickening alveolar septa by chronic inflammatory cells No differences between exposed and control dogs, so intercurrent disease may have been present Intercurrent disease may have masked effects of NO₂ At 5 ppm 6 mo no pathology, 12 mo dilated alveoli and alveolar ducts, 18 mo edema, congestion, and thickened alveolar septa due to inflammatory cells</td>
<td>Wagner et al (1965)</td>
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<tr>
<td>1,880 µg/m³ 1.0 ppm</td>
<td>6 h/day, 5 days/week, 18 mo</td>
<td>LM M NS Guinea pig (English)</td>
<td>Evidence of intercurrent disease that may have masked NO₂ morphological effects</td>
<td>Wagner et al (1965)</td>
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<tr>
<td>NO₂ Concentration</td>
<td>ppm</td>
<td>Exposure Procedures</td>
<td>Gender</td>
<td>Age (Species)</td>
<td>Response Description</td>
<td>Reference</td>
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<tr>
<td>1,880 ppm</td>
<td>10</td>
<td>7 h/day,</td>
<td>M/F</td>
<td>14-16 Rat</td>
<td>No lesions observed</td>
<td>Gregory et al (1983)</td>
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<td>5 days/week,</td>
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<td>15 weeks</td>
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<td>9,400 ppm</td>
<td>50</td>
<td>Base 7 h/day,</td>
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<td>9,400 ppm</td>
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<td>2 1 5-h peaks/day</td>
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<td>15 weeks</td>
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<tr>
<td>3,760 ppm</td>
<td>20</td>
<td>Continuous,</td>
<td>LM</td>
<td>4 weeks Rat</td>
<td>Loss of cilia in</td>
<td>Stephens et al (1971a,b)</td>
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<td></td>
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<td>2 years</td>
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<td></td>
<td>terminal bronchioles,</td>
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<td>TEM</td>
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<td>abnormal cilogenesis,</td>
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<td>NS</td>
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<td>crystalloid</td>
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<td>4 weeks</td>
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<td>inclusions in</td>
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<td>bronchiolar epithelial</td>
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<td>cells, increased</td>
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<td>fibrils and basement</td>
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<td>bronchioles</td>
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<td>3,760 ppm</td>
<td>20</td>
<td>Continuous, up to</td>
<td>LM</td>
<td>4 weeks Rat</td>
<td>Loss of cilia by 72 h,</td>
<td>Stephens et al (1972)</td>
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<td>12 mo</td>
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<td>decreased number of</td>
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<td>TEM</td>
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<td>ciliated cells by 7</td>
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<td>M</td>
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<td>days, hypertrophy and</td>
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<td>4 weeks</td>
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<td>hyperplasia of</td>
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<td>bronchiolar epithelium</td>
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<td>return to normal after</td>
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<td>21 days exposure</td>
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<td>3,760 ppm</td>
<td>20</td>
<td>Continuous, up to</td>
<td>LM</td>
<td>4 weeks Rat</td>
<td>No change in turnover</td>
<td>Evans et al (1972)</td>
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<td>360 days</td>
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<td></td>
<td>of terminal bronchiole</td>
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<td>TEM</td>
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<td>cells, increase in</td>
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<td>M</td>
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<td>turnover of Type 2 cells</td>
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<td>alveoli by 1 day, but</td>
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<td>normal by 7 days</td>
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<td>3,760 ppm</td>
<td>20</td>
<td>Continuous,</td>
<td>LM</td>
<td>NS Monkey</td>
<td>Bronchiolar epithelial</td>
<td>Furioso et al (1973)</td>
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<td>14 mo</td>
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<td>(Macaca</td>
<td>hypertrophy, especially</td>
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<td>speciosa)</td>
<td>adjacent to alveolar</td>
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<td>ducts, change to</td>
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<td>cuboidal cells in</td>
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<td>proximal bronchiolar</td>
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<td>Minimal effect</td>
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<td>some terminal bronchiol</td>
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<td>epitelial hypertrophy</td>
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<tr>
<td>NO₂ Concentration</td>
<td>Exposure</td>
<td>Procedures</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Effects</td>
<td>Reference</td>
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<tr>
<td>3,760 ppm 20 mg/m³</td>
<td>Continuous, lifetime (up to 763 days), 0 8 ppm for 69 days, then 2 0 ppm</td>
<td>LM</td>
<td>M</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>Alveolar distension, especially near alveolar duct level, increased variability in alveolar size, hypertrophy in terminal bronchiolar cells, no inflammation</td>
<td>Freeman et al (1968b)</td>
</tr>
<tr>
<td>7,520 ppm 40 mg/m³</td>
<td>Continuous, 16 weeks</td>
<td>LM</td>
<td>M</td>
<td>4 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>Bronchial epithelial hyperplasia</td>
<td>Haydon et al (1965)</td>
</tr>
<tr>
<td>9,400 ppm 50 mg/m³</td>
<td>6 h/day, 5 days/week, 14 mo</td>
<td>LM</td>
<td>M</td>
<td>NS</td>
<td>Mouse (C57BL/6, Webster)</td>
<td>No lesions observed</td>
<td>Wagner et al (1965)</td>
</tr>
<tr>
<td>9,400 ppm 50 mg/m³</td>
<td>4-7 5 h/day, 5 days/week, 5 5 mo</td>
<td>LM</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (New England)</td>
<td>Some dilation of terminal bronchioles, tracheal inflammation, desquamative pneumonitis</td>
<td>Balchum et al (1965)</td>
</tr>
<tr>
<td>17,900 ppm 95 mg/m³</td>
<td>7 h/day, 5 days/week, 6 mo</td>
<td>LM</td>
<td>M</td>
<td>in utero and 6 mo</td>
<td>Rat (Fischer 344)</td>
<td>No histological changes, no effects on mean linear intercepts</td>
<td>Mauderly et al (1987) Mauderly (1989)</td>
</tr>
<tr>
<td>17,900 ppm 95 mg/m³</td>
<td>7 h/day, 5 days/week, 24 mo</td>
<td>LM</td>
<td>M</td>
<td>18 weeks</td>
<td>Rat (Fischer 344)</td>
<td>Minimal inflammatory response, mild hyperplasia of bronchial epithelium extending into proximal alveoli; Slight thickening of terminal bronchiolar walls; No change in mean linear intercept; Emphysemic rats had similar response</td>
<td>Mauderly et al (1989, 1990)</td>
</tr>
</tbody>
</table>

LM = Light microscopy
TEM = Transmission electron microscopy
M = Male
F = Female
NS = Not stated
The large degree of interspecies variability in responsiveness to NO₂ is clearly evident from those few studies (Table 13-9 and 13-10) where different species were exposed under identical conditions and examined using the same methods (Wagner et al., 1965; Furioso et al., 1973, Azoulay-Dupuis et al., 1983) Such differences in response may be due to inherent species differences in sensitivity of cells at target sites, to differences in effective dose of NO₂ reaching target sites due to anatomical and ventilatory differences, or to a combination of these factors Thus, the choice of experimental animal affects the magnitude of changes observed following exposure Morphological lesions may not be detectable in some species by less sensitive methods of evaluation However, in most cases, the sites and types of morphological lesions produced by NO₂ inhalation are similar in all species when effective concentrations are used and appropriate tissues are examined using sensitive methods In direct comparisons, the guinea pig, hamster, and monkey all appear to be more severely affected by equivalent exposure to NO₂ than is the rat, which is the most commonly used experimental animal

Sites Affected

The extent of injury to an individual cell is related to both the sensitivity of that cell type and the dose of NO₂ delivered to the site occupied by that cell Among cells of a specific type, several factors (e.g., the maturity of the cell and its antioxidant capacity) may influence its sensitivity The dose of NO₂ to which an individual cell is exposed is determined by the concentration of NO₂ at the site in the respiratory system occupied by that individual cell and the surface area of that cell that is exposed to that concentration Thus, sensitivity of cells and the magnitude of morphologically detectable injury are not the same

In lungs, morphological evidence of injury is first noted, and is usually most severe, in the epithelium of the centriacinar region (i.e., at the junction of the conducting airways with the gas exchange area) The centriacinar region includes the terminal or respiratory bronchioles and the immediately adjacent alveoli, often called proximal alveoli Within this region, those cells that are most sensitive to NO₂-induced injury are the ciliated cells of the bronchiolar epithelium and the Type 1 cells of the alveolar epithelium Ciliated cells located in terminal or respiratory bronchioles lose some or all of their cilia or become necrotic and are shed from the epithelium Nonsecretory bronchiolar cells
Clara cells in rodents) appear less sensitive to NO₂, but they do lose secretory granules and surface projections. With continued exposure, nonciliated bronchiolar cells increase in number (hyperplasia) and in size (hypertrophy). These cells are the progenitor cells for replacement of the ciliated cells that were sloughed from the airway epithelium. Following chronic exposure, there is also increased loss of cilia both in bronchioles and more proximal conducting airways. Remaining cilia may have abnormal structure and location.

In centriacinar (proximal) alveoli, but not in alveoli located at the periphery of acini, short-term (acute) exposure to NO₂ results in necrosis and sloughing of Type 1 alveolar epithelial cells, which leaves the underlying basal lamina bare. This is followed by proliferation of, and replacement by, Type 2 alveolar epithelial cells, which are progenitor cells for Type 1 cells. Because Type 2 cells have a cuboidal shape, rather than the squamous shape of Type 1 cells, this may result in a few centriacinar alveoli with a thickened epithelial component of the air-blood barrier.

Bronchiolar epithelium was observed in centriacinar alveoli by Mauderly et al. (1990) in rats exposed to 17,900 μg/m³ (9.5 ppm) NO₂, 7 h/day, 5 days/week for 24 mo. In addition to the usually described mild hyperplasia of the terminal bronchiolar epithelium, they reported an extension of bronchiolar cell types into centriacinar alveoli, giving the appearance of respiratory bronchioles which was not observed in controls. They also reported a slight progression of this lesion, but not of the epithelial hyperplasia. Nettesheim et al. (1970) described this lesion as "alveolar bronchiolization" and reported it in mice chronically exposed to a synthetic smog. Although it may appear to be only a slight modification of alveolar epithelial hypertrophy, replacement of one type of epithelium by another (e.g., alveolar by bronchiolar), and the progression of the lesion with increasing length of exposure, may have quite different consequences.

Although the centriacinar epithelial changes have received the most attention, as they are easily seen and quantitated using a variety of methods, there have also been reports of changes in the centriacinar basal lamina and connective tissue interstitium that underlie the epithelium. In earlier studies, Freeman et al. (1969, 1972) reported proliferation of new connective tissue at the junction of terminal bronchioles and alveolar ducts from rats exposed to 28,200 μg/m³ (15 ppm) NO₂. These interstitial changes persisted following postexposure "recovery" periods of up to 52 weeks. In more recent studies, Kyono and Kawai (1982)
reported morphometric increases in the interstitium following continuous exposure of 1-mo-old rats to 940, 5,640, and 18,800 μg/m³ (0.5, 3.0, and 10.0 ppm) NO₂ for 1 mo Chang et al. (1986), in a TEM morphometric study of centriacinar alveoli from rats exposed to an urban pattern consisting of a baseline of 940 or 3,760 μg/m³ (0.5 or 2.0 ppm) with 1-h peaks twice a day to 3 times these levels NO₂, reported an increase in mean volume, but not number, of interstitial fibroblasts and in volume of interstitial matrix. These interstitial changes were statistically significant in rats exposed to the lower concentration (940 μg/m³ with peaks to 2,820 μg/m³) NO₂ for 6 weeks. In the same study, they also reported increased surface density of the basement membrane (basal lamina) in the rats exposed to 3,760 μg/m³ (2.0 ppm) baseline with peaks to 11,300 μg/m³ (6.0 ppm) for 6 weeks. Kubota et al. (1987) reported morphometric increases in the interstitium of rats exposed to 752 μg/m³ (0.4 ppm) for 27 mo and to 7,520 μg/m³ (4.0 ppm) for 18 mo. These qualitative and quantitative morphological studies correlate well with the increased collagen synthesis rate in NO₂-exposed rats discussed in Section 13.2.2 on lung biochemistry.

Chronic exposures may result in airspace enlargement characteristic of animal models of emphysema. In most, but not all cases, tissue destruction is not present, so the condition does not meet the 1985 National Heart, Lung and Blood Institute (NHLBI) definition of human emphysema (National Institutes of Health, 1985). A detailed discussion of emphysema in relation to NO₂ exposure follows the discussion on susceptibility to NO₂-induced morphological changes.

**Progression and Regression of Morphological Changes**

The temporal progression of early events due to NO₂ exposure focusing on centriacinar epithelial cells has best been described in the rat (e.g., Evans et al., 1972, 1973a, 1974, 1975, 1977, Freeman et al., 1966, 1968b, Stephens et al., 1971a, 1972). The earliest alterations resulting from exposure to concentrations of ≥3,760 μg/m³ (2.0 ppm) are seen within 24 to 72 h of continuous exposure and include desquamation of the Type 1 cells and ciliated bronchiolar cells, resulting in bare basal lamina in the centriacinar region and accumulation of fibrin in small airways. Accumulations of AMs were also reported. Epithelial repair by replacement of destroyed cells begins within 24 to 48 h of continuous exposure. The new cells in the bronchioli are derived from nonciliated cells, whereas in the
alveoli, the damaged Type 1 cells are replaced with Type 2 cells. Cell renewal, as measured by incorporation of tritiated thymidine ($^3$H-thymidine) by Type 2 cells, is observed within 12 h after the initial NO$_2$ exposure. During continued exposure, the number of $^3$H-thymidine labeled cells becomes maximal by about 48 h and decreases to preexposure levels by about 6 days (Evans et al., 1975). If exposure levels are very high ($>18,800 \mu g/m^3$, 10 ppm), however, resolution and return to normal-appearing centriacinar epithelial cells may be delayed or prevented, and the presence of increased numbers of Type 2 cells may be prolonged or permanent.

The resolution of NO$_2$-induced morphologic changes may be complete after the exposure ends or some lesions may remain, depending upon details of the exposure regime, species of animal studied, and the methods of evaluation. For example, Rombout et al. (1986) exposed rats to 20,000 $\mu g/m^3$ (10 ppm) continuously for 28 days. By LM and SEM, broncholar epithelial hyperplasia appeared totally resolved beyond 4 days after cessation of exposure, and hypertrophy was totally resolved after 16 days of postexposure recovery. After a 1-week postexposure period, all changes seen by LM and SEM appeared completely recovered. However, some abnormal cilia were observed by TEM 4 weeks after the exposure ended and some remnants of the NO$_2$-induced lesions were still seen by TEM 56 days after the exposure ended. Kubota et al. (1987) examined the time course of alveolar epithelial lesions in small groups of rats exposed to 7,520 $\mu g/m^3$ (4.0 ppm) NO$_2$, 24 h/day for up to 27 mo. In the quantitative or morphometric portions of this study, Kubota et al. (1987) deliberately avoided centriacinar or proximal alveoli, so the changes they describe represent those in the overall gas exchange area rather than only those in the most involved alveoli (i.e., centriacinar or proximal alveoli), which are the only alveoli usually studied.

One phase, which lasted for 9 to 18 mo of exposure, consisted of a decrease in number and an increase in cell volume of Type 1 epithelium, an increase in the number and volume of Type 2 cells, and an increase in the relative ratio of Type 2 to Type 1 cells. A second phase, which occurred at 18 to 27 mo of exposure, showed some recovery of alveolar epithelium. Thus, epithelial changes tend to resolve, at least partially, during continued chronic exposure to low concentrations and to resolve rapidly during postexposure periods.

Changes in the interstitium, the basal lamina and connective tissues under the epithelium, develop much more slowly and resolution, if it occurs, is prolonged. In the two
studies of epithelium discussed above, Rombout et al (1986) reported TEM observations of increased collagen and elastin in the interstitium of rats exposed to 20,000 \( \mu g/m^3 \) (10 6 ppm) for 28 days, and Kubota et al (1987) reported morphometric increases of the mean alveolar air-blood barrier in rats exposed to 752 \( \mu g/m^3 \) (0 4 ppm) for 27 mo and in rats exposed to 7,520 \( \mu g/m^3 \) (40 ppm) for 18 mo These authors considered the interstitial changes progressive and leading to fibrosis, rather than resolving as do epithelial changes

In summary, epithelial changes have been the focus of most studies Epithelial changes progress rapidly and regress rapidly and relatively completely both during chronic exposures and during postexposure periods In contrast, changes in the interstitium have been the subject of fewer studies, even though significant interstitial changes have been reported following chronic exposure to low concentrations of NO\(_2\) Interstitial changes develop more slowly, may progress during exposure, and regress slowly, if at all, during postexposure periods

**Effects of Nitrogen Dioxide as a Function of Exposure Pattern**

Although the extent and degree of morphologic alterations in the epithelium and interstitium appear to correspond to NO\(_2\) exposure concentrations, little is actually known about effects of other modifying factors, such as the exposure duration and concentration relationship, short-term peaks in concentration, or cycles of exposure and postexposure

The relative roles of C and T in response to subchronic exposure were examined by Rombout et al (1986) These researchers exposed rats to 1,000 to 5,000 \( \mu g/m^3 \) (0 53 to 2 66 ppm) NO\(_2\) for up to 28 days, or to 20,000 \( \mu g/m^3 \) (10 6 ppm) for either 6 h, 6 h/day for 28 days, or 24 h/day for 28 days They concluded that concentration played a more important role in inducing lesions than did exposure duration, as long as the product of \( C \times T \) was constant, and that the effect of concentration was stronger with intermittent exposure than with continuous exposure They also reported that continuous exposure was important to the development of strong AM response These findings are similar to those using the infectivity model discussed in Section 13 2 2 1

Ambient concentrations of NO\(_2\) are often characterized by transient peaks superimposed upon a lower and relatively constant baseline concentration, unlike experimental exposures, which are most commonly at a constant concentration The morphological effects of
exposure patterns involving transient peaks were examined in a number of studies. However, some studies lacked a group exposed to the constant baseline concentration for comparison with those exposed to the baseline concentration plus transient peaks. Thus, the findings of these studies do not elucidate the relative contributions of the baseline and peaks to the responses.

A study that included a baseline concentration group was that by Gregory et al. (1983). They exposed rats for 7 h/day, 5 days/week for up to 15 weeks to atmospheres consisting of the following concentrations of NO₂: (1) 1,880 µg/m³ (1.0 ppm), (2) 9,400 µg/m³ (5.0 ppm), or (3) 1,880 µg/m³ (1.0 ppm) with two 1.5-h peaks of 9,400 µg/m³ (5.0 ppm) per day (i.e., animals were exposed to NO₂ at 1,880 µg/m³ for 1.5 h, 9,400 µg/m³ for 1.5 h, 1,880 µg/m³ for 3 h, 9,400 µg/m³ for 1.5 h, and 1,880 µg/m³ for 0.5 h). No change in lung weight was found in any exposure group. After 15 weeks of exposure, routine LM histopathology showed minimal effects, with focal hyperinflation and areas of subpleural accumulation of macrophages found in some of the animals exposed either to a constant level of 9,400 µg/m³ or to 1,880 µg/m³ with the 9,400-µg/m³ peaks. Because the 1,880 µg/m³ group without peak exposures did not have these changes, the peak exposures appear to have contributed to increased morphological effects. Changes were also reported in the lung biochemistry of the NO₂-exposed animals as discussed in Section 13.2.2.2.

Miller et al. (1987) exposed mice for 1 year (23 h/day, 7 days/week) either to a continuous baseline concentration of 380 µg/m³ (0.2 ppm) NO₂, or to this baseline onto which was superimposed, for 5 days/week, two 1-h peaks (given in the morning and afternoon) of 1,470 µg/m³ (0.78 ppm). Morphologic examination (LM) performed after 32 and 52 weeks of exposure, and then 1 mo after all exposures had ended, revealed no treatment-related lesions in either exposure group, although host defense and pulmonary function changes were noted (Sections 13.2.2.1 and 13.2.2.3).

Crapo et al. (1984) and Chang et al. (1986) reported on the NO₂-induced changes in the centriacinar (proximal) alveolar region of 1-day and 6-week-old rats exposed for 6 weeks to a baseline concentration of 940 or 3,760 µg/m³ (0.5 or 2.0 ppm), 23 h/day for 7 days/week onto which were superimposed two daily 1-h peaks of three times the baseline concentration for 5 days/week. The rats were studied using TEM morphometric analyses. In the older rats (6 weeks old at the start of the exposure) at both exposure levels, Type 2
cells increased in total volume and mean cell volume, but not in number/mm³, and they occupied a larger percentage of the basal lamina (basement membrane). In the older rats exposed to 3,760 µg/m³, but not 940 µg/m³, with peaks, Type 1 cells had a larger total volume and increased numbers/mm³, with a smaller mean surface area. The percentage of the basal lamina occupied by Type 1 cells was decreased in older rats of both exposure groups. They also had increased total volume and number of AMs, but the mean cell volume was increased only in the 940-µg/m³ group. The total volume of interstitial matrix and of fibroblasts increased in the 940-µg/m³ older group, but not the 3,760-µg/m³ older group. The number of interstitial fibroblasts did not change, but their mean cell volume increased in the older groups. Both age groups of rats reacted in a generally similar manner.

Pulmonary function changes in similarly exposed animals were assessed by Stevens et al. (1988), indicating a correlation between decreased compliance and thickening of the alveolar interstitium (Section 13.2.2.3).

The terminal bronchiolar region of these rats was also examined morphometrically (Chang et al., 1988). The lower exposure level caused no effects. At the higher level, there was a 19% decrease in ciliated cells per unit area of the epithelial basement membrane and a reduction in the mean surface area of 29% in the remaining cilia in the high exposure group. In the Clara cells, the size of the dome protrusions were decreased, giving the bronchial epithelium a flattened appearance.

**Susceptibility to Nitrogen Dioxide-Induced Morphological Changes**

Susceptibility to morphological effects may be influenced by many factors. Some factors that have been studied include age, compromised lung function, and acute infections. Age of the animal at the time of exposure may be responsible for some of the variability in morphological response seen in the same species exposed to comparable concentrations. Stephens et al. (1978) exposed rats from 1 to 40 days of age to 26,320 µg/m³ (14 ppm) NO₂ for 24, 48, or 72 h. Using LM and TEM, they found only minor injury and loss of cilia in terminal bronchioles of rats exposed before weaning at 20 days. After weaning, there was a progressive increase in cellular response in both terminal bronchioles and alveoli, with a plateau reached at about 35 days of age. Other investigators expanded on these observations using lower concentrations.
Azoulay-Dupuis et al (1983) exposed both rats and guinea pigs aged 5 to \( \geq 60 \) days to 3,760 or 18,800 \( \mu g/m^3 \) (2 or 10 ppm) for 3 days. No rats of either age died during the exposure, but in the guinea pigs exposed to 18,800 \( \mu g/m^3 \) (10 ppm), mortality increased with increasing age from 4\% in the 5-day-old group to 60\% in the 55-day-old group and 67\% in the mothers. In both species, older animals showed greater effects of exposure than did the newborns. At 3,760 \( \mu g/m^3 \) (2.0 ppm), no histological effects were observed in rats of any age. Neither did exposure of guinea pigs < 45 days old to this level produce histological effects. The 45-day-old guinea pigs exposed to 3,760 \( \mu g/m^3 \) had thickened alveolar walls, alveolar edema, and inflammation, whereas animals older than 45 days showed similar, but more frequent, alterations that seemed to increase with age. The mother guinea pigs exposed to 3,760 \( \mu g/m^3 \) had focal loss of cilia in bronchioli. At 18,800 \( \mu g/m^3 \), only rats \( \geq 45 \) days old responded, confirming the observations of Stephens et al (1978). Rats in this group had fibrinous deposits in alveoli and focal loss of cilia in bronchioles. Guinea pigs of all ages were affected by exposure to 18,800 \( \mu g/m^3 \). All guinea pigs in that exposure group had loss of cilia in bronchioles and the trachea, edema, an increase in number of Type 2 cells, and alveolar inflammation. Guinea pigs greater than 60 days old and mother guinea pigs exposed to 18,800 \( \mu g/m^3 \) had the most severe lesions, including foci of interstitial pneumonia and emphysema, presumably enlarged airspaces. This study clearly demonstrated the relative insensitivity of newborn rats and guinea pigs to \( \text{NO}_2 \) and the greater sensitivity of guinea pigs compared to rats of the same age. The investigators noted that the "lungs of newborn guinea pigs at birth were more mature than newborn rat lungs," which may explain some, but probably not all, of the species differences in response of similarly aged rats and guinea pigs. (See Section 13.2.2.1 on host defense mechanisms.)

An extensive series of exposures designed to relate morphometric changes in the air-blood barrier to age was performed by Kyono and Kawai (1982). Rats at 1, 3, 12, and 21 mo of age were exposed continuously for 1 mo to 207, 865, 5,260, or 16,500 \( \mu g/m^3 \) (0.11, 0.46, 2.8, or 8.8 ppm) \( \text{NO}_2 \). Light and electron microscopic analyses were used to evaluate the exposure effects. Various morphometric parameters were assessed, including arithmetic mean thickness of the air-blood barrier (i.e., the thickness of the tissue between the surfaces of the alveolar and capillary lumens) and the volume density of various alveolar wall components. Because these investigators were interested in effects on the overall gas
exchange area, they deliberately excluded centriacinar alveoli, the site of major NO₂ damage, from the morphometric analyses. The arithmetic mean thickness of the air-blood barrier tended to increase in an exposure-dependant manner in all age groups. The arithmetic mean thickness of the air-blood barrier was significantly increased in all age groups in rats exposed to 16,544 μg/m³ or 5,264 μg/m³, with the exception of 12-mo-old rats, and in 3-mo-old rats exposed to 207 μg/m³. Response of arithmetic mean thickness to NO₂ was greatest in the 1-mo-old rats, decreased from 1 to 12 mo and increased again at 21 mo. The total interstitium (matrix and cells) increased with NO₂ concentrations over 5,264 μg/m³ and was a major component of the increase in volume density of alveolar wall tissue. Alveolar Type 1 and 2 cells showed various degrees of response, depending on both age at onset of exposure and exposure concentration. In general, the response of each lung component did not always show a simple concentration-dependent increase or decrease, but suggested a multiphasic reaction pattern. The investigators suggested that part of this observation may have been due to varying stages of impairment and repair. Further, they concluded that age-dependent differences in response to the same concentration of NO₂ occurred, and that the degree of response decreased with aging from 1 to 12 mo, after which it increased at 21 mo.

This study by Kyono and Kawan (1982) is especially important, as it is one of the few studies that examined the interstitium, and all studies of the interstitium (Chang et al., 1986, Kubota et al., 1987) have reported increases in one or both components of the interstitium. These morphological studies correlate well with reports of increased collagen synthesis rates discussed in Section 13.2.2.3 on lung biochemistry.

A possible concern in assessing NO₂ toxicity is the effect on adults from exposure during early life, especially during the period of lung development. Unfortunately, there are few data to allow evaluation of this. Mauderly et al. (1987) compared developing rats (exposure began in utero) and 6-mo-old animals exposed to 17,900 μg/m³ (9.5 ppm) NO₂ for 7 h/day, 5 days/week for 6 mo. Lung development, as determined at young adulthood, was not significantly affected by earlier exposures. There was no significant LM evidence of lung injury in either group of animals, and there were no exposure-related differences in the morphometric parameters studied (i.e., alveolar mean linear intercept or the internal surface area of the lungs). Thus, the available data base indicates that alveoli of NO₂-exposed rats
develop normally, but does not provide information concerning other morphological parameters of lung growth and development.

Chang et al. (1986, 1988) exposed 1-day- or 6-week-old rats for 6 weeks to a baseline of 940 μg/m³ (0.5 ppm) NO₂ for 23 h/day, 7 days/week, with two 1-h peaks (given in the morning and afternoon) of 2,820 μg/m³ (1.5 ppm), 5 days/week, and examined the proximal alveolar and the terminal broncholar regions. The detailed results are described in the previous section. The older animals had an increase in the surface density of the alveolar basement membrane that was not found in the younger animals. Although both age groups responded in a generally similar manner, the 6-week-old animals seemed to be generally more susceptible to injury than were the 1-day-old animals, as they had more variables that were significantly different from their control groups. Although there was no qualitative evidence of morphological injury in the terminal bronchioles of the younger rats, there was a 19% increase in the average ciliated cell surface that was not evident in the older rats. The authors also reported a 13% increase in the mean luminal surface area of Clara cells in the younger animals versus control animals of the same age.

Although the investigators concluded that the 6-week-old rats were as sensitive or more sensitive than 1-day-old rats, an alternative conclusion that the reverse is true cannot be ruled out by this study. The 1-day-old rats were probably not sensitive to the effects of NO₂ until they were 20 days old (Stephens et al., 1978, Azoulay-Dupuis et al., 1983), after which their sensitivity would increase until they were 35 days old. Thus, the effects measured in the 1-day-old rats may have resulted only from the last 22 days of exposure rather than the entire 42 days of effective exposures of the older rats. If the measured effects were essentially the same as the investigators concluded, the 1-day-old rats would be more sensitive than the older animals as they responded quantitatively similarly to about one half the exposure time as the older animals. Another alternative conclusion is that the 1-day-old rats, due to the 20 nonreactive exposures, were in a different phase of damage and repair at the time of examination. The exposure method may also have had an influence. Prior to weaning, neonates are exposed with their mother, often on bedding. The resulting huddling behavior and reactivity of NO₂ with the bedding could affect exposure of the neonates.

In general, it seems that neonates, specifically prior to weaning, are resistant to the morphological effects of NO₂, and that responsiveness increases with age after weaning until...
a plateau is reached. Furthermore, the responsiveness of mature animals appears to decline somewhat with age, until an increase in responsiveness occurs at some point in senescence. However, the morphological response to NO₂ in animals of different ages involves similarities in the cell types affected and in the nature of the damage incurred. Age-related differences occur in the extent of damage and in the time required for repair, the latter taking longer in older animals. The reasons for age differences in sensitivity are not known, but may involve differences in diet, variable sensitivity of target cells during different growth phases, or differences in site-specific doses.

Preexisting respiratory disease as a susceptibility factor has been studied rarely in animals, however, an animal model of emphysema (i.e., with enlarged airspaces but not airspace wall destruction) has been used. Lafuma et al. (1987) exposed both normal and elastase-induced emphysematous hamsters to 3,760 µg/m³ (20 ppm) NO₂, 8 h/day, 5 days/week for 8 weeks and analyzed lung tissue with morphometric assays. The emphysematous lesions produced by elastase appeared to be aggravated by subsequent exposure to NO₂. When compared to hamsters treated with elastase and exposed to clean air, elastase-treated hamsters exposed to NO₂ had increases in mean linear intercept and pulmonary volume (volume of fixed lung as measured by saline displacement) and a decrease in internal alveolar surface area. The investigators suggested that these results may imply a role for NO₂ in enhancing preexisting emphysema. However, there no changes in pulmonary mechanics of the animals (Section 13.2.2.3). In a more comprehensive, longer term study, Mauderly et al. (1989, 1990) exposed elastase-treated and control rats to 17,900 µg/m³ (9.5 ppm) NO₂, 7 h/day, 5 days/week for 2 years or to filtered air. They evaluated pulmonary function, clearance, lung collagen, BAL, and histopathology (by LM), including quantitative methods for alveolar size and internal surface area. There were no additive morphological effects of NO₂ on the emphysematous rat lungs. In summary, it appears that elastase-induced emphysema in rats does not increase susceptibility to NO₂ exposure, but that the similar procedures using hamsters may result in enhancement of the emphysema.

Acute infectious lung disease before and during NO₂ exposure could also affect morphologic responses to NO₂ exposure. Fenters et al. (1973) challenged squirrel monkeys with an influenza virus at various times during continuous exposure to 1,880 µg/m³ (1.0 ppm) NO₂ for 16 mo, and compared the response to that seen in animals not challenged.
but exposed to NO\textsubscript{2}. Only the virus-challenged animals showed responses to NO\textsubscript{2}, which included thickening of bronchial and bronchiolar epithelium. The investigators also reported "slight emphysema" in viral-challenged exposed monkeys. However, the lungs were not fixed in a manner appropriate for the diagnosis of emphysema using the current definition for human emphysema (National Institutes of Health, 1985). See the following subsection addressing NO\textsubscript{2}-induced emphysema in experimental animals. This study suggests that acute lung disease may increase responses to NO\textsubscript{2}. The effects of NO\textsubscript{2} on infectivity due to challenges with microorganisms are discussed in Section 13.2.2.1.

**Emphysema Following Nitrogen Dioxide Exposure**

In evaluating the reports of emphysema following NO\textsubscript{2} exposure, it is necessary to consider both the current and previous definitions of emphysema and to try to determine the morphological lesions the investigators observed that led them to the diagnosis of emphysema. Several professional groups have presented definitions of emphysema. Those definitions have changed significantly from those proposed by a group of British physicians meeting under the auspices of Ciba in 1959 (Fletcher et al., 1959). Presumably, both investigators who wrote manuscripts and reviewers for journals that published manuscripts concerning emphysema in NO\textsubscript{2}-exposed animals were aware of, and used, the latest definition at the time the manuscripts were written and reviewed. Thus, the date of publication of the reports of effects of NO\textsubscript{2} inhalation relative to the dates of publication of the definitions of emphysema must be considered in the diagnosis of emphysema. The most recent definition, by NHLBI, Division of Lung Diseases Workgroup (National Institutes of Health, 1985), differentiates between emphysema in human lungs and animal models of emphysema. When reports of emphysema following NO\textsubscript{2} exposures of animals are to be extrapolated to potential hazards for humans, the definition of human emphysema, rather than that for animal models of emphysema, must be used. Thus, the current definitions of emphysema in human lungs and in animal models are critical to this review.

The report from the National Institutes of Health (1985) first defines respiratory airspace enlargement. "Respiratory airspace enlargement is defined as an increase in airspace size as compared with the airspace size of normal lungs. The term applies to all varieties of airspace enlargement distal to the terminal bronchioles, whether occurring with
or without fibrosis or destruction" Emphysema is one of several forms of airspace enlargement. In human lungs, "Emphysema is defined as a condition of the lung characterized by abnormal, permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by destruction of their walls, and without obvious fibrosis." Destruction is further defined "Destruction in emphysema is further defined as nonuniformity in the pattern of respiratory airspace enlargement so that the orderly appearance of the acinus and its components is disturbed and may be lost." The report also indicates that "Destruction may be recognized by subgross examination of an inflation-fixed lung slice." Emphysema in animal models was defined differently. The stated reason for this difference in the definitions of emphysema in humans and in animal models was "In order to foster the development of new knowledge, animal models of emphysema are defined as nonrestrictively as possible. An animal model of emphysema is defined as an abnormal state of the lungs in which there is enlargement of the airspaces distal to the terminal bronchiole. Airspace enlargement should be determined quantitatively in appropriate specimens and qualitatively by stereologic methods." Thus, in animal models of emphysema, airspace wall destruction need not be present. "Appropriate specimens" presumably refers to lungs fixed in the inflated state and is similar to the 1962 American Thoracic Society Committee's requirement for tissue fixation. This document states "It is still not clear whether the airspace enlargement of age is due to age alone or to the combination of age and environmental history, but the occurrence of these changes in nearly all subjects suggests that the changes are normal" (Meneely et al., 1962). Control animals of the same age as the experimental animals appear necessary to avoid potential confusion due to age. This National Institutes of Health committee also noted that, to date, animal models of emphysema fall into two general classes. "The first class centers on testing the pathogenicity of agents suspected of being relevant to the genesis of emphysema, models produced by NO₂, cadmium, and tobacco smoke are examples of this type. The second class of models is analytical, for testing specific hypotheses of the pathogenesis of emphysema." Both classes of studies are in this review.

These definitions of emphysema in human lungs and of animal models of emphysema are significantly different from the 1959 Ciba definitions (Fletcher et al., 1959). One of the lasting benefits of these Ciba definitions was the development of the concept that emphysema
should be defined in terms of morbid anatomy rather than altered physiology or clinical observations (Fletcher and Pride, 1984) The Meneely et al (1962) definition continued that concept and expanded on the most useful instruments for morphological examination of properly fixed lungs All of the definitions since 1959 have required that lungs be distended and fixed before they are cut so the airspaces can be examined in the inflated state

Thus, in reviewing reports of emphysema following experimental NO₂ exposure, important considerations include (1) whether the tissue was fixed in an inflated state, (2) whether airspaces distal to the terminal bronchiole were enlarged beyond normal and whether that enlargement was determined quantitatively by stereologic methods (control animals of identical age as exposed animals should be used for stereologic studies to exclude the possibility that airspace enlargement was due to age), and (3) whether or not airspace wall destruction, as defined by the NHLBI workgroup (National Institutes of Health, 1985), was present. The presence of airspace wall destruction, as defined by the NHLBI workgroup, is critical In published reports of emphysema following NO₂ exposure, evidence of airspace wall destruction can only be obtained by careful review of the authors’ description of the lesions or by examining the micrographs the author selected for publication In reviewing the research reports, the authors’ descriptions of tissue changes relative to the definition of destruction in the NHLBI workgroup document (National Institutes of Health, 1985) are quoted, and all published micrographs were examined for evidence of destruction as defined by this 1985 NHLBI document Because of the changes in the definitions of emphysema and in the methods used for evaluation of results of NO₂ exposures, the studies are reviewed chronologically

Freeman et al (1964) reported emphysema in rats exposed to 47,000 µg/m³ (25 ppm) NO₂ for 32 to 65 days Control rats of the same age were maintained in an identical chamber They reported that "alveolar ducts and alveoli in experimental rats are more variable in size and many are much larger than in controls " The methods for fixation of the lungs are not mentioned, nor are the methods for evaluating size of airspaces Although the size of the lungs from exposed rats was stated to be increased, lung weights rather than lung volumes were reported The conclusion that emphysema was present was presumably based on the large size of the lungs and the observation of enlarged airspaces that were variable in size, as no evidence of destruction of airspace walls was presented In terms of the 1985
NHLBI definitions, these lesions appear to represent airspace enlargement rather than emphysema of the type seen in human lungs.

Wagner et al. (1965) exposed dogs, rabbits, guinea pigs, rats, hamsters, and mice to 9,400 μg/m³ (5 0 ppm) NO₂ for up to 18 mo. They did not observe morphological effects due to the exposure.

Haydon et al. (1965) reported emphysema in rats exposed for varying periods from 51 to 813 days to 22,600 μg/m³ (12 ppm) NO₂, but not in other rats exposed to 7,520 or 1,500 μg/m³ (4 0 or 0 8 ppm) NO₂. The lungs were fixed via the trachea in the inflated state. The alveoli were described as "quite variable in size, being either dilated or collapsed." The most striking microscopic abnormality was the "persistent variation in size of alveoli." The diagnosis of emphysema was based on alveolar size and variation in size and on the "grossly distended, air-filled lungs that fail to collapse when removed from the thorax." They reported "occasional rupture of alveolar walls." In terms of the 1985 NHLBI definitions, these lesions appear to be primarily airspace enlargement rather than emphysema of the type seen in human lungs.

Haydon et al. (1967) also reported emphysema in rabbits exposed continuously (presumably 24 h/day) for 3 to 4 mo to 15,000 or 22,600 μg/m³ (8 or 12 ppm) NO₂. The lungs were fixed via the trachea in an expanded state. They reported enlarged lungs that failed to collapse when the thorax was opened. In 100-μm thick sections from formaldehyde-fixed dried lungs, they reported "dilated" airspaces with "distorted architecture." In those and other tissue preparations, they reported that the airspaces appeared "grossly enlarged and irregular, which appears to be due to disrupted alveoli and the absence of adjacent alveolar collapse." Thus, in appropriately fixed lungs, they reported evidence of enlarged airspaces with destructive changes in alveolar walls. Although no stereology was done, this appears to be emphysema of the type seen in human lungs. Davidson et al. (1967) reported physiologic changes in these rabbits, but no new observations related to the criteria for emphysema.

Unlike their previous reports of emphysema in rats exposed to higher concentrations of NO₂, Freeman et al. (1968b) reported that rats exposed continuously (24 h/day) to 3,760 μg/m³ (2 0 ppm) NO₂ for 112 to 763 days had only equivocal increases in lung weight and distension of airspaces. These lungs were fixed in a distended state via the trachea. These NO₂ exposures did not result in emphysema.
Freeman et al (1968a) summarized many of their previously published NO₂ exposures in a manuscript resulting from a meeting (symposium) presentation. They again followed the definition of emphysema proposed by the 1959 Ciba Symposium and did not present new data relative to enlarged airspaces or destruction of alveolar walls. No new information relevant to emphysema following NO₂ exposure was presented.

At the same meeting (symposium), Kleinerman and Cowdrey (1968), reported results of exposures of hamsters to 84,600 to 103,000 µg/m³ (45 to 55 ppm) NO₂, 22 to 23 h daily for 10 continuous weeks. Some of the hamsters were held in room air for a 4-week postexposure period. The lungs were fixed in a distended state by formaldehyde fumes via the trachea. Fixed-lung volumes were estimated by water displacement. Fixed-lung volumes of exposed and postexposed hamsters were significantly larger than similar-aged controls. Although the size of alveolar spaces appeared enlarged in the exposed animals, as compared to similarly fixed controls, there was no evidence of destruction. The authors concluded that "emphysema had not been produced in this experiment." These authors also reported previously unpublished observations that 21 to 23 h/day exposure of guinea pigs, rabbits, and rats to concentrations of NO₂ resulting in a mortality of approximately 35% does not result in emphysema due to the nondestructive character of the tissue response. They based their conclusions on the definition of emphysema proposed by the 1962 American Thoracic Society and discussed the necessity for inflation fixation of lungs using standard techniques. The conclusion that emphysema of the type seen in human lungs was not produced appears appropriate.

Gross et al (1968) studied the effects of NO₂ on control and pneumoconiotic lungs using hamsters and guinea pigs. Most exposures were for 2 h/day for 5 days/week. The NO₂ concentration appears to have been planned for 41,360 µg/m³ (22 ppm), but varied with an initial range of 94,000 to 169,000 µg/m³ (50 to 90 ppm) during the first 4 weeks and then was reduced to ranges of 56,400 to 94,000 µg/m³ (30 to 50 ppm) for a total exposure period of 12 mo. The lungs were fixed in a distended state via the trachea with formaldehyde fumes. No morphometry of airspaces was reported. The complex experimental design and deaths of animals during exposure made interpretation difficult. In hamsters, "since more animals with emphysema were found in the group not exposed to NO₂ it can also be concluded that long-term exposure of hamsters to NO₂ did not cause emphysema." In guinea...
pigs, this exposure resulted in "multiple small foci of emphysema with a prevalence of only 15%". More animals (guinea pigs) without pneumoconiosis developed this emphysema than did animals with pneumoconiosis. Both enlarged airspaces and destruction of alveolar walls can be seen in some of the published micrographs. Although emphysema with alveolar wall destruction was present, the relationship of the emphysema to NO$_2$ exposure is not clear.

Blair et al. (1969) exposed mice to 940 $\mu$g/m$^3$ (0.5 ppm) NO$_2$, 6, 18, or 24 h/day for 1 to 12 mo. The method of fixation of the lungs is not entirely clear. They were fixed by immersion, presumably by immersion of slices of lung, which would be collapsed rather than fixed in a distended state as recommended by the American Thoracic Society in 1962 (Meneely et al., 1962). It is possible that the lungs might have been distended with air, the trachea tied and then immersed in fixative that would diffuse into the lung through the thin pleura. Control unexposed mice had pneumomitis. Although these investigators made an attempt to measure alveolar size, they properly conclude that their measurements "did not represent quantitatively whole lung structure." Thus, the data concerning enlarged airspaces is not reliable due to the types of lung fixation and alveolar morphometry. They also mentioned alveolar "septal breakage", but not destruction as defined by the 1985 NHLBI definition, and the septal breakage was not a factor in the increased size of alveoli. No evidence of emphysema was presented in this publication.

Buckley and Loosh (1969) studied the effects of 75,200 $\mu$g/m$^3$ (40 ppm) NO$_2$ for 6 or 8 weeks on germ-free mice. Following exposure, the mice were either sacrificed and the lungs were examined, or the mice were infected with microorganisms for additional studies. The lungs were fixed by inflation via the trachea. They made no mention of lung or alveolar size or of the presence or absence of alveolar destruction, even though they cited two of the earlier studies of Freeman and Haydon. In the published micrographs of control- and NO$_2$-exposed mice, the airspaces appear to be the same size and evidence of destruction was not seen. No evidence of emphysema was presented in this publication.

Freeman et al. (1972) exposed rats to 37,600 $\mu$g/m$^3$ (20 ppm) NO$_2$, which was reduced during the exposure to 28,200 $\mu$g/m$^3$ (15 ppm) or to 18,800 $\mu$g/m$^3$ (10 ppm) for varying periods up to 33 mo. Following removal at necropsy, the lungs were fixed via the trachea at 25 cm of fixative pressure. Morphometry of lung and alveolar size was performed in a suitable, although unconventional, manner. The morphometry indicated enlargement of 13-115.
alveoli and reduction in alveolar surface area. They also both reported alveolar destruction and illustrated alveolar destruction in their figures. They correctly conclude that they have demonstrated emphysema in their NO2-exposed rats. However, it is not entirely clear whether both experimental groups or only the group exposed to 28,200 μg/m³ NO2 had emphysema.

Ehrlich and Fenters (1973) exposed squirrel monkeys to 9,400 or 18,800 μg/m³ (5 or 10 ppm) NO2 for 3 mo or to 1,880 μg/m³ (1 0 ppm) NO2 for 16 mo and then challenged the monkeys with influenza virus. Pieces of lung were probably fixed in a collapsed condition by immersion, as they state: "At autopsy representative lung tissues were obtained for histopathological examination." Neither airspace wall destruction nor morphometry are mentioned in the article. They concluded that "slight emphysema" was present in the monkeys exposed to the two highest exposure levels and to the virus and "slight to moderate emphysema" was present in those exposed to 1,880 μg/m³ NO2 and to the virus. They also reported emphysema was not present in monkeys exposed to 1,880 μg/m³ NO2 without the viral challenge. The morphological methods used preclude useful information concerning emphysema following NO2 exposure.

Stephens et al. (1976), in a long abstract of papers presented at that year's Aspen conference, stated that "Rats exposed for long periods (3 to 5 mo) to 28,200 μg/m³ (15 ppm) NO2 or 0.8 ppm O3 develop a disease which closely resembles emphysema." Because it is only a long abstract and the emphasis of the paper was cellular injury, there were no data relative to alveolar size, nor was there information concerning the presence or absence of alveolar destruction. Thus, this paper does not provide new data relative to the presence of emphysema of the type seen in human lungs.

Port et al. (1977) studied experimental and spontaneous emphysema in rat, mouse, hamster, horse, and human lungs and compared them with normal control lungs from the same species. Only six mice and one rat had been exposed to NO2. The mice were exposed to 188 μg/m³ (0 1 ppm) NO2 with a 2-h peak of 1,880 μg/m³ (1 0 ppm) NO2 daily for 6 mo. Control mice of the same age were also examined. Only one NO2-exposed and one control rat were studied. The exposed rat breathed 28,200 μg/m³ (15 ppm) NO2 from 35 days to approximately 5 mo of age, when clinical illness became apparent. The NO2 was then administered intermittently, based on the clinical signs, to permit survival for at least
2 years The control rat was approximately the same age as the exposed one. The lungs were fixed in a distended condition via the trachea at a constant pressure. The lungs were examined by LM and SEM. The NO₂-exposed rat had distended alveoli and evidence of airspace wall destruction. Both dilated airspaces and evidence of alveolar wall destruction were reported in the NO₂-exposed mice lungs. Although airspace wall destruction was demonstrated, the small number of animals studied severely limits the value of this study.

Hyde et al. (1978) studied dogs that had been exposed 16 h daily for 68 mo to either filtered air or to 1,210 μg/m³ (0.6 ppm) NO₂ with 310 μg/m³ (0.16 ppm) NO or to 270 μg/m³ (0.14 ppm) NO₂ with 2,050 μg/m³ (1.1 ppm) NO. The dogs then breathed clean air during a 32- to 36-mo postexposure period. The right lungs were fixed in a distended state via the trachea at 30 cm fixative pressure. Semiautomated image analysis was used for morphometry of airspaces. The dogs exposed to 1,210 μg/m³ NO₂ with 310 μg/m³ NO had statistically significantly larger lungs with enlarged airspaces and evidence of destruction of alveolar walls. These effects were not observed in dogs exposed to 270 μg/m³ NO₂ with 2,050 μg/m³ NO, implying a significant role of the NO₂ in the production of the lesions. The lesions in dogs exposed to the higher NO₂ concentration meet the criteria of the 1985 NHLBI workshop for emphysema of the type seen in human lungs.

Lam et al. (1983) exposed 3- and 21-day-old hamsters to 56,400 to 65,800 μg/m³ (30 to 35 ppm) NO₂, 23 h/day for 7 days. The hamsters then breathed room air until they were 1 year old, when they were killed and examined. The lungs were fixed via the trachea at 25 cm of fixative pressure and fixed lung volumes were determined by displacement. Appropriate stereologic methods were used to determine the mean linear intercept and internal surface area. The authors did not report emphysema or evidence of alveolar destruction, nor was emphysema demonstrated in the published micrographs of exposure-related lesions. The group exposed starting at 3 days old, but not those exposed starting at 21 days old, had longer mean linear intercepts, indicating larger alveoli. Although they indicated that these changes were "compatible" with "early emphysema", they did not specifically conclude that the hamsters had emphysema. This conclusion is appropriate as no evidence of emphysema of the type seen in human lungs was presented.

Kleinerman et al. (1985) describe the effects following exposure of hamsters to 56,400 μg/m³ (30 ppm) NO₂, 22 h/day for 12 mo. The authors did not describe the
A statistically significant longer mean linear intercept was noted in the exposed hamsters, indicating larger alveoli. However, there was no indication that destruction was or was not present. The authors concluded that "a small definable degree of emphysema developed" using the 1985 National Institutes of Health workgroup's definition of emphysema in animal models. Evidence of emphysema of the type seen in humans was not presented.

Stavert et al. (1986) exposed rats that had received a single intratracheal instillation of sterile normal saline to either 65,800 µg/m³ (35 ppm) NO₂ for 6 h/day or to filtered air for 25 days. The rats were held an additional 10 weeks and then were examined. The lungs were fixed by intratracheal formalin at 25 cm water pressure and morphometry was performed according to standard, acceptable techniques. They reported that, microscopically, the lungs from these two groups appeared identical. The mean linear intercepts of these two groups were nearly identical, indicating similar-sized alveoli. They did not report destruction, nor is it evident in their published micrographs. They concluded that this exposure regimen "does not bring about irreversible changes in the lungs of rats which are consistent with either centrilobular or panlobular emphysema." This conclusion is appropriate.

Glasgow et al. (1987) exposed rats to 56,400 µg/m³ (30 ppm) NO₂, 24 h/day for up to 140 days. The primary objective of this study was to evaluate neutrophil recruitment and degranulation from NO₂-induced emphysema. The lungs were fixed via the trachea in an appropriate manner and the mean linear intercept was determined by semiautomatic image analysis, which was compared to manual methods. Exposed rats had significantly longer mean linear intercepts, indicating larger alveoli and alveolar wall destruction. The authors concluded that the exposed rats had emphysema based on the 1985 National Institutes of Health's workgroup definition of emphysema in animal models. However, it appears that they either pooled data from several ages of control rats or terminated all of the control rats at or before the day the exposures were started. Thus, controls may have been younger than the exposed rats. In addition, there may have been differences in the methods by which the lung tissues were processed, exposed and control rats may not have been processed at the same time. For example, in their Figure 1 (the example of alveolar wall destruction), the alveoli in the control lung do not appear to be as fully distended, as the alveolar walls are
less straight than those of the lungs from the exposed rat. The figure legend does not indicate the ages of either the control or exposed rat. Although these problems may not influence the investigators' main objectives, they are troublesome with respect to the presence or absence of emphysema. The results are inconclusive with respect to the production of emphysema of the type seen in human lungs.

Blank et al. (1978) used NO2 to produce an animal model of emphysema based on the 1985 National Institutes of Health's workgroup definition. The objective of this study was to determine the effect of beta-aminopropionitrile, which inhibits cross-linking of collagen and elastin, on that animal model. They exposed rats to 56,400 μg/m3 (30 ppm) NO2, 24 h/day for up to 8 weeks. The lungs were fixed by appropriate methods, and the mean linear intercept was determined by semiautomatic image analysis. Control and exposed rats fed the usual rat chow were terminated at the same time and age. They reported longer mean linear intercepts in the exposed rats, indicating larger alveoli. They mentioned that the lungs were examined for alveolar wall destruction, but do not clearly indicate whether destruction was present. They properly refer to the NO2-induced lesions as "emphysema-like" rather than emphysema of the type seen in human lungs.

Lafuma et al. (1987) studied the effect of exposure to 3,760 μg/m3 (20 ppm) NO2, 8 h/day, 5 days/week for 8 weeks on control hamsters and on hamsters with elastase-induced emphysema. The lungs were fixed via the trachea at a constant pressure. Relative alveolar sizes were estimated using standard stereological methods. They found statistically significantly larger lung volumes and mean linear intercepts and smaller internal surface areas, indicating larger alveoli in hamsters exposed to NO2 alone. They did not state whether airspace wall destruction was or was not found. There is no evidence of destruction in the published micrographs of lungs from that group of hamsters. There is no evidence from this publication that NO2 alone produced emphysema of the type seen in human lungs.

The study by Mauderly et al. (1989, 1990), discussed in the previous section, is also relevant to emphysema in that the findings were negative. They studied the mean linear intercept and internal surface areas of rats exposed to 17,900 μg/m3 (95 ppm) NO2, 7 h/day, 5 days/week for 2 years. There was no significant effect on either of these parameters.
Table 13-12 provides an overview of the results of the studies discussed in this section.

**Summary**

The anatomic region most sensitive to NO$_2$, and within which injury is generally first noted, is the area that encompasses the terminal conducting airways and adjacent alveolar ducts and alveoli. Within this region, those cells most sensitive to NO$_2$-induced injury are the ciliated cells of the bronchiolar epithelium and the Type 1 cells of the alveolar epithelium. There is, however, a large degree of interspecies variability in responsiveness to NO$_2$ exposure, with guinea pigs, hamsters, and monkeys being more severely affected than the rat when exposed under the same conditions and examined using the same techniques (Azoulay-Dupuis et al., 1983, Wagner et al., 1965, Furiosi et al., 1973). When effective NO$_2$ concentrations are used and appropriate tissues are examined using sensitive methods, similar morphological lesions are found in all species. In alveoli, necrosis and sloughing of Type 1 cells, leaving bare basal lamina, is followed by proliferation of Type 2 cells that replace damaged Type 1 cells. In bronchioles, ciliated cells lose some or all of their cilia and nonciliated bronchiolar cells (Clara cells in rodents) lose their dome-like luminal surface projections. Ciliated cells may become necrotic and sloughed from the basal lamina. Nonciliated bronchiolar cells, progenitor cells for ciliated cells, proliferate and become larger. Such effects have been reported following acute exposure to $\geq 3,760$ $\mu$g/m$^3$ (2.0 ppm) NO$_2$, depending on the species tested (Azoulay-Dupuis et al., 1983, Rombout et al., 1986). As the exposure duration increases, similar effects are noted, but hyperplasia and hypertrophy of Type 2 cells and nonciliated bronchiolar cells are the predominant epithelial changes (Sherwin and Richters, 1982, Rombout et al., 1986, Nakajima et al., 1980, Sherwin et al., 1973, Yamamoto and Takahashi, 1984). By LM, these changes may appear to regress during an exposure to low concentrations, or soon after the exposure ends (Kubota et al., 1987). Increases in the thickness of the basal lamina and interstitium are slower to develop and probably slower to resolve (Kubota et al., 1987).

Both exposure concentration and duration are important factors affecting response, with concentration perhaps playing a more significant role (Rombout et al., 1986). Age may also be a factor affecting response, with neonates being more resistant to the morphological effects of NO$_2$ and responsiveness increasing with age until weaning or shortly after weaning.
<table>
<thead>
<tr>
<th>NO$_2$ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Emphysema$^b$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>188 with 2-h peaks to 1,880</td>
<td>0 1 with peaks to 1 0</td>
<td>Daily, 6 mo</td>
<td>NS</td>
<td>4 weeks</td>
<td>Mouse (NS)</td>
<td>±</td>
<td>Port et al (1977)</td>
</tr>
<tr>
<td>270 plus 2,050 μg/m$^3$ NO</td>
<td>0 14</td>
<td>16 h/day, 68 mo</td>
<td>F</td>
<td>6 mo</td>
<td>Dog (Beagle)</td>
<td>−</td>
<td>Hyde et al (1978)</td>
</tr>
<tr>
<td>1,210 plus 310 μg/m$^3$ NO</td>
<td>0 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>940</td>
<td>0 5</td>
<td>6, 18, or 24 h/day, 1-12 mo</td>
<td>NS</td>
<td>NS</td>
<td>Mouse (NS)</td>
<td>−</td>
<td>Blair et al (1969)</td>
</tr>
<tr>
<td>1,504 7,520</td>
<td>0 8 4 0</td>
<td>51-813 days</td>
<td>M</td>
<td>4 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>−</td>
<td>Haydon et al (1965)</td>
</tr>
<tr>
<td>1,880</td>
<td>1 0</td>
<td>16 mo, with and without viral challenge</td>
<td>NS</td>
<td>NS</td>
<td>Monkey (Squirrel)</td>
<td>±</td>
<td>Ehrlich and Fenters (1973)</td>
</tr>
<tr>
<td>3,760</td>
<td>2 0</td>
<td>Continuous, 112-763 days</td>
<td>M</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>−</td>
<td>Freeman et al (1968b)</td>
</tr>
<tr>
<td>3,760</td>
<td>2 0</td>
<td>8 h/day, 5 days/week, 2 mo</td>
<td>M</td>
<td>8 weeks</td>
<td>Hamster (Golden Syrian)</td>
<td>−</td>
<td>Lafuma et al (1987)</td>
</tr>
<tr>
<td>9,400 18,800</td>
<td>5 0 10 0</td>
<td>3 mo</td>
<td>NS</td>
<td>NS</td>
<td>Monkey (Squirrel)</td>
<td>−</td>
<td>Ehrlich and Fenters (1973)</td>
</tr>
<tr>
<td>NO$_2$ Concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Emphysema$^b$</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------</td>
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</tr>
<tr>
<td>9,400</td>
<td>5 0</td>
<td>6 h/day, 5 days/week, up to 18 mo</td>
<td>M</td>
<td>NS</td>
<td>Dog (Mongrel) Rabbit (NS) Guinea pig (English) Rat (Sherman) Hamster (Golden Syrian) Mouse (C57BL/6, CAF$_1$/JAX, HLA)</td>
<td>-</td>
<td>Wagner et al (1965)</td>
</tr>
<tr>
<td>15,040</td>
<td>8 0</td>
<td>3-4 mo (presumably 24 h/day)</td>
<td>NS</td>
<td>6-12 mo</td>
<td>Rabbit (NS)</td>
<td>+</td>
<td>Haydon et al (1967)</td>
</tr>
<tr>
<td>22,600</td>
<td>12 0</td>
<td>3-5 mo</td>
<td>NS</td>
<td>NS</td>
<td>Rat (NS)</td>
<td>-</td>
<td>Stephens et al (1976)</td>
</tr>
<tr>
<td>28,200</td>
<td>15 0</td>
<td>Continuously for 35 days, then intermittently for at least 2 years</td>
<td>NS</td>
<td>16 weeks</td>
<td>Rat (NS)</td>
<td>±</td>
<td>Port et al (1977)</td>
</tr>
<tr>
<td>37,600 reduced to either 28,200 or 18,800</td>
<td>20 reduced to 15 or 10</td>
<td>Up to 33 mo</td>
<td>M</td>
<td>4 weeks</td>
<td>Rat (Wistar)</td>
<td>+</td>
<td>Freeman et al (1972)</td>
</tr>
<tr>
<td>17,900</td>
<td>9 5</td>
<td>7 h/day, 5 days/week, 2 years</td>
<td>M</td>
<td>18 weeks</td>
<td>Rat (Fischer 344)</td>
<td>-</td>
<td>Mauerley et al (1989, 1990)</td>
</tr>
<tr>
<td>47,000</td>
<td>25</td>
<td>32-65 days</td>
<td>NS</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>-</td>
<td>Freeman et al (1964)</td>
</tr>
<tr>
<td>56,400</td>
<td>30</td>
<td>22 h/day, 12 mo</td>
<td>NS</td>
<td>NS</td>
<td>Hamster (NS)</td>
<td>-</td>
<td>Kleinerman et al (1985)</td>
</tr>
<tr>
<td>56,400</td>
<td>30</td>
<td>Continuous, up to 140 days</td>
<td>M</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>-</td>
<td>Glasgow et al (1987)</td>
</tr>
</tbody>
</table>
Emphysema IS defined accordmgto the 1985 NatIonal Heart, Lung and Blood Institute Workshop cntena for human emphysema. Although several of the papers reviewed reported findmg emphysema, some of these studies (especially the early studies) were reported accordmg to preVIOUS, dIfferent cntena, some reports dId not fully descnbe the methods used, and/or the results obtamed were not In suffICIent detaIl to allow mdependent confinnatlOn of the presence of emphysema. Thus, a "-" (I e., no emphysema) should only be interpreted as lack of proof of emphysema, because it IS conceivable that if the study were repeated with current methods and the current criteria were applied, some results might be positive.
Responsiveness in mature animals appears to decline with age until an increase occurs at some point in senescence (Kyono and Kawai, 1982)

Several groups of investigators who experimentally exposed different species of laboratory animals to NO\(_2\) have reported emphysema of the type seen in human lungs as defined by the NHLBI workgroup (National Institutes of Health, 1985). In human lungs, the workgroup defined emphysema as "a condition of the lung characterized by abnormal, permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by destruction of their walls, and without obvious fibrosis." "Destruction in emphysema is defined as nonuniformity in the pattern of respiratory airspace enlargement so that the orderly appearance of the acinus and its components is disturbed and may be lost." Studies in this group include those by Haydon et al (1967), Freeman et al (1972), and Hyde et al (1978). Results of studies by several additional groups of investigators are inconclusive because, although they demonstrated enlargement of airspaces, they did not document or report whether or not destruction as defined by the NHLBI workgroup (National Institutes of Health, 1985) occurred. The latter group includes several studies testing specific hypotheses of the pathogenesis of emphysema, which, as appropriate to their research objectives, used the NHLBI workgroup's definition of animal models of emphysema rather than the definition of emphysema in human lungs. The definition of emphysema in animal models requires airspace enlargement, but not destruction. Several studies were considered inconclusive due to problems with control animals or to insufficient numbers of animals. It is important to note that a 2-year exposure of rats to 17,900 \(\mu\)g/m\(^3\) (9.5 ppm) NO\(_2\) did not result in evidence of emphysema, even though Mauderly et al (1987, 1990) used appropriate morphologic and morphometric methods.

### 13.2.3 Extrapulmonary Effects

Exposure to NO\(_2\) produces a wide array of health effects beyond the confines of the lung. Although the aggregate data are inconclusive and do not portray any single issue as paramount, the evidence suggests that NO\(_2\) and/or some of its reactive products penetrate the lung epithelial and endothelial layers to enter the blood and produce alterations in blood
and various other organs. Effects on the systemic immune system are included in Section 13.2.2.1 (Host Defense Mechanisms).

13.2.3.1 Body Weight

Traditionally, the measurement of body weight in animal toxicology studies has been considered a primary and sensitive end point. However, its biological significance and extrapolation to humans are still generally uninterpretable. The measurement of body weight may be useful in examining questions related to differences in species sensitivity, age, and different exposure scenarios. A compilation of the effects of NO\textsubscript{2} on body weight can be found in Table 13-13.

The most comprehensive study was performed by Wagner et al. (1965), who exposed rabbits, guinea pigs, rats, hamsters, and four strains of mice to 1,880, 9,400, or 47,000 \(\mu g/m^3\) (1, 5, or 25 ppm) and dogs to 1,880 or 9,400 \(\mu g/m^3\) NO\textsubscript{2}. For all species examined, no significant differences in body weight were observed after 6, 12, and 18 mo of exposure. Similarly, a study by Steadman et al. (1966) indicated that 90 days of continuous exposure to between 900 and 21,600 \(\mu g/m^3\) (0.48 and 11.5 ppm) NO\textsubscript{2} resulted in scattered reductions in body weight gain in five species of animals (dogs, rabbits, squirrel monkeys, guinea pigs, and rats) evaluated at three intervals (30, 60, and 90 days). The authors concluded that there was no significant weight loss, however, no statistical analysis of the data was presented. Also, increased mortality was observed at the 9,200-\(\mu g/m^3\) (4.9-ppm) concentration in guinea pigs and rabbits, and in all species at 21,600 \(\mu g/m^3\) (11.5 ppm) NO\textsubscript{2}.

The possibility that newborn mice were more sensitive to ambient levels of NO\textsubscript{2} than similarly exposed adults was investigated by Richters et al. (1987), who exposed 7-day gravid mice to 470 \(\mu g/m^3\) (0.25 ppm) NO\textsubscript{2}. Examination of the male offspring born during NO\textsubscript{2} exposure and exposed for 12 additional weeks showed less of an increase in body weight gain after 3 and 12 weeks of exposure than did air-exposed rats. However, at the 6-week measurement interval, the body weights of air- and NO\textsubscript{2} fetally exposed rats were not statistically different. Neonates similarly exposed to 564 \(\mu g/m^3\) (0.3 ppm) NO\textsubscript{2} did not show a reduction in body weight gain at 3 weeks, but did after 6 weeks of exposure, whereas adult mice exposed to 320 to 1,500 \(\mu g/m^3\) (0.17 to 0.8 ppm) NO\textsubscript{2} for periods ranging from 1 to 12 weeks showed no significant body weight changes.
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>0.05</td>
<td>90 days</td>
<td>NS</td>
<td>NS</td>
<td>Rat</td>
<td>No effect</td>
<td>Shalamberdze (1969)</td>
</tr>
<tr>
<td>320-1,504</td>
<td>0.17-0.8</td>
<td>8 h/day, 5 days/week, 1-12 weeks</td>
<td>M</td>
<td>Adult</td>
<td>Mouse (Swiss Webster)</td>
<td>No effect</td>
<td>Richters et al (1987)</td>
</tr>
<tr>
<td>470-564</td>
<td>0.25-0.30</td>
<td>8 h/day, 5 days/week, 3-12 weeks</td>
<td>M</td>
<td>in utero</td>
<td>Mouse (Swiss Webster)</td>
<td>Reduced body weight gain compared to controls at 3 and 12 weeks, but not at 6 weeks</td>
<td>Kuraitis et al (1981)</td>
</tr>
<tr>
<td>658</td>
<td>0.35</td>
<td>8 h/day, 5 days/week, 6 weeks</td>
<td>M</td>
<td>in utero and older mouse</td>
<td>Mouse (Swiss Webster)</td>
<td>Reduced body weight gain in newborns, but not older rats</td>
<td>Kuraitis et al (1981)</td>
</tr>
<tr>
<td>900-21,600</td>
<td>0.48-11.5</td>
<td>Continuous, 90 days</td>
<td>NS</td>
<td>NS</td>
<td>Dog (Beagle) Rabbit (New Zealand) Guinea pig (NS) Rat (Sprague-Dawley) Monkey (Squirrel)</td>
<td>No effect</td>
<td>Steadman et al (1966)</td>
</tr>
<tr>
<td>940-3,760</td>
<td>0.5-2.0</td>
<td>22 h/day base + 2 1-h peaks/day, 5 days/week, 1, 3, and 6 weeks</td>
<td>M</td>
<td>1 day and 7 weeks</td>
<td>Rat (Fischer 344)</td>
<td>Reduced body weight gain in older rats after 3 and 6 weeks exposure to 1 0 or 2 0 ppm No effects in younger rats</td>
<td>Stevens et al (1988)</td>
</tr>
<tr>
<td>1,300-1,500</td>
<td>0.7-0.8</td>
<td>30 days</td>
<td>F</td>
<td>8 weeks</td>
<td>Mouse (ICR)</td>
<td>No effect</td>
<td>Nakajima et al (1969)</td>
</tr>
<tr>
<td>1,504</td>
<td>0.8</td>
<td>Continuous, lifetime</td>
<td>M</td>
<td>4 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>No effect</td>
<td>Freeman et al (1966)</td>
</tr>
<tr>
<td>1,880</td>
<td>1.0</td>
<td>8 h/day, 6 mo</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Reduced body weight gain</td>
<td>Kosmider et al (1973b)</td>
</tr>
<tr>
<td>NO$_2$ Concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Effects</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------</td>
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</tr>
<tr>
<td>1,900-47,000</td>
<td>1.0-25</td>
<td>18 mo</td>
<td>M</td>
<td>NS</td>
<td>Rabbit (NS) Guinea pig (English) Rat (Sherman) Hamster (Golden Syrian) Mouse (C57BL/6, CAF$_1$/JAX, Webster)</td>
<td>No effect</td>
<td>Wagner et al (1965)</td>
</tr>
<tr>
<td>9,400</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td>Dog (Mongrel)</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>2,400-5,680</td>
<td>1.3-3.0</td>
<td>2 h/day, 15-17 weeks</td>
<td></td>
<td></td>
<td>Rabbit</td>
<td>Reduced body weight gain</td>
<td>Mitina (1962)</td>
</tr>
<tr>
<td>3,760</td>
<td>2.0</td>
<td>Continuous, up to 6 weeks</td>
<td>M</td>
<td>8 weeks</td>
<td>Rat (Wistar)</td>
<td>No effect</td>
<td>Azoulay et al (1978)</td>
</tr>
<tr>
<td>3,760</td>
<td>2.0</td>
<td>Continuous, lifetime</td>
<td>M</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>No effect</td>
<td>Freeman et al (1968c)</td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>Up to 62 days</td>
<td>M/F</td>
<td>in utero</td>
<td>Rat (NS)</td>
<td>Reduced body weight gain and length of pups</td>
<td>Freeman et al (1974)</td>
</tr>
</tbody>
</table>

$^a$M = Male  
F = Female  
NS = Not stated
Kuratsis et al. (1981) also reported that the body weights of newborn mice exposed to 658 µg/m³ (0.35 ppm) NO₂ for 6 weeks were significantly less than age-matched controls. No decrease in body weight was observed when older mice were exposed. Also, Nakajima et al. (1969) found no effects in 8-week-old mice exposed for 30 days to 1,316 or 1,504 µg/m³ (0.7 or 0.8 ppm) NO₂.

Only very high exposure concentrations of NO₂ have been found to cause reduced body weights in rats. No exposure-related effects were found in rats exposed for 90 days to 94 µg/m³ (0.05 ppm) NO₂ (Shalamberidze, 1969). Freeman et al. (1966, 1968b) found no effects on body weight after lifetime exposure to 1,504 or 3,760 µg/m³ (0.8 or 2.0 ppm) NO₂.

Although the data in mice suggest that newborns may be more sensitive to NO₂ exposure than older mice, other evidence (see Section 13.2.2.4), including body weight data, do not support this contention for rats. In contrast to the effects on body weight observed in newborn mice, Stevens et al. (1988) reported that 1-day-old rats were less responsive to NO₂ than 7-week-old rats exposed for 6 weeks to baselines of 940, 1,880, and 3,760 µg/m³ (0.5, 1.0, and 2.0 ppm) NO₂ with two 1-h peaks/day (5 days/week) to three times the baseline concentration. To simulate ambient exposure patterns, the NO₂ exposure was increased to three times the baseline concentration for 1 h twice daily. The body weight of the 7-week-old rats was less than the baseline concentration for 1 week and significantly less at 3 and 6 weeks when the baseline exposure concentration was 1,880 or 3,760 µg/m³. Rats exposed to 940 µg/m³ with peaks to 2,820 µg/m³ NO₂ showed a transitory decrease at 3 weeks that was no longer evident at 6 weeks. The 1-day-old rats, exposed within 36 h after birth to the same NO₂ concentrations, showed no changes in body weight after 1, 3, or 6 weeks of exposure.

Kosmider et al. (1973a) reported a reduction in body weight gain in guinea pigs exposed continuously to 1,880 µg/m³ (1.0 ppm) NO₂ for 6 mo. Mitina (1962) exposed rabbits to 2,400 and 5,680 µg/m³ (1.3 and 3.0 ppm) NO₂ for 15 and 17 weeks and found a significant reduction in body weight gain that persisted 5 to 7 weeks after the exposure ended.
13.2.3.2 Hematologic Changes

Alterations of blood constituents as a result of NO$_2$ exposure may be due to a variety of causes. Direct effects of NO$_2$, formation of nitrates and nitrates, or secondary effects emanating from other organs such as the lung, liver, heart, kidneys, and spleen could all result in alterations of blood content and chemistry. However, the significance of many of these hematological changes is uncertain.

**Effects on Blood Cell Counts and Hemoglobin**

Several authors have shown effects on the number of red blood cells (RBCs) and hemoglobin concentration, although the results have been inconsistent. A summary of these studies can be found in Table 13-14. In some of these studies, leukocytes (white blood cells [WBCs]) and platelet counts were also examined (Table 13-15).

Shalamberidze (1969) exposed rats continuously to 94 µg/m$^3$ (0.05 ppm) NO$_2$ for 90 days, causing no change in blood hemoglobin or RBC counts. Yakimchuk and Chelkanov (1972) reported that during a 3-mo continuous exposure to 600 µg/m$^3$ (0.32 ppm) NO$_2$, rats showed a significant increase in the number of leukocytes, and a tendency toward decreased hemoglobin and RBCs. However, by the end of exposure, these changes returned to within the control range. Plasma cholinesterase (ChE) was also measured, but no significant effects were found.

Fenters et al. (1973) showed that exposing male squirrel monkeys to 1,880 µg/m$^3$ (1.0 ppm) NO$_2$ continuously for 16 mo had no significant effect on hematocrit, hemoglobin, total protein, globulins, chloride, sodium, calcium, potassium, glucose, blood urea nitrogen, glutamic-pyruvic transaminase, LDH, and LDH isoenzymes. Challenge with influenza A/PR/8/34 virus increased the leukocyte number in NO$_2$-exposed animals above the levels in similarly challenged controls. A study by Steadman et al. (1966) indicated that 90 days of continuous exposure to between 900 and 21,600 µg/m$^3$ (0.48 and 11.5 ppm) NO$_2$ resulted in no significant changes in number of WBCs, percent hemoglobin, or percent hematocrit in five species of animals (dogs, rabbits, squirrel monkeys, guinea pigs, and rats).

Dogs exposed to 1,880 or 9,400 µg/m$^3$ (1.0 or 5.0 ppm) NO$_2$ for 18 mo showed no significant change in hemoglobin, hematocrit, WBC count, or serum alkaline phosphatase.
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>0.05</td>
<td>Continuous, 90 days</td>
<td>NS</td>
<td>NS</td>
<td>Rat</td>
<td>No effect on blood hemoglobin or RBCs</td>
<td>Shalamberdze (1969)</td>
</tr>
<tr>
<td>940-1,500 +</td>
<td>0.5-0.8 +</td>
<td>Continuous, 1 to 1.5 mo</td>
<td>M/F</td>
<td>4 weeks</td>
<td>Mouse (ICR JCL)</td>
<td>Addition of 50 ppm CO to NO₂ failed to affect carboxyhemoglobin</td>
<td>Nakajima and Kusumoto (1970)</td>
</tr>
<tr>
<td>1,500</td>
<td>0.8</td>
<td>Continuous, 5 days</td>
<td>M</td>
<td>7 weeks</td>
<td>Mouse (ICR)</td>
<td>No effect on methemoglobin</td>
<td>Nakajima and Kusumoto (1968)</td>
</tr>
<tr>
<td>1,880</td>
<td>1.0</td>
<td>Continuous, 16 mo</td>
<td>M</td>
<td>NS</td>
<td>Monkey (Squirrel)</td>
<td>No effect on hematocrit or hemoglobin with NO₂ and influenza exposure</td>
<td>Fenters et al (1973)</td>
</tr>
<tr>
<td>1,880</td>
<td>1.0</td>
<td>Continuous, 18 mo</td>
<td>M</td>
<td>NS</td>
<td>Dog (Mongrel)</td>
<td>No changes in hemoglobin or hematocrit</td>
<td>Wagner et al (1965)</td>
</tr>
<tr>
<td>1,880-56,400</td>
<td>1-30</td>
<td>18 h</td>
<td>NS</td>
<td>NS</td>
<td>Mouse (NS)</td>
<td>Concentration-related increase in methemoglobin and nitrosylhemoglobin</td>
<td>Case et al (1979)</td>
</tr>
<tr>
<td>2,400-5,640</td>
<td>1-3-0</td>
<td>2 h/day, 15 and 17 weeks</td>
<td>Rabbit</td>
<td>Decreased RBCs</td>
<td>Mitina (1962)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,760</td>
<td>2.0</td>
<td>Continuous, 14 mo</td>
<td>M/F</td>
<td>NS</td>
<td>Monkey (Macaca speciosa)</td>
<td>With or without NaCl (330 μg/m³) polycythemia with reduced mean corpuscular volume and normal mean corpuscular hemoglobin</td>
<td>Furioso et al (1973)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>8 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,760</td>
<td>2.0</td>
<td>Continuous, up to 6 weeks</td>
<td>M</td>
<td>8 weeks</td>
<td>Rat (Wistar)</td>
<td>No effect on hemoglobin, hematocrit or RBC count, no methemoglobin was observed</td>
<td>Azoulay et al (1978)</td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Effects</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----</td>
<td>----------</td>
<td>--------</td>
<td>-----</td>
<td>-----------------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>9,400-75,200</td>
<td>5-40</td>
<td>1 h</td>
<td>F</td>
<td>4 mo</td>
<td>Mouse (JCL ICR)</td>
<td>No increase in methemoglobin Increased nitrite and especially nitrate</td>
<td>Oda et al (1981)</td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>2 h/day, 5 days/week, up to 30 weeks</td>
<td>F</td>
<td>6-8 weeks</td>
<td>Mouse (BALB/c)</td>
<td>Small decrease in hemoglobin and mean corpuscular hemoglobin concentration</td>
<td>Holt et al (1979)</td>
</tr>
</tbody>
</table>

a NS = Not stated
RBCs = Red blood cells
M = Male
F = Female
CO = Carbon monoxide
NaCl = Sodium chloride
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 0.32</td>
<td></td>
<td>Continuous, 3 mo</td>
<td>M</td>
<td>NS</td>
<td>Rat (NS)</td>
<td>Increased leukocytes after 8-10 weeks, no difference after 3 mo of exposure</td>
<td>Yakimchuk and Chekanov (1972)</td>
</tr>
<tr>
<td>1,880 1.0</td>
<td></td>
<td>Continuous, 16 mo, followed by viral challenge</td>
<td>M</td>
<td>NS</td>
<td>Monkey (Squirrel)</td>
<td>Increased leukocyte count in viral-challenged NO₂-exposed animals</td>
<td>Fenters et al (1973)</td>
</tr>
<tr>
<td>1,880 1.0</td>
<td></td>
<td>Continuous, 18 mo</td>
<td>M</td>
<td>NS</td>
<td>Dog (Mongrel)</td>
<td>No effect on leukocyte count</td>
<td>Wagner et al (1965)</td>
</tr>
<tr>
<td>9,400 5.0</td>
<td></td>
<td>Continuous, 2 h/day, 15 and 17 weeks</td>
<td>M</td>
<td>NS</td>
<td>Rabbit</td>
<td>Increased leukocytes followed by decreased phagocytic activity</td>
<td>Mitma (1962)</td>
</tr>
<tr>
<td>2,400-5,640 1.3-3.0</td>
<td></td>
<td>Continuous, 14 mo</td>
<td>M/F</td>
<td>NS</td>
<td>Monkey (Macaca speciosa) Rat (Sprague-Dawley)</td>
<td>Neutrophil/lymphocyte ratio tendency to shift upwards in both animal species tested</td>
<td>Furosi et al (1973)</td>
</tr>
<tr>
<td>18,800 10</td>
<td></td>
<td>Continuous, 14 days</td>
<td>M</td>
<td>18 weeks</td>
<td>Rat (Wistar)</td>
<td>Decreased platelets at 1 to 7 days of exposure, but not after 14 days</td>
<td>Kobayashi et al (1983)</td>
</tr>
<tr>
<td>18,800 10</td>
<td></td>
<td>2 h/day, 5 days/week, up to 30 weeks</td>
<td>F</td>
<td>6-8 weeks</td>
<td>Mouse (BALB/c)</td>
<td>Increased leukocytes at 5 weeks, but not at 15 or 30 weeks</td>
<td>Holt et al (1979)</td>
</tr>
</tbody>
</table>

*M = Male
NS = Not stated
F = Female
and magnesium-activated phosphatase activity (Wagner et al., 1965). Rabbits exposed to 2,400 to 5,680 µg/m³ (13 to 30 ppm) NO₂ for 15 or 17 weeks had a decrease in the number of RBCs and a significant increase in the number of WBCs (Mitina, 1962).

Furiosi et al. (1973) exposed rats to 3,760 ± 1,880 µg/m³ (20 ± 10 ppm) NO₂ for 14 mo and found polycythemia with reduced mean corpuscular volume, but normal mean corpuscular hemoglobin concentrations. Exposure to NO₂ also increased the ratio of PMNs to lymphocytes. Because exposures occurred while the rats were in plastic cages inside the exposure chamber, the actual concentration of NO₂ would probably be less than the stated concentration. However, the reported observations are supported by similar findings in monkeys that were simultaneously exposed in wire cages.

Azoulay et al. (1978) reported no effects on rat RBC parameters (hemoglobin, hematocrit, and RBC count) after a continuous NO₂ exposure of 3,760 µg/m³ (20 ppm) lasting between 1 day and 6 weeks. Other factors that index or potentially alter oxygen affinity to hemoglobin (the partial oxygen pressure at which hemoglobin is half-saturated with O₂, n Hill factor [hemoglobin binding affinity], pH, oxygen combining capacity, and 2,3-diphosphoglycerate [a measure of tissue deoxygenation]) were not affected.

Although not a direct measure of RBC content, the number of RBCs in the red pulp of the spleen of mice was increased by a 6-week, 5-day/week, 8-h/day exposure to 658 µg/m³ (0.35 ppm) NO₂ (Kurattis et al., 1981). This finding could be interpreted as supporting the polycythemia that was sometimes observed in NO₂-exposed animals. Spleen weights and the size of spleen lymphoid nodules were also increased.

Three studies examined the production of physiologically inactive hemoglobin (methemoglobin) that might be produced if nitrates or nitrates reacted with hemoglobin. Nakajima and Kusumoto (1968) found that the amount of methemoglobin was not increased when mice were exposed to 1,504 µg/m³ (0.8 ppm) NO₂ for 5 days. Methemoglobin was not detected after a 6-week exposure of rats to 3,760 µg/m³ (0.2 ppm) (Azoulay et al., 1978). Oda et al. (1981) also found no increase in methemoglobin, but nitrates and especially nitrates were elevated in the blood of mice exposed for 1 h to between 9,400 and 75,200 µg/m³ (5 and 40 ppm) NO₂. In contrast, Case et al. (1979) showed that mice exposed to 1,880 to 56,400 µg/m³ NO₂ (1 to 30 ppm) exhibited a concentration-related
increase in methemoglobin and nitrosylhemoglobin and decreased ferric catalase and iron transferrin activities.

**Effects on Red Blood Cell Membranes**

Several studies have examined changes in RBC membranes of rats after NO₂ exposure (see Table 13-16). In a preliminary report, Mersch et al. (1973) showed that RBC D-2,3-diphosphoglycerate was increased in all four guinea pigs continuously exposed to 677 µg/m³ (0.36 ppm) NO₂ for 1 week. However, as previously mentioned, Azoulay et al. (1978) reported no changes in 2,3-diphosphoglycerate levels after a 3,760-µg/m³ (2.0-ppm) continuous NO₂ exposure lasting between 1 day and 6 weeks. The authors attribute the difference between their results and the results of Mersch et al. (1973) to their use of a more precise enzymatic assay and to a larger study population. Additionally, there may be species-related differences because Azoulay et al. (1978) examined rats and Mersch et al. (1973) examined guinea pigs.

Changes in the contents of RBC membranes were detected after exposure to 7,520 µg/m³ (4.0 ppm) NO₂ (Kaya et al., 1980). Increased amounts of sialic acid were noted in rats exposed between 1 and 10 days to 7,520 µg/m³ NO₂. Increased sialic acid, a glycosidic residue distributed on the outer surface of the RBC, is found in younger RBCs (Durocher et al., 1975), suggesting that NO₂ inhalation may have stimulated renewal of the RBC population (increased population of immature cells). An increased amount of lyso-phosphatidylethanolamine, known to increase cell membrane fragility, was found on Days 5, 7, and 10 after exposure to 7,520 µg/m³ and after 1, 5, and 7 days of exposure to 18,800 µg/m³ (10 ppm) NO₂. The protein content of RBCs was slightly decreased at 18,800 µg/m³ after 1, 5, and 7 days of exposure, but not after 3 days.

The possibility of a younger circulating RBC population was investigated by Kunimoto et al. (1984), who showed that after 1 and 4 days of exposure to 7,520 µg/m³ (4.0 ppm) NO₂, the activity and content of Na⁺, K⁺-ATPase, and the amount of sialic acid were increased in RBC membranes. These changes have also been associated with a younger population of RBCs (Cohen et al., 1976).

In contrast, Mochitate and Mura (1984) found that after 7 days of continuous exposure to 7,520 µg/m³ (4.0 ppm) NO₂, there was a decreased population of younger RBCs.
### TABLE 13-16. EFFECTS OF NITROGEN DIOXIDE ON RED BLOOD CELL MEMBRANES$^a$

<table>
<thead>
<tr>
<th>NO$_2$ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>677</td>
<td>0.36</td>
<td>Continuous, 7 days</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Increased RBC d-2,3-DPG</td>
<td>Mersch et al (1973)</td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>8 h/day, 7 days</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (Hartley)</td>
<td>Decrease in RBC GSH peroxidase</td>
<td>Menzel et al (1976)</td>
</tr>
<tr>
<td>3,760</td>
<td>2.0</td>
<td>Continuous, up to 6 weeks</td>
<td>M</td>
<td>8 weeks</td>
<td>Rat (Wistar)</td>
<td>No effect on RBC 2,3-DPG</td>
<td>Azoulay et al (1978)</td>
</tr>
<tr>
<td>7,520</td>
<td>4.0</td>
<td>Continuous, 1-10 days</td>
<td>M</td>
<td>16-21 weeks</td>
<td>Rat (JCL Wistar)</td>
<td>Increased sialic acid, Na$^+$, and K$^+$-ATPase in RBC membranes associated with an increased proportion of younger RBCs</td>
<td>Kunimoto et al (1984a)</td>
</tr>
<tr>
<td>7,520</td>
<td>4.0</td>
<td>Continuous, 1-10 days</td>
<td>M</td>
<td>16-20 weeks</td>
<td>Rat (JCL Wistar)</td>
<td>At Day 7, the fraction of young RBCs was reduced and the fraction of older RBCs increased. Activities of pyruvate kinase and phosphofructokinase were increased in young RBCs</td>
<td>Mochitate and Miura (1984)</td>
</tr>
<tr>
<td>7,520</td>
<td>4.0</td>
<td>Continuous, 10 days</td>
<td>M</td>
<td>8-18 weeks</td>
<td>Rat (JCL Wistar)</td>
<td>Increased arachidonic acid in membranes and serum Stearic palmitic acid decreased at 10 and 4 ppm in RBC membranes</td>
<td>Kaya and Miura (1982)</td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>Continuous, 7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,520-37,780</td>
<td>4-20</td>
<td>Continuous, 1-10 days</td>
<td>M</td>
<td>8-14 weeks</td>
<td>Rat (JCL Wistar)</td>
<td>Decreased RBC membrane protein at Day 1, 5, and 7, but not 3. Lyso-phosphatidyl-ethanolamine, sialic acid, and hexose increased at 4 and 10 ppm</td>
<td>Kaya et al (1980)</td>
</tr>
</tbody>
</table>

$^a$NS = Not stated
M = Male
RBCs = Red blood cells
GSH = Glutathione
However, the activity of two glycolytic enzymes (pyruvate kinase and phosphofructokinase) was elevated in NO₂-exposed animals on Days 5 and 7, but returned to control levels on Day 10. The authors concluded that there was not a corresponding reduction in the activity of the glycolytic pathway with the NO₂-induced increase in the apparent aging of the RBCs.

Kaya and Miura (1982) investigated the effects of NO₂ on fatty acids in RBCs, sera, and liver. They found a net increase in unsaturated fatty acids (predominately arachidonic acid) occurred in RBC membranes from rats after exposure to 7,520 μg/m³ (4.0 ppm) NO₂ for 10 days.

**Effects on Serum and Plasma**

As mentioned in the previous section, Kaya and Miura (1982) showed that arachidonic acid was increased in the RBC membrane after exposure to NO₂. Because de novo synthesis of fatty acids is not possible in the mature RBC, composition changes in the membrane usually reflect exchange with serum fatty acids, probably originating in the liver. In fact, Kaya and Miura (1982) found that after exposing rats to 7,520 μg/m³ (4.0 ppm) NO₂ for 10 days, arachidonic acid was elevated in serum and in liver homogenates. However, exposure to 18,800 μg/m³ (10 ppm) NO₂ for 7 days increased RBC and serum arachidonic acid, but liver concentrations were decreased. The authors suggested that the rat cannot completely overcome the consequences of a 18,800-μg/m³ NO₂ exposure, but could metabolically compensate for the effects of exposure to 7,520 μg/m³ NO₂.

Menzel et al (1977) demonstrated that acute effects do not necessarily predict chronic injury by contrasting the serum changes of guinea pigs after short-term (7-day) and long-term (4 mo) continuous exposure to 940 μg/m³ (0.5 ppm) NO₂. Plasma ChE was elevated after a 7-day exposure, but was decreased compared to control values with a long-term exposure (4 mo). This depression in ChE is suggestive of a hepatic lesion (Moore et al., 1957). A depression in RBC GSH peroxidase activity was also initially observed, but the effect did not persist after 4 mo of exposure. Similarly, several indices of nonspecific tissue damage (serum creatine phosphokinase, LDH, serum glutamic oxaloacetic transaminase, and serum glutamic pyruvic transaminase) were also increased after 7 days, but were not altered by long-term exposure.
The following studies all indicate a general decrease in serum proteins and lipoproteins and an increase in serum globulins, thus suggesting hepatic damage. Drozdz et al. (1976) reported decreased serum total protein, albumin, and seromucoid concentrations in guinea pigs exposed to 2,000 µg/m³ (1.05 ppm) NO₂, 8 h/day for 180 days. However, serum levels of α₁- and β-globulins were increased. These authors also found that serum alanine and aspartate aminotransferase activity was increased in the mitochondrial fraction, but was decreased in the cytoplasmic fraction. In agreement with the Menzel et al. (1977) subchronic data, Drozdz et al. (1976) also observed decreased plasma ChE levels. However, the meaning of the cytoplasmic and mitochondrial fraction of serum is not clear from the translation of the article.

Kosmider et al. (1973a) reported a general decrease in protein synthesis evidenced by decreased serum proteins in guinea pigs after continuous exposure to 1,880 µg/m³ (1 ppm) NO₂ for 6 mo. Following exposure to 1,000 µg/m³ NOₓ (mainly NO₂, ≈0.5 ppm), 8 h/day for 120 days, Kosmider (1975) reported decreased serum cholesterol, total lipids, β (low density lipids) and gamma lipoproteins, and sodium, and increased serum α (high density lipids)-lipoproteins in guinea pigs. Similarly, Mitina (1962), after exposing rabbits to 2,400 to 5,680 µg/m³ (1.3 to 3.0 ppm) NO₂ for 15 and 17 weeks, found reduced amounts of albumin, but increased serum globulins. Table 13-17 summarizes the data on NO₂-induced changes in serum proteins and clinical chemistries.

13.2.3.3 Hepatic Function

As described in the above section, changes in serum chemistries suggest that NO₂ exposure may affect the liver. Several studies have examined hepatic function either directly or by indirect means. These studies are cataloged in Table 13-18.

One important function of the liver is detoxification of xenobiotic compounds. Measurement of the duration of barbiturate-induced sleeping time has been used as an indirect measurement of hepatic mixed-function oxidase activity, the enzymes responsible for xenobiotic metabolism. Nitrogen dioxide has been shown to increase pentobarbital-induced sleeping times in mice (Miller et al., 1980, Graham et al., 1982). The effect was observed in female mice, but not in males, occurred only at specified time intervals after exposure, and usually did not persist beyond 1 day postexposure. However, the effects reliably
**TABLE 13-17. EFFECTS OF NITROGEN DIOXIDE ON SERUM PROTEINS AND CLINICAL CHEMISTRIES**

<table>
<thead>
<tr>
<th>NO$_2$ Concentration</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 µg/m$^3$</td>
<td>Continuous, 3 mo</td>
<td>M</td>
<td>NS</td>
<td>Rat (NS)</td>
<td>Cholinesterase was not affected</td>
<td>Yakimchuk and Chelikanov (1972)</td>
</tr>
<tr>
<td>940 µg/m$^3$</td>
<td>8 h/day, 7 days, 8 h/day, 4 mo</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (Hartley)</td>
<td>At 4 days serum LDH, total creatinine phosphokinase, SGOT and SGPT, plasma cholinesterase, and lysozyme elevated. At 4 mo lysozyme and plasma cholinesterase depressed</td>
<td>Menzel et al (1977)</td>
</tr>
<tr>
<td>1,000 NO$_x$</td>
<td>8 h/day, 120 days</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Serum cholesterol and total lipids depressed</td>
<td>Kosmider (1975)</td>
</tr>
<tr>
<td>1,880 µg/m$^3$</td>
<td>Continuous, 6 mo</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Protein synthesis inhibited, total serum proteins and immunoglobulins decreased</td>
<td>Kosmider et al (1973a)</td>
</tr>
<tr>
<td>1,880 µg/m$^3$</td>
<td>Continuous, 16 mo</td>
<td>M</td>
<td>NS</td>
<td>Monkey (Squirrel)</td>
<td>Animals challenged with virus. No effect on clinical biochemical parameters</td>
<td>Fenters et al (1973)</td>
</tr>
<tr>
<td>2,000 µg/m$^3$</td>
<td>8 h/day, 180 days</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Plasma changes decreased albumin, serum mucoid, choline-sterase, alanine, and aspartate transaminases. Increased $\alpha_1$ and $\beta_2$ globulins</td>
<td>Drozdz et al (1976)</td>
</tr>
<tr>
<td>1,880-56,400 µg/m$^3$</td>
<td>18 h</td>
<td>NS</td>
<td>NS</td>
<td>Mouse (NS)</td>
<td>Decreased catalase and iron transferrin activity</td>
<td>Case et al (1979)</td>
</tr>
<tr>
<td>11,700 µg/m$^3$</td>
<td>Continuous, 8 days</td>
<td>M</td>
<td>8 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>No effect on serum lysozyme</td>
<td>Chow et al (1974)</td>
</tr>
</tbody>
</table>

$^a$M = Male
NS = Not stated
LDH = Lactate dehydrogenase
SGOT = Serum glutamic oxaloacetic transaminase
SGPT = Serum glutamic pyruvic transaminase
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.03</td>
<td>6 h/day,</td>
<td>M/F</td>
<td>in utero</td>
<td>Rat</td>
<td>Increased hexobarbital-induced sleeping time at 0.5 and 5.0 ppm Cytochrome P-450 level and aminopyrine N-demethylase activity were decreased, whereas lipid peroxides and O₂ consumption were increased at 5.0 ppm</td>
<td>Tabacova et al (1985)</td>
</tr>
<tr>
<td>100</td>
<td>0.05</td>
<td>7 days/week,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,000</td>
<td>0.5</td>
<td>21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10,000</td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>470-9,400</td>
<td>0.25-5.0</td>
<td>3 h/day, 1, 2, or M/F</td>
<td>5-7 weeks</td>
<td>Mouse</td>
<td>Increase in pentobarbital-induced sleeping time in female mice only, repeated daily exposures caused no effect</td>
<td>Miller et al (1980)</td>
<td></td>
</tr>
<tr>
<td>235</td>
<td>0.125</td>
<td>3 h/day, 1-2 days</td>
<td></td>
<td></td>
<td>No effect on pentobarbital-induced sleeping time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>Continuous, 14 weeks</td>
<td>M</td>
<td>22-24 weeks</td>
<td>Rat</td>
<td>Cytochrome P-450 level decreased during first 8 weeks of exposure, but returned to control levels with continued exposure (4.0 ppm)</td>
<td>Takahashi et al (1986)</td>
</tr>
<tr>
<td>2,256</td>
<td>1.2</td>
<td></td>
<td></td>
<td>(JCL Wistar)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,520</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>Continuous, 7 days</td>
<td>M</td>
<td>23-26 weeks</td>
<td>Rat</td>
<td>Succinate-cytochrome c reductase activity was reduced at 10 ppm only, on Days 3 and 5 Cytochrome P-450 level was reduced at 10, 40, and 0.4 ppm but not at 1.2 ppm NADPH-cytochrome c reductase activity was reduced at 4.0 ppm only</td>
<td>Mochtate et al (1984)</td>
</tr>
<tr>
<td>3,260</td>
<td>1.2</td>
<td></td>
<td></td>
<td>(Wistar)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,520</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7,520</td>
<td>4.0</td>
<td>10 days</td>
<td></td>
<td>21-24 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,000 NOₓ (mainly NO₂)</td>
<td>0.5</td>
<td>8 h/day, 120 days</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig</td>
<td>Depleted liver magnesium and zinc, swollen liver mitochondria</td>
<td>Kosimder (1975)</td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>Continuous</td>
<td>F</td>
<td>weanling</td>
<td>Mouse</td>
<td>No effect on lipofusin pigment in liver or other organs</td>
<td>Azaz and Csallany (1977, 1978)</td>
</tr>
<tr>
<td>1,880</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 13-18. Effects of Nitrogen Dioxide on the Liver**
TABLE 13-18 (cont’d). EFFECTS OF NITROGEN DIOXIDE ON THE LIVER\textsuperscript{a}

<table>
<thead>
<tr>
<th>NO\textsubscript{2} Concentration</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,000 ( \mu g/m^3 ) 1.05 ppm</td>
<td>8 h/day, 180 days</td>
<td>M NS</td>
<td>Guinea pig (NS)</td>
<td>Decreased liver protein and cytoplasmic aspartate transaminase activity, increased mitochondrial alanine and aspartate transaminase activities, intracellular edema, and inflammatory and parenchymal changes observed</td>
<td>Drodz et al (1976)</td>
<td></td>
</tr>
<tr>
<td>7,520</td>
<td>Continuous, 1, 14, or 30 days</td>
<td>M</td>
<td>4 weeks</td>
<td>Rat (Wistar)</td>
<td>Cytochrome P-450 level and aminopyrine N-demethylase activity increased and aniline hydroxylase activity was decreased. No effect at Day 1</td>
<td>Takano and Miyazaki (1984)</td>
</tr>
<tr>
<td>9,400</td>
<td>3 h</td>
<td>F</td>
<td>6-7 weeks</td>
<td>Mouse (CD-1)</td>
<td>No decrease in cytochrome P-450 levels or mixed function oxidase activities</td>
<td>Graham et al (1982)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}M = Male  
F = Female  
O\textsubscript{2} = Oxygen  
NADPH = Reduced nicotinamide adenine dinucleotide phosphate  
NS = Not Stated
occurred after a 3-h exposure to concentrations as low as 470 μg/m³ (0 25 ppm) NO₂
No significant effects were detected after 1- or 2-day exposure to 235 μg/m³ (0 125 ppm)
In an attempt to examine the mechanism of this response, the level of hepatic cytochrome
P-450 and the activities of aminopyrine N-demethylase, p-nitroanisole O-demethylase, and
aniline hydroxylase were measured in the livers of mice exposed for 3 h to 9,400 μg/m³
(5 0 ppm) NO₂, however, no NO₂-related effects were found (Graham et al , 1982)

Increased hexobarbital-induced sleeping times have also been reported in the progeny of
maternally exposed rats (Tabacova et al , 1985) This effect was measured in the offspring
exposed to 10,000 μg/m³ (5 3 ppm) NO₂ at 7, 14, and 21 days postexposure Additionally, lipids increased and O₂ consumption decreased in liver homogenates Cytochrome
P-450 content and aminopyrine-N-demethylase activity were decreased on Postnatal Day 30
In the animals exposed to 1,000 μg/m³ (0 5 ppm) NO₂, increased hexobarbital-induced
sleeping times occurred on Days 7 and 21, but not on Day 14 Liver lipid peroxides were
also increased on Postnatal Day 30 in this exposure group No exposure monitoring method
was cited and the details of the biological methods used were not available

Components of the rat microsomal electron-transport system, especially cytochrome
P-450, were generally decreased after continuous exposure to 752 to 7,520 μg/m³ (0 4 to
4 0 ppm) NO₂ during the first 8 weeks of exposure, but with continued exposure, the levels
returned to control values (Takahashi et al , 1986) Takano and Miyazaki (1984) exposed
rats to 7,520 μg/m³ NO₂ for 1, 14, or 30 days After 14 days, cytochrome P-450 levels and
aminopyrine N-demethylase activity were increased, whereas aniline hydroxylase activity was
decreased Aminopyrine N-demethylase activity was also increased at 30 days, but none of
the other measures were affected at Day 1 or 30 Mochutate et al (1984) also observed a
duration-dependence of effects Reductions of succinate-cytochrome c reductase activity in
rat liver homogenates were found during the third and fifth day of an 18,800 μg/m³ (10 ppm)
NO₂ exposure, but not during the first and seventh day, or with exposure to 7,520 μg/m³
(4 0 ppm) NO₂ Decreases in cytochrome P-450 levels from liver microsomes were also
found after 7 days of exposure to 752 or 7,520 μg/m³ (0 4 or 4 0 ppm) NO₂, but not after
exposure to 2,256 μg/m³ (1 2 ppm) NO₂ A reduction in NADPH-cytochrome c reductase
activity was found after 5 days of exposure to 7,520 and 18,800 μg/m³ NO₂
Drozdz et al (1976) found decreased total liver protein and sialic acid, but increased protein-bound hexoses in guinea pigs exposed to 2,000 \( \mu g/m^3 \) (1.05 ppm) NO\(_2\), 8 h/day for 180 days. Liver alanine and aspartate aminotransferase activity was increased in the mitochondrial fraction. In contrast to the effect seen in the cytoplasmic fraction of the serum, aspartate aminotransferase activity was decreased in the cytoplasmic fraction of the liver. Electron micrographs of the liver showed intracellular edema and inflammatory and parenchymal degenerative changes.

Kosmider (1975) reported liver magnesium and zinc stores were depleted in guinea pigs following exposure to 1,000 \( \mu g/m^3 \) NO\(_x\) (mainly NO\(_2\), \( \approx 0.5 \) ppm), 8 h/day for 120 days. Swollen liver mitochondria were also observed.

Ayaz and Csallany (1978) and Csallany and Ayaz (1978) exposed weanling mice to 940 or 1,880 \( \mu g/m^3 \) (0.5 or 1.0 ppm) NO\(_2\) continuously for 17 mo. Animals were divided into three groups receiving the basal diet with either a normal supplement of vitamin E (30 mg/kg), 300 mg/kg vitamin E, or 30 mg/kg of the synthetic antioxidant \( N,N \)-diphenyl-phenylene diamine. After 17 mo of exposure, the presence of lipofuscin pigment in the liver, lungs, spleen, heart, brain, kidney, and uterus was determined. No effect could be ascribed to NO\(_2\) exposure.

13.2.3.4 Effects on the Kidney and on Urine Content

The direct effects of NO\(_2\) exposure on the kidney and spleen have been described, and several studies have explored the composition of urine during and after exposure. These studies are summarized in Table 13-19 and are discussed below.

Takahashi et al (1986) found that continuous exposure to 2,256 and 7,520 \( \mu g/m^3 \) (1.2 and 4.0 ppm) NO\(_2\) increased the amount of cytochrome P-450 and cytochrome b\(_5\) in the kidney after 8 weeks of exposure. Continuous exposure for 12 weeks resulted in less substantial increases in the amount and activity of the microsomal electron-transport enzymes. This is in contrast to the decreased activity these authors reported for the liver, as discussed in Section 13.2.3.3.

Yakimchuk and Chelikanov (1972) reported that during a 3-mo continuous exposure to 600 \( \mu g/m^3 \) (0.32 ppm) NO\(_2\), rats showed a significant increase in the urinary excretion of
### TABLE 13-19. EFFECTS OF NITROGEN DIOXIDE ON THE KIDNEY AND ON URINE CONTENTS<sup>a</sup>

<table>
<thead>
<tr>
<th>NO₂ Concentration (µg/m³)</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>0.32</td>
<td>Continuous, 3 mo</td>
<td>M</td>
<td>NS</td>
<td>Rat (NS)</td>
<td>Increased urinary coproporphyrins</td>
<td>Yakimchuk and Chebkanov (1972)</td>
</tr>
<tr>
<td>940</td>
<td>0.5</td>
<td>Continuous, 7 or 14 days</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Increased urinary protein and specific gravity, proteins characteristic of the nephrotic syndrome</td>
<td>Sherwin and Layfield (1974)</td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>Continuous,</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Increased urinary nitrite, nitrate, and coproporphyrin</td>
<td>Kosmider (1975)</td>
</tr>
<tr>
<td>1,000</td>
<td>0.5</td>
<td>8 h/day, 120 days</td>
<td>NS</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Increased cytochrome P-450 and b5 after 8 weeks of exposure, less of an effect after 12 weeks</td>
<td>Takahashi et al. (1986)</td>
</tr>
<tr>
<td>2,260-7,520</td>
<td>1.2</td>
<td>Continuous, 3 mo</td>
<td>M</td>
<td>22-24 weeks</td>
<td>Rat (JCL Wistar)</td>
<td>Increased urinary nitrate, urinary nitrite increase appeared to be artifactual</td>
<td>Saul and Archer (1983)</td>
</tr>
</tbody>
</table>

<sup>a</sup>M = Male  
NS = Not stated
coproporphyrins  Kosmider (1975) also reported increased levels of urinary coproporphyrin in guinea pigs exposed to 1,000 \( \mu g/m^3 \) NO\(_x\) (mainly NO\(_2\), \( \approx 0.5 \) ppm), 8 h/day for 120 days. Increased coproporphyrins can indicate increased heme synthesis, which might occur if an increased number of RBCs were synthesized. As discussed in Section 13.2.3.2, NO\(_2\) exposure has been reported to cause polycythemia and an increase in the number of RBCs in the red pulp of the spleen.

Increases in urinary protein and specific gravity of the urine were reported by Sherwin and Layfield (1974) in guinea pigs exposed continuously to 940 \( \mu g/m^3 \) (0.5 ppm) NO\(_2\) for 14 days. Proteinuria was detected in another group of animals when the exposure was reduced to 752 \( \mu g/m^3 \) (0.4 ppm) NO\(_2\) for 4 h/day. Disc electrophoresis of the urinary proteins demonstrated the presence of albumin and alpha, beta, and gamma globulins. The presence of high molecular weight proteins in urine is characteristic of the nephrotic syndrome. Differences in water consumption or in the histology of the kidney were not found.

In a more comprehensive study of the relationship between inhaled NO\(_2\) and urinary nitrite and nitrate, Saul and Archer (1983) exposed rats for 24 h to 2,256 to 16,544 \( \mu g/m^3 \) (1.2 to 8.8 ppm) NO\(_2\). They demonstrated that mostly nitrate, with very little nitrite, was excreted in the urine. The small amount of urinary nitrite appeared to be an artifact that originated from an in vitro reaction with urine. The rate and linearity of the conversion of NO\(_2\) to urinary nitrate suggested that NO\(_2\) does not form nitrate by reacting with respiratory water, but reacts with oxidizable tissue to form nitrite. Nitrite is then further oxidized in the blood by oxyhemoglobin (Kosaka et al., 1979) to form nitrate, which is excreted in the urine. Nitrite and nitrate were also found in the urine of guinea pigs exposed to 1,000 \( \mu g/m^3 \) NO\(_x\) (mainly NO\(_2\), \( \approx 0.5 \) ppm), 8 h/day for 120 days (Kosmider, 1975).

13.2.3.5 Cardiovascular Effects

Few papers have reported the effects of NO\(_2\) exposure on the heart. Potential changes in hemoglobin and RBCs as well as lung edema could reduce oxygen uptake and affect cardiovascular performance. Because many of the NO\(_2\)-induced cardiovascular effects are secondary to pulmonary edema or stimulation of sensory receptors in the respiratory tract,
some of the studies addressing effects on the cardiovascular system are addressed in the discussion on pulmonary function (see Section 13.2.2.3)

Suzuki et al (1981) exposed rats for up to 3 mo to between 752 and 7,520 µg/m³ (0.4 and 4.0 ppm) NO₂. After 3 mo of exposure to 7,520 µg/m³ NO₂, anesthetized rats, artificially ventilated at high frequencies, had a significant reduction in PaO₂. A reduction in heart rate was reported in unanesthetized mice exposed to 2,250 or 7,520 µg/m³ (1.2 or 4.0 ppm) NO₂ for 1 mo (Suzuki et al, 1984).

Messina et al (1983) examined rats, which previously had been maintained on 10% ethanol or drinking water as the sole drinking fluid, after 3 days of exposure to 9,400 µg/m³ (5.0 ppm) NO₂. Heart LDH was significantly elevated in NO₂-exposed rats maintained on water, but not in NO₂-exposed rats maintained on ethanol. Because no changes in LDH were found in liver or serum, the authors suggested that NO₂ may be responsible for the induction of LDH, however, induction by lactate could not be excluded.

Tsubone and Suzuki (1984) examined the effects of NO₂ exposure on phenyl diguanide-induced cardiopulmonary changes. Rats were preexposed to 18,800 µg/m³ (10 ppm) NO₂ for 24 h, 7,520 µg/m³ (4.0 ppm) for 1 week, or 752 µg/m³ (0.4 ppm) for 4 weeks prior to phenyl diguanide injection. The cardiopulmonary effects of phenyl diguanide (decreased heart rate and respiratory rate) were enhanced by exposure to 18,800 µg/m³ NO₂, but not by lower NO₂ exposures.

Dowell et al (1971) showed decreased cardiac output, blood pressure, PaO₂, and pH in dogs after a 1-h exposure to between 13,160 and 30,080 µg/m³ (7 and 16 ppm) NO₂. This is in contrast to the findings in another experimental animal species exposed to higher concentrations of NO₂ (Abraham et al, 1980). However, exposures in the Dowell et al (1971) study were delivered via an endotracheal tube in anesthetized dogs, thereby bypassing any scrubbing effects of the upper airways.

### 13.2.3.6 Effects on the Central Nervous System and Behavioral Effects

Information regarding the effects of NO₂ on development and animal behavior is limited to a few studies (see Table 13-20), most of which have uncertain relationships to humans. Shalamberidze (1969) exposed rats to 100 µg/m³ (0.05 ppm) NO₂ for 3 mo with no demonstrated effects on the central nervous system. Yakimchuk and Chelikanov (1972)
<table>
<thead>
<tr>
<th>NO$_2$ Concentration</th>
<th>ppm Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-10,000</td>
<td>0.03-5.3</td>
<td>M/F</td>
<td>in utero</td>
<td>Rat (Wistar)</td>
<td>Significant defects in posture and gait were detected at 9 and 14 days at 0.05 ppm, additional effects at higher levels, no monitoring method was described</td>
<td>Tabacova et al (1985)</td>
</tr>
<tr>
<td>100</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
<td>Rat</td>
<td>No effect on CNS</td>
<td>Shalamberidze (1969)</td>
</tr>
<tr>
<td>600</td>
<td>0.32</td>
<td>M</td>
<td>NS</td>
<td>Rat (NS)</td>
<td>Decreased conditioned reflexes to sound and light</td>
<td>Yakimchuk and Chehkanov (1972)</td>
</tr>
<tr>
<td>845</td>
<td>0.45</td>
<td>M</td>
<td>NS</td>
<td>Mouse (Swiss Webster)</td>
<td>Increased 5-HT, 5-HIAA, and turnover</td>
<td>Sherwin et al (1986)</td>
</tr>
<tr>
<td>1,000</td>
<td>0.53</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Decreased malate, sorbitol, LDH, alanine aminotransferase, ATPase, and 5'-nucleotidase homogenate, increased 1,6-diphosphofructose aldolase, isocitrate, α-hydroxybutyrate dehydrogenase, phosphocreatine kinase, and cholinesterase</td>
<td>Drozdz et al (1975)</td>
</tr>
<tr>
<td>1,880</td>
<td>1.0</td>
<td>F</td>
<td>12 weeks</td>
<td>Rat (Wistar)</td>
<td>More or less constant swimming performance in only 1.0-ppm group, with a slight tendency to deterioration, decrease of 25% by fifth and sixth week of exposure to 5.0 ppm, declined from first month at 20 ppm</td>
<td>Tusl et al (1973)</td>
</tr>
<tr>
<td>6,580</td>
<td>5.0</td>
<td>F</td>
<td>up to 6 mo</td>
<td>Rat (Wistar)</td>
<td>Decreased swimming performance at 3.5 ppm</td>
<td></td>
</tr>
<tr>
<td>37,600</td>
<td>20</td>
<td>F</td>
<td>12 weeks</td>
<td>Rat (Wistar)</td>
<td>More or less constant swimming performance in only 1.0-ppm group, with a slight tendency to deterioration, decrease of 25% by fifth and sixth week of exposure to 5.0 ppm, declined from first month at 20 ppm</td>
<td>Tusl et al (1973)</td>
</tr>
<tr>
<td>6,580</td>
<td>3.5</td>
<td>M</td>
<td>6 h/day, 8 weeks</td>
<td>Rat (Wistar)</td>
<td>Decreased swimming performance at 3.5 ppm</td>
<td></td>
</tr>
<tr>
<td>9,400-75,200</td>
<td>5.40</td>
<td>M</td>
<td>15-16 weeks</td>
<td>Mouse (JCL ICR)</td>
<td>Decreased swimming performance at 10 ppm, increased blood lactate compared to similarly exercised controls at 5.0 ppm</td>
<td>Suzuki et al (1982a)</td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>ppm</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Effects</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----</td>
<td>----------</td>
<td>--------</td>
<td>-----</td>
<td>-----------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>14,000</td>
<td>7</td>
<td>6 h</td>
<td>NS</td>
<td>NS</td>
<td>Mouse</td>
<td>Decreased voluntary running activity, return to normal within 24 h postexposure</td>
</tr>
<tr>
<td>9,400</td>
<td>5</td>
<td>2 h/day, 5 weeks</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Depleted total lipids, phospholipids, and cholesterol in all brain regions, except increased cholesterol in spinal cord, increased lipid peroxidation in all brain regions</td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td>5 weeks</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Decreased total and protein bound sulphydryls, increased nonprotein bound sulphydryls</td>
</tr>
<tr>
<td>9,400</td>
<td>5</td>
<td>2 h/day, 5 weeks</td>
<td>M</td>
<td>NS</td>
<td>Guinea pig (NS)</td>
<td>Depleted total and protein bound sulphydryls, increased nonprotein bound sulphydryls</td>
</tr>
</tbody>
</table>

*M = Male
F = Female
NS = Not stated
CNS = Central nervous system
5-HT = 5-hydroxytryptamine
5-HIAA = 5-hydroxyindole acetate acid
LDH = Lactate dehydrogenase
reported that during a 3-mo continuous exposure to 600 μg/m³ (0.32 ppm) NO₂, rats developed an increased latency of response to conditioned sound and light stimuli.

Exposure of guinea pigs to 1,000 μg/m³ (0.53 ppm) NO₂, 8 h/day for 180 days affected brain enzyme activity levels (Drozdz et al., 1975). Decreased activities in brain protein metabolism enzymes were seen in brain malate dehydrogenase, alanine aminotransferase, sorbitol dehydrogenase, LDH, ATPase, 5'-nucleotidase, and asparagine aminotransferase. Increases in brain glycolytic enzyme activities were seen in 1,6-diphosphofructose aldolase, isocitrate dehydrogenase, alpha-hydroxybutyrate dehydrogenase, phosphocreatine kinase, and ChE.

A study by Sherwin et al. (1986) indicated that the brain content of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA, the primary metabolite of 5-HT) increased in mice exposed to 846 μg/m³ (0.45 ppm) NO₂, 7 h/day for 4 weeks. The ratio of 5-HIAA 5-HT was also increased. The authors did not speculate as to what these observations mean, however, they noted that increased turnover, as reflected in the increased 5-HIAA 5-HT ratio, have also been observed in trimethyltin and chlordecone exposure.

Vyskocil et al. (1985) measured a variety of hormone levels and organ weights after continuous exposure to 6,580 μg/m³ (3.5 ppm) NO₂ for 1 or 2 mo. The only significant effect reported was a decrease in the hypothalamic concentration of noradrenaline at both exposure durations.

Two recent papers by the same group of authors (Faraham and Hasan, 1990, 1991) reported neurotoxic changes in guinea pigs exposed 2 h/day for 35 days to 9,400 or 18,800 μg/m³ (5 or 10 ppm) NO₂. Although the report contains insufficient information to adequately evaluate the exposure and t-tests were used for all comparisons, the effects were substantial, as well as brain region- and concentration-dependent. In the first study (Faraham and Hasan, 1990), total lipids, cholesterol, and phospholipids were found to be decreased in a concentration-dependent manner by 4.9 to 41.1% in three brain regions: cerebral hemisphere, cerebellum, and midbrain. Similar decrements in lipids were observed in the spinal cord, except that cholesterol was significantly increased. Lipid peroxidation, as measured by malonaldehyde formation, was increased in all four brain regions from 7.5 to 46.5%, again in a region-dependent and concentration-dependent manner. In the second report (Faraham and Hasan, 1991), using the same exposure regimen, total nonprotein bound...
(mostly GSH) and protein bound sulfhydryl groups in the same four brain regions were affected by NO₂ exposure in a concentration-dependent manner. Nonprotein bound sulfhydryls significantly increased after 9,440 μg/m³ (5 0 ppm) exposure, whereas protein bound sulfhydryls decreased in the cerebellum and especially in the midbrain. The increased nonprotein sulfhydryls may be a protective, compensatory response to the lipid peroxidation described above, further corroborating that finding, however, the degree of protection from neurotoxic injury was not evaluated.

As discussed in the section on reproductive, developmental, and heritable mutagenic effects (Section 13 2 3 7), Tabacova et al. (1985) reported significant postnatal deficits in the onset of normal neuromotor development and reduced open field activity in the progeny of maternally exposed rats 2 mo after the dams were exposed to 1,000 or 10,000 μg/m³ (0.5 or 5.3 ppm) NO₂, 6 h/day for 7 days/week. Postural and gait defects were also reported at 100 μg/m³ (0.05 ppm). No NO₂ monitoring method was specified.

Tusl et al. (1973) exposed rats to 9,400 μg/m³ (5.0 ppm) NO₂ for 8 weeks. The influence of NO₂ on forced swimming endurance time was measured. By the fifth and sixth weeks of exposure, swimming performance had decreased 25%. In rats exposed to 1,880 μg/m³ (1.0 ppm) NO₂, performance was maintained with a slight tendency toward deterioration.

A concentration-dependent decrease in forced swimming endurance time after a single 24-h exposure to between 9,400 and 75,200 μg/m³ (5 and 40 ppm) NO₂ was reported by Suzuki et al. (1982a). Significant decrements in performance were reported at exposure concentrations as low as 18,000 μg/m³ (10 ppm). Recovery from exposure required 5 to 6 days, 7 to 8 days, and over 9 days for the 9,400, 18,800, and 37,600 μg/m³ (5, 10, and 20 ppm) groups, respectively. In an attempt to examine the mechanism that produced the decrement in performance, it was observed that as forced swimming endurance time decreased, lung edema increased. Furthermore, compared to similarly exercised control rats, blood lactate concentration was increased in rats exposed to 9,400 μg/m³ both immediately and 24 h after exposure. These two findings suggest that lung edema prevented sufficient O₂ from entering the blood during exercise to meet aerobic demands. See Section 13 2 2 3 on pulmonary function.

13-149
13.2.3.7 Reproductive, Developmental, and Heritable Mutagenic Effects

As summarized in Table 13-21, few studies have examined the effects of NO₂ on reproduction and development or the heritable mutagenic potential of NO₂ in vivo. Exposure to 1,880 μg/m³ (1.0 ppm) NO₂, 7 h/day, 5 days/week for 21 days resulted in no alterations in spermatogenesis, germinal cells, or interstitial cells of the testes in six rats (Kripke and Sherwin, 1984). Additionally, the level of vitamin B₁₂, a coenzyme in folate metabolism that is used for DNA synthesis, was not affected by NO₂ exposure. Similarly, breeding studies by Shalamberdze and Tsereteli (1971) found that long-term NO₂ exposure had no effect on fertility. However, there was a decrease in litter size and neonatal weight when male and female rats exposed to 2,360 μg/m³ (1.3 ppm) NO₂, 12 h/day for 3 mo were bred. In utero death due to NO₂ exposure resulted in smaller litter sizes, but no direct teratogenic effects were observed in the offspring. In fact, after several weeks, NO₂-exposed litters approached weights similar to controls.

In the only study that has examined postnatal development, a significant delay in eye opening and incisor eruption was observed in the progeny of maternally exposed Wistar rats (Tabacova et al., 1985). The dams were exposed to 50, 100, 1,000, or 10,000 μg/m³ (0.03, 0.05, 0.5, or 5.3 ppm) NO₂ for 6 h/day, 7 days/week throughout gestation and the offspring were studied for 2 mo postexposure. Significant effects were detected in the offspring of dams exposed to 1,000 and 10,000 μg/m³ NO₂. There were also concentration-related increases in neurobehavioral development reported in the offspring of the maternally exposed animals. These findings are discussed in the section on NO₂ effects on the central nervous system and behavior (Section 13.2.3.6). The method of monitoring NO₂ was not reported.

Balabaeva and Tabakova (1985) exposed pregnant and nonpregnant albino rats to 1,000 or 10,000 μg/m³ (0.5 or 5.3 ppm) NO₂, 5 h/day for 21 days and examined lipid peroxidation in lung, liver, and placenta. Nonpregnant rats had greater lipid peroxidation in the liver than in the lung, whereas the opposite was true in pregnant rats. Even more surprising was a fourfold increase in lipid peroxidation in the placenta of 10,000-μg/m³ NO₂-exposed rats compared to unexposed pregnant controls. The authors then examined the offspring of the pregnant rats. The 1-mo-old F₁ nonpregnant rats, exposed to air or the same concentrations of NO₂, showed similar changes as were observed in their mothers.
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 0.03 6 h/day, M/F in utero Rat</td>
<td>Concentration-dependent delay in eye opening and incisor eruption in progeny of dams exposed to 0.5 or 5.3 ppm during gestation</td>
<td>Monitoring method not described</td>
<td>Tabacova et al (1985)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 0.05 7 days/week, (Wistar)</td>
<td>eye opening and incisor eruption in progeny of dams exposed to 0.5 or 5.3 ppm during gestation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,000 0.5 21 days</td>
<td>eruption in progeny of dams exposed to 0.5 or 5.3 ppm during gestation</td>
<td>Monitoring method not described</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10,000 5.3</td>
<td>exposed to 0.5 or 5.3 ppm during gestation</td>
<td>Monitoring method not described</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>188 0.1 6 h</td>
<td>NS Mouse (C3H)</td>
<td>No increase in chromatid- or chromosome-type alterations in leukocytes or primary spermatocytes immediately and 1 to 2 weeks postexposure</td>
<td>No mutagenic effects</td>
<td>Gooch et al (1977)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,880 1.0</td>
<td>NS Rat (LEW/fmat)</td>
<td>No alterations in spermatogenesis, germina, or interstitial testicular cells, no effect on vitamin B₁₂</td>
<td>No mutagenic effects</td>
<td>Kripke and Sherwin (1984)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400 5.0</td>
<td>Continuous, 7 h/day, 5 days/week, 21 days</td>
<td>M NS Rat (LEW/fmat)</td>
<td>No effect on fertility, but litter size and weight were decreased</td>
<td>No teratogenic effects</td>
<td>Shalamberidze and Tsereteli (1971)</td>
<td></td>
</tr>
<tr>
<td>13,600 1.3</td>
<td>12 h/day, 3 mo</td>
<td>F NS Rat (NS)</td>
<td>Decreased litter size and mortality of neonates up to 15 days postdelivery</td>
<td>No teratogenic effects noted</td>
<td>Freeman et al (1974b)</td>
<td></td>
</tr>
<tr>
<td>18,800 10</td>
<td>Continuous, from pregnancy to 3 mo after delivery</td>
<td>M/F in utero Rat (NS)</td>
<td>Decreased litter size and mortality of neonates up to 15 days postdelivery</td>
<td>No teratogenic effects noted</td>
<td>Freeman et al (1974b)</td>
<td></td>
</tr>
</tbody>
</table>

*M = Male  
F = Female  
NS = Not stated
However, pregnant F₁ rats, exposed to 1,000 or 10,000 μg/m³, had a 9- or 17-fold increase in placental lipid peroxides, respectively. The authors report that increased placental formation of toxic lipid peroxides in the F₁ rats could be due to decreased blood GSH (no measurements presented) and that such levels of lipid peroxides could be fetotoxic. However, the methods of monitoring NO₂ and lipid peroxides were not reported, nor were the statistical methods.

Potential mutagenic effects were investigated by Gooch et al. (1977), who reported that exposure to 188, 1,880, 9,400, and 18,800 μg/m³ (0.1, 1.0, 5.0, and 10 ppm) NO₂ for 6 h did not increase either chromatid or chromosome aberrations in the leukocytes of mice. Blood samples were obtained immediately after exposure and 1 and 2 weeks postexposure. Similarly, no increase in the number of translocations in primary spermatocytes was detected. Therefore, the authors concluded that NO₂ exposure did not induce mutagenesis in these experiments.

13.2.3.8 Potential Carcinogenic or Cocarcinogenic Effects

No direct evidence indicates that tumors may be produced by NO₂ exposure alone. Several studies have evaluated the issue of carcinogenesis and cocarcinogenesis, but results are often unclear or conflicting. Insofar as we are aware, there are no published reports on studies using classical carcinogenesis whole-animal bioassays. An excellent critical review and discussion of some of the important theoretical issues in interpreting these types of studies was written by Witschi (1988). Table 13-22 summarizes information on the carcinogenic or cocarcinogenic potential of NO₂.

Studies of Hyperplasia and Enhanced Retrovirus Expression

Hyperplasia of the lung epithelium, although a common response to lung injury, could be construed as suggesting a potential carcinogenic or cocarcinogenic effect of NO₂. However, the relatively frequent reports of hyperplasia, as discussed in Section 13.2.2.4 on morphological effects, did not include the observation of any tumors. It should be noted that these studies were not designed to detect tumors, so it is not surprising that none were found. Nakajima et al. (1972) found hyperplastic foci due to proliferation of epithelial cells of the terminal bronchioles and alveoli in mice exposed to 940 to 1,504 μg/m³ (0.5 to 0.8 ppm).
<table>
<thead>
<tr>
<th>NO₂ Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>0.04</td>
<td>Continuous, 17 mo</td>
<td>Rat</td>
<td></td>
<td></td>
<td>Nonsignificant increase in BHPN-induced tumors with exposure to 4.0 ppm</td>
<td>Ichmose et al (1991)</td>
</tr>
<tr>
<td>752</td>
<td>0.4</td>
<td>0.5-4 h</td>
<td>M</td>
<td>7 weeks</td>
<td>Mouse (ICR)</td>
<td>Mice exposed to DMA had whole-body concentration-related increase in DMN</td>
<td>Iqbal et al (1981)</td>
</tr>
<tr>
<td>188</td>
<td>0</td>
<td>7 h/day, 5 days/week, up to 181 days</td>
<td>F</td>
<td>5 weeks</td>
<td>mice (AKR/cum)</td>
<td>Fewer spontaneous lymphomas and increased survival time</td>
<td>Richters and Dangji (1990)</td>
</tr>
<tr>
<td>658</td>
<td>0.35</td>
<td>7 h/day, 5 days/week, 6 or 12 weeks</td>
<td>M</td>
<td>5 weeks</td>
<td>Mouse (C57BL/6J)</td>
<td>Significant increase in lung tumors in mice injected with melanoma cells at 6 weeks</td>
<td>Richters and Richters (1989)</td>
</tr>
<tr>
<td>752</td>
<td>0</td>
<td>8 h/day, 5 days/week, 10-12 weeks</td>
<td>M</td>
<td>NS</td>
<td>Mouse (Swiss Webster, C57BL/6J)</td>
<td>Increased lung tumors in mice injected with melanoma cells after NO₂ exposure</td>
<td>Richters and Kuraits (1981)</td>
</tr>
<tr>
<td>940-1,504</td>
<td>0.5-0.8</td>
<td>Continuous, 30 days</td>
<td>F</td>
<td>4 weeks</td>
<td>Mouse (ICR JCL)</td>
<td>Hyperplastic foci identical to those observed after exposure to known carcinogens</td>
<td>Nakajima et al (1972)</td>
</tr>
<tr>
<td>1,504</td>
<td>0.8</td>
<td>8 h/day, 5 days/week, 18 weeks</td>
<td>M/F</td>
<td>6 and 10 weeks</td>
<td>Mouse (Swiss Webster, AKR)</td>
<td>Enhanced retrovirus expression in two strains of mice</td>
<td>Roy-Burman et al (1982)</td>
</tr>
<tr>
<td>1,800</td>
<td>1</td>
<td>6 h/day, 5 days/week, 6 weeks</td>
<td>Mouse (AJ)</td>
<td></td>
<td></td>
<td>No effect at 1.0 or 5.0 ppm at 10 ppm, spontaneous adenomas in strain A/J mice increased only when compared to pooled control group</td>
<td>Adkins et al (1986)</td>
</tr>
<tr>
<td>9,400</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,000</td>
<td>1</td>
<td>Continuous, lifetime</td>
<td>M/F</td>
<td>NS</td>
<td>Rat (NS)</td>
<td>DMA plus NO₂ did not produce tumors Nitroso-DMA, DMA, and NO₂ produced excess tumors</td>
<td>Benemansku et al (1981)</td>
</tr>
<tr>
<td>3,000</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age (Strain)</td>
<td>Effects</td>
<td>Reference</td>
<td></td>
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<tr>
<td>9,400 µg/m³ 50 ppm</td>
<td>Continuous, up to 11 weeks</td>
<td>NS 5 weeks</td>
<td>Rat (NS)</td>
<td>Hyperplastic foci at 3 weeks Decreased ciliated cells Extensive hyperplasia, cuboidal metaplasia by 5 weeks Decreased bronchiolar lumen and polymorphous epithelium by 7 weeks Increased ciliated cells and decreased epithelial layers at 9 weeks By 11 weeks, return to one-layer epithelium</td>
<td>Rejthar and Rejthar (1975)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,400-18,800 µg/m³ 5-10 ppm</td>
<td>2 h/day, 5 days/week, 50 weeks</td>
<td>NS NS Mouse (NS)</td>
<td>4-Nitroquinoline-1-oxide during NO₂ exposure had no effect on tumor production</td>
<td>Ide and Otsu (1973)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18,800 µg/m³ 10 ppm</td>
<td>2 h/day, 5 days/week, 50 weeks</td>
<td>NS 4 weeks Mouse (NS)</td>
<td>Mice given 4-nitroquinoline-1-oxide and NO₂. NO₂ decreased incidence of lung tumors</td>
<td>Otsu and Ide (1975)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28,200-94,000 µg/m³ 15-50 ppm</td>
<td>1-4 h</td>
<td>M NS Mouse (ICR)</td>
<td>Mice gavaged with morpholine had concentration-dependent increase in whole-body content of NMOR</td>
<td>Iqbal et al. (1980)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31,000-38,500 µg/m³ 5-20 5 ppm</td>
<td>5-6 h/day, 4 days, plus 3 h on fifth day</td>
<td>M NS Mouse (CD-1)</td>
<td>In vivo production of NMOR when 1 g/kg of morpholine was administered each day prior to exposure</td>
<td>Van Stee et al. (1983)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aBHPN = N-bis(2-hydroxypropyl) nitrosamine
M = Male
DMA = Dimethylamine
DMN = Dimethylnitrosamine
F = Female
NS = Not stated
NMOR = N-nitrosomorpholine*
NO$_2$ for 30 days. The authors reported that these lesions were completely identical to early changes that appeared in the development of pulmonary adenomas after administration of known carcinogenic chemicals such as isoniazid, urethane, and 4-nitroquinoline-1-oxide (4NQO). However, no adenomas were detected.

Rejthar and Rejthar (1975) exposed rats to 9,400 $\mu$g/m$^3$ (5 0 ppm) NO$_2$ continuously for periods of 3, 5, 7, 9, or 11 weeks. After 3 weeks of exposure, the bronchioles had a uniform cuboidal one-layer epithelium composed of nonciliated cells. The cells showed vacuolization, and hyperplastic foci appeared in the bronchiolar epithelium. The foci were two- to four-layer pyramidal formations. By 5 weeks, extensive hyperplasia composed of three to four layers of epithelial cells was apparent. Centers of cuboidal metaplasia were found in adjacent alveoli. By 7 weeks, hyperplasia was apparent in all bronchioles, thereby narrowing the bronchiolar lumen. The polymorphous epithelium was extensive with a few ciliated cells in hyperplastic areas. After 9 weeks, the terminal bronchiolar epithelium generally showed two or three irregular layers. The number of ciliated cells increased, but cilia were often located atypically in intercellular spaces. A return to a single layer of epithelium without cilia was observed after 11 weeks. Seven weeks after exposure to NO$_2$, the lungs appeared to be in a state of repair, moving towards reversal of the lesions.

The possibility that NO$_2$ may facilitate the production of tumors has been suggested and examined by several authors. Endogenous retrovirus expression was enhanced in the spleen of low-expressor Swiss Webster mice after exposure to 1,500 $\mu$g/m$^3$ (0.8 ppm) NO$_2$, 8 h/day, 5 days/week for 1 or 18 weeks (Roy-Burman et al., 1982). However, measurements taken at intermediate time points were not different from controls. High-expressor AKR mice also showed an increase in the concentration of virus-specific RNA in the spleen after 8, 12, or 15 weeks of exposure to 564 $\mu$g/m$^3$ (0.3 ppm) NO$_2$. The authors suggest that such data may indicate inappropriate or inordinate expression of genes that could potentially influence genetically controlled diseases, such as cancer.

**Studies with Nitrogen Dioxide Plus Known Carcinogens**

Ide and Otsu (1973) studied tumor production in conventional mice receiving five weekly injections of 0.25 mg 4NQO (a lung-tumor-specific carcinogen) when the animals were exposed from birth to a NO$_2$ concentration between 9,400 and 18,800 $\mu$g/m$^3$ (5 and
10 ppm), 2 h/day, 5 days/week for 50 weeks. There was no difference in the number of tumors produced by 4NQO alone (6 of 10) and the number produced in combination with NO2 (6 of 13). Mice exposed to NO2 alone had a similar number of tumors as the air controls. Thus, NO2 did not facilitate the production of tumors.

One of the goals of a study by Benemansky et al. (1981) was to evaluate the potential of NO2 to influence the production of tumors during coexposure to a known carcinogen, nitrosodimethylamine (NDMA) or its precursor, dimethylamine (DMA). No excess tumors were observed in rats during a continuous lifetime exposure to the combination 0.07 mg/m³ DMA + 2,000 µg/m³ (1.1 ppm) NO2. This suggested that NO2 did not convert DMA to NDMA, which alone was shown to produce tumors. However, when the rats were exposed to NDMA at a concentration that alone did not produce tumors (0.06 mg/m³), an excess of tumors, especially in males, was observed when DMA (0.05 mg/m³) plus NO2 (3,000 µg/m³, 1.6 ppm) was added to the exposure. Appropriate statistical techniques and control groups were not incorporated into the design, and the methods of exposure and monitoring of NO2 were not reported, making the study difficult to evaluate.

In a similarly designed study, Ichinose et al. (1991) evaluated rats injected with N-N-bis(2-hydroxypropyl) nitrosamine (BHPN) and continuously exposed to 75, 752, or 7,520 µg/m³ (0.04, 0.4, or 4.0 ppm) NO2 for 17 mo. Although their data indicated five times as many lung adenomas or adenocarcinomas in the rats injected with BHPN and exposed to 7,520 µg/m³ (4.0 ppm) NO2, the results failed to achieve statistical significance using a Chi-square test. Nitrogen dioxide exposure alone caused no significant increase in tumors.

**Facilitation of Metastases**

Richters and Kuraitis (1981) performed two experiments in which mice were exposed to either 752 or 1,500 µg/m³ (0.4 or 0.8 ppm) NO2, 8 h/day, 5 days/week for 10 or 12 weeks, respectively. After exposures were terminated, the mice were injected intravenously with a cultured-derived melanoma cell line (B16). The first experiment suggested that there was an increased tumor yield if tumors were counted at 21 days postinjection, however, they did not observe a significant interaction or main time effect in the analysis of variance. For the second experiment, they chose a 3-week time period to count tumors. The results indicated
an increased number of tumors in the NO₂ group compared to filtered chamber and room air control groups. The authors concluded that NO₂ might facilitate the metastases of tumors, and these conclusions were based on inappropriate statistics. In more recent experiments, consistent effects have not been observed. For example, tumor facilitation was observed when mice were exposed to 564 or 752 µg/m³ (0.3 or 0.4 ppm) NO₂ for 12 weeks (Richters and Kuraitis, 1983). However, when mice were exposed to 940 µg/m³ (0.5 ppm) for 8 weeks (Richters and Kuraitis, 1983) or 752 µg/m³ (0.4 ppm) for 12 weeks with intermittent air exposures (Richters and Richters, 1983), facilitation was not observed. Richters et al. (1985) attempted to extend their findings by showing that, if allowed, the increased metastases from exposure to 752 µg/m³ (0.4 ppm) NO₂ for 12 weeks led to increased mortality in the mice. However, their post hoc analysis of the data precludes this conclusion. More recently, Richters and Richters (1989) exposed mice to 658 µg/m³ (0.35 ppm) NO₂ for 6 or 12 weeks, and examined tumor facilitation or lung injury after B16 melanoma injection. The authors reported increased facilitation at 6 weeks (p = 0.04, t-test), however, no statistical evaluation of the 12-week results were reported. The authors further claim that NO₂-induced injury to pulmonary endothelium may facilitate the retention of the injected melanoma. Again, this result (number of microthrombi in lung, p = 0.10, t-test) was found only after 6 weeks of exposure (not at 12 weeks) and only when examined 24 h after melanoma injection (not at 4 h). Pulmonary endothelial injury from NO₂ alone was not examined. Furthermore, the actual experimental design used in these studies probably did not evaluate metastases formation, as the term is generally understood, but more correctly, evaluated colonization of the lung by tumor cells. Studies in a true tumor metastases model, such as the Lobin Wistar rat, should be performed.

An abstract by Weinbaum et al. (1987) indicated that NO₂ could inhibit metastases formation if exposure occurred after injection of the B16F10 tumor cell suspension. Thus, studies showing facilitation of tumor colonization in the lung after NO₂ exposure should be viewed with caution because NO₂ may inhibit metastases as well as facilitate their colonization.
Studies in Animals with Spontaneously High Tumor Rates

A study by Wagner et al (1965) suggested that NO₂ may accelerate the production of tumors in CAF₁/JAX mice (a strain that is genetically susceptible to pulmonary tumors) after continuous exposure to 9,400 µg/m³ (5.0 ppm) NO₂. At the 12-mo evaluation, 7 of 10 mice had tumors in the exposed group, compared to 4 out of 10 in the controls. The number of tumors per animal was not reported. At the 14- and 16-mo evaluation, no differences in tumor production were observed. A statistical evaluation of the data was not presented.

The frequency and incidence of spontaneously occurring pulmonary adenomas was found to increase in strain A/J mice after exposure to 18,800 µg/m³ (10 ppm) NO₂ for 6 h/day, 5 days/week for 6 mo (Adkins et al., 1986). These small, but statistically significant, increases were only detectable when the control response from nine groups (N=400) were pooled. Exposure to 1,880 and 9,400 µg/m³ (1.0 and 5.0 ppm) NO₂ did not increase the number of spontaneous adenomas in this in vivo short-term model for predicting carcinogenicity.

A study by Richters and Damji (1990) evaluated the effect of exposure to 470 µg/m³ (0.25 ppm) NO₂, 7 h/day, 5 days/week for up to 181 days on the development and progression of spontaneous T-cell lymphomas in AKR/cum mice. Their results indicated that control animals developed lymphomas earlier and their survival time was less than NO₂-exposed mice. The reason for the increased incidence and progression of the lymphoma in control animals over that seen in NO₂-exposed animals was attributed to the decrease in T-helper/inducer (CD4⁺) lymphocytes, which produce growth factors for lymphomas, in the spleen of NO₂-exposed mice. A discussion of NO₂-induced effects on host lymphocyte populations appears in Section 13.2.2.1 on host defense mechanisms.

Production of N-Nitroso Compounds

Because of evidence that NO₂ could produce nitrile and nitrates in the blood, and nitrates is known to react with amines to produce animal carcinogens (nitrosamines), the possibility that NO₂ could produce cancer via nitrosoamine formation has been investigated.

Iqbal et al. (1980) was the first to demonstrate a linear time-dependent and concentration-dependent relationship in the amount of N-nitrosomorpholine (NMOR), an animal carcinogen, found in whole-mouse homogenates after the mice were gavaged with...
2 mg of morpholine (an exogenous amine that is rapidly nitrosated) and exposed for between 1 and 4 h to 28,200 to 94,000 µg/m³ (15 to 50 ppm) NO₂. Thus, because NMOR (a nitrosamine) is an animal carcinogen, these studies are sometimes used to suggest that NO₂ exposure could theoretically react with amines in the body to produce tumors.

Iqbal et al. (1981), using DMA, an amine that is slowly nitrosated to dimethylnitrosamine (DMN), found a concentration-related increase in biosynthesis of DMN at NO₂ concentrations as low as 188 µg/m³ (0.1 ppm), however, the rate was significantly greater at concentrations above 18,800 µg/m³ (10 ppm) NO₂. Increased length of exposure also increased DMN formation between 0.5 and 2 h, but synthesis of DMN was less after 3 and 4 h of exposure than after 0.5 h.

Mirvish et al. (1981) concluded that the results of Iqbal et al. (1980) were technically flawed. According to these researchers, the Iqbal et al. (1980, 1981) method, which involved homogenization of the whole frozen mouse, did not use an adequate stopping solution to prevent in vitro production of nitrosamines. According to Mirvish et al. (1981), they could verify the results of Iqbal et al. (1981) by eliminating the use of the stopping solution, but found no in vivo production of NMOR when in vitro production was eliminated. However, they did find that in vivo exposure to NO₂ could produce a nitrosating agent (NSA) that would nitrosate morpholine when morpholine was added in vitro. Additional experiments showed that NSA was localized in the skin (Mirvish et al., 1983) and that mouse skin cholesterol was a likely NSA (Mirvish et al., 1986). It has also been reported that only very lipid soluble amines, which can penetrate the skin, would be available to the NSA. Compounds such as morpholine, which is not lipid-soluble, could only react with NO₂ when it was painted directly on the skin (Mirvish et al., 1988).

Iqbal (1984), responding to the criticisms of Mirvish et al. (1981), concluded after the completion of several control experiments that in vitro nitrosation could only account for between 1 to 2% of the total amount of NMOR collected using his previous technique (Iqbal et al., 1980). Several control experiments further suggested that the effects in the original experiments were due to in vivo nitrosation. One experiment showed that nitrosamine biosynthesis could be inhibited in vivo with the addition of sulfamate, ascorbate, or α-tocopherol prior to NO₂ exposure. Another experiment indicated that the rapid half-life of morpholine (48 to 54 min) might explain why significant levels were not found by Mirvish.
et al. (1981) because they transferred the NO$_2$-exposed rats to room air for 30 min prior to sacrifice. In vivo nitrosation was also demonstrated by Norkus et al. (1984) after morpholine administration and a 2-h exposure to 84,600 µg/m$^3$ (45 ppm) NO$_2$.

Postlethwait and Mustafa (1983) examined this problem of in vivo production of nitrosamines using an isolated perfused rat lung. Rat lungs were ventilated with 37,400 µg/m$^3$ (19.9 ppm) NO$_2$ and the perfusion media was supplemented with 10 mM of morpholine. An excess of NMOR was found in the NO$_2$-exposed group when lung tissue and perfusate were combined. Control experiments could not exclude the possibility that NMOR was produced in the perfusate.

Another study (Van Stee et al., 1983) reported that NMOR was produced in mice gavaged with 1 g of morpholine/kg of body weight/day and then exposed to 31,020 to 38,540 µg/m$^3$ (16.5 to 20.5 ppm) NO$_2$, 5 to 6 h/day for 5 days. The single site containing the greatest amount of NMOR was the gastrointestinal tract. Regardless of whether in vivo nitrosation can occur, the relative significance of nitrile from NO$_2$ compared to nitrile resulting from food, tobacco, and nitrile-reducing oral bacteria is questionable (Murdia et al., 1982).

Aside from nitrosamines, other evidence suggests the possibility that inhaled NO$_2$ may be involved in the production of other potentially hazardous N-nitroso compounds. Protein and peptides may undergo nitrosation to produce diazo derivatives, most of which are mutagenic and/or carcinogenic. Challis et al. (1987) suggested, based on in vivo studies, that diazopeptides could be produced from inhaled NO$_2$ that is absorbed into the blood. These diazopeptides would be relatively stable at blood pH so as to allow them to act as circulating carcinogens.

**Summary**

Exposure to NO$_2$ produce a wide array of health effects beyond the confines of the lung. Evidence suggests that NO$_2$ and/or some of its reactive products penetrate the lung and enter the blood, producing alterations in the blood and other organs.

Conflicting results have been reported on whether NO$_2$ affects body weight gain in experimental animals. One study reported that NO$_2$ did not affect body weight gain in rabbits, guinea pigs, rats, hamsters, and mice at exposure concentrations of up to...
47,000 μg/m³ (25 ppm) and dogs at 9,400 μg/m³ (50 ppm) for up to 18 mo (Wagner et al., 1965) However, a decline in body weight in guinea pigs exposed continuously to 1,880 μg/m³ (1.0 ppm) for 6 mo (Kosmider et al., 1973b) and in rabbits exposed to 2,400 μg/m³ (1.3 ppm) for 15 to 17 weeks (Mitma, 1962) has been reported Newborn mice appear to be more sensitive to NO₂ exposure than adult mice (Kuraitis et al., 1981, Richters et al., 1987), but based on limited data, juvenile rats appear to be less sensitive to the effects of NO₂ exposure than young adult rats (Stevens et al., 1988).

Nitrogen dioxide-induced changes in blood constituents may result from the direct effect of NO₂, formation of nitrate and nitrite, or secondary effects emanating from other organs such as the lung, liver, heart, kidneys, and spleen. No effect on hematocrit and hemoglobin have been reported in squirrel monkeys exposed to 1,880 μg/m³ (1.0 ppm) NO₂ for 16 mo (Fenters et al., 1973) and in dogs exposed to up to 9,400 μg/m³ (5.0 ppm) for 18 mo (Wagner et al., 1965). There was, however, polycythemia and an increased ratio of PMNs to lymphocytes found in rats exposed to 3,760±1,880 μg/m³ (2.0±1.0 ppm) NO₂ for 14 mo (Furrosi et al., 1973). There have also been reported changes in the RBC membranes of experimental animals following NO₂ exposure. Red blood cell d-2,3-diphosphoglycerate was reportedly increased in guinea pigs exposed to 677 μg/m³ (0.36 ppm) NO₂ for 1 week (Mersch et al., 1973). An increase in RBC sialic acid, indicative of a younger population of RBCs, was reported in rats exposed to 7,520 μg/m³ (4.0 ppm) continuously for 1 to 10 days (Kumimoto et al., 1984), but in another study, exposure to the same concentration of NO₂ produced a decrease in RBCs (Mochitate and Mura, 1984).

Decreases in serum proteins and lipoproteins and increases in serum globulins, indicating NO₂-induced hepatic damage, have also been reported (Drozdz et al., 1976, Menzel et al., 1977, Kosmider et al., 1973a, Kosmider, 1975). Nitrogen dioxide increased pentobarbital-induced sleeping times in female mice after a 3-h exposure to 470 μg/m³ (0.25 ppm) (Miller et al., 1980), suggesting effects on hepatic xenobiotic metabolism. The effects only occurred at specified time periods after exposure ended and did not persist beyond 1 day. Similar effects (increased hexobarbital-induced sleeping time) were reported in the progeny of maternally exposed rats on Postexposure Days 7 and 21, but not on Day 14, after being exposed to 1,000 μg/m³ (0.5 ppm) NO₂ (Tabacova et al., 1985). Decreases in cytochrome P-450 levels in rat liver microsomes have been found after 7 days.
of exposure to 752 or 7,520 \( \mu g/m^3 \) (0.4 or 4.0 ppm), but not after exposure to 2,260 \( \mu g/m^3 \)
(1.2 ppm) NO\(_2\) (Mochutate et al., 1984)

Contrary to the finding of decreased amounts of cytochrome P-450 in liver homogenate following NO\(_2\) exposure, cytochrome P-450 and cytochrome b\(_5\) levels were increased in the kidney of rats after 8 weeks of exposure to both 2,260 and 7,520 \( \mu g/m^3 \) (1.2 and 4.0 ppm) NO\(_2\) (Takahashi et al., 1986). Nitrogen dioxide has also been reported to increase urinary concentrations of coproporphyrins, indicating a possible increase in heme synthesis, at NO\(_2\) exposure concentrations of 600 \( \mu g/m^3 \) (0.32 ppm) over a 3-mo period (Yakimchuk and Chelikanov, 1972) and has increased urinary alpha, beta, and gamma globulins in guinea pigs exposed to 752 \( \mu g/m^3 \) (0.4 ppm) NO\(_2\), 4 h/day for 14 days (Sherwin and Layfield, 1974).

Only limited information is available on the effect of NO\(_2\) on the heart. Nitrogen dioxide-induced effects on cardiac performance are suggested by a significant reduction in PaO\(_2\) in rats exposed to 7,520 \( \mu g/m^3 \) (4.0 ppm) NO\(_2\) for 3 mo. When exposure was decreased to 752 \( \mu g/m^3 \) (0.4 ppm) over the same exposure period, PaO\(_2\) was not affected (Suzuki et al., 1981). Also, a reduction in heart rate has been shown in mice exposed to both 2,250 and 7,520 \( \mu g/m^3 \) (1.2 and 4.0 ppm) NO\(_2\) for 1 mo (Suzuki et al., 1984). However, whether these effects are the direct result of NO\(_2\) exposure or secondary to lung edema and changes in blood hemoglobin content, is not known.

From the limited information available, it would appear that NO\(_2\) affects the central nervous system. Decreased activity of protein metabolizing enzymes, increased glycolytic enzymes; changes in neurotransmitter levels (5-HT and noradrenaline), and increased lipid peroxidation, accompanied by lipid profile and antioxidant changes, have been reported (Farahani and Hasan, 1990, 1991, Sherwin et al., 1986, Drozdz et al., 1975). Unfortunately, none of these effects have been replicated and all reports lack sufficient methodological rigor, thus, the implications of these findings, albeit important, are not clear and require further investigation.

The available data do not support the possibility that NO\(_2\) is a direct acting carcinogen. The data that suggest that NO\(_2\) may act as a promoter or facilitator of neoplastic disease are fraught with methodological and interpretive problems. The evidence suggests that further study may be warranted.
13.3 EFFECTS OF MIXTURES CONTAINING NITROGEN DIOXIDE

Exposure to pollutant mixtures in ambient air provides a basis for possible toxicologic interactions, whereby combinations of pollutants may behave differently than would be expected from consideration of the action of each constituent separately. In many cases, the study of mixtures containing NO₂ involved exposures to only two pollutants, and the role played by each can be elucidated with the appropriate experimental design. However, there is a fairly large data base that involves mixtures of more than two components, often with no single pollutant control, so the contribution of each individual agent to overall response is often obscure. In some cases, the NO₂ (or NOₓ) may have varied between exposure groups, or NO₂ was present in one group and not in another, so its relative influence could be assessed. This section focuses on those studies where the role of NO₂ (or NOₓ) can be elucidated.

**Simple Mixtures Containing Nitrogen Dioxide**

Table 13-23 outlines those studies in which experimental animals were exposed to constant levels of an atmosphere containing NO₂ with only one other pollutant (binary mixtures). The table is categorized by pollutants in the mixture and further subdivided by class of effect. By far, the largest data base is for NO₂ plus O₃. Examination of these studies indicates that various degrees and types of interaction may occur. The morphologic response to an NO₂/O₃ mixture, as reported by Freeman et al. (1974a) and Yokoyama et al. (1980), was primarily that due to O₃ alone, although in these studies, the levels of NO₂ used would have produced only small changes (by light microscopy) that would easily be obscured by the more potent O₃. Acute lethality and some biochemical responses to NO₂/O₃ mixtures involve synergism (e.g., Diggle and Gage, 1955, Mustafa et al., 1984, Sagai and Ichinose, 1991, Lee et al., 1989), some have ascribed this interaction to the production of new reaction products in the exposure atmosphere. On the other hand, antagonism has also been reported for effects of NO₂ and O₃ in some enzyme systems (Takahashi and Miura, 1989).

In terms of host antimicrobial defense, toxicologic interactions involving NO₂ and O₃ are generally additive after acute exposures. It seems that each pollutant contributes to
<table>
<thead>
<tr>
<th>Pollutant Concentration</th>
<th>Exposure</th>
<th>Gender</th>
<th>Species (Strain)</th>
<th>End Points</th>
<th>Response to Mixture</th>
<th>Interaction</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₂ (3,390-30,200 µg/m³, 1 8-4 75 ppm) + O₃ (11,000-23,300 µg/m³, 5 0-10 7 ppm)</td>
<td>4 h</td>
<td>M</td>
<td>NS Rat (NS)</td>
<td>Mortality, dyspnea</td>
<td>Increase</td>
<td>Synergistic</td>
<td>Interaction due to production of nitrogen pentoxide</td>
<td>Diggle and Gage (1955)</td>
</tr>
<tr>
<td>NO₂ (4,700 µg/m³, 25 ppm) + O₃ (490 µg/m³, 0 25 ppm)</td>
<td>6 mo M</td>
<td>4 weeks Rat (Sprague-Dawley)</td>
<td>Morphology</td>
<td>Hypertrophy of alveolar duct epithelium</td>
<td>None</td>
<td>Lesion due primarily to O₃</td>
<td>Freeman et al (1974)</td>
<td></td>
</tr>
<tr>
<td>NO₂ (1,690 µg/m³, 0 9 ppm) + O₃ (1,760 µg/m³, 0 9 ppm)</td>
<td>60 days</td>
<td>Emphysema</td>
<td>None</td>
<td>Lesion due primarily to O₃</td>
<td>None</td>
<td>Lesion due primarily to O₃</td>
<td>Freeman et al (1974)</td>
<td></td>
</tr>
<tr>
<td>NO₂ (10,300 µg/m³, 5 5 ppm) + O₃ (2,160 µg/m³, 1 1 ppm)</td>
<td>3 h/day, 14 days</td>
<td>M</td>
<td>8 weeks Rat (Wistar)</td>
<td>Enzyme activity</td>
<td>Increase</td>
<td>Synergistic</td>
<td>Yokoyama et al (1980)</td>
<td></td>
</tr>
<tr>
<td>NO₂ (10,200 µg/m³, 5 4 ppm) + O₃ (1,960 µg/m³, 1 0 ppm)</td>
<td>3 h/day, 14 or 30 days</td>
<td>7 weeks</td>
<td>Morphology pulmonary mechanics</td>
<td>Increase no change</td>
<td>None or additive</td>
<td>None or additive</td>
<td>Yokoyama et al (1980)</td>
<td></td>
</tr>
<tr>
<td>Pollutant Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>Response to Mixture</td>
<td>Interaction</td>
<td>Remarks</td>
<td>Reference</td>
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<tr>
<td>NO$_2$ (75 µg/m$^3$, 0.04 ppm) + O$_3$ (98 µg/m$^3$, 0.05 ppm)</td>
<td>NO$_2$ continuous, 5-22 mo (O$_3$ was intermittent)</td>
<td>M</td>
<td>7 weeks (Wistar)</td>
<td>Lipid peroxidation, antioxidants, antioxidant enzyme activity</td>
<td>Increase in lipid peroxides, no change in enzyme of activity</td>
<td>Synergistic (for peroxides up to 9 mo exposure only)</td>
<td>NO$_2$ or O$_3$ alone showed no change in peroxides</td>
<td>Sagai and Ichnose (1991)</td>
</tr>
<tr>
<td>NO$_2$ (752 µg/m$^3$, 0.4 ppm) + O$_3$ (98 µg/m$^3$, 0.05 ppm)</td>
<td>NO$_2$ continuous, 5-22 mo (O$_3$ was intermittent)</td>
<td>M</td>
<td>24 h/day for 2 weeks (Wistar)</td>
<td>Lipid peroxide production and activity of antioxidant enzymes</td>
<td>Increased lipid peroxides only in guinea pigs</td>
<td>Synergistic</td>
<td>Relation between antioxidant production and peroxide formation</td>
<td>Ichnose and Sagai (1989)</td>
</tr>
<tr>
<td>NO$_2$ (2,260 µg/m$^3$, 1.2 ppm) + O$_3$ (588 µg/m$^3$, 0.3 ppm)</td>
<td>Continuous, 3 days (Sprague-Dawley)</td>
<td>M</td>
<td>3 mo</td>
<td>Lung weight, activity of various enzymes</td>
<td>Increases in enzyme activity and lung weight</td>
<td>None or synergistic (end point dependent)</td>
<td>Synergistic for some end points, additive for others, same as O$_3$ for others</td>
<td>Lee et al (1990)</td>
</tr>
<tr>
<td>NO$_2$ (3,380 µg/m$^3$, 1.8 ppm) + O$_3$ (882 µg/m$^3$, 0.45 ppm)</td>
<td>Continuous, 3 days (Sprague-Dawley)</td>
<td>M</td>
<td>3 mo</td>
<td>Lung enzymes, lung weight</td>
<td>Increases in enzyme activity</td>
<td>Synergistic (for some enzymes, additive for others, and same as O$_3$ for lung weight and G-6-P dehydrogenase activity)</td>
<td>Lee et al (1989)</td>
<td></td>
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<tr>
<td>Pollutant Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>End Points</td>
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<tr>
<td>NO₂ (7,520 μg/m³, 4.0 ppm) + O₃ (392 μg/m³, 0.2 ppm)</td>
<td>Continuous, 1-2 mo</td>
<td>M</td>
<td>22 weeks</td>
<td>Rat (Wistar)</td>
<td>Xenobiotic metabolism system in lungs</td>
<td>(see Remarks)</td>
<td>Antagonistic</td>
<td>Mixture with NO₂ reduces any increased metabolism produced by O₃ alone for various enzyme activities</td>
</tr>
<tr>
<td>NO₂ (13,200 and 28,200 μg/m³, 7 and 15 ppm) + O₃ (980 and 1,960 μg/m³, 0.5 and 1.0 ppm)</td>
<td>3 h/day, 7 days</td>
<td>M</td>
<td>4 weeks</td>
<td>Mouse (ICR JCL)</td>
<td>Level of reduced glutathione in lung</td>
<td>Increase</td>
<td>Additive</td>
<td>Watanabe et al (1980)</td>
</tr>
<tr>
<td>NO₂ (9,020 μg/m³, 4.8 ppm) + O₃ (880 μg/m³, 0.45 ppm)</td>
<td>8 h/day, 7 days</td>
<td>M</td>
<td>8 weeks</td>
<td>Mouse (Swiss Webster)</td>
<td>Lung weight, rate of O₂ consumption (in lung homogenate), sulfhydryl metabolism in lung, activity of NADP reducing enzymes</td>
<td>Increase</td>
<td>Synergistic</td>
<td>Mustafa et al (1984)</td>
</tr>
</tbody>
</table>

Lung DNA content, lung protein content | No change | None | No effect with mixture or either alone
TABLE 13-23 (cont’d). TOXICOLOGIC INTERACTIONS TO SIMPLE MIXTURES CONTAINING NITROGEN DIOXIDEa

<table>
<thead>
<tr>
<th>Pollutant Concentration</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>End Points</th>
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<th>Interaction</th>
<th>Remarks</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>NO2 (5,640 µg/m³, 3 0 ppm) + O3 (588 µg/m³, 0 3 ppm)</td>
<td>2 h</td>
<td>M</td>
<td>5 mo</td>
<td>Rabbit (New Zealand)</td>
<td>Effects on arachidonic acid metabolites</td>
<td>Increase</td>
<td>Synergistic</td>
<td>Increases in PGE2 and PGE2α (Response driven by O3)</td>
<td>Schlesinger et al (1990)</td>
</tr>
<tr>
<td>NO2 (5,640 µg/m³, 3 0 ppm) + O3 (588 µg/m³, 0 3 ppm)</td>
<td>2 h/day, up to 14 days</td>
<td>M</td>
<td>5 mo</td>
<td>Rabbit (New Zealand)</td>
<td>Prostanoids in lavage</td>
<td>Decrease in selected prostanoids</td>
<td>None</td>
<td>Depending on prostanoid, and number of days of exposure mixture was additive or similar to O3 or NO2 when given alone</td>
<td>Schlesinger et al (1991)</td>
</tr>
<tr>
<td>NO2 (3,760-18,800 µg/m³, 2-10 ppm) + O3 (980 and 1,960 µg/m³, 0 5 and 1 0 ppm)</td>
<td>1-2 h</td>
<td>NS</td>
<td>NS</td>
<td>Mouse (NS)</td>
<td>Creatinine phosphokinase in plasma</td>
<td>Increase or decrease, depending on concentration</td>
<td>None</td>
<td>Effect due to O3</td>
<td>Venninga et al (1982)</td>
</tr>
<tr>
<td>NO2 (6,770-27,100 µg/m³, 3 6-14 4 ppm) + O3 (392-1,570 µg/m³, 0 2-0 8 ppm)</td>
<td>6-24 h/day, 3 days</td>
<td>M</td>
<td>10-12 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>BAL protein, cell types in BAL</td>
<td>Increased protein at ≥10 8 ppm NO2 + ≥0 6 ppm O3, increased epithelial cells at all concentrations, increase neutrophils at ≥10 8 ppm NO2 + ≥0 6 ppm O3</td>
<td>Additive or synergistic</td>
<td>Additive at low dose-rate (3 6 ppm NO2 + 0 2 ppm O3) and synergistic at higher dose-rate</td>
<td>Gelzleichter et al (1992a)</td>
</tr>
<tr>
<td>Pollutant Concentration</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
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<tr>
<td>NO₂ (6,770-27,100 μg/m³, 3 6-14 4 ppm) + O₃ (392-1,570 μg/m³, 0 2-0 8 ppm) (concurrent and sequential)</td>
<td>6 h/day, 3 days</td>
<td>M</td>
<td>10-12 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>BAL protein, cell types</td>
<td>Increased in all endpoints</td>
<td>Additive, synergistic, or antagonistic</td>
<td>For BAL protein and PMNs, additivity with sequential exposure, synergism for concurrent mixture for epithelial cells, O₃ and then NO₂ caused additivity, NO₂ then O₃ caused antagonism, concurrent mixture caused synergism</td>
<td>Gelzleichter et al (1992b)</td>
</tr>
<tr>
<td>NO₂ (7,520 μg/m³, 4 0 ppm) + O₃ (1,568 μg/m³, 0 8 ppm)</td>
<td>Continuous, 3, 7, 14, or 56 weeks</td>
<td>M</td>
<td>8-10 weeks</td>
<td>Mouse BALB/c</td>
<td>Organ weights, antibody response to SRBCs and to DNP-Ficoll</td>
<td>Decrease in spleen weight and increase in lung weight, no effect on response to DNP, but response to SRBCs was depressed with 3-14 day exposure only</td>
<td>None</td>
<td>Additive for spleen weight, similar to O₃ for lung weight, antibody response similar to O₃</td>
<td>Fujimaki (1989)</td>
</tr>
</tbody>
</table>
TABLE 13-23 (cont’d). TOXICOLOGIC INTERACTIONS TO SIMPLE MIXTURES CONTAINING NITROGEN DIOXIDE

<table>
<thead>
<tr>
<th>Pollutant Concentration</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>End Points to Mixture</th>
<th>Interaction</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₂ (2,820 µg/m³, 1.5 ppm) + O₃ (200 µg/m³, 0.1 ppm)</td>
<td>4 h</td>
<td>M</td>
<td>NS Mouse (Swiss albino)</td>
<td>Bactericidal</td>
<td>Decrease in bactericidal activity when NO₂ &gt; 40 ppm + O₃ &gt; 0.36 ppm</td>
<td>Additive</td>
<td>Bacterial challenge after exposure</td>
</tr>
<tr>
<td>NO₂ (2,800-7,860 µg/m³, 1.49-4.18 ppm) + O₃ (220-530 µg/m³, 0.11-0.27 ppm)</td>
<td>17 h</td>
<td></td>
<td></td>
<td>Additive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ (2,820-9,400 µg/m³, 1.5-5 ppm) + O₃ (100-980 µg/m³, 0.05-0.5 ppm)</td>
<td>3 h</td>
<td>M</td>
<td>6-10 weeks (CD₂F₁)</td>
<td>Bacterial infectivity</td>
<td>Decreased survival time with 0.5 ppm O₃ and any NO₂, and with 0.1 ppm O₃ and 3.5 ppm NO₂</td>
<td>Additive</td>
<td>Bacterial challenge after exposure</td>
</tr>
<tr>
<td>NO₂ (3,760 µg/m³, 2.0 ppm) + O₃ (100 µg/m³, 0.05 ppm)</td>
<td>3 h/day, 5 days/week, 1-4 weeks</td>
<td></td>
<td></td>
<td>Excess mortality at all times</td>
<td>Synergistic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 13-23 (cont’d). **TOXICOLOGIC INTERACTIONS TO SIMPLE MIXTURES CONTAINING NITROGEN DIOXIDE**\(^a\)

<table>
<thead>
<tr>
<th>Pollutant Concentration</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age</th>
<th>Species (Strain)</th>
<th>End Points</th>
<th>Response to Mixture</th>
<th>Interaction</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{NO}_2 (2,260-\mu g/m^3, 1.2-\text{ppm base with } 4,700-\mu g/m^3, 2.5 \text{ ppm peak}) + \text{O}_3 (196-\mu g/m^3, 0 1-\text{ppm base with } 588-\mu g/m^3, 0 3-\text{ppm peak}))</td>
<td>15 days</td>
<td>F</td>
<td>4-6 weeks</td>
<td>Mouse (CD-1)</td>
<td>Bacterial infectivity</td>
<td>Increased infectivity</td>
<td>Synergistic</td>
<td>(\text{NO}_2 \text{ and } \text{O}_3 \text{ alone increased infectivity})</td>
<td>Graham et al (1987)</td>
</tr>
<tr>
<td>(\text{NO}_2 (940-\mu g/m^3, 0 5-\text{ppm base with } 1,880-\mu g/m^3, 1 0-\text{ppm peak}) + \text{O}_3 (98-\mu g/m^3, 0 05-\text{ppm base with } 196-\mu g/m^3, 0 1-\text{ppm peak}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Synergistic</td>
<td>(\text{NO}_2 \text{ alone increased infectivity, } \text{O}_3 \text{ alone did not})</td>
<td>Gardner et al (1982)</td>
</tr>
<tr>
<td>(\text{NO}_2 (94-\mu g/m^3, 0 05-\text{ppm base with } 188-\mu g/m^3, 0 1-\text{ppm peak}) + \text{O}_3 (98-\mu g/m^3, 0 05-\text{ppm base with } 196-\mu g/m^3, 0 1-\text{ppm peak}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>(\text{NO}_2 \text{ or } \text{O}_3 \text{ alone had no effect})</td>
<td></td>
</tr>
<tr>
<td>Pollutant Species</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>End Points</td>
<td>Response to Mixture</td>
<td>Interaction</td>
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</tr>
<tr>
<td>NO₂ (3,760 µg/m³) + SO₂ (5,240 µg/m³)</td>
<td>Continuous, up to 13 weeks</td>
<td>NS</td>
<td>8 weeks</td>
<td>Rat (Wistar)</td>
<td>Morphology</td>
<td>No change</td>
<td>None</td>
<td>No effect of either SO₂ or NO₂</td>
<td>Azoulay et al (1980)</td>
</tr>
<tr>
<td>NO₂ (8,000-11,000 µg/m³), 2-5 8 ppm + SO₂ (9,000-11,000 µg/m³), 3 4-2 ppm</td>
<td>24 h/day, F</td>
<td>NS</td>
<td>Guinea pig</td>
<td>Respiratory mechanics (frequency, flow rate, minute volume)</td>
<td>No change</td>
<td>None</td>
<td>No effect of either SO₂ or NO₂</td>
<td>Antweiler and Brockhaus (1976)</td>
<td></td>
</tr>
<tr>
<td>NO₂ (3,760 µg/m³), 2 0 ppm + NaCl (330 µg/m³)</td>
<td>Continuous, M/F</td>
<td>NS</td>
<td>14 mo</td>
<td>Monkey (Macaca speciosa)</td>
<td>Morphology</td>
<td>Respiratory bronchiolar epithelial hypertrophy</td>
<td>None</td>
<td>Effect due to NO₂</td>
<td>Furiosi et al (1973)</td>
</tr>
<tr>
<td>NO₂ (3,760 µg/m³), 2 0 ppm + NaCl (330 µg/m³)</td>
<td>Continuous, 18 mo</td>
<td></td>
<td></td>
<td></td>
<td>Hematology</td>
<td>Polycythemia</td>
<td>None</td>
<td>Effect due to NO₂</td>
<td>Furiosi et al (1973)</td>
</tr>
<tr>
<td>NO₂ (3,760 µg/m³), 2 0 ppm + NaCl (330 µg/m³)</td>
<td>Continuous, M</td>
<td>4 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>Hematology</td>
<td>Polycythemia</td>
<td>None</td>
<td>Effect due to NO₂</td>
<td>Furiosi et al (1973)</td>
<td></td>
</tr>
<tr>
<td>Pollutant</td>
<td>Concentration (µg/m³, ppm)</td>
<td>Exposure</td>
<td>Gender</td>
<td>Age</td>
<td>Species (Strain)</td>
<td>End Points</td>
<td>Response to Mixture</td>
<td>Interaction</td>
<td>Remarks</td>
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<tr>
<td>NO₂ (9,400 µg/m³, 5 0 ppm) + NaCl (1,000 µg/m³, 0 4 µm)</td>
<td>7 days</td>
<td>M</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>Rate of collagen synthesis by lung mincs</td>
<td>Increase</td>
<td>Synergistic</td>
<td></td>
<td>Last and Warren (1987)</td>
</tr>
<tr>
<td>NO₂ (9,400 µg/m³, 5 0 ppm) + NaCl (1,000 µg/m³, 0 4 µm)</td>
<td>1, 3 days</td>
<td>3</td>
<td></td>
<td>Protein content of lung lavage fluid</td>
<td>Increase at 1 and 3 days</td>
<td>Synergistic at 3 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂ (47,000-56,400 µg/m³, 25-30 ppm) + carbon</td>
<td>6 h/day, 5 days/week, 3 mo</td>
<td>NS</td>
<td>NS</td>
<td>Mouse (Swiss albino)</td>
<td>Morphology</td>
<td>Focal parenchymal lesions</td>
<td>Carbon acted as carrier for localized NO₂ deposition</td>
<td>Boren (1964)</td>
<td></td>
</tr>
<tr>
<td>NO₂ (9,400-47,000 µg/m³, 5-25 ppm) + (NH₄)₂SO₄ (5,000 µg/m³, 0 8-1 µm MMAD)</td>
<td>23 5 h/day, 7 days</td>
<td>M</td>
<td>10-11 weeks</td>
<td>Rat (Sprague-Dawley)</td>
<td>Rate of collagen synthesis by lung mincs</td>
<td>Increase</td>
<td>Synergistic</td>
<td>Last et al (1983)</td>
<td></td>
</tr>
<tr>
<td>NO₂ (3,760 and 9,400 µg/m³, 2 0 and 5 0 ppm) + H₂SO₄ (890 µg/m³, 0 4 µm MMAD)</td>
<td>23 5 h/day, 7 days</td>
<td>M</td>
<td>NS</td>
<td>Rat (Sprague-Dawley)</td>
<td>Rate of collagen synthesis by lung mincs</td>
<td>Increase</td>
<td>Synergistic</td>
<td>Last and Warren (1987) Last (1989)</td>
<td></td>
</tr>
</tbody>
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### TABLE 13.23 (cont’d). TOXICOLOGIC INTERACTIONS TO SIMPLE MIXTURES CONTAINING NITROGEN DIOXIDE

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<th>Interaction</th>
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</tr>
</thead>
<tbody>
<tr>
<td>( \text{NO}_2 ) (560 and 1,880 ( \mu \text{g/m}^3 ), 0.3 and 1.0 ppm) ( + ) ( \text{H}_2\text{SO}_4 ) (500 ( \mu \text{g/m}^3 ), 0.4 ( \mu \text{m} ) MMAD)</td>
<td>2 h/day, M 5 mo Rabbit (New Zealand)</td>
<td>Particle clearance Decrease from respiratory region</td>
<td>None at 0.3 ppm, response due to ( \text{H}_2\text{SO}_4 ), at 1.0 ppm, response different from both ( \text{NO}_2 ) and ( \text{H}_2\text{SO}_4 )</td>
<td>Schlesinger and Gearhart (1987) Schlesinger et al (1987a)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mucociliary clearance</td>
<td></td>
<td></td>
<td>With 0.3 ppm NO(_2) in mixture, clearance faster, no change with 1 ppm NO(_2)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>( \text{NO}_2 ) (560 and 1,880 ( \mu \text{g/m}^3 ), 0.3 and 1.0 ppm) ( + ) ( \text{H}_2\text{SO}_4 ) (500 ( \mu \text{g/m}^3 ), 0.3 ( \mu \text{m} ) MMAD)</td>
<td>2 h/day, M 5 mo Rabbit (New Zealand)</td>
<td>Alveolar macrophage function and numbers Variable, depending on NO(_2) concentration and end point</td>
<td>Additive or synergistic depending on NO(_2) concentration and end point</td>
<td>Schlesinger (1987a)</td>
<td></td>
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</tbody>
</table>

M = Male  
NS = Not stated  
NADP = Nicotinamide adenine  
PGE\(_2\) = Prostaglandin \( \text{E}_2 \)  
PGE\(_{2\alpha}\) = Prostaglandin \( \text{E}_{2\alpha} \)  
F = Female  
SO\(_3\) = Sulfur dioxide  
oxHb = Oxyhemoglobin  
RBC = Red blood cell  
Mthb = Methemoglobin  
NaCl = Sodium chloride  
(NH\(_4\))\(_2\)SO\(_4\) = Ammonium sulfate  
MMAD = Mass median aerodynamic diameter  
H\(_2\)SO\(_4\) = Sulfuric acid  
G-6-P dehydrogenase = Glucose-6-phosphate dehydrogenase  
SRBCs = Sheep red blood cells  
BAL = Bronchoalveolar lavage
the observed response when its concentration reaches a level at which it would have affected bacterial resistance when administered alone (Goldstein et al., 1974). The mouse subchronic infectivity study conducted by Ehrlich et al. (1977) provided a suggestion of synergism with exposure to 3,760 μg/m³ (2.0 ppm) NO₂ and 97.5 μg/m³ (0.05 ppm) O₃.

Simulation of urban patterns involving NO₂ and O₃ have also been performed by examining the effects, on bacterial resistance, of a continuous baseline exposure, with superimposed short-term peaks to a higher level. Ehrlich et al. (1979) exposed mice for 1 to 6 mo (24 h/day, 7 days/week) to a baseline concentration of 0 (air) or 188 μg/m³ (0.1 ppm) NO₂, upon which was superimposed 3-h/day, 5-day/week peak exposures of 940 μg/m³ (0.5 ppm) NO₂, or a combination of 940 μg/m³ NO₂ and 200 μg/m³ O₃, bacterial exposure followed pollutant exposure, and animals were then observed for 14 days. A significant and similar increase in percentage mortality was found by 6 mo in all groups, with no evidence that exposure to the NO₂/O₃ peaks altered the response, there was also no change in survival time. In another experiment, mice were reexposed for 14 days (after 1- to 3-mo pollutant exposure and bacterial challenge) to the same pollutant concentrations as above, and mortality was examined during this time. Animals preexposed for a least 2 mo either to NO₂/O₃ peaks over the air baseline or to NO₂/O₃ peaks over the 188 μg/m³ NO₂ baseline showed significant reductions in survival time. Although no conclusions were drawn as to the efficacy of the mixture, the investigators concluded that the sequence of peak exposure was important in altering resistance to infection.

Ehrlich et al. (1979) also examined the effect of the same baseline and peak exposures (for 1 to 3 mo) on AMs. Cell viability was decreased after 3 mo of exposure only when the NO₂/O₃ peaks were superimposed on continuous exposure to clean air. There also was a general increase in blood enzyme activity, but continuous exposure to 188 μg/m³ (0.1 ppm) NO₂ with superimposed peaks of NO₂ and O₃ was the most effective in this regard.

In another study, Ehrlich (1983) examined the effects of continuous exposure (24 h/day, 5 days/week) to a baseline level of 376 μg/m³ (0.2 ppm) NO₂, with two daily peaks given 5 days/week as follows: 1,880 μg/m³ (1.0 ppm) NO₂ for 1 h in the morning and a mixture of 200 μg/m³ (0.1 ppm) O₃ plus 1,880 μg/m³ NO₂ given for 1 h in the afternoon. Exposures lasted for 9 mo, followed by bacterial challenge. Other groups were exposed continuously to 376 μg/m³ NO₂ either with no peak or with a 1,880-μg/m³ peak given for
1 h in both the morning and the afternoon. The only group that showed a significant increase in mortality was that exposed for 9 mo to 376 μg/m³ NO₂ with daily peaks of NO₂ in the morning and NO₂ and O₃ in the afternoon. In addition, only this group showed a change (increase) in cellular ATP levels in AMs. By 8 mo of exposure, this group also showed an increase in counts of RBCs, leukocytes, and lymphocytes, and a decrease in mean hemoglobin concentration. The other pollutant exposure groups showed increases only in leukocyte count.

Gardner et al. (1982), Gardner (1980), and Graham et al. (1987) examined bacterial resistance in mice continuously exposed (15 days, 24 h/day) to a baseline level of an NO₂/O₃ mixture with two daily 1-h peaks of the mixture, as follows: (1) high exposure level 2,260 μg/m³ (1.2 ppm) NO₂ plus 196 μg/m³ (0.1 ppm) O₃ baseline with 4,700 μg/m³ (2.5 ppm) NO₂ plus 588 μg/m³ (0.3 ppm) O₃ peak, (2) intermediate exposure level 940 μg/m³ (0.5 ppm) NO₂ plus 98 μg/m³ (0.05 ppm) O₃ baseline with 1,880 μg/m³ (1.0 ppm) NO₂ plus 196 μg/m³ (0.1 ppm) O₃ peak, or (3) low exposure level 94 μg/m³ (0.05 ppm) NO₂ plus 100 μg/m³ (0.05 ppm) O₃ baseline with 188 μg/m³ (0.1 ppm) NO₂ plus 196 μg/m³ (0.1 ppm) O₃ peak. Animals were also exposed to the same baseline levels of either NO₂ or O₃ onto which were superimposed twice daily, 1-h peaks of either NO₂ or O₃ at the concentrations described above. The low concentrations of either given alone, or in combination, did not significantly increase mortality. At the intermediate exposure levels, the mixture was synergistic, whereas NO₂ alone increased mortality and O₃ had no effect. At the high exposure level, the combined exposure was again synergistic, exposure to each pollutant given separately also increased mortality.

Sagai et al. (1987) exposed mice, hamsters, guinea pigs, and rats to a mixture of 750 μg/m³ (0.4 ppm) NO₂ and 780 μg/m³ (0.4 ppm) O₃, 24 h/day for 2 weeks, to assess effects on lipid peroxidation in the lungs. Although the two gases were not also administered singly to allow assessment of effects due to each alone, the study showed species differences in lipid peroxide formation following exposure that were related to the relative content of antioxidants and the specific composition of phospholipids and their fatty acids. The guinea pig was the most sensitive animal and the hamster was the most resistant. In follow-up studies, Sagai and Ichinose (1991) exposed rats for 22 mo to mixtures of NO₂ and O₃.
increase in lipid peroxidation was synergistic and maximal at 9 mo, later examinations revealed no effects.

Ichinose and Sagai (1989) also observed a species dependence in the interaction of NO$_2$ (752 $\mu$g/m$^3$, 0.4 ppm) and O$_3$ (784 $\mu$g/m$^3$, 0.4 ppm) after 2 weeks of continuous exposure. Guinea pigs, but not rats, had a synergistic increase in lung lipid peroxides, expressed as TBA reactants. Rats, but not guinea pigs, had synergistic increases in nonprotein sulfhydryls, vitamin C, G-6-P dehydrogenase, and GSH peroxidase.

Duration of exposure can also have an impact. Schlesinger et al. (1990) observed a synergistic increase in prostaglandins E$_2$ and F$_{2\alpha}$ in the lung lavage of rabbits exposed for 2 h to 5,640 $\mu$g/m$^3$ (3.0 ppm) NO$_2$ plus 588 $\mu$g/m$^3$ (0.3 ppm) O$_3$, the response appeared to have been driven by O$_3$. However, with 7 of 14 days of repeated 2-h exposures, only prostaglandin E$_2$ was decreased, apparently due to NO$_2$, there was no synergism (Schlesinger et al., 1991).

The studies described above involved simultaneous exposure to both NO$_2$ and another gas. However, "real world" exposures to these pollutants typically have temporal patterns, and exposure to one agent may then alter the response to another subsequently inhaled. Thus, order of exposure to inhaled NO$_2$ may be important in toxic interactions. Yokoyama et al. (1980) exposed rats to either NO$_2$ or O$_3$ for 3 h or to NO$_2$ for 3 h followed by O$_3$ for 3 h, and assessed lung mechanics in postmortem lungs, lung histology, and enzyme activity in subcellular fractions of lung tissue. In one series of exposures, rats were exposed for 7 or 14 days to NO$_2$ and O$_3$ at concentrations of 10,300 $\mu$g/m$^3$ (5.5 ppm) and 2,160 $\mu$g/m$^3$ (1.1 ppm), respectively. The activity of phospholipase A$_2$ in the mitochondrial fraction of lung homogenate was only increased in those animals exposed to O$_3$ after NO$_2$, for 14 days. A decrease in activity of lysolecithin acyltransferase in the supernatant fraction was found after 7 and 14 days in all groups of animals. In a second study, rats were exposed for 14 or 30 consecutive days to 10,200 $\mu$g/m$^3$ (5.4 ppm) NO$_2$ followed by 1,960 $\mu$g/m$^3$ (1.0 ppm) O$_3$. Pulmonary mechanics tests performed on the postmortem lung indicated an increase in pulmonary flow resistance in the O$_3$- and sequential NO$_2$/O$_3$-exposed animals. There were no changes in volume-pressure curves in any of the groups. Histologically, the lungs of the animals exposed to both NO$_2$ and O$_3$ appeared similar to those exposed to O$_3$ alone. However, a slight degree of epithelial necrosis in the medium.
bronch1, not found with either NO\textsubscript{2} or O\textsubscript{3} alone, was seen in the animals exposed to both pollutants. In addition, damage at the bronchoalveolar junction appeared to be somewhat more marked in animals exposed to both gases than in those exposed to O\textsubscript{3} alone. These studies suggest that sequential exposures produced responses that were, in most cases, not greatly different from those due to O\textsubscript{3} alone.

Gelzleichter et al. (1992b) also evaluated sequential exposure. Rats were exposed for 3 days for 6 h/day to O\textsubscript{3} (392 to 1,570 μg/m\textsuperscript{3}, 0.2 to 0.8 ppm) or NO\textsubscript{2} (6,770 to 27,100 μg/m\textsuperscript{3}, 3.6 to 14.4 ppm) or their combinations. Combinations were either concurrent or sequential (O\textsubscript{3} first and then NO\textsubscript{2} or vice versa). For either of the sequential exposures, the increase in BAL protein and PMNs was additive, it was synergistic for the concurrent mixture. For lavageable epithelial cells, the O\textsubscript{3} then NO\textsubscript{2} group showed additivity, whereas the NO\textsubscript{2} then O\textsubscript{3} group displayed antagonism, this endpoint exhibited synergism when the O\textsubscript{3} and NO\textsubscript{2} were concurrent. The synergisms observed were concentration-dependent. Effects on epithelial cell numbers were most sensitive, showing synergism at 392 μg/m\textsuperscript{3} (0.2 ppm) O\textsubscript{3} with 27,100 μg/m\textsuperscript{3} (14.4 ppm) NO\textsubscript{2}. The authors postulate that the synergism may be due to chemical reactivity of O\textsubscript{3} and NO\textsubscript{2} within the exposure chamber and the subsequent formation of nitrogen pentoxide.

An important consideration in examining responses to air pollutants is the relative roles of exposure C and T on response. The roles of C and T in responses to mixtures of NO\textsubscript{2} and O\textsubscript{3} were examined by Gelzleichter et al. (1992a). Rats were exposed to various concentrations of each gas (6,770 to 27,100 μg/m\textsuperscript{3} [3.6 to 14.4 ppm] NO\textsubscript{2} and 392 to 1,570 μg/m\textsuperscript{3} [0.2 to 0.8 ppm] O\textsubscript{3}) for various durations, such that the product of C × T was constant in all cases. They found that the response to these mixtures could not be related to the product of C × T but, rather, seemed to be more dependent upon actual concentration than exposure duration. The responses were disproportionately greater at the higher concentrations of these gases.

Some limited data exist for combinations of NO\textsubscript{2} with gases other than O\textsubscript{3}. In the two reported studies with sulfur dioxide (SO\textsubscript{2}), neither SO\textsubscript{2} nor NO\textsubscript{2} given alone, or together, produced any response. The concentrations used by Azoulay et al. (1980) were quite low, and the respiratory mechanical end points assessed by Antweiler and Brockhaus (1976) were likely not very sensitive to pollutant-induced changes.
Trzeciak et al (1977) exposed guinea pigs to 940 \( \mu g/m^3 \) (0.5 ppm) \( NO_2 \) plus 61 \( \mu g/m^3 \) (0.05 ppm) \( NO \), or this \( NO_x \) mixture plus an equal amount of ammonia, for 8 h/day for a total of 122 days and analyzed lung phospholipids. There was no difference in the phospholipid content, expressed as milligrams per gram of wet tissue, of exposed versus control lungs. Significant alterations were found in the individual phospholipid classes. Decreases were noted in phosphatidylethanolamine, sphingomyelin, phosphatidylinerine, phosphatidylylglycerol-3-phosphate, and phosphatidic acid. Increases were noted in the lysophosphatidyl-ethanolamine content, whereas the lecithin content remained constant or was slightly depressed. Such changes could be indicative of changes in the permeability of the cell wall and subsequently changes in the cell content. The presence of ammonia did not significantly influence the results.

One major interaction that may occur in ambient air is that between \( NO_2 \) and particles. Particle contact may result in gas adsorption and subsequent transport to target sites where the gas normally would not deposit in concentrated amounts. Boren (1964) adsorbed \( NO_2 \) onto carbon to determine whether this carrier changed the toxicity of the \( NO_2 \). Mice were exposed 6 h/day, 5 days/week for 3 mo to carbon (38% of particles were <2 \( \mu m \), 16,000 particles/cm\(^3\)) onto which 553 mg \( NO_2 \) was adsorbed per gram, the exposure air also contained 47,000 to 56,400 \( \mu g/m^3 \) (25 to 30 ppm) free \( NO_2 \) as well. The exposed animals showed focal changes in the lung parenchyma. These lesions contained carbon particles, and were characterized by enlarged airspaces and loss of alveolar walls. Exposure solely to \( NO_2 \) resulted in edema and inflammation, but no parenchymal lesions, and no lesions were found due to carbon-only exposure. Thus, Boren (1964) concluded that the carbon particles served as a carrier for \( NO_2 \), delivering high concentrations of \( NO_2 \) to localized areas in the lungs where the carbon deposited.

The role of adsorbed \( NO_2 \) in the toxicity of mineral dusts was addressed by Robertson et al. (1982). They examined the effects of \( NO_2 \) adsorption on the cytotoxicity of coal, quartz, or kaolinite on P388D1 cells exposed in vitro to mineral dusts for 48 h. Viability and enzyme release (e.g., LDH) were used as end points. The amount of \( NO_2 \) absorbed was 5 to 10 \( \mu g/mg \) dust. Although a small decrease in cytotoxicity was found after adsorption of \( NO_2 \), the investigators concluded that there was no systematic or significant difference in biochemical measures of toxicity from cells exposed to dust with or without \( NO_2 \). On the
other hand, Shevchenko (1971) noted an increase in the fibrogenicity of quartz dust in albino rats following adsorption of 0.36 μg NO₂/mg dust, a lower level than that used by Robertson et al. (1982). These different results may be due to differences in particle residence time. Robertson et al. (1982) exposed the cells for only 48 h, whereas dust was present in the lungs in the Shevchenko (1971) study for months, allowing a greater time for gas desorption.

Other aerosols, although not necessarily acting as carriers, may potentiate response to NO₂ by producing local changes in the lungs that enhance the toxic action of co-inhaled NO₂. Last et al. (1983) and Last and Warren (1987) have examined the effects of inhalation of acidic sulfate aerosols plus NO₂ on biochemical end points, using minces prepared from the lungs of rats after various exposure regimes. Last et al. (1983) exposed rats to 9,400 to 47,000 μg/m³ (5 to 25 ppm) NO₂ alone, or in combination with 5,000 μg/m³ (ammonium sulfate (NH₄)₂SO₄) (1 μm mass median aerodynamic diameter [MMAD]), for up to 7 days, and examined the rate of collagen synthesis by lung minces. Ammonium sulfate alone caused no effects. Analysis of the slope of the exposure concentration-response curve for NO₂ indicated an approximate doubling of the synthesis rate when the mixture was employed compared to NO₂ alone, examination of responses at individual NO₂ concentrations showed that the mixture clearly began to increase the synthesis rate (above NO₂ alone) when NO₂ concentrations exceeded 18,800 μg/m³ (10 ppm). The investigators also noted that there was a tendency towards a reduction in lethal concentration for 75% of the animals when exposures were to (NH₄)₂SO₄ plus NO₂, compared to that for NO₂ alone. On the other hand, there was no difference in the level of pulmonary edema between animals exposed to NO₂ alone or to NO₂ in combination with (NH₄)₂SO₄.

In a later study, Last and Warren (1987) exposed rats to 9,400 μg/m³ (5 0 ppm) NO₂ alone or in combination with either 1,000 μg/m³ sulfuric acid (H₂SO₄) (0.4 μm MMAD) or sodium chloride (NaCl) (0.4 μm MMAD) for up to 7 days. A synergistic interaction for collagen synthesis rate was found when either aerosol was used with NO₂. Reduction of the NO₂ level to 3,760 μg/m³ (2 0 ppm) also resulted in a synergistic increase in the collagen synthesis rate when combined with 1,000 μg/m³ H₂SO₄ (Last, 1989). Changes in protein content of the lavage fluid (an index of lung edema) showed evidence of synergism at 1 day with H₂SO₄ or 3 days with NaCl. The investigators suggested that the interaction with NaCl was due to the formation of acids (e.g., hydrogen chloride, HNO₃, HONO) from nitrosyl
chloride following its hydrolysis after deposition in the deep lung, the latter may be formed from a chemical reaction between NO₂ and NaCl. Similarly, potentiation with the acid sulfate aerosols was likely due to localized effects following their deposition. It has been proposed that the acid aerosols would produce a shift in local pH within the alveolar milieu. This shift would result in a change in the reactivity or residence time of reactants involved in oxidant-induced pulmonary effects (Last et al., 1984).

The effects of exposure to mixed atmospheres of NO₂ and H₂SO₄ on lung host defenses have been examined by Schlesinger and Gearhart (1987) and Schlesinger (1987a). In the former study, rabbits were exposed for 2 h/day, 5 days/week for 14 days to either 564 or 1,880 μg/m³ (0.3 or 1.0 ppm) NO₂ or 500 μg/m³ H₂SO₄ (0.3 μm) alone, or to mixtures of the low and high NO₂ concentrations with the acid. After the first exposure, an inert tracer aerosol was administered to assess clearance from the respiratory region of the lungs. In the single-pollutant groups, both concentrations of NO₂ accelerated clearance, whereas H₂SO₄ retarded clearance, compared to air-exposed controls. Exposure to the combination of 564 μg/m³ NO₂ plus H₂SO₄ resulted in a response that was not different from that due to the acid alone. However, exposure to 1,880 μg/m³ NO₂ plus H₂SO₄ resulted in a clearance pattern that differed from that of both NO₂ and H₂SO₄, but was more similar to that of the H₂SO₄.

Schlesinger (1987a) exposed rabbits to the same NO₂/H₂SO₄ atmospheres as above, but then examined the animals 24 h after 2, 6, or 13 exposures and recovered cells from the lungs by bronchopulmonary lavage. Exposure to 1,880 μg/m³ (1.0 ppm) NO₂ with acid resulted in an increase in PMNs at all time points (not seen with either pollutant alone), and an increase in the phagocytic capacity of AMs after two or six exposures. In contrast, exposure to 564 μg/m³ (0.3 ppm) NO₂ with acid resulted in depressed phagocytic capacity and mobility. A comparison of responses due to exposure to the NO₂/H₂SO₄ mixture with those due to either pollutant alone showed that the effects of the combined atmospheres were generally either additive or synergistic, depending on the specific cellular end point being examined.

Furiosis et al. (1973) exposed rats and monkeys continuously to a combination of 3,760 μg/m³ (2.0 ppm) NO₂ and 330 μg/m³ NaCl. Histological response after 14 mo of exposure in monkeys (respiratory bronchiolar epithelial hypertrophy) was similar in groups.
exposed to NO\textsubscript{2} alone or to NO\textsubscript{2} with NaCl. Hematologic changes (polycythemia) in both monkeys, after 18 mo, and rats, after 6 mo, were similar for groups exposed to NO\textsubscript{2} with or without NaCl. Thus, in this study, the NaCl did not potentiate response to NO\textsubscript{2}. Perhaps the end points were not sensitive to the effects of any reaction products of NO\textsubscript{2} and NaCl, or the concentration of NaCl was too low to allow production of significant amounts of such products.

**Complex Mixtures Containing Nitrogen Dioxide**

Although many studies have examined the response to NO\textsubscript{2} with only one additional pollutant, the atmosphere in most environments is a complex mixture of more than two materials. A number of studies have attempted to examine the effects of multicomponent atmospheres containing NO\textsubscript{2}. But, as mentioned, in many cases, the exact role played by NO\textsubscript{2} in the observed responses is not always clear.

Klemman et al (1985a,b) exposed rats for 4 h to atmospheres consisting of various combinations of NO\textsubscript{2} (4,700 \(\mu\)g/m\textsuperscript{3}, 2.5 ppm), O\textsubscript{3} (1,180 \(\mu\)g/m\textsuperscript{3}, 0.6 ppm), SO\textsubscript{2} (13,100 \(\mu\)g/m\textsuperscript{3}, 5.0 ppm), and particles. The particles consisted of 1 mg/m\textsuperscript{3} (0.2 \(\mu\)m MMAD) of either H\textsubscript{2}SO\textsubscript{4} or (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, laced with iron and manganese sulfates. The metallic salts act as catalysts for the conversion of sulfur IV into sulfur VI and the incorporation of gases into the aerosol droplets. The respiratory region was examined for morphological effects. A confounding factor in these studies was the production of HNO\textsubscript{3} in atmospheres that contained NO\textsubscript{2} and O\textsubscript{3}, and nitrates in atmospheres that contained O\textsubscript{3} and (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, but not NO\textsubscript{2}. Nevertheless, a significant enhancement of tissue damage was produced by exposure to atmospheres containing H\textsubscript{2}SO\textsubscript{4} or HNO\textsubscript{3}, compared to those containing (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}. In addition, it was suggested that the former atmospheres resulted in a greater area of the lung becoming involved in lesions, which were characterized by a thickening of alveolar walls, cellular infiltration in the interstitium, and an increase in free cells within alveolar spaces. Exercise seemed to potentiate the histological response to the complex mixtures containing acids (Klemman et al., 1980).

One of the more common complex mixtures studied is that of combustion exhaust emissions from automobiles. In some cases (see below), the exhaust was irradiated to produce a reactive mixture that is a model for photochemical smog.

Coffin and Blommer
(1967) exposed mice for 4 h to irradiated gasoline-engine exhaust to assess the effects on bacterial resistance. Levels of NO\textsubscript{x} in the atmosphere were as follows: NO\textsubscript{2}, 200 to 1,600 \(\mu g/m^3\) (0.11 to 0.85 ppm), and NO, 20 to 180 \(\mu g/m^3\) (0.02 to 0.15 ppm). Exposure was found to result in an increase in bacterial-induced mortality, but the investigators were not able to clearly ascribe the results to any one pollutant. However, they noted that the exposure levels of NO\textsubscript{2} were less than those that were known to alter resistance when NO\textsubscript{2} was given alone and, thus, they suggested that the effect of the exhaust mixture was due to other oxidants, such as O\textsubscript{3}.

Stupfel et al. (1973) exposed rats for 6 h/day, 5 days/week for 2.5 mo to 2 years to gasoline-engine exhaust mixtures for morphologic analysis. The atmosphere contained CO\textsubscript{2}, aldehydes, carbon monoxide (CO), and either 0.2 or 23 ppm NO\textsubscript{x}. Only the mixture with the higher NO\textsubscript{x} concentration produced any significant toxic response, namely a decrease in body weight and increase in spontaneous tumors. However, the latter was ascribed to the hydrocarbon component of the exhaust mixture.

Cooper et al. (1977) exposed rats continuously for 38 or 88 days to three gasoline-engine exhaust atmospheres that differed in their component concentrations, all contained H\textsubscript{2}SO\textsubscript{4}, SO\textsubscript{2}, and CO, as well as NO (8,700 to 13,300 \(\mu g/m^3\), 7.1 to 10.8 ppm) and NO\textsubscript{2} (564 to 9,590 \(\mu g/m^3\), 0.3 to 5.1 ppm). All exposures resulted in a significant depression of spontaneous locomotor activity not seen with exposure to either H\textsubscript{2}SO\textsubscript{4} or CO alone, the investigators concluded that this response was due to either the hydrocarbon or the NO\textsubscript{x} components of the mixture.

The results of a long-term exposure of dogs to gasoline-engine exhaust emissions have been described by several investigators (Stara et al., 1980). Animals were exposed for 68 mo (16 h/day) to various atmospheres, which included raw exhaust, irradiated exhaust, or two mixtures of NO\textsubscript{x}—one with high NO\textsubscript{2} (1,210 \(\mu g/m^3\), 0.64 ppm) and low NO (310 \(\mu g/m^3\); 0.25 ppm), and one with low NO\textsubscript{2} (270 \(\mu g/m^3\), 0.14 ppm) and high NO (2,050 \(\mu g/m^3\); 1.67 ppm). Following the end of exposure, the animals were maintained for about 3 years in normal indoor air. Numerous pulmonary function, hematologic, and histologic end points were examined after various times of exposure (Lewis et al., 1974, Vaughan et al., 1969, Stara et al., 1980, Bloch et al., 1973). Only results related to NO\textsubscript{x} will be described. Vaughan et al. (1969) reported no alterations in CO-diffusing capacity,
dynamic compliance, or total expiratory resistance to flow after 18 mo of exposure. However, by 36 mo, a significant number of animals exposed to high NO₂/low NO had an abnormally low CO diffusing capacity (as a ratio of total lung capacity) (Lewis et al., 1974). Additional changes were observed after 61 mo of exposure, in the dogs breathing low NO₂/high NO or raw auto exhaust, residual volume was increased compared to animals exposed to control or high NO₂/low NO. The common treatment factor causing this effect appeared to be the higher concentration of NO. A significant number of dogs exposed to high NO₂/low NO had a lower mean CO diffusing capacity/total lung capacity ratio, and a lower peak flow rate, compared to controls. The investigators attributed the change in diffusing capacity to an alteration in the alveolocapillary membrane. Bloch et al. (1973) reported no significant change in hematocrit, blood viscosity, or level of methemoglobin due to any of the exposure atmospheres after 48 mo of exposure.

After all exposures were terminated, the animals were allowed to recover for 2 years before pulmonary function measurements were made again (Stara et al., 1980). In all pollutant-exposed dogs, total lung capacity was increased relative to the control group of animals. Those animals that received the NO₂/NO mixtures experienced modest increases in inspiratory volume, vital capacity, and total lung capacity.

Orthoefer et al. (1976) evaluated biochemical alterations 2.5 to 3 years after the end of all exposures. In groups exposed to irradiated exhaust or high NO₂/low NO, there was a rise in lung propyl hydroxylase, an enzyme involved in collagen synthesis. In addition, a correlation was found between lung weight and hydroxyproline content in animals exposed to the NOₓ atmospheres.

Lung morphology of the dogs was evaluated by Hyde et al. (1978) 32 to 36 mo after 68 mo of exposure. In the high NO₂/low NO group, there were increases in total lung capacity and lung volume, and decreases in the surface density of the alveoli and the volumetric density of parenchymal tissue. Alveoli were enlarged in both the high NO₂ and high NO groups. In the high NO₂, but not the high NO group, there was cilia loss and hyperplasia of nonciliated broncholar cells. In the high NO group, there were lesions in the interalveolar pores. In the most severely affected dogs in the high NO₂ group, morphological changes considered to be analogous to centrilobular emphysema were present (see Section 13.2 discussing NO₂-induced emphysema in experimental animals). Because
these morphologic measurements were made after a 2.5- to 3-year holding period in clean air, it cannot be determined with certainty whether these disease processes abated or progressed during this time. However, indications were that the long-term exposures produced persistent damage that was indeed progressive even after exposures ended.

Another complex mixture involving NO₂ is diesel-engine exhaust. Like gasoline-engine exhaust, this contains a number of gases and particles. Numerous toxicologic studies have been performed with acute, subchronic, and chronic exposure protocols (U.S. Environmental Protection Agency, 1991). In acute exposures, toxic effects appear to be associated with high concentrations of CO, NO₂, and various other gases. On the other hand, comparison of responses in laboratory animals repeatedly exposed to whole diesel exhaust or filtered exhaust containing no particles appears to demonstrate that the particles are the principal etiologic agent of noncancerous health effects resulting from exposure (U.S. Environmental Protection Agency, 1991). Whether these particles act additively or synergistically with the gases in the exhaust mixture cannot, however, be determined from the designs of the available studies. Thus, the diesel studies do not provide additional information concerning the toxicity of NO₂ over and above that which is already available in the data base.

Summary

Exposures to mixtures containing NO₂ are quite common and provide a basis for toxicological interactions whereby combinations of pollutants may behave differently than would be expected from consideration of the action of each constituent separately. The largest data base exists for the combination of NO₂ and O₃. Morphologic response to exposure to this mixture is generally due to O₃ (Freeman et al., 1974a, Yokoyama et al., 1980), but biochemical effects may involve synergism (Yokoyama et al., 1980, Ichinose and Sagai, 1989, Sagai and Ichinose, 1991, Mustafa et al., 1984, Schlesinger et al., 1990). Reactions of host defenses, specifically antibacterial activity, may be additive or synergistic (Goldstein et al., 1974, Graham et al., 1987, Ehrlich et al., 1977). Mixtures of NO₂ and acid sulfates result in additive to synergistic effects (Last, 1989, Last et al., 1983, Last and Warren, 1987, Schlesinger and Gearhart, 1987, Schlesinger, 1987a, Schlesinger et al., 1987a). Although many studies examined responses to simple mixtures of NO₂ with one other material, the atmosphere in most environments is a complex mix of more than two.
pollutants The effects of complex mixtures have been examined to some extent, however, the exact role played by NO$_2$ in the observed responses is not always clear.

13.4 NITRIC OXIDE

The toxicologic data base for NO is not extensive, except for those studies examining its interaction with blood. One problem is that it is often difficult to obtain pure NO in air without some contamination with NO$_2$. In recent years, much research has increased the understanding of the role of endogenous NO as a mediator of vascular tone, macrophage cytotoxicity of microorganisms and tumors, and platelet disaggregation (Moncada et al., 1991). However, this research and findings are not directly related to NO as an air pollutant.

Little is actually known about NO absorption in the respiratory tract, and nothing is known on its subsequent intrapulmonary distribution. Because NO is less water soluble and less reactive than NO$_2$, it follows that its absorption from inhaled air should be less. Yoshida et al. (1981) found that <10% of the NO "inhaled" by isolated, perfused lungs of rabbits was absorbed. On the other hand, absorption in normal breathing humans in vivo was 85 to 92% for NO concentrations ranging from 400 to 6,100 $\mu$g/m$^3$ (0.33 to 5.0 ppm) (Wagner, 1970, Yoshida and Kasama, 1987), values for NO$_2$ were 81 to 90% (Wagner, 1970). Absorption of NO with exercise was 91 to 93% in humans (Wagner, 1970). Yoshida et al. (1980a) found the percentage of NO absorbed in rats acutely exposed to 169,300 $\mu$g/m$^3$ (138 ppm), 331,300 $\mu$g/m$^3$ (270 ppm), and 1,079,800 $\mu$g/m$^3$ (880 ppm) to be 90%, 60%, and 20%, respectively. The lower absorption at the two highest concentrations was ascribed to an exposure-induced decrease in ventilation. Vaughan et al. (1969) exposed dogs to auto exhaust mixtures and found that 73% of the constituent NO was removed when the mixture was passed through the nose and out through a tracheostomy tube, this compared to 90% removal for NO$_2$. Thus, respiratory tract absorption of NO has some similarities to that of NO$_2$ in spite of solubility differences. The lower solubility of NO may, however, result in greater amounts reaching the pulmonary region, where it then diffuses into blood and reacts with hemoglobin (Yoshida and Kasama, 1987). In fact, exposures in vivo do seem to

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indicate that NO has a faster rate of diffusion through tissue than does NO₂ (Chiodi and Mohler, 1985).

High exposure levels of NO are apparently needed to be lethal. Pflesser (1935) reported that mice exposed to 380,400 µg/m³ (310 ppm) NO for 8 h showed no mortality, whereas 50% mortality was seen during similar exposures to 392,600 µg/m³ (320 ppm), however, possible NO₂ contamination was not accounted for. Greenbaum et al. (1967) reported that dogs exposed to 2% NO (24,540 mg/m³, 20,000 ppm) for 7 to 50 min all died within 15 min after exposures ended, a single dog exposed to 0.5% (5,000 ppm) NO also died. Death was due to pulmonary edema. No increase in death rate over control was found in mice exposed to 12,270 µg/m³ (10 ppm) NO for 6.5 mo (Oda et al., 1976), or to 2,940 µg/m³ (2.4 ppm) NO for their lifetime (23 to 29 mo) (Oda et al., 1980b).

The few studies that have examined histologic response to nonlethal levels of NO are outlined in Table 13-24. With chronic exposure, the morphologic changes seen are similar to those discussed in the section on the morphological effects of NO₂, except that the NO levels needed to produce them are higher. In terms of pulmonary effects with high level acute exposure, NO is estimated to be approximately 30 times less toxic than NO₂ (Stavert and Lehnert, 1990). Additionally, Hugod (1979) noted that the absence of NO-induced alterations in the alveolar epithelium suggested that the observed responses occurred after absorption of NO, that is, they were not due to direct action of deposited NO. Perhaps higher exposure concentrations of NO are needed for direct toxic action (e.g., results of Holt et al., 1979). Some of the effects seen by Oda et al. (1976) with 12,270 µg/m³ (10 ppm) NO may be due to the presence of 1,880 to 2,820 µg/m³ (1.0 to 1.5 ppm) NO₂ in the exposure atmosphere.

Data concerning the physiological effects of inhaled NO are sparse. Murphy (1964) found no changes in respiratory function of guinea pigs exposed for 4 h to NO at 19,600 or 61,300 µg/m³ (16 or 50 ppm). Yoshida et al. (1980b) reported that guinea pigs exposed twice per week (30 min each) for 7 weeks to 5,900 µg/m³ (5.02 ppm) NO and challenged during exposure to aerosolized albumin exhibited dyspneic breathing patterns and an increased responsiveness to acetylcholine. These results were not substantially different from...
<table>
<thead>
<tr>
<th>NO Concentration</th>
<th>ppm</th>
<th>Exposure</th>
<th>Gender</th>
<th>Age (Strain)</th>
<th>Species (Strain)</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,460 (NO₂ = 0.08 ppm)</td>
<td>2.0</td>
<td>Continuous, 6 weeks</td>
<td>NS</td>
<td>8 weeks</td>
<td>Rat (Wistar)</td>
<td>Slight emphysema-like alterations of alveoli</td>
<td>Azoulay et al (1977)</td>
</tr>
<tr>
<td>2,950 (NO₂ = 0.01-0.04 ppm)</td>
<td>2.4</td>
<td>Continuous, 23-29 mo</td>
<td>F</td>
<td>12 weeks</td>
<td>Mouse (JCL-ICR)</td>
<td>No difference from control</td>
<td>Oda et al (1980a)</td>
</tr>
<tr>
<td>6,150 (NO₂ = ≤0.1 ppm)</td>
<td>5.0</td>
<td>Continuous, 14 days</td>
<td>M</td>
<td>NS</td>
<td>Rabbit (Danish)</td>
<td>Edema, thickening of alveolo-capillary membrane due to fluid in interstitial space, fluid-filled vacuoles seen in arteriolar endothelial cells and at junctions of endothelial cells, no changes in alveolar epithelium, no inflammation</td>
<td>Hugod (1979)</td>
</tr>
<tr>
<td>12,300</td>
<td>10</td>
<td>2 h/day, 5 days/week, up to 30 weeks</td>
<td>F</td>
<td>6-8 weeks</td>
<td>Mouse (BALB/c)</td>
<td>Enlarged airspaces in lung periphery, paraseptal emphysema, some hemorrhage, some congestion in alveolar septa, increased concentration of goblet cells in bronchi</td>
<td>Holt et al (1979)</td>
</tr>
<tr>
<td>12,300 (NO₂ = 1-1.5 ppm)</td>
<td>10</td>
<td>Continuous, 65 mo</td>
<td>F</td>
<td>12 weeks</td>
<td>Mouse (JCL-ICR)</td>
<td>Bronchial epithelial hyperplasia, hyperemia, congestion, enlargement of alveolar septum, increase in ratio of lung to body weight</td>
<td>Oda et al (1976)</td>
</tr>
</tbody>
</table>

NS = Not stated
F = Female
M = Male

*b Represents reported NO₂ levels measured during exposure
those in guinea pigs exposed to 9,400 \( \mu g/m^3 \) (5 0 ppm) \( NO_2 \) (details of the study are reported in Section 13 2 2 3 addressing \( NO_2 \) exposure-related effects on pulmonary function)

The effects of \( NO \) on defense function of the lungs has been examined in two studies Holt et al. (1979) examined immunological end points in mice exposed to 12,270 \( \mu g/m^3 \) (10 ppm) \( NO \), 2 h/day, 5 days/week for up to 30 weeks. Leukocytosis was evident by 5 weeks of exposure, and a decrease in mean hemoglobin content of RBCs was found by 30 weeks. A decrease in RBC count at Week 15 was not found at 30 weeks. An enhancement of the humoral immune response to SRBCs was seen at 10 weeks, but this was not evident at the end of the exposure series. Spleen cell response to phytohemagglutinin was decreased after 15 weeks of exposure, but mitogenesis then recovered and became greater than control. The ability of spleen cells to mount a graft versus host reaction was stimulated by 20 weeks of exposure, but was suppressed by 26 weeks. Finally, the ability of mice to reject virus-induced tumors was assessed, only 40% of the \( NO \)-exposed animals survived tumor challenge, compared with 66% for control animals. This study suggests that \( NO \) exposure may have affected the immunologic competence of exposed animals.

Effects of \( NO \) on bacterial defenses were examined by Azoulay et al. (1981). Male and female mice were exposed continuously to 2,450 \( \mu g/m^3 \) (2 0 ppm) \( NO \) for 6 h to 4 weeks, to assess the effect on resistance to infection induced by a bacterial aerosol (\( Pasteurella multocida \)) administered after each \( NO \) exposure. Although there appeared to be somewhat of an increase in bacterial-induced mortality in each group of females exposed to \( NO \) for at least 1 week, there was no statistically significant difference for either sex. Likewise, each group of females exposed to \( NO \) for at least 1 week showed a slight decrease in mean survival time, but this change was not statistically significant, nor was there any observable difference in males exposed to \( NO \). When the data for those groups exposed for 1 to 4 weeks were combined, \( NO \)-exposed females showed a significant increase in percentage mortality and a significant decrease in survival time, this was not seen for males. Thus, this study suggests some gender-related difference in response, at least to the one level of \( NO \) examined.

One possible mechanism of toxic action of \( NO \) is lipid peroxidation. The GSH transferase system serves to protect vital molecules from peroxidative damage. Thus,
changes in constituents of this system may serve as a marker of effects from inhaled NO. However, mice exposed to 12,300 to 25,800 \( \mu g/m^3 \) (10 to 21 ppm) NO, 3 h/day for 7 days showed no change in lung levels of reduced GSH, a cofactor for GSH peroxidase (Watanabe et al., 1980).

There is some evidence that NO may alter the activity of other enzymes. A number of in vitro studies (Arnold et al., 1977, Braughler, 1982, Katsuki et al., 1977) have indicated that NO may affect guanylate cyclase, the enzyme that catalyzes the formation of cyclic-guanosine monophosphate and guanosine triphosphate. They have shown, based upon exposure of purified enzymes or tissue minces from various organs, that NO increases enzyme activity in a concentration-dependent fashion, and that the activation is reversible when NO is removed from the preparation. Although variable degrees of activation were seen in different tissues, lung tissue showed one of the highest degrees of activation. It is, however, not known whether NO would alter guanylate cyclase activity with in vivo exposure.

The bulk of the toxicologic data base for NO biochemistry concerns its reaction with hemoglobin. Inhaled NO that enters the bloodstream through the lungs binds to hemoglobin, producing nitrosylhemoglobin (Oda et al., 1975, 1980a, 1980b, Case et al., 1979, Nakajima et al., 1980). This may, in fact, be a major mechanism of action, and in vitro studies have suggested that NO may severely reduce the ability of RBCs to carry \( O_2 \). These studies have shown that the affinity of hemoglobin for NO is very high, much higher even than that for \( O_2 \) (Gibson and Roughton, 1957; Moore and Gidson, 1976). In addition, in vitro measurements of \( O_2 \)-dissociation curves for partially NO-ligated human hemoglobin have shown that NO binding tends to reduce dissociation of bound \( O_2 \) on the molecule (Kon et al., 1977). Finally, nitrosylhemoglobin is easily and rapidly oxidized to methemoglobin in the presence of \( O_2 \) (Chiodi and Mohler, 1985, Kon et al., 1977), further reducing the ability of RBCs to transport \( O_2 \).

Following in vivo exposures, a linear relationship was found between the exposure concentration of NO (24,500 to 98,200 \( \mu g/m^3 \), 20 to 80 ppm) for 1 h in mice and blood content of nitrosylhemoglobin, however, levels of methemoglobin were found to increase exponentially with NO concentration, resulting in greater blood levels of methemoglobin than nitrosylhemoglobin (Oda et al., 1980b). After exposure of mice to 49,100 \( \mu g/m^3 \) (40 ppm),
for 1 h, concentrations of both methemoglobin and nitrosylhemoglobin decreased rapidly, with half-times of only a few minutes (Oda et al., 1980b). Thus, the steady-state concentration of nitrosylhemoglobin during NO exposure would be fairly low, whereas that for methemoglobin would be somewhat higher (Maeda et al., 1987).

Studies of animals exposed to NO in vivo have shown that the amount of nitrosylhemoglobin in blood was much less than would be expected from in vitro exposure data (Oda et al., 1980b, 1975). Lifetime (23 to 29 mo) exposures of mice to 2,940 \( \mu g/m^3 \) (2.4 ppm) NO resulted in the blood content of nitrosylhemoglobin remaining relatively steady at 0.01%, whereas the maximum amount of methemoglobin was 0.3% (Oda et al., 1980a). Mice exposed to 12,300 \( \mu g/m^3 \) (10 ppm) NO for 6.5 mo showed nitrosylhemoglobin at 0.13% and methemoglobin at 0.2% (Oda et al., 1976). These results suggest that a steady-state concentration of methemoglobin may be reached with exposures to different concentrations. Furthermore, although the results of various studies have shown that the final product of NO reaction with hemoglobin is methemoglobin, with some persistent nitrosylhemoglobin, this effect of NO is not generally lethal because of a number of factors, these include the conversion of inhaled NO to NO\(_2\) in the airways, the rapid oxidation of nitrosylhemoglobin into methemoglobin, and the subsequent reduction of methemoglobin into ferrous hemoglobin by methemoglobin reductase, an enzyme present in RBCs (Kon et al., 1980, Maeda et al., 1984b, 1987). As long as the activity of methemoglobin reductase is maintained, the conversion of nitrosylhemoglobin to methemoglobin should mitigate any potentially toxic effect on hemoglobin due to NO inhalation (Kon et al., 1980). In long-term exposure studies, Oda et al. (1976, 1980a) exposed mice to 4,512 or 18,800 \( \mu g/m^3 \) (2.4 or 10 ppm) NO and after examination of organs sensitive to \( O_2 \) depletion (e.g., brain and heart), found no evidence of hypoxic damage, which would have been expected if methemoglobin levels were substantially increased. Azoulay et al. (1977) exposed rats to 2,450 \( \mu g/m^3 \) (2.0 ppm) NO continuously for 6 weeks to examine various hematologic parameters, including blood-\( O_2 \) affinity. No exposure-related changes were found in hemoglobin content, hematocrit, RBC count, red cell glucose metabolism, or in the oxyhemoglobin dissociation curve. In addition, no methemoglobin was detected in either exposed or control animals. This showed that low-level NO exposure did not alter the blood-\( O_2 \) affinity. On the other hand, the same

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investigation reported that in vitro studies had shown that blood-O₂ transport was altered by high levels of NO (>12,300 μg/m³, 10 ppm) in both human and rat blood.

In addition to interaction with hemoglobin, exposure to NO may alter other aspects of blood. Case et al. (1979) exposed mice to 11,070 μg/m³ (90 ppm) NO for 16 h and found a decrease in the level of iron transferrin. Mice exposed to 12,300 μg/m³ (10 ppm) NO for 6.5 mo showed increased WBC counts and an increase in the ratio of PMNs to lymphocytes (Oda et al., 1976). These investigators noted that 11% of the RBCs obtained from NO-exposed mice contained Heinz bodies, whereas the control group showed none. Coupled with an increase in spleen weight and bilirubin, the investigators suggested that this indicated that NO facilitated the destruction of RBCs.

A slight increase in RBC hemolysis was seen in mice exposed to 2,940 μg/m³ (2.4 ppm) NO for their lifetime (Oda et al., 1980a). Rat RBCs exposed to NO in vitro, showed oxidative cross-linking between cell membrane proteins and hemoglobin (Maeda et al., 1984a), an alteration that could change the cells' rheological properties. However, in an in vivo exposure study, no cross-linking of membrane proteins was detected in rats exposed to 30.7 to 254.4 mg/m³ (25 to 200 ppm) NO for 1 h (Maeda et al., 1987), the investigators suggested that this may have been due to rapid repair mechanisms operating in vivo.

The pH of blood has been shown to be reduced by NO, but only with very high exposure levels (e.g., 0.5 to 2.0%, 5,000 to 20,000 ppm) (Toothill, 1967, Greenbaum et al., 1967). Rats exposed to 2,450 μg/m³ (2.0 ppm) NO continuously for 6 weeks showed no change in blood pH (Azoulay et al., 1977).

An examination of the mutagenicity of NO was performed by Arroyo et al. (1992). Salmonella typhimurium TA1535 was exposed for 30 min to 6,150 to 110,700 μg/m³ (5 to 90 ppm) NO, and mutagenic potential was assessed using a modified Ames reversion assay. The number of revertant colonies increased roughly in proportion to the square of the NO concentration up to 24,600 μg/m³ (20 ppm), and then remained relatively constant or slightly decreased at >24,600 μg/m³. It was also noted that the observed mutagenicity required that the bacteria were actively dividing at the time of exposure to NO. These results suggested that NO can act as a direct-acting mutagen.

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A few studies have examined the response to inhalation of mixtures of NO plus one other component. Watanabe et al. (1980) exposed mice to NO (12,300 μg/m³, 10 ppm) plus O₃ (1,960 μg/m³, 10 ppm), 3 h/day for 7 days; they observed an increase in the level of lung GSH, but this was due solely to the O₃. Azoulay et al. (1980) exposed rats to NO (2,460 μg/m³, 20 ppm) with SO₂ (5,240 μg/m³, 20 ppm) for 13 weeks; no change in blood-O₂ affinity, methemoglobin level, RBC count, or lung histology was noted. Finally, in an in vitro study, Robertson et al. (1982) adsorbed NO onto mineral dusts (2 to 5 μg NO/mg dust), no change in the cytotoxicity of coal, quartz, or kaolinite to P388D₁ cells was found, compared to dust without adsorbed NO, suggesting no interaction of NO and dust. The effects of chronic exposures to NO and NO₂ in dogs were comprehensively examined (Stara et al., 1980). The results of the study, summarized in Section 13.3, showed a variety of morphological and physiological effects on the lungs. However, because there were no NO₂- or NO-only groups, the nature of the interaction is not clear.

McFaul and McGrath (1985) examined the effect of inhalation exposure to NO at levels of 18.4 to 78.6 mg/m³ (15 to 64 ppm) for up to 38 h on the reduction of methemoglobin produced initially in the blood of rats by injection of sodium nitrate. They found the methemoglobin reduction was impaired at all of the NO levels used, and suggested that exposure to NO may modulate certain repair processes following exposure to other oxidant pollutants.

Summary

The toxicologic data base for NO is not extensive, except for its interaction with blood. Fairly high levels, ≥2,460 μg/m³ (20 ppm), are needed for morphologic changes in the lungs following subchronic or chronic inhalation (Azoulay et al., 1977, Holt et al., 1979, Oda et al., 1976). Inhaled NO that is absorbed into the bloodstream results in production of nitrosylhemoglobin which, in turn, is oxidized to methemoglobin (Oda et al., 1975, 1980a,b, Case et al., 1979, Nakajima et al., 1980). This has the potential to reduce the ability of RBCs to transport O₂ (Gibson and Roughton, 1957, Moore and Gibson, 1976). But as with other effects, very high exposure levels are needed for significant changes. It should be noted that a number of cell types have recently been shown to produce NO, which seems to
have various biological functions as an inter- and intracellular messenger (Curran et al., 1991)

13.5 NITRIC ACID AND NITRATES

13.5.1 Nitric Acid

There are only a few toxicologic studies of HNO₃, which exists in ambient air generally as a highly water soluble vapor. In an early study, Diggle and Gage (1954) noted that a single exposure to HNO₃ vapor at 63,000 μg/m³ (25 ppm) had no "obvious effect on rats", exposure duration and end points examined were unspecified.

More recent studies have examined the histological response to instilled HNO₃ (usually 1%), a procedure used in developing models of bronchiolitis obliterans in various animals, namely the dog, rabbit, and rat (Totten and Moran, 1961, Greenberg et al., 1971, Mink et al., 1984). The major changes noted were degeneration of alveolar Type 2 cells and alveolar cell hyperplasia. In a somewhat similar study, Peters and Hyatt (1986) delivered 1% HNO₃ into a catheter positioned in the main bronchi of the dog, however, in this case, the acid was delivered via nebulization, alternately (every other day) as either a coarse spray or as a fine mist, for 2 h/day for 4 weeks. Pulmonary function testing after 4 weeks of exposure indicated decreases in expiratory flow rate, dynamic compliance, total lung capacity, and vital capacity, and increases in pulmonary resistance, closing capacity, the ratio of functional residual capacity to total lung capacity, and phase III of the single breath nitrogen washout curve. Histologically, there was widespread chronic inflammation of conducting airways, especially medium and small ones, peribronchial fibrosis, focal hemorrhage, edema, and hyperplasia of goblet cells in the trachea and bronchi.

Gardiner and Schanker (1976) examined the effect of HNO₃-induced damage on drug absorption from the lungs of rats. Instillation of 1% HNO₃ produced bronchiolitis and alveolitis and increased the rate of pulmonary absorption of various drugs up to 1.6 times control values. This change was ascribed to an increase in the permeability of the alveolocapillary barrier.

Only two studies were designed specifically to examine the pulmonary response to pure HNO₃ vapor. Abraham et al. (1982) exposed both normal sheep and allergic sheep (i.e.,
those having airway responses similar to that occurring in humans with allergic airway disease) for 4 h to 4,120 μg/m³ (1.6 ppm) HNO₃ vapor. The exposure, which was performed using a "head-only" chamber, resulted in a decrease in specific pulmonary flow resistance, compared to preexposure control values, in both groups of sheep, this indicated the absence of any bronchoconstriction. To assess airway reactivity, pulmonary resistance was also measured after challenge with a bronchoconstrictor aerosol (carbachol). Allergic sheep showed increased reactivity, both immediately and 24 h after HNO₃ exposure. Although there was no significant change in reactivity in the normal groups as a whole, two of the animals showed an increase in reactivity to carbachol after HNO₃ exposure, according to the investigators, this suggested that some individuals in the normal population may be more sensitive than others.

Nadziejko et al. (1992) exposed rats for 4 h to HNO₃ at either 644 μg/m³ (0.25 ppm) or 2,575 μg/m³ (1 mg/m³). Exposures were nose-only, and bronchopulmonary lavage was performed 18 h after the exposure ended. Exposure to HNO₃ had no effect on total number of cells recovered, numbers of macrophages recovered, or protein content in lavage fluid. Exposure to the lower acid level did result in a reduction in respiratory burst activity of macrophages (which was not similarly measured at the higher concentration), and exposure to the higher concentration resulted in an increase in the lavage fluid elastase inhibitory capacity.

13.5.2 Nitrates

The toxicologic data base for inhaled nitrates is quite sparse. Ehrlich (1979) examined the effect of nitrates on resistance to respiratory infection. Mice were exposed for 3 h to various nitrate salts at maximal concentrations as follows: lead nitrate, 2,000 μg/m³; calcium nitrate, 2,800 μg/m³; NaNO₃, 3,100 μg/m³; potassium nitrate, 4,300 μg/m³; ammonium nitrate (NH₄NO₃), 4,500 μg/m³; and zinc nitrate (Zn(NO₃)₂), 1,250 μg/m³. Following exposure, the animals were challenged with a bacterial aerosol, and mortality determined after 14 days. Only the Zn(NO₃)₂ exposure resulted in any significant mortality increase, the extent of which seemed to be concentration related, the highest concentration increased mortality by ≈20%. However, since the response was similar to that seen with zinc sulfate, the investigator ascribed the effect to the zinc ion, rather than to the nitrate.
Busch et al (1986) exposed rats and guinea pigs with either normal lungs or lungs with elastase-induced emphysema to 1 mg/m³ NH₄NO₃, 6 h/day, 5 days/week for 4 weeks. Using both LM and electron microscopy, the investigators concluded that there were no significant effects of exposure on lung structure due to the nitrate exposure.

Charles and Menzel (1975) examined the effects of nitrate on the release of histamine by guinea pig lung fragments. Response to some pollutants may be a function of their ability to elicit histamine release. Lung fragments were incubated for 30 min with 20 to 200 mM NH₄NO₃. Histamine was released in proportion to the concentration of salt present. However, the response was not totally due to nitrate, ammonium ion was also a possible contributor.

**Summary**

Inhalation studies with HNO₃ are limited and no conclusions can be reached. Likewise, the toxicologic data base for inhaled nitrates is sparse, with no conclusions possible.

### 13.6 SUMMARY

The vast majority of animal toxicology studies of NOₓ are on NO₂, which apparently is more toxic than other nitrogen species (NO, HNO₃, and particulate nitrates) that commonly occur in the ambient air. However, direct comparative studies of NOₓ species are rare, more new information could challenge assumptions of relative potency. Given the current weight of evidence, this summary will only address NO₂, alone and in mixtures. Ambient and indoor levels of NO₂ are ordinarily below 1,880 µg/m³ (1 0 ppm) acutely and 94 µg/m³ (0.05 ppm) chronically, making high-concentration animal studies difficult to interpret for assessment of ambient air. Thus, with rare exception, only studies below 9,400 µg/m³ (5.0 ppm) are summarized here. Although a wide array of systemic effects have been observed after NO₂ exposure, their interpretation for risk assessment is quite difficult and unclear (with the possible exception of immune system effects) compared to respiratory tract effects. Thus, for more discussion of systemic effects, see the summaries within the chapter. This summary is organized to focus attention on key issues pertaining to respiratory tract effects. They include animal-to-human extrapolation, mechanisms of effects, effects on

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host defenses, relative influences of concentration and time (duration) of exposure and exposure patterns, impact of exposure duration on effects, and effects of pollutant mixtures. For summaries of each end point, see the summaries within the main text.

13.6.1 Animal-to-Human Dosimetric Extrapolation Estimates

Qualitatively, most experts would agree that a class of effects of NO₂ observed in several animal species could also occur in humans, if exposures were adequate to induce the effect. Such a qualitative extrapolation is founded on the interspecies commonality in molecular mechanisms of toxicity and in targets of toxicity. For example, small laboratory animals, nonhuman primates, and humans all have AMs with susceptible membrane components. However, quantitative extrapolation requires quantitative knowledge of interspecies commonalities and differences in dosimetry and species sensitivity. Although some information is available on these two elements, it is not yet sufficient for quantitative extrapolation. Nevertheless, the state of knowledge facilitates the interpretation of animal studies in terms of potential human risks.

Total NO₂ respiratory tract uptake in humans depends on the experimental methods used, the health status of the subjects, and breathing state (Wagner, 1970, Bauer et al., 1986). Total respiratory tract uptake ranged from 81 to 90% in normally breathing healthy subjects and increased to 91 to 92% during maximum respiration (Wagner, 1970). The average total uptake in resting asthmatics was 72%, and as respiration increased, the percent total uptake of NO₂ increased to 87% (Bauer et al., 1986). Roughly similar findings were made in dogs (Kleinman and Mautz, 1991). At rest, total respiratory tract uptake was 78%, during exercise, it was 94%. Exercise obviously increases the total uptake of NO₂, but it also alters the regional distribution of dose. Generally, increased ventilation decreases the percent uptake in the upper respiratory tract and increases the percent uptake in the total and lower respiratory tract. Theoretical models based on O₃ predict that the increased dose to the lower respiratory tract is predominantly to the pulmonary region (Miller et al., 1985).

A wider range of lower respiratory tract uptake values has been observed in animals as a result of differences in species and methods (Postlethwait and Mustafa, 1981, 1989, Kleinman and Mautz, 1991). However, mathematical modeling of lower respiratory tract uptakes in humans and animals (rats, guinea pigs, and rabbits) revealed that the greatest dose
is delivered to the centriacinar region (i.e., junction between the conducting airways and the gas-exchange region) in all these species (Miller et al., 1982, Overton, 1984). This is the site where NO₂-induced lesions are observed morphologically in animal species, lending credence to the mathematical model. Once deposited and bound, NO₂ reacts with fluids and tissues, forming other products that can be transported systemically. Various theories and experimental findings are available suggesting that HONO and HNO₃ can be produced in the lung from NO₂ exposure (Goldstein et al., 1977b) or that nitrite is produced in the lungs, enters the bloodstream, and reacts with hemoglobin to form nitrate and methemoglobin (Postlethwait and Mustafa, 1981, 1989, Saul and Archer, 1983).

### 13.6.2 Biochemical and Cellular Mechanisms

Acute exposure to NO₂ at or below 9,400 µg/m³ (5 ppm) can oxidize unsaturated fatty acids in cell membranes as well as functional groups of proteins (either soluble proteins in the cell, such as enzymes, or structural proteins, such as components of cell membranes), producing cell injury or death and the toxic symptoms associated with NO₂ inhalation (Menzel, 1976; Freeman and Mudd, 1981). Such a proposed mechanism of action is supported by data showing an initial increase in lipid peroxidation products and some protective lung antioxidant enzymes after NO₂ exposure (Sagai et al., 1984), as well as an increased susceptibility to NO₂ in animals deficient in vitamins C and E (Selgrade et al., 1981, Sevanian et al., 1982). The direct cytotoxic effect of NO₂ on epithelial cell membranes may be the fundamental mechanism of edemagenesis in response to NO₂ exposure, whereas the direct cytotoxicity of NO₂ to membranes of AMs could well be the mechanism underlying increased infectivity of bacteria and viruses in the lungs of exposed animals. A large number of studies have suggested that various enzymes in the lung, including GSH peroxidase, SOD, and catalase, may also serve to defend the lung against oxidant attack. One may speculate that were there to be a threshold level for NO₂ toxicity to the lung, it would be that concentration of NO₂ that was able to overwhelm these endogenous defense systems of the lung.

The biochemical study using the lowest concentration of NO₂ was conducted by Sagai et al. (1984), who reported that 9 and 18 mo of exposure to ≥75 µg/m³ (0.04 ppm) NO₂ increased ethane exhalation (exhaled ethane is an in vivo indicator of lipid peroxidation).
in rats; at 752 μg/m³ (0.4 ppm), they observed this response at 6 mo. Although this chronic study showed increases in lipid peroxidation with increasing concentration and duration of exposure, a shorter term (4-mo) exposure revealed that ethane exhalation increased after 1 week of exposure, decreased to control levels by 4 weeks, and then rose again (Ichinose and Sagai, 1982). Sagai et al. (1984) also observed that lipid peroxidation had an inverse relationship with changes in lung antioxidant metabolism.

13.6.3 Effects on Host Defenses

Host defenses are a broad category of functions, encompassing defenses against infectious (bacterial and viral) disease, neoplastic disease, and inhaled particulate matter. The immune system, a major component of host defense, is compartmentalized physiologically (e.g., pulmonary and systemic immune system). Effects of NO₂ on the respiratory tract defense mechanisms will be presented first, followed by a discussion of systemic effects.

Studies of respiratory tract host defenses have shown that NO₂ enhances susceptibility to bacterial and viral disease, probably through effects on AMs and possible through changes in the immune system and other defense mechanisms not yet adequately investigated. The mucociliary escalator, an important component of defenses, is not functionally affected in rabbits exposed for 14 days (2 h/day) to 1,880 μg/m³ (1 ppm) or for 2 h to 18,800 μg/m³ (10 ppm) NO₂ (Schlesinger et al., 1987a,b), although there are numerous reports of structural changes in the ciliated epithelium at levels below 9,400 μg/m³ (5 ppm) (Rombout et al., 1986, Stephens et al., 1972, Yamamoto and Takahashi, 1984).

Various acute and subchronic exposure regimens, generally >1,889 μg/m³ (1.0 ppm) NO₂, increase the number of AMs in the lung (Mochitate et al., 1992, Gregory et al., 1982, Rombout et al., 1986). Structure, function, and metabolic activity of AMs are also affected by NO₂ exposure. Pulmonary bactericidal activity, often interpreted as representative of AM activity, is decreased in mice by a 17-h exposure to ≥4,320 μg/m³ (2.3 ppm) NO₂ (Goldstein et al., 1973), however, effects on AM phagocytosis are complex. For example, exposure to 560 μg/m³ (0.3 ppm) NO₂, 2 h/day for 13 days, initially decreased AM phagocytosis in rabbits, whereas AMs exposed to 1,880 μg/m³ (1.0 ppm) showed an initial increase in phagocytosis (Schlesinger, 1987b). However, exposure of rabbits to these NO₂
concentrations for 2 h/day for 14 days increased alveolar clearance, which also represents AM function (Schlesinger and Gearhart, 1987, Vollmuth et al., 1986) Acute NO₂ exposure decreases superoxide anion radical production by AMs, and longer exposures cause morphological changes in AM membranes, metabolic changes, an increase in AM numbers, a decreased responsiveness to migration inhibitory factor, and a decrease in AM random mobility (Mochitate et al., 1986, Aranyi et al., 1976, Greene and Schneider, 1978, Amoruso et al., 1981, Schlesinger, 1987b)

One of the most widely applied methods to investigate effects on defenses in experimental animals is the infectivity model Using this model, experimental animals are exposed to NO₂ and then are challenged with viable bacteria or viruses, microbial-induced mortality is measured. The mortality reflects the net impairment of host defense mechanisms. The sensitivity of this model to detect NO₂-induced changes in host susceptibility to infectious disease is influenced significantly by the microbial species, the animal species, and exposure regimen. After acute exposure, the sensitivity ranking was mice > hamsters > monkeys (Ehrlich, 1975) In mice exposed for 2 to 3 h, the lowest concentration that enhanced mortality was 6,580 μg/m³ (3.5 ppm) using Klebsiella pneumonia and 3,760 μg/m³ (2.0 ppm) using Streptococcus sp (Ehrlich, 1975, Purvis and Ehrlich, 1963, Ehrlich et al., 1977) Long-term, intermittent exposure of mice to 940 μg/m³ (0.5 ppm) has been reported to decrease resistance to bacterial infections within 6 mo, however, continuous exposure decreases resistance to bacterial infections within 3 mo (Ehrlich and Henry, 1968) Extensive studies of C × T relationships observed in the infectivity model are summarized in Section 13.6.4

Few studies with other microbes have been conducted, but they show that repeated exposures can increase susceptibility to influenza virus or cytomegalovirus infection in mice and monkeys (Ito, 1971, Henry et al., 1970, Rose et al., 1988, 1989) Acute, high-concentration exposure (9,400 μg/m³, 5.0 ppm) of mice increases the incidence and severity of Mycoplasma pulmonis lesions (Parker et al., 1989)

The pulmonary immune system is rarely investigated, and NO₂ reports with modern methods and appropriate experimental designs and analyses have not appeared. Systemic immune responses to antigens delivered via the respiratory tract are altered by NO₂ For example, monkeys exposed to 1,880 μg/m³ (1.0 ppm) NO₂ for 16 mo or 9,400 μg/m³
(5.0 ppm) for 6 mo and immunized with influenza experienced alterations in circulating antibody titers (Fenters et al., 1973, Ehrlich and Fenters, 1973) Several other investigations show that NO₂ can alter systemic humoral and cell-mediated immunity Using examples from studies at lower concentrations, a 7-week intermittent exposure to 470 μg/m³ (0.25 ppm) altered percentages of splenic T-cell subpopulations in mice (Richter and Damji, 1988, 1990), a 4-week exposure to 752 μg/m³ (0.4 ppm) decreased splenic primary PFC responses in mice (Fujimaki et al., 1982), and a 12-mo exposure to 940 μg/m³ (0.5 ppm) caused a linear decrease in PHA-induced mitogenesis of mouse spleen cells with NO₂ duration (Malgetter et al., 1978) Selgrade et al. (1991) found no effects on splenic or circulating lymphocytic responses to B- or T-cell mitogens after up to 78-weeks of exposure of mice to an urban exposure pattern of NO₂ (940-μg/m³ baseline with 2,820-μg/m³ peaks, 0.5 ppm and 1.5 ppm).

13.6.4 Influence of Concentration, Duration, and Exposure Regimen

An extensive body of research on the exposure-response of NO₂ indicates the importance of understanding the complexity of the exposure used in the study Two classes of studies have contributed to this topic C × T examinations and investigation of other exposure patterns, both of which are discussed here

Studies directly comparing C × T responses focus on host defenses and, to a limited degree, lung morphology and are discussed here Other work that allows interpretation about the progression of effects with exposure duration is summarized Section 13.6.5 Most of the C × T findings described here are a result of using the mouse streptococcal infectivity model. In one series of studies, Gardner et al. (1977a,b, 1979), Gardner et al. (1982), and Coffin et al. (1977) varied the concentration of NO₂ from 1,880 to 26,320 μg/m³ (1 to 14 ppm) and the exposure duration from 0.5 to 7 h so that the C × T product was 7 ppm-h The bacterial-induced mortality was enhanced more by concentration than by time An investigation of six concentrations of NO₂, ranging from 940 to 52,670 μg/m³ (0.5 to 28.0 ppm), for various durations (30 min at the highest concentration and 12 mo at the lowest) showed that bacterial-induced mortality increased linearly with length of exposure, the slope of the line was steeper at higher concentrations (Gardner et al., 1977) As before, concentration had more influence than time in eliciting the response For example, at a
constant C $\times$ T of 14 ppm-h, a 9 3-h exposure to 2,820 $\mu$g/m$^3$ (1.5 ppm) increased mortality by 10.2%, whereas a 10-h exposure to 27,300 $\mu$g/m$^3$ (14.0 ppm) enhanced mortality by 44.9%. Intermittent (7-h/day) and continuous (22- to 24-h/day) exposures were also compared in the streptococcal infectivity model (Gardner et al., 1979). Mice were exposed to 2,820 or 6,580 $\mu$g/m$^3$ (1.5 or 3.5 ppm) NO$_2$ for up to 15 days. All exposures increased mortality. At the higher concentration, there were no significant differences between the two exposure groups. However, when the concentration was reduced, a longer duration of exposure (14 days) was required for intermittent exposure to produce a level of effect equivalent to continuous exposure.

In mice exposed to 940 $\mu$g/m$^3$ (0.5 ppm) for 6 mo, intermittent exposure (6 or 18 h/day) was equivalent to continuous exposure (24 h/day) in increasing mortality due to *Klebsiella pneumoniae*. However, after 12 mo of exposure, effects were only observed in the continuous exposure. As duration increased to 12 mo, the decreased bacterial clearance was equivalent in the two intermittent and continuous groups (Ehrlich and Henry, 1968).

Rombout et al. (1986) evaluated C $\times$ T impacts on lung morphology. Rats were exposed from 1,000 to 20,000 $\mu$g/m$^3$ (0.53 to 10.6 ppm) for up to 28 days. Epithelial changes were more related to exposure concentration than to duration.

In ambient air, there is a low baseline concentration of NO$_2$ on which are superimposed one or two peaks of higher concentrations (primarily Monday through Friday) resulting from the influence of vehicular traffic. The impacts of such patterns have been investigated using the infectivity model, pulmonary function, and lung morphology/morphometry. Using the mouse streptococcal infectivity model, mice were exposed to a series of regimens, with and without a continuous baseline of 2,820 $\mu$g/m$^3$ (1.5 ppm) and with and without peaks (1, 3.5, or 7 h) of 8,460 $\mu$g/m$^3$ (4.5 ppm), mice were challenged with bacteria immediately or 18 h after the peak exposures, total exposure durations varied between 1 day and 2 weeks (Graham et al., 1987, Gardner, 1980, Gardner et al., 1982). The baseline exposure alone caused no effects, whereas peaks alone enhanced mortality when the bacterial challenge was immediately after the peak exposure. With both the baseline and peak exposures, the effect persisted 18 h after the peak exposure. When these data were compared to a 2-week continuous exposure to 2,800 $\mu$g/m$^3$ (1.5 ppm), there was no apparent trend towards a C $\times$ T relationship. In a 1-year exposure study, continuous exposure to 376 $\mu$g/m$^3$.
(0.2 ppm) did not affect streptococcal-induced mortality (Miller et al., 1987) However, in mice exposed to this baseline plus two daily 1-h peaks of 1,500 μg/m³ (0.8 ppm) for 5 days/week, mortality was enhanced over air controls and the baseline NO₂ group. Pulmonary function was also affected (decreased vital capacity) more in the baseline-plus-peak group compared to baseline only and air-control groups. Pulmonary function and morphometric studies using urban patterns of NO₂ are described further in the next section (Section 13.6.5).

The body of work comparing different exposure regimens clearly shows the dependence of effects on the concentration and duration and the exposure profile, rather than the cumulative C × T. This illustrates the difficulty of extrapolating from a laboratory exposure to a complex ambient pattern of NO₂.

13.6.5 Impact of Exposure Duration

As exposure duration increases, generally lower concentrations of NO₂ are needed to produce effects, and different types of effects may occur. The effects of exposure duration on increasing susceptibility to pulmonary infection have been described above (Section 13.6.4). Thus, other chronic studies that principally focused on lung morphology/morphometry will be discussed here, along with supporting research on pulmonary function.

Although NO₂ produces morphological changes in the respiratory tract, the data base is sometimes confusing due to quantitative and qualitative variability in responsiveness between species, and even within the same species. For example, the rat appears to be relatively resistant to NO₂, although this is the most commonly used experimental animal involved in morphological assessments of exposure. In any case, when effective levels are used, the target site is the region that includes the terminal and/or respiratory bronchioles, alveolar ducts, and alveoli. Sensitive cells are the ciliated cells of the bronchiolar epithelium and the Type 1 cells of the alveolar epithelium. As these cells are sloughed off, they are replaced by nonciliated bronchiolar (Clara) cells and Type 2 cells, respectively.

Acute exposures to concentrations of ≤9,400 μg/m³ (50 ppm) generally produce minimal or no morphological effects in the rat; however, similar exposures in the guinea pig may result in some epithelial damage (Azoulay-Dupuis et al., 1983). Thus, prolonged
exposures are of more interest. Longer term exposures result in lesions in some species with NO₂ concentrations as low as 560 to 940 μg/m³ (0.3 to 0.5 ppm) (Sherwin and Richters, 1982, Kubota et al., 1987, Yamamoto and Takahashi, 1984; Hayashi et al., 1987). Lesions are characterized by epithelial damage similar to that described following acute exposure to ≤9,400 μg/m³ (5.0 ppm), but with the involvement of more proximal airways, the thickness of the basal lamina and interstitium can increase. Many of these changes, however, will resolve even with continued exposure, and long-term exposures to levels above about 3,760 μg/m³ (2.0 ppm) are required for more extensive and permanent changes in the lungs. Some effects are relatively persistent, for example, bronchiolitis and collagen deposition, whereas others, such as epithelial cell hyperplasia, tend to be reversible and limited even with continued exposure (Kubota et al., 1987, Yamamoto and Takahashi, 1984). In any case, it seems that for both acute or longer term exposure regimes, the response is more dependent on concentration than on exposure duration (Rombout et al., 1986).

Results from rats exposed to 940, 1,880, and 3,760 μg/m³ (0.5, 1.0, and 2.0 ppm) NO₂ with two daily 1-h peaks at three times the baseline concentration provide an example of subchronic effects of NO₂ on pulmonary function and lung morphometry (at the electron microscopic level) (Stevens et al., 1988, Chang et al., 1986, 1988). Pulmonary function (decreased respiratory system compliance) was only affected by 6 weeks (and not 1 or 3 weeks) or exposure to the highest concentration, recovery occurred by 3 weeks after exposure ceased. Morphometric measurements were only made at 6 weeks of exposure. Animals exposed to the 940-μg/m³, but not the 3,760-μg/m³, base (plus peaks) had a thickening of the alveolar interstitium in the proximal alveolar region due to the increase in total volume of fibroblasts. At the lowest concentration of NO₂, Type 2 cells were spread over more surface area and exhibited hypertrophy, the number of AMs increased. There were no effects in the terminal bronchial region. At the highest NO₂ concentration, the proximal alveolar region had similar changes in Type 2 cells as well as an increase in the number of Type 1 cells, which were smaller in size, the terminal bronchial region had fewer ciliated cells and alterations in nonciliated (Clara) cells. A study by Hayashi et al. (1987) provides an example of chronic effects of rats exposed to 940 μg/m³ (0.5 ppm) continuously for up to 19 mo. At 4 mo of exposure, Type 2 cell hypertrophy was observed.
by 6 mo, the thickness of alveolar septa had increased, and at the end of exposure, there was fibrous pleural thickening.

One of the major factors determining responsiveness within a particular species is age at time of exposure. Compared to adults, neonatal animals seem to be more resistant to pulmonary function or structural changes caused by NO₂, however, interpretation is confounded by difficulty in exposing animals prior to weaning (Stevens et al., 1988, Chang et al., 1986, 1988, Mauderly et al., 1987, Azoulay-Dupuis et al., 1983, Kyono and Kawai, 1982). Kyono and Kawai (1982) observed a complex interrelationship between NO₂ concentration and age that cannot be interpreted clearly. However, for some end points (e.g., air-blood barrier thickness), there was a decrease from 1 to 12 mo of age and an increase in 21-mo-old rats exposed for 1 mo to ≥207 μg/m³ (0.11 ppm).

There is very substantial evidence that long-term exposure of several species of laboratory animals to high concentrations of NO₂ (>9,400 μg/m³, 5.0 ppm) results in morphologic lung lesions, which meet the current NHLBI criteria for an animal model of emphysema (National Institutes of Health, 1985). Those criteria are "An animal model of emphysema is defined as an abnormal state of the lungs in which there is enlargement of the airspaces distal to the terminal bronchiole. Airspace enlargement should be determined qualitatively in appropriate specimens and quantitatively by stereologic methods.

Destruction of alveolar walls, an essential additional criterion for human emphysema, has been reliably reported in lungs from animals in a limited number of studies (Haydon et al., 1967, Freeman et al., 1972, Hyde et al., 1978). The only one of these studies conducted at NO₂ exposure levels of less than 9,400 μg/m³ (5.0 ppm) involved coexposure of Beagle dogs to 1,210 μg/m³ (0.6 ppm) NO₂ and 310 μg/m³ (0.16 ppm) NO or to 270 μg/m³ (0.14 ppm) NO₂ with 2,050 μg/m³ (1.1 ppm) NO (Hyde et al., 1978). Animals were exposed 16 h/day for 68 mo and then breathed clean air during a 32- to 36-mo postexposure period. The dogs exposed to the higher level of NO₂ had emphysema of the type seen in human lungs. Although the lowest NO₂ concentration and the shortest exposure duration that will result in emphysematous lung lesions cannot be reliably determined from these published studies, the NO₂ concentrations and exposure durations used are far greater than those currently reported in ambient air.
The susceptibility to NO\textsubscript{2} of animals with experimentally induced emphysema has also been examined. Nitrogen dioxide (3,760 \(\mu\)g/m\textsuperscript{3}, 2.0 ppm, intermittent, 8 weeks) appeared to exacerbate emphysema in hamsters using morphological methods, pulmonary function was not affected (Lafuma et al., 1987). However, elastase-induced emphysema in rats was not affected, even though the exposure was high (17,900 \(\mu\)g/m\textsuperscript{3}, 9.5 ppm, 7 h/day, 5 days/week, 24 mo, Mauderly et al., [1990]).

The literature provides no evidence that NO\textsubscript{2} is a direct-acting carcinogen, but no classical chronic inhalation bioassays have been reported. Other reports that NO\textsubscript{2} may act as a promoter or facilitator of neoplastic disease are fraught with methodological and interpretative problems.

### 13.6.6 Effects of Pollutant Mixtures

Nitrogen dioxide exists in the ambient air with other pollutants, especially NO and O\textsubscript{3}, which are part of the photochemistry of NO\textsubscript{x}. Animal toxicology research has addressed complex mixtures (e.g., exposure to ambient air containing NO\textsubscript{2}, automobile exhaust), but the contribution of NO\textsubscript{2} to the mixtures effect(s) cannot be determined due to the study designs used. Binary mixture studies primarily include O\textsubscript{3} and, to a lesser extent, H\textsubscript{2}SO\textsubscript{4}. Even with the numerous studies available (described in Section 13.3), interpretation of interactions is unclear. In binary mixtures, NO\textsubscript{2} either makes no contribution, is additive, or is synergistic, depending on exposure regimen and end point. For example, in a 6-mo exposure study of rats, 4,700 \(\mu\)g/m\textsuperscript{3} (2.5 ppm) NO\textsubscript{2} did not affect the lung lesion induced by 490 \(\mu\)g/m\textsuperscript{3} (0.25 ppm) O\textsubscript{3} (Freeman et al., 1974a). Mustafa et al. (1984) found no effect of an O\textsubscript{3}-NO\textsubscript{2} mixture on lung DNA or protein content of mice exposed for 1 week, however, the mixture caused a synergistic increase of oxygen consumption, sulfhydryl metabolism, and activities of NADP-reducing enzymes. Ehrlich et al. (1977) reported that a 3-h exposure to O\textsubscript{3} plus NO\textsubscript{2} caused an additive response in the mouse infectivity model, whereas longer exposure (4 weeks) appeared to result in synergism. When mixtures of NO\textsubscript{2} and H\textsubscript{2}SO\textsubscript{4} were examined, Schlesinger and Gearhart (1987) and Schlesinger (1987a) found additive or synergistic effects on host defense mechanisms, depending on the NO\textsubscript{2} concentration and end point.
The findings of either additivity or synergism are of concern because of the ubiquitous, cooccurring nature of O₃ and NO₂ and the type of effects observed. For example, if one of these pollutants is causing a decrease in host defenses, even an additive response to the other pollutant would likely increase the incidence or severity of the effect. Precise interpretation of these findings to ambient scenarios is confounded, however. In the ambient air, the common diurnal pattern is a series of peaks of the photochemical oxidants and their precursors (e.g., NO, NO₂, O₃), there is some mixing between the peaks. Such a "real-world" pattern has been approximated by Gelzleichter et al. (1992b), who examined the effects of O₃ and NO₂ in mixture and in sequence. Acute exposure of rats to the mixture caused a synergistic increase in lavage fluid protein, PMNs, and epithelial cells. Sequential exposures generally caused an additive response, with one exception. When the sequence was NO₂ first and O₃ second, there was an antagonistic response for the number of lavagable epithelial cells. The body of work with NO₂ and NO₂-O₃ mixtures illustrates the importance of exposure patterns, so extrapolating laboratory binary mixture study results to ambient patterns raises concern, but does not allow precise conclusions.
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14. EPIDEMIOLOGY STUDIES OF OXIDES OF NITROGEN

14.1 INTRODUCTION

This chapter discusses the epidemiological evidence for the effects of nitrogen oxides (NO\textsubscript{x}) on human health. Major emphasis is placed on discussion of the effects of nitrogen dioxide (NO\textsubscript{2}) because it is the NO\textsubscript{x} compound measured in most epidemiological studies and because it is the NO\textsubscript{x} compound currently of greatest concern from a public health perspective. Human health effects associated with exposure to NO\textsubscript{2} have been the subject of several literature reviews since 1970. National Research Council (1971, 1977), World Health Organization (1977), Samet et al (1987, 1988), and Graham et al (1990) Oxides of nitrogen have also been reviewed previously by the U.S. Environmental Protection Agency (1982a), which presented a comprehensive review of studies conducted up to 1980. This chapter focuses mainly on studies conducted since 1980, while also utilizing some key information from earlier literature.

Studies discussed in the chapter text are those that provide useful quantitative information on exposure-effect relationships for health effects associated with ambient air levels of NO\textsubscript{2} likely to be encountered in the United States. In addition, some studies that do not provide quantitative information are briefly discussed in the main text as appropriate to help elucidate particular points concerning NO\textsubscript{2} health effects. Both the quantitative studies discussed in the main text and the additional qualitative studies evaluated here but found to be of limited usefulness for present criteria development purposes are concisely summarized in Appendix 14A.

The present chapter is organized as follows. First, studies of respiratory symptoms and illnesses that meet criteria (see Section 14.6.4) for use in a quantitative analysis are discussed, followed by studies that provide qualitative information. The respiratory illness section is divided into indoor and outdoor subsections. Next, studies are described that examine effects of NO\textsubscript{2} exposure on pulmonary function. Then, a short discussion of occupational studies is provided. Finally, a quantitative analysis is presented that synthesizes the available evidence on respiratory illness.
In U.S. Environmental Protection Agency (1982a), a group of studies examining the relationship between respiratory illness and exposure in the home to gas (cooking fuel) combustion products, notably including NO₂, were evaluated. At that time, those studies inferred the presence of NO₂ by the presence of gas combustion emission sources. Since then, new studies and updates of earlier ones have been conducted. Many of these studies provide data on NO₂ concentrations and estimates of human exposure.

14.2 METHODOLOGICAL CONSIDERATIONS

Studies assessed here were evaluated for several factors noted earlier by Hill (1965) and U.S. Environmental Protection Agency (1982b) to be of importance for interpreting epidemiological studies. Factors considered here in evaluating epidemiological studies of the health effects of NO₂ include (1) exposure measurement errors, (2) misclassification of health outcomes, (3) adjustments for covariates, (4) selection bias, (5) internal consistency, and (6) plausibility of observed effects, based on other evidence. Because these factors are common to the evaluation of all epidemiology studies, a brief discussion follows.

14.2.1 Measurement Error

Measurement error in exposure is potentially one of the most important methodological problems in epidemiological studies of NO₂. Ideally, personal monitors would be placed on all subjects for the entire period of a study. Even then, some error associated with the monitoring device itself would remain. Such intensive personal monitoring is not feasible. Even personal monitoring, because of the integrated multiday sampling, does not adequately measure short-term peaks nor long-term chronic exposures. Instead, NO₂ exposure may be estimated by source description, personal monitors, in-home monitors, and fixed-site outdoor monitors. In most of the early studies, gas stove presence was related to health outcomes without any direct exposure estimates. Additionally, a by-product of nitrogen dioxide, nitrous acid (HONO), may be a factor contributing to observed effects, however, very limited aerometric or health effects data are available that examine this possibility.

The effect of exposure measurement error on estimation has been studied by several authors, including Shy et al. (1978), Gladen and Rogan (1979), Clark (1982), Stefanski and
Carroll (1985), Walker and Blettner (1985), Fuller (1987), Lebret (1987), Schafer (1987), Whittemore and Keller (1988), Samet and Utell (1990), and Yoshinura (1990) In general, exposure measurement error that is independent of the health outcome results in estimated effects being biased towards the null. For example, Whittemore and Keller (1988) specifically consider the data of Melia et al (1980) as described by Florey et al (1979) and show that a 20% misclassification rate of the exposure category would result in an underestimate of the logistic regression coefficient by as much as 50%. Also, Stefanski and Carroll (1985) have shown that even without the independence of error related to outcome, the bias is towards the null in situations where the probabilities of response are not extremely close to 0 or 1. The use of the presence of a gas stove as a surrogate for an actual NO<sub>2</sub> exposure can introduce measurement misclassification error. Clark (1982) studied the effect of measurement error, which was towards the null in logistic regression when that error was sampled from a normal or logistic regression, and also found bias towards the null for certain multiple logistic models.

If the observed health effects (see Chapter 13 and Section 14.3) result from peaks (1-h or less) generated during source use rather than longer term averages, then the use of estimated averages creates another source of exposure measurement error. However, adequate data are available to adequately evaluate the relative contributions of personal exposures to peak versus average NO<sub>2</sub> values to health effects studied in epidemiology studies. Peak levels in bedrooms and other locations are not as high as in kitchens (see Chapter 7), and most indoor activity occurs in locations other than the kitchens (see Chapter 8). Harlos et al (1987) state that NO<sub>2</sub> concentrations in the kitchen are different for each cooking event in a 12- or 24-h period. To improve exposure measurement estimates, NO<sub>2</sub> concentrations during room occupancy are needed. The average bedroom NO<sub>2</sub> concentration already contains most of the time-location information by virtue of being a primary daily location, especially for infants. In most homes, peak values may be related to average values such that reducing peaks reduces the average concentration. Average values may serve as surrogates for the peaks, however, if effects are associated with the peaks, then the use of averages will increase measurement error.
14.2.2 Misclassification of Health Outcomes

Misclassification of the health outcome can occur whether the outcome is continuous, such as a measure of pulmonary function, or dichotomous, such as the presence or absence of respiratory symptoms. Lung function is typically measured with spirometry, a well-standardized (Ferris, 1978) technique. The measurement errors of the instruments collecting the data have also been carefully estimated, and random errors will simply add to the error variance. On the other hand, respiratory symptoms and disease are usually measured by a questionnaire. Responses to symptom questions are typically positively correlated and depend on the interpretation of the respondent. As noted later in the chapter, a specific respiratory disease is likely to be reflected by reporting of a constellation of symptoms, it is therefore appropriate to consider aggregate rather than, or as well as, single specific symptom reports. Obviously, questionnaire measurements that depend on recent recall are better than those based on recall of events that occurred several years in the past. Questionnaires for cough and phlegm production have been standardized, such as the British Medical Research Council (BMRC) questionnaire (American Thoracic Society, 1969) and revisions of the BMRC questionnaire (Ferris, 1978, Samet, 1978). These questionnaires and modifications of them have been used extensively.

14.2.3 Adjustments for Covariates

It is common when analyzing a data set to discover that one or more key covariates for the analysis were not measured. Schenker et al. (1983) discuss socioeconomic status (SES), passive smoking, and gender as important covariates in childhood respiratory disease studies. Other covariates include age, humidity, and pollutants, such as particulate matter. The concern is that, had an omitted covariate been measured, then the estimate of the regression coefficient for a dependent variable of interest would have been significantly different. Although the problem is faced by many investigators, the literature on the field is relatively sparse. For example, Kupper (1984) shows that high correlations between the variables just described will result in "unreliable parameter estimates with large variances." Gail (1985) considered the special case of omitting a balanced covariate from the analysis of a cohort study and concluded that "In principle, the bias may be either toward or away from zero, though in typical examples—the bias is toward zero." In applications with additive or
multiplicative regression, there is no bias" Neither paper provided information on how to attempt to correct for the bias or on approaches for investigating the possible bias in a given situation.

Most studies of respiratory disease and NO₂ exposures discussed here measured important covariates such as age, socioeconomic level of the parents, gender, and parental smoking habits. The estimated effect (regression coefficient of disease on NO₂ exposure) will be overestimated if a missing covariate is positively (or negatively) correlated with both exposure and outcome. The estimated effect will be underestimated if positively correlated with either exposure or outcome and negatively correlated with the other. Ware et al. (1984), for example, found that parents with some college education were more likely to report respiratory symptoms and were less likely to use a gas stove, leading to an underestimate of the health effect if education were left out of the analysis.

14.2.4 Selection Bias

The possibility of selection bias, although a concern of every study, seems very low for the epidemiologic studies of NO₂. Selection bias would require selection of participants based on exposure (e.g., use of gas stove) and also health outcome. Because most epidemiologic studies of these exposures are population based, there is little possibility of selection based on health end points. Nevertheless, the loss of subjects by attrition associated with both exposure and health studies must be considered.

14.2.5 Internal Consistency

Internal consistency is always a check on the validity of a study, but often the authors do not report sufficient detail by which to check for such consistency. For known risk factors for respiratory effects, a study should provide evidence of expected associations (e.g., between passive smoking and increased respiratory illness among exposed coworkers or children or more wheeze in exposed asthmatic children). Furthermore, certain patterns of age or gender relationships to observed health outcomes should be expected. On the other hand, study results suggesting a significant beneficial effect of NO₂ amid other deleterious effects must be viewed with extreme caution in the absence of independent animal toxicologic
or other types of evidence for plausible mechanisms to account for such effects. Consistency between studies provides a further indication of the overall strength of the total data base.

14.2.6 Plausibility of Effects

Health outcomes measured should be ones for which there are plausible bases to suspect that they could be affected by NO$_2$ exposure. Two health outcome measures have been most extensively considered in the NO$_2$ epidemiologic studies reviewed here: lung function measurements and respiratory illness occurrence. Human clinical and animal toxicological studies have not indicated a demonstrated effect on lung function at ambient levels in normal subjects (see Chapters 13 and 15). However, in contrast, animal toxicological studies in Chapter 13 have shown that NO$_2$ exposure can impair components of the respiratory host defense system, resulting in the host being more susceptible to respiratory infection. Thus observed increases in respiratory symptoms and disease among children in epidemiologic studies of NO$_2$ exposure may be more plausibly hypothesized to be the result of an increase in respiratory infection.

Special attention is accorded to considering all of the above factors in evaluating the studies reviewed below. Those studies that address these factors most appropriately provide a stronger basis for accepting conclusions based on their results.

14.3 STUDIES OF RESPIRATORY ILLNESS

Respiratory illness and the factors determining occurrence and severity are important public health concerns. This section discusses epidemiological findings relating estimates of NO$_2$ exposure to respiratory illness. This effect is of public health importance because of the widespread potential for exposure to NO$_2$ and because the occurrence of childhood respiratory illness is common (Samet et al., 1983, Samet and Utell, 1990). This takes on added importance because recurrent childhood respiratory illness (independent of NO$_2$) may be a risk factor for later susceptibility to lung damage (Glezen, 1989, Samet et al., 1983, Gold et al., 1989).

The NO$_2$ studies used standard respiratory questionnaires that evaluated respiratory health by asking questions about each child’s respiratory disease and symptom experience.
The reported symptoms and diseases (typically based on parental recall) characterize lower respiratory morbidity in the cohorts studied. A brief discussion of aspects of epidemiology of lower respiratory morbidity in children provides a background for studies examining NO₂ exposure in relation to lower respiratory health. Lower respiratory morbidity in children typically includes asthma, bronchitis, croup, tracheobronchitis, bronchiolitis, and pneumonia. Asthma and bronchitis are briefly discussed individually below, and the latter four are discussed together as part of lower respiratory illness syndromes.

Asthma is characterized by reversible airway obstruction, airway inflammation, and increased airway responsiveness to stimuli (National Institutes of Health, 1991). Schenker et al. (1983) report a prevalence of approximately 3.5/100 for MD-diagnosed asthma in children 5 to 9 years of age. The Centers for Disease Control (1990) indicate that, for those less than 20 years of age, the prevalence of asthma increased from approximately 3.5/100 persons in 1980 to 5.0/100 persons in 1987. Asthma patients develop such clinical symptoms as wheezing and dyspnea after exposure to allergens, environmental irritants, viral infections, cold air, or exercise. Exacerbations of asthma are acute or subacute episodes of progressively worsening shortness of breath, cough, wheezing, chest tightness, or some combination of these symptoms. Although viral respiratory tract infections are common asthma triggers, especially in young children (National Institutes of Health, 1991), symptoms such as wheezing may occur without an infectious cause.

Chronic bronchitis is defined in adults as a clinical disorder characterized by excessive mucous secretion in the bronchial tube with an associated chronic productive cough on most days for a minimum of 3 mo of the year for not less than 2 successive years (American Thoracic Society, 1962). The diagnosis can only be made after excluding other disorders with similar symptoms. In contrast, Morgan and Taussig (1984) state that a clear definition and etiology of chronic bronchitis in childhood have not yet been described. They characterize chronic bronchitis in children as a symptom complex consisting of a chronic or recurrent "wet" cough, increased phlegm production, and wheezing that may be associated with evidence of airway inflammation. A rational approach would be to view it as a clinical presentation of chronic or recurrent airway disease.

Symptoms and findings observed in children with physician-diagnosed chronic bronchitis commonly include recurrent respiratory infections and wheezing, with chronic
phlegm production and chronic cough being less prevalent (Burrows and Lebowitz, 1975) Schenker et al (1983) report a prevalence of approximately 22/100 for M D -diagnosed bronchitis in children 5 to 9 years of age. Respiratory syncytial virus (RSV) and paramfluenza virus are isolated in cases of bronchitis (Chanock and Parrott, 1965), but symptoms of bronchitis may occur without an infectious cause.

Lower respiratory illnesses are generally classified into one of four clinical syndromes: croup (laryngotracheobronchitis), tracheobronchitis, bronchiolitis, and pneumonia (Glezen and Denny, 1973, Wright et al, 1989, McConnochie et al, 1988). In a study in Tucson, the most common diagnosis during the first year of life was bronchiolitis, which accounts for 60% of all lower respiratory illness (Wright et al, 1989). The most common signs and symptoms associated with lower respiratory illnesses were wet cough (85%), wheeze (77%), tachypnea (48%), fever (54%), and croupy cough (38%) as reported by Wright et al (1989). A few infectious agents are presumed to cause the majority of childhood lower respiratory illness. Bacteria are not thought to be common causes of lower respiratory illness in nonhospitalized infants in the United States (Wright et al, 1989). Seventy-five percent of the isolated microbes were one of four types: RSV, paramfluenza virus types 1 and 3, and Mycoplasma pneumoniae (Glezen and Denny, 1973, McConnochie et al, 1988). Respiratory syncytial virus is particularly likely to cause lower respiratory illness during the first 2 years of life. More than half of all illnesses diagnosed as bronchiolitis, for which an agent was identified, were positive for RSV (Wright et al, 1989). Wright et al (1989) noted that studies that rely on parental reports of symptoms may underestimate illness. Asking parents about illnesses at the end of the first year of life revealed that one-third of them failed to report illnesses diagnosed by pediatricians and evaluated by study nurses.

Various studies of lower respiratory illness have reported rates based on visits to physicians ranging from about 20 to 30 illnesses/100 children in the first year of life (Glezen and Denny, 1973, Wright et al, 1989, Denny and Clyde, 1986, McConnochie et al, 1988). Glezen and Denny (1973) reported that the rate for lower respiratory illnesses ranged from 24/100 person-years in infants under 1 year of age and decreased steadily each year through the preschool years, tending to level off in school children (age 12 to 14 years) to about 7.5 illnesses/100 person-years. Several factors affect the rate of lower respiratory illness in children, including age, immunologic status, prior viral infections, level of health, SES.
(Chanock et al, 1989), day care attendance, home dampness and humidity, environmental tobacco smoke, NO₂, particulate matter, and other pollutants. Rates also depend on method of illness ascertainment. Studies in the United States (Wright et al, 1989, Denny and Clyde, 1986, McConnochie et al, 1988) indicated that the overall pattern and incidence of lower respiratory illness is consistent in different geographic regions during the two decades covered by the studies, suggesting that diagnosis and infectious agents have changed little in that time period. In summary, lower respiratory illness remains one of the major causes of childhood morbidity in the United States (McConnochie et al, 1988).

A large number of factors affect the susceptibility of children and, thus, the subsequent occurrence of respiratory symptoms. Special attention is directed at viral lower respiratory morbidity in the first 2 years of life, because the highest incidence and rate of hospitalization for lower respiratory illnesses are found at this time and because of the risk of chronic sequelae from lower respiratory morbidity in early childhood. There is an immunologic basis for increased susceptibility of the neonate to infection (Wilson, 1986). Full-term infants are immune-deficient (as compared with older children and adults) in essentially all measured immunologic parameters due to lack of prior exposure and subsequent development of immunity, thus rendering them susceptible to serious infections (Bernbaum et al, 1984, Kibler et al, 1986).

The occurrence of lower respiratory morbidity in early childhood may be associated with impaired lung function and growth that appears to persist through adolescence. Early insult from virus infection in the lower respiratory tract may be an essential element in the development of chronic and persistent lung function impairment (Glezen, 1989, Gold et al, 1989). Britten et al (1987) reported that the extension to age 36 of the earlier work of BMRC's National Survey of Health and Development of the 1946 Great Britain Cohort indicates that there can be little doubt in this cohort of the existence of an association between childhood respiratory experience and adult respiratory morbidity. They comment that their study, coupled with evidence from Colley et al (1976), lends support to the model of acquired lung damage predisposing individuals to increased respiratory diseases during adulthood, with genetic susceptibility to respiratory disease being less of a factor. Denny and Clyde (1986) stated that it is now recognized that infections, reactive airways, and inhaled pollutants (mostly cigarette smoke) are the most important risk factors in the
development of chronic lung disease. Thus, factors such as the presence of NO₂ (which increases the risk for respiratory symptoms and related respiratory morbidity) are important because of associated public health concern with regard to both the immediate symptoms produced and the longer term potential for increases in the development of chronic lung disease.

The rest of this section examines epidemiological studies relating NO₂ exposures to respiratory illness. The respiratory illness studies in this section are divided into indoor and outdoor subsections.

### 14.3.1 Indoor Studies

In this section, studies that meet criteria (see Section 14.6) for use in a quantitative analysis are presented. Studies conducted by Melia and colleagues in Great Britain are discussed first. Next, two large studies conducted in six United States cities are examined. Then, other quantitative studies are presented that were conducted by different authors in various locations. These are followed by a quantitative study of infants in Albuquerque, NM. Finally, a discussion of selective studies that provide useful information concerning NO₂ relationships to respiratory illness is presented.

Many indoor studies report the results of their analyses as odds ratios. The odds ratio is defined as \( \frac{p_1 (1 - p_c)}{p_c (1 - p_1)} \), where \( p_1 \) is the probability of disease in the exposed group, and \( p_c \) is the probability of disease in the control group. For small probabilities, the odds ratio approaches the relative risk, \( \frac{p_1}{p_c} \). Odds ratios or relative risks greater than one suggest an adverse effect of the exposure. Although the odds ratio is more difficult to interpret than the relative risk, it is a natural measure resulting from many epidemiological analyses.

#### 14.3.1.1 United Kingdom Studies

Results of several British studies have been reported by Melia et al. (1977, 1978, 1979, 1980, 1982, 1985, 1988), Goldstein et al. (1979, 1981), and Florey et al. (1979, 1982). Aspects of these studies were reviewed previously (U S Environmental Protection Agency, 1982a), but their importance requires a further, more complete discussion of them here.
The initial study, reported by Mella et al. (1977), was based on a survey of 5,658 children (excludes asthmatics, thus 100 less than the number reported), aged 6 to 11 years, with sufficient questionnaire information in 28 randomly selected areas of England and Scotland. The study included a self-administered questionnaire (completed by a parent) that obtained information on the presence of morning cough, day or night cough, colds going to chest, chest sounds of wheezing or whistling, and attacks of bronchitis. The questionnaire was distributed in 1973 and asked about symptoms during the previous 12 mo. Colds going to the chest accounted for the majority of the symptoms reported. Information about cooking fuel (gas or electric), age, gender, and social class (manual versus nonmanual labor) was obtained, but information on parental smoking was not. Mella et al. (1977) note that, although they could not include family smoking habits in the analysis, the known relation between smoking and social class (Tobacco Research Council, 1976) allowed them to avoid at least some of the potential bias from this source. It seemed unlikely to the authors that within the social class groups there was a higher prevalence of smoking in homes where gas was used for cooking. No measurements of NO\textsubscript{2}, either indoors or outdoors, were given.

The authors presented the results in the form of a contingency table for nonasthmatics with complete covariate information. Table 14-1 is a summary of that data for nonasthmatic children. The authors indicated that there was a trend for increased symptoms in homes with gas stoves, but the increase was only significant for girls in urban areas. The authors gave no measures of increased risk.

Hasselblad et al. (1992) reanalyzed the data in Table 14-1 using a multiple logistic model, with the results as shown in Table 14-2. Because it had been suggested that gender had an effect on the relationship with "gas cooker", interaction terms for gender were included in the original model. None of these proved to be significant, and they were subsequently dropped from the model. When separate terms for each gender were used for the effect of "gas cooker", an estimated odds ratio of 1.25 was obtained for boys and an odds ratio of 1.39 was obtained for girls. The combined odds ratio for both genders was 1.31 (95% confidence limits of 1.16 and 1.48) and was statistically significant (p < 0.0001). The other main effects of gender, SES, and age were all statistically significant. This reanalysis suggests that gas stove use in this study is associated with an estimated 31% increase in the odds for children of having respiratory illness symptoms.
TABLE 14-1. RESPIRATORY SYMPTOM RATES OF UNITED KINGDOM CHILDREN BY GENDER, SOCIAL CLASS, AND COOKING TYPE<sup>a</sup>

<table>
<thead>
<tr>
<th>Social Classes I-III (Nonmanual)</th>
<th>Social Classes III-V (Manual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 8 years</td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td></td>
</tr>
<tr>
<td>Electric</td>
<td>25.6% (203)</td>
</tr>
<tr>
<td>Gas</td>
<td>26.1% (88)</td>
</tr>
<tr>
<td>Girls</td>
<td>22.2% (171)</td>
</tr>
<tr>
<td>Electric</td>
<td>30.4% (112)</td>
</tr>
<tr>
<td>Age ≥ 8 years</td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td></td>
</tr>
<tr>
<td>Electric</td>
<td>20.8% (365)</td>
</tr>
<tr>
<td>Gas</td>
<td>23.3% (189)</td>
</tr>
<tr>
<td>Girls</td>
<td>18.1% (303)</td>
</tr>
<tr>
<td>Electric</td>
<td>19.2% (187)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Numbers in parentheses refer to number of subjects

Source: Melia et al (1977)

---


<table>
<thead>
<tr>
<th>Factor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Regression Coefficient</th>
<th>Standard Error</th>
<th>Likelihood Ratio Chi-Square</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SES and age</td>
<td>2.46</td>
<td>0.72</td>
<td>19.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>by gender interactions (2 d f)</td>
<td>0.2733</td>
<td>0.0616</td>
<td>19.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gas by gender interaction (1 d f)</td>
<td>0.0612</td>
<td>15.48</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Gas cooker</td>
<td>0.1531</td>
<td>0.0612</td>
<td>6.29</td>
<td>0.0121</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.2730</td>
<td>0.0702</td>
<td>15.48</td>
<td>0.0001</td>
</tr>
<tr>
<td>SES (manual)</td>
<td>0.3864</td>
<td>0.0626</td>
<td>37.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (&lt;8 years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>SES = Socioeconomic status  
<sup>d f</sup> = Degrees of freedom
Meha et al (1979) report further results of the national survey covering a new cohort of 4,827 boys and girls, aged 5 to 10 years, from 27 randomly selected areas that were examined in 1977. The 1977 study collected information on the number of smokers in the home. In the 1977 cross-sectional study, only the prevalence of day or night cough in boys ($p = 0.02$) and colds going to the chest in girls ($p < 0.05$) were found to be significantly higher in children from homes where gas was used for cooking compared with children from homes where electricity was used. Grouping responses according to the six respiratory questions into (a) none or (b) one or more symptoms or diseases yielded a prevalence higher in children from homes where gas was used for cooking than in those from homes where electricity was used ($p = 0.01$ in boys, $p = 0.07$ in girls). The results of this analysis are presented in Table 14-3. The effects of gender, social class, use of pilot lights, and number of smokers in the house were examined.

The reanalysis of the data in Table 14-3 by Hasselblad et al (1992), applying a multiple logistic model, is given in Table 14-4. This model contained the same terms as the analysis in Table 14-2. As in the previous analysis, none of the interaction terms proved to be significant, and they were subsequently dropped from the model. When separate terms for each gender were used for the effect of "gas cooker", an estimated odds ratio of 1.29 was obtained for boys and an odds ratio of 1.19 was obtained for girls. The combined odds ratio for both genders was 1.24 (95% confidence limits of 1.09 and 1.42). This effect was statistically significant ($p < 0.0002$). The other main effects of gender, SES, and age were all statistically significant. This reanalysis suggests that gas stove use in this study is associated with an estimated 24% increase in the odds of having symptoms.

This study was followed by a study in 1978 of 808 schoolchildren (Meha et al., 1980), aged 6 to 7 years, in Middlesbrough, an urban area of northern England. Respiratory illness was defined in the same manner as in the previous study. Weekly indoor NO$_2$ measurements were collected from 66% of the homes, with the remaining 34% refusing to participate. Nitrogen dioxide was measured weekly by triethanolamine diffusion tubes (Palmes tubes) attached to walls in the kitchen area and in the children’s bedrooms. In homes with gas stoves, weekly levels of NO$_2$ in kitchens ranged from 0.005 to 0.317 ppm (10 to 596 µg/m$^3$) with a mean of 0.112 ppm (211 µg/m$^3$), and levels in bedrooms ranged from 0.004 to 0.169 ppm (8 to 318 µg/m$^3$) with a mean of 0.031 ppm (56 µg/m$^3$). In homes
### TABLE 14-3. UNADJUSTED RATES OF ONE OR MORE RESPIRATORY SYMPTOMS AMONG UNITED KINGDOM CHILDREN BY GENDER, SOCIAL CLASS, AND COOKING TYPE

<table>
<thead>
<tr>
<th>Age</th>
<th>Social Classes I-III (Nonmanual)</th>
<th>Social Classes III-V (Manual)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Electric</td>
<td>Gas</td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt; 8 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls 27 4%</td>
<td>31 7%</td>
<td></td>
</tr>
<tr>
<td>(277) (145)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls 24 4%</td>
<td>27 6%</td>
<td></td>
</tr>
<tr>
<td>(291) (134)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age ≥ 8 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls 19 2%</td>
<td>28 3%</td>
<td></td>
</tr>
<tr>
<td>(286) (113)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls 14 8%</td>
<td>18 6%</td>
<td></td>
</tr>
<tr>
<td>(243) (118)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses refer to number of subjects*

Source: Melia et al. (1979)

### TABLE 14-4. HASSELBLAD ET AL. (1992) MULTIPLE LOGISTIC ANALYSIS OF DATA FROM MELIA ET AL. (1979) STUDY

<table>
<thead>
<tr>
<th>Factor</th>
<th>Regression Coefficient</th>
<th>Standard Error</th>
<th>Likelihood Ratio Chi-Square</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SES and Age by gender interactions (2 d f )</td>
<td>1.11</td>
<td>0.5749</td>
<td>1.11</td>
<td>0.5749</td>
</tr>
<tr>
<td>Gas by gender interaction (1 d f )</td>
<td>0.35</td>
<td>0.5566</td>
<td>0.35</td>
<td>0.5566</td>
</tr>
<tr>
<td>Gas cooker</td>
<td>0.2183</td>
<td>0.0674</td>
<td>10.43</td>
<td>0.0012</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>-0.1970</td>
<td>0.0664</td>
<td>8.81</td>
<td>0.0030</td>
</tr>
<tr>
<td>SES (manual)</td>
<td>0.2225</td>
<td>0.0764</td>
<td>8.60</td>
<td>0.0034</td>
</tr>
<tr>
<td>Age (&lt;8 years)</td>
<td>0.5253</td>
<td>0.0675</td>
<td>61.48</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*aSES = Socioeconomic status  
d f = Degrees of freedom*
with electric stoves, weekly levels of NO$_2$ in kitchens ranged from 0.006 to 0.188 ppm (11 to 353 µg/m$^3$) with a mean of 0.018 ppm (34 µg/m$^3$), and levels in bedrooms ranged from 0.003 to 0.037 ppm (6 to 70 µg/m$^3$) with a mean of 0.014 ppm (26 µg/m$^3$). Outdoor levels of NO$_2$ were determined using diffusion tubes systematically located throughout the area, and the weekly average ranged from 0.014 to 0.024 ppm (26 to 45 µg/m$^3$).

One analysis by the authors was restricted to those 103 children in homes where gas stoves were present and where bedroom NO$_2$ exposure was measured, the data are shown in Table 14-5. A linear regression model was fit to the logistic transformation of the symptom or illness rates. Cooking fuel was found to be associated with respiratory illness, independent of social class, age, gender, or presence of a smoker in the house (p = 0.06). However, when social class was excluded from the regression, the association was weaker (p = 0.11). For the 6- to 7-year-old children living in gas stove homes, there appeared to be an increase of respiratory illness with increasing levels of NO$_2$ in their bedrooms (p = 0.10), but no significant relationship was found between respiratory symptoms in those children or their siblings or parents and levels of NO$_2$ in kitchens.

### TABLE 14-5. UNADJUSTED RATES OF ONE OR MORE RESPIRATORY SYMPTOMS AMONG UNITED KINGDOM BOYS AND GIRLS BY BEDROOM LEVELS OF NITROGEN DIOXIDE$^a$

<table>
<thead>
<tr>
<th>Bedroom Levels of NO$_2$ (ppm)</th>
<th>&lt;0.020</th>
<th>0.020-0.039</th>
<th>&gt;0.039</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>43.5%</td>
<td>57.9%</td>
<td>69.2%</td>
<td>54.5%</td>
</tr>
<tr>
<td></td>
<td>(23)</td>
<td>(19)</td>
<td>(13)</td>
<td>(55)</td>
</tr>
<tr>
<td>Girls</td>
<td>44.0%</td>
<td>60.0%</td>
<td>75.0%</td>
<td>54.2%</td>
</tr>
<tr>
<td></td>
<td>(25)</td>
<td>(15)</td>
<td>(8)</td>
<td>(48)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>43.7%</td>
<td>58.8%</td>
<td>71.4%</td>
<td>54.4%</td>
</tr>
<tr>
<td></td>
<td>(48)</td>
<td>(34)</td>
<td>(21)</td>
<td>(103)</td>
</tr>
</tbody>
</table>

$^a$Numbers in parentheses refer to number of subjects.

Source: Melia et al. (1980)
Because no concentration-response estimates were given by the authors, a multiple logistic model was fitted by Hasselblad et al. (1992) to the data in Table 14-5, using a linear slope for NO$_2$ and separate intercepts for boys and girls. Nitrogen dioxide levels for the groups were estimated by fitting a lognormal distribution to the grouped NO$_2$ data, and the average exposures within each interval were estimated (see Hasselblad et al., 1980). The estimated logistic regression coefficient for NO$_2$ (in $\mu g/m^3$) was 0.015 with a standard error of 0.007. The likelihood ratio test for NO$_2$ yielded a chi-square of 4.94 with one degree of freedom, with a corresponding p-value of 0.03.

The study was repeated in January to March of 1980 by Meha et al. (1982a). This time, children aged 5 to 6 years were sampled from the same neighborhood as the previous study, but only families with gas stoves were recruited. Environmental measurements were made and covariate data were collected in a manner similar to the previous study (Mehta et al., 1980). Measurements of NO$_2$ were available for 54% of the homes. The unadjusted rates of one or more symptoms by gender and exposure level are shown in Table 14-6. The authors concluded that "no relation was found between the prevalence of respiratory illness and levels of NO$_2".

The reanalysis by Hasselblad et al. (1992) of the data in Table 14-6 was made using a multiple logistic model similar to the one used for the previous study (Mehta et al., 1980). The model included a linear slope for NO$_2$ and separate intercepts for boys and girls. Nitrogen dioxide levels for the groups were estimated by fitting a lognormal distribution to the grouped bedroom NO$_2$ data. The estimated logistic regression coefficient for NO$_2$ (in $\mu g/m^3$) was 0.0037 with a standard error of 0.0052. The likelihood ratio test for the effect of NO$_2$ gave a chi-square of 0.51 with one degree of freedom ($p = 0.48$).

Mehta et al. (1982b) report an association between the prevalence of respiratory symptoms in children and relative humidity in bedrooms. Florey et al. (1979) had hypothesized that the respiratory health effects seen at the observed NO$_2$ levels may be a proxy for some other factor such as temperature or humidity. After further study, these researchers (Mehta et al., 1982b) conclude that this study did not support the hypothesis that high humidity or low temperature are associated with levels of NO$_2$ within homes with a gas cooker and state that these two environmental variables are thus unlikely to explain their original observation of an association between respiratory illness among primary school students.
TABLE 14-6. UNADJUSTED RATES OF ONE OR MORE RESPIRATORY SYMPTOMS AMONG UNITED KINGDOM BOYS AND GIRLS BY BEDROOM LEVELS OF NITROGEN DIOXIDE

<table>
<thead>
<tr>
<th>Bedroom Levels of NO₂ (ppm)</th>
<th>&lt;0.020</th>
<th>0.020-0.039</th>
<th>&gt;0.039</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.020</td>
<td>56.4%</td>
<td></td>
<td>72.0%</td>
<td></td>
</tr>
<tr>
<td>(39)</td>
<td></td>
<td>(25)</td>
<td></td>
<td>(101)</td>
</tr>
<tr>
<td>0.020-0.039</td>
<td>67.6%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(37)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;0.039</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(23)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(101)</td>
<td></td>
<td>(25)</td>
<td></td>
<td>(87)</td>
</tr>
</tbody>
</table>

| Girls                      |        |             |        |       |
| <0.020                     | 60.0%  |             | 52.2%  |       |
| (25)                       |        | (23)        |        | (87)  |
| 0.020-0.039                | 41.0%  |             |        |       |
| (39)                       |        |             |        |       |
| >0.039                     |        |             |        |       |
| (23)                       |        |             |        |       |
| Total                      |        |             |        |       |
| (87)                       |        | (25)        |        | (101) |

*Numbers in parentheses refer to number of subjects*

Source: Melia et al. (1982a)

children and NO₂. Also, Melia et al. (1982b) note that, contrary to their original hypothesis, homes with an electric cooker tended to have slightly higher relative humidity than homes with a gas cooker. Arundel et al. (1986) comment in general that the majority of health effects related to relative humidity would be minimized by maintaining indoor levels between 40 and 60% and that this would require humidification during winter because indoor relative humidities below 40% are widespread in winter.

Melia et al. (1983) investigated the association between gas cooking in the home and respiratory illness in a study of 390 infants born between 1975 and 1978. When the child reached 1 year of age, the mother was interviewed by a trained field worker to complete a questionnaire. The mother was asked whether the child usually experienced morning cough, day or night cough, wheeze, or colds going to the chest, and whether the child had experienced bronchitis, asthma, or pneumonia during the past 12 mo. No relationship was found between type of fuel used for cooking at home and the prevalence of respiratory symptoms and diseases recalled by the mother after allowing for the effects of gender, social class, and parental smoking. The authors reported prevalence rates for children having at least one symptom by gas stove use and gender. The combined odds ratio for presence of symptoms by gas stove use was 0.63 with 95% confidence interval of 0.36 to 1.10.

Melia et al. (1988) studied factors affecting respiratory morbidity in 1,964 primary school children living in 20 inner city areas of England in 1983 as part of a national study of health and growth. Data on age, gender, respiratory illness, cooking fuels, mother’s
education, and size of family were obtained by questionnaire. Smoking was not studied.

The same respiratory questions were asked as in the previous studies. Melia et al. (1990) reported indoor levels of NO$_2$ associated with gas stoves in inner city areas of England in 1987. The mean weekly NO$_2$ level measured in 22 bedrooms of homes with gas stoves was $0.0241 \pm 0.013$ ppm. The mean weekly NO$_2$ level measured in four bedrooms of homes without gas stoves was $0.0207 \pm 0.0118$ ppm. Melia et al. (1988) reported a relative risk of 1.06 (95% confidence interval of 0.94 to 1.17) for one or more respiratory conditions relative to risk in white boys aged 8 years with mothers educated up to secondary school level, one child in family, two-parent family, and no gas or kerosene fuel used in the home.

14.3.1.2 United States Six Cities Studies

Several authors (Spengler et al., 1979, Speizer et al., 1980, Ferris et al., 1983, Spengler et al., 1986, Berkey et al., 1986, Ware et al., 1984, Quackenboss et al., 1986, Dockery et al., 1989a, Neas et al., 1990, Neas et al., 1991) have reported on two cohorts of children studied in six different U.S. cities (Watertown, MA, Kingston and Harriman, TN, southeast St. Louis, MO, Steubenville, OH, Portage, WI, and Topeka, KS). The six cities were selected to represent a range of air quality based on their historic levels of outdoor pollution. In each community during the period 1974 through 1977, approximately 1,000 first- and second-grade schoolchildren were enrolled in the first year and an additional 500 first graders were enrolled during the following year (Ferris et al., 1979). Families reported the number of persons living in the home and their smoking habits, parental occupation and educational background, and the fuels used for cooking and heating. Outdoor pollution was measured at fixed sites in the communities as well as at selected households. Indoor pollution, including NO$_2$, was measured in several rooms of selected households. Spengler et al. (1979) show that a striking difference in NO$_2$ levels exists between homes with gas versus electric cooking. Later results of monitoring in Portage, WI, verify the fact that the presence of a gas stove contributes to the indoor NO$_2$ levels. Table 14-7 is taken from Quackenboss et al. (1986) based on data collected in 1981 and 1982. These results clearly show that gas stoves increase indoor concentrations and therefore also increase the personal exposures of children.
TABLE 14-7. NITROGEN DIOXIDE CONCENTRATIONS (ppm) BY SEASON AND STOVE TYPE IN PORTAGE, WISCONSIN

| Season | Stove  | Indoor |  | Outdoor |  | Personal |  |
|--------|--------|--------|  |        |  |         |  |
|        |        | Mean   | Std Dev | Mean   | Std Dev | Mean   | Std Dev |
| Summer | Gas    | 0.016  | 0.006 | 0.006  | 0.003 | 0.014  | 0.004 |
|        | Electric | 0.007 | 0.003 | 0.008  | 0.003 | 0.009  | 0.003 |
| Winter | Gas    | 0.027  | 0.013 | 0.008  | 0.003 | 0.023  | 0.009 |
|        | Electric | 0.005 | 0.003 | 0.009  | 0.003 | 0.008  | 0.003 |

Source: Quackenboss et al (1986)

Speizer et al (1980) first reported on results from the six cities studies, based on evaluations of 8,120 children (aged 6 to 10 years) who had been followed for 1 to 3 years. Health end points were measured by a standard respiratory questionnaire, completed by the parents of the children. The authors used log-linear models to estimate the effect of current gas stoves versus electric stoves on the rates of serious respiratory illness before age 2. The analysis gave an odds ratio of 1.12 (95% confidence limits of 1.00 and 1.26) for gas stove use. The results were adjusted for the presence of adult smokers, presence of air conditioning, and SES of the family.

Ware et al (1984) later reported results from a larger cohort of 10,160 white children, aged 6 to 9 years, in the same six communities over a longer period (1974 to 1979). Directly standardized rates of reported illnesses and symptoms did not show any consistent pattern of increased risk for children from homes with gas stoves. Logistic regression analyses controlling for age, gender, city, and maternal smoking level gave estimated odds ratios for the effect of gas stoves ranging from 0.93 to 1.07 for bronchitis, chronic cough, persistent wheeze, lower respiratory illness index, and illness for the last year. The lower respiratory illness index indicated the presence of bronchitis, restriction of activity due to chest illness, or chronic cough during the past year. None of these symptom-specific odds ratios were statistically different from 1. Only two odds ratios approached statistical significance: (1) history of bronchitis (odds ratio = 0.86, 95% confidence interval 0.74 to 1.00) and (2) respiratory illness before age 2 (odds ratio = 1.13, 95% confidence interval 0.99 to 1.28). When the odds ratio for respiratory illness before age 2 was adjusted...
for parental education, the odds ratio was 1.11 with 95% confidence limits of 0.97 and 1.27 ($p = 0.14$). Thus, the study suggests an increase of about 11% in respiratory illness before age 2 years, which is nearly the same as that reported by Speizer et al. (1980), although the increase was not statistically significant at the $p < 0.05$ level. The end point in the Ware et al. (1984) study most similar to that of the Meha studies was the lower respiratory illness index. The authors gave the unadjusted prevalence, and from those data, an estimated odds ratio of 1.08 with 95% confidence limits of 0.97 and 1.19 was calculated by Hasselblad et al. (1992). This rate was not adjusted for other covariates. The analysis of Ware et al. (1984) on the other end points found that the effect of adjustment for covariates was minimal.

During the period 1983 through 1986, a new cohort of approximately 1,000 second-through fifth-grade schoolchildren in each community were enrolled and given an initial symptom questionnaire. Dockery et al. (1989a) evaluated reported respiratory symptoms on a subsequent symptom questionnaire (second annual) for 5,338 white children who were aged 7 to 11 years at the time of enrollment. The end points of chronic cough, bronchitis, restriction of activity due to chest illness, and persistent wheeze were not found to be associated with gas stove use in the home. But the health end point of doctor-diagnosed respiratory illness prior to age 2, yielded an odds ratio of 1.15 with 95% confidence limits of 0.96 and 1.37. The odds ratio for chronic cough was 1.15 with 95% confidence limits of 0.89 and 1.91 and was adjusted for age, sex, parental education, city of residence, and use of unvented kerosene heaters.

Neas et al. (1990, 1991) studied a stratified one-third random sample of the children that were part of the Dockery et al. (1989a) analysis. The sample was restricted to 1,286 white children 7 to 11 years of age at enrollment having complete covariate information and at least one valid indoor measurement of both NO$_2$ and respirable particles. Methods for measuring indoor pollutants were described by Spengler et al. (1986). Indoor pollutants were measured in each child's home for 2 weeks during the heating season and 2 weeks during the cooling season. Nitrogen dioxide was measured by Palmes passive diffusion tubes at three locations: kitchen, activity room, and the child's bedroom.

Brunekreef et al. (1989) examined children studied in the six cities studies and concluded that home dampness is a strong predictor of respiratory symptoms among 8- to
12-year-old children. Dampness was determined by response to these three questions on a questionnaire: (1) Does water ever collect on the basement floor? (2) Has there ever been water damage to the building? and (3) Has there ever been mold or mildew on any surface inside the home? Brunekreef et al. (1989) comment that relative humidity of the indoor air is less important for the growth of mites and fungi than the dampness of specific surfaces or parts of the building structure. Dampness did not confound the gas stove (Dockery et al., 1989a) nor the NO\textsubscript{2} association (Neas et al., 1991).

The analyses by Neas et al. (1990, 1991) were based on the final symptom questionnaire (third annual), which was completed by parents following the indoor measurements. The questionnaire reported symptoms during the previous year, including attacks of shortness of breath with wheeze, persistent wheeze, chronic cough, chronic phlegm, and bronchitis. The authors used a multiple logistic model with separate city intercepts, indicator variables for gender and age, parental history of chronic obstructive pulmonary disease, parental history of asthma, parental education, and single parent family status. The increases in symptoms were estimated for an additional 0.015 ppm (28.3 μg/m\textsuperscript{3}) NO\textsubscript{2} exposure. Table 14-8 shows the odds ratios for the five separate symptoms associated with the increase in NO\textsubscript{2} exposure. All of these odds ratios are consistent with the size of effect seen in the other analyses of the Six City data and the analyses of the British studies.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortness of breath</td>
<td>1.23</td>
<td>0.93 to 1.61</td>
</tr>
<tr>
<td>Persistent wheeze</td>
<td>1.16</td>
<td>0.89 to 1.52</td>
</tr>
<tr>
<td>Chronic cough</td>
<td>1.18</td>
<td>0.87 to 1.60</td>
</tr>
<tr>
<td>Chronic phlegm</td>
<td>1.25</td>
<td>0.94 to 1.66</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>1.05</td>
<td>0.75 to 1.47</td>
</tr>
</tbody>
</table>

Source: Neas et al. (1991)
Neas et al (1990, 1991) defined a combined symptom measure, which was the presence of any of the above-noted symptoms. A multiple logistic regression of this combined lower respiratory symptom measure, equivalent to the single response regressions, gave an estimated odds ratio of 1.40 with a 95% confidence interval of 1.14 to 1.72. The odds ratio for the combined symptom score was slightly higher than in other studies, but is not inconsistent with those results. The reference category for the symptom-specific odds ratios included some children with the other lower respiratory symptoms, whereas the children in the reference category for combined lower respiratory symptoms were free of any of these symptoms. When split by gender, the odds ratio was higher in girls, and, when split by smoking versus nonsmoking homes, the odds ratio was higher in smoking homes.

When separate logistic analyses were performed for each community, the adjusted odds ratios ranged from 1.26 for Topeka, KS, to 1.86 for Portage, WI. When the cohort was restricted to the 495 children in homes with a gas stove, the adjusted odds ratio was 1.37 with a 95% confidence interval of 1.02 to 1.84.

Table 14-9 provides the adjusted odds ratios for combined lower respiratory symptoms across ordered NO₂ exposure categories. The association is statistically significant for the upper exposure category, and the lower exposure categories are consistent with a linear dose-response relationship between NO₂ and lower respiratory symptoms in children.

<table>
<thead>
<tr>
<th>NO₂ Level (ppm)</th>
<th>Range</th>
<th>Mean</th>
<th>Number of Children</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 to 0.0049</td>
<td>0.0037</td>
<td>263</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0050 to 0.0099</td>
<td>0.0073</td>
<td>360</td>
<td>1.06</td>
<td>0.71 to 1.58</td>
</tr>
<tr>
<td></td>
<td>0.0100 to 0.0199</td>
<td>0.0144</td>
<td>317</td>
<td>1.36</td>
<td>0.89 to 2.08</td>
</tr>
<tr>
<td></td>
<td>0.0200 to 0.0782</td>
<td>0.0310</td>
<td>346</td>
<td>1.65</td>
<td>1.03 to 2.63</td>
</tr>
</tbody>
</table>

Neas et al (1992) reported that the estimated effect of exposure to an additional 0.015 ppm (28.3 μg/m$^3$) NO$_2$ on lower respiratory symptoms was consistent across the seasons and sampling locations. Table 14-10 provides the odds ratios and 95% confidence intervals for this association by season and sampler location. The NO$_2$ levels measured by the activity room and bedroom sampler were more strongly associated with lower respiratory symptoms than those in the kitchen. The NO$_2$ measurements in the kitchen were suggested to be influenced more by the transient peak levels associated with meal preparation on gas stoves, whereas the other sampling locations were more reflective of the child's long-term average exposures to NO$_2$ in the home. Spengler et al (1992) indicated that children spend relatively little time (0.5 hours per day) in the kitchen when the range is operating.

### TABLE 14-10. ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR THE EFFECT OF AN ADDITIONAL 0.015 ppm NITROGEN DIOXIDE ON THE PREVALENCE OF LOWER RESPIRATORY SYMPTOMS BY SAMPLING LOCATION AND SEASON

<table>
<thead>
<tr>
<th>Sampler Location and Season</th>
<th>Mean Difference (Gas vs Electric) ppm</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household annual average</td>
<td>0.016</td>
<td>1.40</td>
<td>1.14 to 1.72</td>
</tr>
<tr>
<td>Household winter average</td>
<td>0.018</td>
<td>1.16</td>
<td>1.04 to 1.29</td>
</tr>
<tr>
<td>Household summer average</td>
<td>0.014</td>
<td>1.46</td>
<td>1.13 to 1.89</td>
</tr>
<tr>
<td>Kitchen annual average</td>
<td>0.022</td>
<td>1.23</td>
<td>1.05 to 1.44</td>
</tr>
<tr>
<td>Activity room annual average</td>
<td>0.014</td>
<td>1.50</td>
<td>1.20 to 1.87</td>
</tr>
<tr>
<td>Bedroom annual average</td>
<td>0.013</td>
<td>1.47</td>
<td>1.17 to 1.85</td>
</tr>
</tbody>
</table>

Source: Neas et al (1992)

### 14.3.1.3 Iowa Study

Ekwo et al (1983) surveyed 1,355 children 6 to 12 years of age for respiratory symptoms and lung function in the Iowa City School District. Parents of the school children completed a questionnaire that was a modification of the questionnaire developed by the American Thoracic Society. The children were a random sample from those families whose parents had completed the questionnaire. Eight different measures of respiratory illness were
reported by the authors, but only two of those were similar to the end points used in the British studies and the Six City studies. Parental smoking was also measured and used as a covariate in the analyses. The results of the analyses are presented in Table 14-11, and are based on 1,138 children. No measurements of NO₂ exposure, either inside or outside the homes, were reported.

### TABLE 14-11. ANALYSIS OF IOWA CITY SCHOOL CHILDREN RESPIRATORY SYMPTOMS BY GAS STOVE TYPE AND PARENTAL SMOKING

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hospitalization for Chest Illness Before Age Two</th>
<th>Chest Congestion and Phlegm with Colds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio</td>
<td>SE&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gas stove use</td>
<td>2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.684</td>
</tr>
<tr>
<td>Smoking effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father alone smokes</td>
<td>2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.856</td>
</tr>
<tr>
<td>Mother alone smokes</td>
<td>2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.239</td>
</tr>
<tr>
<td>Both smoke</td>
<td>1.6</td>
<td>0.859</td>
</tr>
</tbody>
</table>

<sup>a</sup>SE = Standard error of the odds ratio

<sup>b</sup>Indicates statistical significance at the 0.05 probability level


#### 14.3.1.4 Dutch Studies

In the Netherlands, Houthuys et al (1987), Brunekreef et al (1987), and Dijkstra et al (1990) studied the effect of indoor factors on respiratory health in children. The population consisted of 6- to 9-year-old children from 10 primary schools in five nonindustrial communities in the southeast region of the Netherlands. Concentrations of NO₂ in the home and personal exposures to NO₂ were measured. An important NO₂ emission/exposure source in these homes were geyser, which are unvented, gas-fired, hot water sources at the water tap. Exposure to tobacco smoke was assessed with a questionnaire that also reported symptom information. The study used Palms diffusion tubes to measure a single weekly average personal NO₂ exposure. In January and February of 1985, the homes of 593 children who had not moved in the last 4 years were measured for 1 week for NO₂ exposure.
Personal exposure was also estimated from time budgets and room monitoring. Estimated and measured exposures to NO$_2$ are given in Table 14-12.

### TABLE 14-12. DUTCH STUDY ESTIMATED AND MEASURED PERSONAL NITROGEN DIOXIDE EXPOSURE (ppm) FOR A SINGLE WEEKLY AVERAGE$^a$

<table>
<thead>
<tr>
<th>NO$_2$ Source</th>
<th>Number</th>
<th>Estimated</th>
<th></th>
<th>Measured</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arth Mean</td>
<td>S D</td>
<td>Arth Mean</td>
<td>S D</td>
</tr>
<tr>
<td>No geyser</td>
<td>370</td>
<td>0.012</td>
<td>0.004</td>
<td>0.012</td>
<td>0.005</td>
</tr>
<tr>
<td>Vented geyser</td>
<td>112</td>
<td>0.015</td>
<td>0.005</td>
<td>0.016</td>
<td>0.006</td>
</tr>
<tr>
<td>Unvented geyser</td>
<td>111</td>
<td>0.021</td>
<td>0.005</td>
<td>0.022</td>
<td>0.006</td>
</tr>
</tbody>
</table>

$^a$Arth Mean = Arithmetic mean  
S D = Standard deviation


Three measures of health were obtained from the questionnaire, which was a modified form of the World Health Organization questionnaire. The different items were combined to create three categories: cough, wheeze, and asthma. Asthma was defined as attacks of shortness of breath with wheezing in the last year. The presence of any of the three symptoms was used as a combination variable. The results are presented in Table 14-13. A logistic regression model was used to fit the combination variable by Hasselblad et al (1992). Exposure was estimated by fitting a lognormal distribution to the grouped data and the mean exposure values for each group were estimated by a maximum likelihood technique (Hasselblad et al., 1980). The estimated logistic regression coefficient was $-0.002$, corresponding to an odds ratio of 0.94 for an increase of 0.015 ppm (28.3 $\mu$g/m$^3$) in NO$_2$, with 95% confidence interval of 0.70 to 1.27. Thus, the Dutch studies did not demonstrate an increase in respiratory disease with increasing NO$_2$ exposure, but the range of uncertainty is quite large and the rates were not adjusted for covariates such as parental smoking and age of the child.

Of several potential explanations for the negative findings of the study with respect to NO$_2$ exposure offered by the authors, one consideration was that the power of the study to
TABLE 14-13. FREQUENCY AND PREVALENCE OF REPORTED RESPIRATORY SYMPTOMS FOR DIFFERENT CATEGORIES OF MEAN INDOOR NITROGEN DIOXIDE CONCENTRATIONS IN A POPULATION OF 775 DUTCH CHILDREN 6 TO 12 YEARS OLD

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Frequency and Prevalence in Category of Indoor NO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-0.011 ppm (n = 336)</td>
</tr>
<tr>
<td>Cough</td>
<td>16 (48%)</td>
</tr>
<tr>
<td>Wheeze</td>
<td>30 (89%)</td>
</tr>
<tr>
<td>Asthma</td>
<td>22 (66%)</td>
</tr>
<tr>
<td>One or more symptoms</td>
<td>36 (10.7%)</td>
</tr>
</tbody>
</table>

Source: Dijkstra et al. (1990)

detect health effects may have been reduced by the smaller sample size of the measured NO₂ data compared to the categorical data (e.g., geyser versus no geyser). They could not estimate whether they gained more precision by measured NO₂ than was lost by the reduction in the sample size. Houthuys et al. (1987) report in an earlier analysis that the presence of an unvented geyser in the kitchen is associated with a higher prevalence of respiratory symptoms and that the difference between no geyser present and an unvented geyser is about 0.010 ppm.

14.3.1.5 Ohio Study

Keller et al. (1979a) and Mitchell et al. (1975) conducted a 12-mo study of respiratory illness and pulmonary function in families in Columbus, OH, prior to 1978. The sample included 441 families divided into two groups: those using gas and those using electric cooking. Participating households were given diaries to record respiratory illnesses for 2-week periods. Respiratory illnesses included colds, sore throat, hoarseness, earache, phlegm, and cough. Only the first incident of illness per person per 2-week period was recorded.

The study measured NO₂ exposure by both Jacobs-Hochheiser and continuous chemiluminescence methods. The electric stove users averaged 0.020 ppm (38 µg/m³) NO₂.
exposure, whereas the gas stove users averaged 0.050 ppm (94 µg/m³). The paper does not report which rooms were measured in order to get this average.

The analysis of incidence rates was done using the "Automatic Interaction Detector." No differences were found in any of the illness rates for fathers, mothers, or children. No analyses were done using multiple logistic regression or Poisson regression (these methods were relatively new at the time). No estimates were made that can be considered comparable to the odds ratios reported in the other studies. The authors did show a bar graph of all respiratory illness for children under 12. The rates were 389 (per 100 person-years) for electric stove use and 377 for gas stove use. These rates were not significantly different even after adjustment for covariates, including family size, age, gender, length of residence, and father's education. No mention was made of adjustments for smoking status or smoking exposure for the children.

In a second, related study (Keller et al., 1979b), 580 persons drawn from households that participated in the earlier study were examined to confirm the reports and to determine the frequency distribution of reported symptoms among parents and children in gas or electric cooking homes. A nurse-epidemiologist examined selected persons reported ill and obtained throat cultures. Unfortunately, these rates were not adjusted for other covariates. The percent of children having respiratory illnesses in homes with a gas stove was 85.1% (n = 87) versus 88.8% (n = 89) in homes with electric stoves. Although the difference is not statistically significant, these rates give an estimated odds ratio of 0.72 with 95% confidence interval of 0.30 to 1.74. Neas et al. (1991) commented that Keller’s model controls for a series of variables that specify the child’s prior illness history and that if chronic exposure to NO₂ is a risk factor for prior illnesses, controlling for the child’s illness history would substantially reduce the estimated effect of current NO₂ exposure.

14.3.1.6 Tayside Study

Ogston et al. (1985) studied infant mortality and morbidity in the Tayside region of northern Scotland. The subjects were 1,565 infants born to mothers who were living in Tayside in 1980. Episodes of respiratory illness were recorded during the first year of life. The information was supplemented by observations made by a health visitor and scrutinized by a pediatrician who checked diagnostic criteria and validity. One health end point assessed
was defined as the presence of any respiratory disease during the year. This end point was analyzed using a multiple logistic regression model that included terms for parental smoking, age of mother, and presence of a gas stove. The results of this analysis are shown in Table 14-14.

**TABLE 14-14. REGRESSION COEFFICIENTS FOR MULTIPLE LOGISTIC ANALYSES OF RESPIRATORY ILLNESS IN TAYSIDE CHILDREN**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Regres Coeff</th>
<th>Odds Ratio</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental smoking</td>
<td>0.429</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td>Age (in 5-year groups)</td>
<td>-0.094</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Presence of gas stove</td>
<td>0.130</td>
<td>1.14</td>
<td>0.86, 1.50</td>
</tr>
</tbody>
</table>

*a* Regres Coeff = Regression coefficient  
*b* NA = Not available  

Source: Ogston et al. (1985)

Only the coefficient for parental smoking was statistically significant (*p < 0.01*). A test for the significance for the coefficient for gas stove use gives a *p*-value of 0.14. The study did not give measured NO₂ exposure values, but referenced the other studies conducted elsewhere in the United Kingdom for exposure estimates.

**14.3.1.7 Albuquerque Study**

Samet et al. (1993, 1992), Lambert et al. (1993), and Samet and Spengler (1989) reported preliminary results of a prospective cohort study of respiratory illness during the first 18 mo of life in relationship to estimates of NO₂ exposure in Albuquerque, NM. Exposure estimates were based on Palms tube and activity data. The study included standardized ascertainment of illness and assessment of potential confounding factors.

Samet et al. (1993) conducted a prospective cohort study between January 1988 and June 1990 to test the hypothesis that exposure to NO₂ increases the incidence and severity of respiratory illness during the first 18 mo of life. A total of 1,315 infants were enrolled into the study at birth in Albuquerque, NM. The subjects were healthy infants from homes.
without smokers and who spent less than 20 h per week in day care. Illness experience was monitored by a daily dairy of symptoms completed by the mother and a telephone interview conducted every two weeks. For a sample of the ill children, a nurse practitioner made a home visit to conduct a standardized history and physical assessment. Exposure to NO\textsubscript{2} was estimated by a two-week average concentration measured in the subjects’ bedrooms with passive samplers. Estimates of exposure based on bedroom concentration were tightly correlated with estimates of exposures calculated as time-weighted averages of the concentrations in the kitchen, bedroom, and activity room. Twenty-six percent of residences had electric cooking ranges, 44\% of homes had gas ranges with continuously burning pilot lights, and 30\% of homes had gas ranges with electronic ignition or burners that were lit with matches. In homes with gas stoves, the subjects’ bedrooms were monitored every 2 weeks, year round. In homes with electric stoves, the child’s bedroom was monitored year-round during every other 2-week cycle. Extensive internal and external quality assurance and control procedures were implemented.

Samet et al. (1993) define illness events as the occurrence of at least two consecutive days of any of the following: runny or stuffy nose, wet cough, dry cough, wheezing, or trouble with breathing. Wheezing was defined as a high-pitched musical sound audible during breathing, and trouble with breathing as the parent’s perception of rapid or labored breathing. Illness events ended with two consecutive symptom-free days. More specific definitions were determined as follows: "upper respiratory tract" was defined as at least 2 consecutive days of any combination of runny or stuffy nose, dry cough, and trouble breathing, "lower respiratory tract" as at least 2 consecutive days of any of the upper respiratory symptoms plus wet cough or wheezing or both being reported on at least 1 day, "lower respiratory tract, wet cough" as any illness meeting the criteria for lower respiratory tract but without wheezing at any time, and "lower respiratory tract, wheezing" as any illness meeting the criteria for lower respiratory tract with wheezing reported for at least 1 day.

The analysis was limited to the 1,205 subjects completing at least 1 month of observation, of these, 823 completed the full protocol. Multivariate methods were used to control for potential confounding factors and to test for effect modification. In analyses of determinants of incident illnesses, the outcome variable was the occurrence of illness during 2 week intervals of days at risk. The independent variables considered in the multivariate
analyses included the fixed factors of birth order (first born versus other), gender, ethnicity (Hispanic versus non-Hispanic), parental asthma and atopic status (considered positive if hay fever or desensitization shots were reported), household income (less than $10,000, $10,000 to $40,000, or greater than $40,000), and maternal education (12 years or less, 13 to 15 years, or 16 years or more) Other variables considered were the temporally varying factors of age (6 mo or less, 7 to 12 mo, or 13 to 18 mo), calendar month, day-care attendance (none, 1 to 4 h, or 5 or more hours per week), and breast feeding (none, partial, or full). Potential confounding and effect modification by cigarette smoking were controlled by excluding subjects from households with smokers

The overall distribution of time at risk by level of bedroom NO₂ level was skewed toward lower contributions, with 22% of the total concentration above 0.02 ppm (Figure 14-1) Lambert et al (1993) reports that during the summer, bedroom NO₂ concentrations in homes with gas stoves averaged 0.014 ppm (standard deviation [SD] = 0.01 ppm) In the bedrooms of homes with electric stoves, the summer average concentrations was 0.007 ppm (SD = 0.006 ppm) During the winter, bedroom concentrations in homes with gas stoves averaged 0.021 ppm (SD = 0.022 ppm) In bedrooms of homes with electric stoves, winter concentrations averaged 0.007 ppm (SD = 0.006 ppm) The exposure estimates were stratified into three classes low (0 to 0.02 ppm), medium (0.02 to 0.04 ppm), and high (greater than 0.04 ppm) For these exposure strata, personal exposures based on bedroom measurements were not substantially different from those derived using the microenvironmental model Approximately 77% of the bedroom NO₂ observations were less than 0.02 ppm, only 5% were greater than 0.04 ppm The 10th and 90th percentiles of the weekly measured concentrations were 0.005 and 0.050 ppm NO₂, respectively, in bedrooms

Samet et al (1993) performed the analysis using the generalized estimated equations described by Zeger and Liang (1986) This takes into account the correlation structure when estimating regression coefficients and their standard errors The multivariate models examined the effects of the unlagged NO₂ exposures, lagged NO₂ exposures, and stove type (Table 14-15) None of the odds ratios was significantly different from unity, the value for the reference category of 0 through 0.02 ppm Additionally, the odds ratios did not tend to increase consistently from the middle category of exposure to the highest category Also,
Figure 14-1. Distribution of time at risk by bedroom NO₂ concentration.

Source Samet et al (1993)

exposure to NO₂ and the durations of the four illness categories were not associated. The authors added NO₂ exposure to the model as a continuous variable, while controlling for the same covariates included in Table 14-15. For each of the five illness variables, the estimated multiplier of the odds ratio per 0.001 ppm increment of NO₂ was 0.999, with confidence limits extending from approximately 0.995 to 1.002.

Health Effects Institute Health Review Committee (1993) noted that although exposure to NO₂ levels in excess of those encountered in this study may be causally related to the incidence or severity of respiratory illness in children, other data indicate that an effect, if it exists, is subtle and may be difficult to distinguish from other environmental risk factors, especially environmental tobacco smoke.

14-31
### TABLE 14-15. ODDS RATIOS\(^a\) FOR EFFECT OF NITROGEN DIOXIDE EXPOSURE ON INCIDENCE OF RESPIRATORY ILLNESS

<table>
<thead>
<tr>
<th>NO(_2) Exposure</th>
<th>All Illnesses</th>
<th>All Lower</th>
<th>Lower, Wet Cough</th>
<th>Lower, Wheezing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odd Ratio 95% CI</td>
<td>Odd Ratio 95% CI</td>
<td>Odd Ratio 95% CI</td>
<td>Odd Ratio 95% CI</td>
</tr>
<tr>
<td>Unlagged(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02–0.04 ppm</td>
<td>1.04 0.96–1.12</td>
<td>0.98 0.89–1.09</td>
<td>1.00 0.89–1.12</td>
<td>0.92 0.73–1.15</td>
</tr>
<tr>
<td>&gt;0.04 ppm</td>
<td>0.94 0.81–1.08</td>
<td>0.93 0.76–1.13</td>
<td>0.94 0.77–1.16</td>
<td>0.88 0.56–1.37</td>
</tr>
<tr>
<td>Lagged(^d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02–0.04 ppm</td>
<td>1.01 0.93–1.10</td>
<td>0.97 0.87–1.08</td>
<td>0.97 0.87–1.09</td>
<td>0.95 0.75–1.19</td>
</tr>
<tr>
<td>&gt;0.04 ppm</td>
<td>0.92 0.77–1.10</td>
<td>0.91 0.72–1.15</td>
<td>0.89 0.68–1.16</td>
<td>0.98 0.66–1.48</td>
</tr>
<tr>
<td>Gas Stove(^d)</td>
<td>0.98 0.90–1.07</td>
<td>0.91 0.81–1.04</td>
<td>0.94 0.82–1.07</td>
<td>0.84 0.64–1.09</td>
</tr>
</tbody>
</table>

\(^a\) Obtained by generalized estimating equation method. Adjusted for season, age, gender, ethnicity, birth order, day care, income, maternal education, breast feeding, parental atopy and asthma, and maternal history of respiratory symptoms.

\(^b\) CI = Confidence interval.

\(^c\) Reference category is 0–0.02 ppm NO\(_2\).

\(^d\) Reference category is electric stove.

Source: Samet et al. (1993)

Health Effects Institute Health Review Committee (1993) commented that performing the multivariate Generalized Estimated Equation analyses without so many covariates being considered simultaneously with an investigation of the separate and combined variables may be informative. In the present analyses, at least 11 variables were entered into the multivariate analyses. These include season, age, gender, ethnicity, birth order, day-care status, income, maternal education, breast feeding, parental atopy and asthma, and a maternal symptom report. These analyses are unable to sort out the effects of the various variables.

The advantage of restrictions in the study population is that the effect of NO\(_2\) exposure could be evaluated in a relatively homogeneous sample of infants. The disadvantages are that the results cannot be generalized to the potentially more susceptible portions of the population, such as infants with parents or care givers who smoke, and infants who attend day care (Health Effects Institute Health Review Committee, 1993).

The study design and implementation represent an effective reduction of misclassification and potential bias. The exposure estimates are a good representative.
estimate of NO$_2$ exposure for the subjects over the time period of the study. The prospective assessment of illness incidence limits potential bias of retrospective ascertainment of illness. The findings indicate that in a population of healthy infants (0 to 18 mo of age), no significant associations between NO$_2$ exposure estimates (in the range of 0 to 0.04 ppm) and respiratory illness were found when precaution was taken to make an accurate assessment of exposures, to validate the measurements of respiratory illness, to eliminate potentially confounding variables, and to adjust for key variables.

14.3.1.8 Chestnut Ridge Study

Schenker et al. (1983) reported a large respiratory disease study of 4,071 children aged 5 to 14 years in the Chestnut Ridge region of western Pennsylvania. The region is predominately rural, and there are numerous underground coal mines and four large coal-fired electricity-generating plants within the area. The standardized ATS-DLD-78-C children's questionnaire (Ferris, 1978) was sent to parents of all children in grades 1 through 6 in targeted schools. An SES scale was derived from the parent's occupation and education and divided into quintiles to provide SES strata V (lowest) through I (highest).

Important confounding factors were evaluated to include gender, SES, and maternal smoking. Persistent wheeze and chronic cough were the most commonly reported symptoms. No relationship was found between persistent wheeze, chronic phlegm, or chest illness in the past year and SES. Significant inverse trends with SES were present for chronic cough and severe chest illness before 2 years of age, whereas physician-diagnosed bronchitis showed significant trends of increased prevalence with higher SES.

Significant linear associations were reported to be present only between the number of parent smokers and the prevalence of chest illness in the past year, and of serious chest illness before 2 years of age, but not with chronic respiratory symptoms. Smoking questionnaires were completed by 1,906 children in grades 4 through 6. Only 53 (2.7%) said that they had ever smoked five or more cigarettes, and currently smoked.

A significant inverse relationship was observed between gas cooking stove use and SES. When gas cooking stove use was tested in the multiple logistic model, a significant association was not found between gas stove use and any of the respiratory or illness variables after adjusting for SES. No odds ratios or other numerical data were reported.
14.3.1.9 Swiss Study

Braun-Fahrlaender et al (1989, 1992) and Rutishauser et al (1990a,b) studied the incidence and duration of common airway symptoms in children up to 5 years old. The study was conducted over a 1-year period in a rural, a suburban, and two urban areas of Switzerland. Parents were asked to record their child's respiratory symptoms (from a list) on a diary form daily over a 6-week period. Additionally, covariates including family size, parental education, living conditions, health status of the child, parents' respiratory health, and smoking habits of the family were assessed by questionnaire.

Nitrogen dioxide was measured weekly during the same 6-week period using Palms tubes, both inside and outside the home of the participants. Meteorological data were obtained from local monitoring stations, but additional air quality data from fixed monitoring sites were only available for the two urban study areas. Figure 14-2 shows the average NO$_2$ concentration outside and indoors and makes it clear that NO$_2$ concentrations inside the home were on average lower than the levels in the outside air. Indoor NO$_2$ levels for Basel, Zurich, Wetzikon, and Rufzerfeld were 33.8, 28.4, 20.5, and 11.2 µg/m$^3$ (0.0179, 0.0151, 0.0109, and 0.0059 ppm), respectively. The indoor NO$_2$ concentration depended to some extent on the concentration of the outside air.

![Figure 14-2. Nitrogen dioxide ambient and indoor concentrations in four Swiss regions with 95% confidence range.](image)

The analysis was restricted to the 1,063 Swiss nationals (from a total of 1,225 participating families) For all four study areas, regional mean incidence rates of upper respiratory illness, cough, breathing difficulties, and total respiratory illness, adjusted for individual covariates and weather data, were regressed (using Poisson regression) against regional differences in annual mean NO₂ concentrations All the relative risks were computed for a 0.0106-ppm (20-μg/m³) increase in pollution concentration Nitrogen dioxide by indoor passive sampler was predictive of the duration of any episode (relative duration of 1.16, 95% confidence interval of 1.12 to 1.21), upper respiratory episodes (relative duration of 1.18, 95% confidence interval of 1.01 to 1.38), and coughing episodes (relative duration of 1.15, 95% confidence interval of 1.03 to 1.29) A discussion of associations with outdoor levels is presented in Section 14.3.2

14.3.1.10 Connecticut Study

Berwick (1987), Berwick et al (1984, 1987, 1989), and Leaderer et al (1986) reported on a 12-week study (six 2-week time periods) of lower and upper respiratory symptoms in 159 women and 121 children (aged 12 or less) living in Connecticut Nitrogen dioxide levels were measured in 91% of the homes, 57 of which had kerosene heaters and 62 of which did not Ambient NO₂ levels ranged from 9 to 19 μg/m³ (0.005 to 0.01 ppm) for the six 2-week time periods Two-week average indoor NO₂ levels in homes of monitored children were highest for homes with kerosene heaters and gas stoves (91 μg/m³, 0.05 ppm, n = 8), second highest for kerosene only (36 μg/m³, 0.02 ppm, n = 45), third highest for gas stoves only (32 μg/m³, 0.02 ppm, n = 13), and lowest for no sources (6 μg/m³, 0.003 ppm, n = 43) Indoor levels did not fluctuate greatly over time, as indicated by the 2-week averages A comparison of personal NO₂ exposures, as measured by Palms’ diffusion tubes, and NO₂ exposures measured in residences had a correlation of 0.94 for a subsample of 23 individuals Results of this comparison are depicted in Figure 14-3 and show an excellent correlation between average household exposure and measured personal exposure The study defined lower respiratory illness as the presence of at least two of the following fever, chest pain, productive cough, wheeze, chest cold physician-diagnosed bronchitis, physician-diagnosed pneumonia, or asthma Upper respiratory illness was defined as the presence of two of the following fever, sore throat, nasal congestion, dry cough, croup, or
head cold. Although both upper and lower respiratory illness were investigated, the major outcome of interest was lower respiratory symptoms. The study obtained information on many potential covariates, which included SES, age, gender, and exposure to environmental tobacco smoke. The covariates having the largest effect were age of the child, SES of the family, and history of respiratory illness. Multiple logistic analysis was used to allow for the various factors.

When controlling for SES and history of respiratory illness, children under the age of 7 exposed to 30 μg/m³ (0.016 ppm) NO₂ or more were found to have an increased risk of lower respiratory symptoms 2.25 times that of children who were not exposed (95% confidence limits of 1.69 and 4.79). They also had an increased risk of upper respiratory symptoms of 1.33 (95% confidence limits of 1.19 and 1.49). Older children and adults showed no increased risk.

Figure 14-3. Total personal exposure to nitrogen dioxide versus nitrogen dioxide levels in Connecticut residences.

Source Leaderer et al (1986)
Although the Berwick study had relatively extensive information on exposure, several problems are evident. The 3-year age-specific relative risks for lower respiratory disease are very unstable, possibly due to the small sample sizes. The rates do not appear to be consistent with the rates for ages 0 to 6 and 7 and above, and it is not clear why a cut-off of 7 years of age was used. The analyses may be sensitive to the adjustment for SES, which can be correlated with exposure. This is less of a problem in studies with larger sample sizes (e.g., Melia et al., 1977, 1979), but may be critical in the Berwick study. Also, Neas et al. (1991) note that the Berwick study controls for prior illnesses, as did the Keller study, which would reduce the estimated effect of current \( \text{NO}_2 \) exposure.

### 14.3.1.11 Maryland Study

Helsing et al. (1982) analyzed the records of 708 nonsmoking white adult residents of Maryland to evaluate the effects of exposure to environmental tobacco smoke at home and use of gas as a cooking fuel. The frequency of cough and/or phlegm among these nonsmokers showed a nonsignificant association with the presence of cigarette smokers in the household. Persons whose households had gas as a cooking fuel reported significantly more chronic cough (relative risk of 2.1) and chronic cough and phlegm (relative risk of 2.2) than those in households using electricity for cooking. The author noted that although gas cooking has been considered by some as simply another indicator of poor social conditions, the multiple adjustments for factors such as years of schooling and persons per room should fully compensate for variations in socioeconomic level. They also noted that all the independent variables combined in the analysis accounted for only 5 to 10% of the variations in symptomatology. Respiratory ventilation function tests gave consistent results with symptom reporting, with those using gas cooking showing impaired pulmonary function.

### 14.3.1.12 German Study

Kuehr et al. (1991) conducted a cross-sectional study on the prevalence of asthma in childhood in relation to \( \text{NO}_2 \) levels in the city of Freiburg and two Black Forest communities. A study group of 704 children aged 7 to 16 years took part in a standardized interview and medical examination. Indoor and outdoor exposure information was taken into
account  Passive smoking exposures were assessed  Stoves used as heating devices carried a
4.8-fold relative risk for asthma compared to other types of heating

14.3.1.13 Canadian Studies

In a case-control study carried out in Montreal, Quebec, Canada, between 1988 and
1990, NO₂ levels measured by passive NO₂ monitoring badge were studied in relation to the
incidence of asthma among 3- and 4-year-old children (Infante-Rivard, 1993)  Multivariate
unconditional logistic regression was carried out for the 140 subjects who had NO₂
measurements, the analysis included NO₂ and the variables retained in the final conditional
model that includes SES and parental smoking  The odds ratios for the NO₂ categories
(defined as >0 0005 to 0 010, >0 010 to 0 015, and >0 015 ppm, in comparison with a
zero level) were 0 95 (95% confidence interval of 0.31 to 2.95), 3.85 (95% confidence
interval of 0.92 to 16.09), and 19.87 (95% confidence interval of 4.75 to 83.03),
respectively

Dekker et al (1991) studied asthma and wheezing syndromes as part of a questionnaire-
based study of 17,962 Canadian school children  The questionnaire was developed from the
1978 American Thoracic Society questionnaire, which was the same one used in the Harvard
Six Cities Study  For analysis, children were restricted to ages 5 through 8 years and those
with cystic fibrosis as well as those living in mobile homes, tents, vans, trailers, and boats
were excluded  The authors calculated odds ratios adjusted for age, race, gender, parental
education, gender of the respondent, region of residence, crowding, dampness, and
environmental tobacco smoke  The adjusted odds ratio of asthma as a function of gas
cooking was 1.95 with 95% confidence limits of 1.41 and 2.68  The adjusted odds ratio of
wheezing as a function of gas cooking was 1.04 with 95% confidence limits of 0.77 and
1.42  The authors note that this finding must be treated with caution, however, because of
the few subjects with asthma in the study who were exposed (n = 60)

14.3.1.14 North Carolina Study

Margolis et al (1992) studied the prevalence of persistent respiratory symptoms in
393 infants of different SES by analyzing data from a community-based cohort study of
respiratory illness in the first year of life in central North Carolina between 1986 and 1988
Infants were limited to those weighing more than 2,000 g and who did not require neonatal care outside the normal newborn nursery. Of those eligible, 47% were enrolled, and of these, 77% completed the study and were included in the analysis. Compared with the 1,241 infants from families refusing enrollment, the 1,091 eligible study infants were more likely to be of high SES and were more often black. Study infants were less likely to have mothers who smoked.

The presence of persistent respiratory symptoms was measured at the 12-mo home interview using an American Thoracic Society children questionnaire (modified for infants) for studies of respiratory illness. Infants who were reported to "usually cough" or "occasionally wheeze" were classified as having persistent respiratory symptoms. The infant's SES was classified into three levels according to the highest level of education achieved by the head of the household. Each infant's exposure to tobacco smoke was measured as the number of cigarettes smoked in the infants presence during the week prior to the 12-mo home visit.

Margolis et al. (1992) used logistic regression to analyze to what extent the relationship between SES and persistent respiratory symptoms could be accounted for by simultaneously considering interactions between SES and other risk factors for lower respiratory illness and confounding by other risk factors. The relationship between the prevalence of persistent respiratory symptoms and SES for infants in the study at low SES was 39%, whereas 14% had persistent symptoms in the high-SES group. Infants in the low-SES group were 2.9 (95% confidence interval of 1.9 to 4.5) times more likely than infants of high SES to have persistent respiratory symptoms. Approximately 224 of the 393 infants in the study were exposed to tobacco smokers. Control for tobacco smoke exposure reduced the relative risk of persistent symptoms among infants of low SES compared with high SES from 2.9 to 2.3. After accounting for all the risk factors, the effect of SES remained significant only for infants not in day care.

Of the 393 infants that Margolis et al. (1992) included in their study, approximately 41 lived in homes with the environmental risk factor of gas cooking. The relative risk of persistent respiratory symptoms among infants exposed to gas cooking unadjusted for any covariates was 1.12 (95% confidence interval of 0.63 to 2.04).
14.3.1.15 United States and Canadian Skating Rink Exposures

Hedberg et al (1989) reported that cough, shortness of breath, and other symptoms among players and spectators of two high school hockey games played at an indoor ice arena in Minnesota were related to emissions from a malfunctioning engine of the ice resurfacer. Although the exact levels of NO$_2$ were not known at the time of the hockey game, levels of 4 ppm (7,500 µg/m$^3$) were detected 2 days later with the ventilation system working, suggesting that levels during the games were higher. Other pollutant levels such as PM$_{10}$ may have also been elevated. Hedberg et al (1989, 1990) reported that pulmonary function testing performed on members of one hockey team with a single exposure demonstrated no decrease in lung function parameters at either 10 days or 2 mo after exposure. Dewailly et al (1988) reported another incident in a skating rink in Quebec, Canada, in 1988 involving referees and employees reporting respiratory symptoms such as coughing, dyspnea, and a suffocating feeling. Five days after the incident, NO$_2$ levels had come down to 3 ppm (5,600 µg/m$^3$), suggesting much higher levels during the incident.

In another skating rink study, Smith et al (1992) report the outcome of a questionnaire administered to all students from two high schools on February 25, 1992, 3 days after 11 students participating in a Wisconsin indoor ice hockey tournament had been treated in emergency rooms for acute respiratory symptoms (i.e., cough, hemoptysis, chest pain, and dyspnea). The game had been attended by 131 students, 57 of whom reported symptoms. A simulation test on February 24 provided levels of NO$_2$ at 1.5 ppm (2,800 µg/m$^3$) in the air over the rink after use of the ice resurfacing machine. Higher levels may have been reached the night of the game. There are more than 800 ice arenas in the United States.

14.3.2 Outdoor Studies

Several studies examined relationships between estimates of ambient NO$_2$ levels and respiratory health measures. Those studies that provide a quantitative estimate of effect are presented in Table 14-16. Health outcome measures include various respiratory symptomologies.
TABLE 14-16. EFFECTS OF OUTDOOR NITROGEN DIOXIDE EXPOSURE ON RESPIRATORY DISEASE

<table>
<thead>
<tr>
<th>Study</th>
<th>Health End Point</th>
<th>NO₂ Levels (ppm)/Period¹</th>
<th>Odds Ratio or Estimate</th>
<th>Odds Ratio Conf Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dockery et al</td>
<td>Bronchitis</td>
<td>0.0065-0.0226/annual average</td>
<td>1.7</td>
<td>0.5 to 5.5</td>
</tr>
<tr>
<td>(1989b)</td>
<td>Chronic cough</td>
<td>0.0065-0.0226/annual average</td>
<td>1.6</td>
<td>0.3 to 10.5</td>
</tr>
<tr>
<td></td>
<td>Chest illness</td>
<td>0.0065-0.0226/annual average</td>
<td>1.2</td>
<td>0.3 to 4.8</td>
</tr>
<tr>
<td></td>
<td>Wheeze</td>
<td>0.0065-0.0226/annual average</td>
<td>0.8</td>
<td>0.4 to 1.6</td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
<td>0.0065-0.0226/annual average</td>
<td>0.6</td>
<td>0.3 to 0.9</td>
</tr>
<tr>
<td>Braun-Fahrlaender</td>
<td>Duration of respiratory</td>
<td>Change of 0.0106/6-week</td>
<td>1.11</td>
<td>1.07 to 1.16</td>
</tr>
<tr>
<td>et al 1992)</td>
<td>episodes</td>
<td>average</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration of</td>
<td>Change of 0.0106/6-week</td>
<td>1.09</td>
<td>0.97 to 1.22</td>
</tr>
<tr>
<td></td>
<td>coughing episodes</td>
<td>average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwartz et al</td>
<td>Croup</td>
<td>0.0053-0.0371/daily average</td>
<td>1.28</td>
<td>1.07 to 1.54</td>
</tr>
<tr>
<td>(1991)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwartz and</td>
<td>Phlegm</td>
<td>0.091/daily 1-h maximum</td>
<td>1.08</td>
<td>1.01 to 1.15</td>
</tr>
<tr>
<td>Zeger (1990)</td>
<td></td>
<td>increase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jaakkola et al</td>
<td>Upper respiratory</td>
<td>Contrasted polluted versus</td>
<td>1.6</td>
<td>1.1 to 2.1</td>
</tr>
<tr>
<td>(1991)</td>
<td>infection</td>
<td>less polluted areas by</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>comparison of annual levels</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Measurement period over which stated nitrogen dioxide (NO₂) level averaged

14.3.2.1 Six City Studies

As part of the Six City Studies, Dockery et al (1989b) obtained respiratory illness and symptom data from questionnaires distributed from September 1980 to April 1981. Indoor air aspects of this study (Dockery et al, 1989a) were described above, in the section on indoor studies. The questionnaires obtained information on bronchitis, cough, chest illness, wheeze, and asthma. A centrally located air monitoring station was established in 1974 where NO₂, sulfur dioxide (SO₂), ozone (O₃), total suspended particulate (TSP), and meteorological variables were measured. The authors used multiple logistic regression analysis in order to adjust for covariates of gender, age, maternal smoking, gas stove use, and separate intercepts for each city. Although the strongest associations were found between respiratory symptoms and particulate matter, there were also increased odds ratios for respiratory symptoms with ambient NO₂. These were not statistically significant, but the
direction for bronchitis, chronic cough, and chest illness was consistent with the studies of indoor exposure. The odds ratios for various health end points for an increase in NO₂ from the lowest exposure city to the highest exposure city (0.0065 to 0.0226 ppm, 12 to 43 µg/m³) are noted in Table 14-16.

14.3.2.2 Swiss Study

Braun-Fahrlaender et al. (1992) studied the incidence and duration of common airway symptoms in children up to 5 years old. This study is also discussed in the earlier section on indoor studies. The study was conducted over a 1-year period in a rural, a suburban, and two urban areas of Switzerland. Parents were asked to record their child's respiratory symptoms (from a list) on a diary form daily over a 6-week period. Additionally, covariates including family size, parental education, living conditions, health status of the child, parents' respiratory health, and smoking habits of the family were assessed by questionnaire. Nitrogen dioxide was measured weekly during the same 6-week period using Palmes tubes, both inside and outside the home of the participants. Meteorological data were obtained from local monitoring stations, but additional air quality data from fixed monitoring sites were only available for the two urban study areas.

The analysis was restricted to the 1,063 Swiss nationals (from a total of 1,225 participating families). For all four study areas, regional mean incidence rates of upper respiratory illness, cough, breathing difficulties, and total respiratory illness, adjusted for individual covariates and weather data, were regressed (using Poisson regression) against regional differences in annual mean NO₂ concentrations. There was no association between long-term differences in NO₂ levels by region and mean annual rates of respiratory incidence.

The adjusted annual mean symptom duration (in days) by region and the corresponding NO₂ levels (measured by passive samplers to produce 6-week averages) are shown in Table 14-17. A second-stage regression of the adjusted natural logarithm of regional mean duration on NO₂ levels yields significant associations between outdoor NO₂ levels (6-week averages) and the average duration (in days) of any respiratory episode (relative duration of 1.11, 95% confidence interval of 1.07 to 1.16) and upper respiratory episodes (relative duration of 1.14, 95% confidence interval of 1.03 to 1.25). A positive trend for the duration.
TABLE 14-17. ADJUSTED ANNUAL RESPIRATORY SYMPTOM DURATION (DAYS) AND NITROGEN DIOXIDE LEVELS BY REGION (n = 1,063)

<table>
<thead>
<tr>
<th>Region</th>
<th>Any Symptom Duration</th>
<th>URI Duration</th>
<th>Cough Duration</th>
<th>Breathing Difficulty Duration</th>
<th>NO₂ In (ppm)</th>
<th>NO₂ Out (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basel</td>
<td>4.50</td>
<td>1.99</td>
<td>2.32</td>
<td>1.55</td>
<td>0.0166</td>
<td>0.0272</td>
</tr>
<tr>
<td>Zurich</td>
<td>4.21</td>
<td>1.85</td>
<td>2.01</td>
<td>1.72</td>
<td>0.0118</td>
<td>0.0248</td>
</tr>
<tr>
<td>Wetzikon</td>
<td>4.00</td>
<td>1.62</td>
<td>2.10</td>
<td>3.47</td>
<td>0.0103</td>
<td>0.0173</td>
</tr>
<tr>
<td>Rafferfeld</td>
<td>3.88</td>
<td>1.72</td>
<td>2.02</td>
<td>1.25</td>
<td>0.0059</td>
<td>0.0133</td>
</tr>
</tbody>
</table>

aURI = Upper respiratory illness

Source: Braun-Fahrlaender et al (1992)

of coughing episodes was also seen (relative duration of 1.09, 95% confidence interval of 0.97 to 1.22). No association was seen with the duration of breathing difficulties. All the relative risks are computed for a 0.0106-ppm (20-μg/m³) increase in pollution concentration. In the suburban and rural areas, NO₂ was the only air quality measure. Correlation between outdoor passive NO₂ sampler and TSP measurements in the two urban study areas was quite high (0.52). The high correlation between NO₂ and TSP suggests that this NO₂ association may reflect confounding with TSP. Unfortunately, the lack of TSP data for the other two regions precludes eliminating TSP as a possible confounder in this analysis.

Although the association with symptom duration in Zurich and Basel may well be due to confounding with TSP, the cross-sectional association across the four regions supports a possible contribution of NO₂. The authors state that, thus, the association between medium and long-term NO₂ exposure and symptom duration deserves consideration as to a possible causal relationship.

14.3.2.3 German Studies

Schwartz et al. (1991) studied respiratory illness in five German communities. Children's hospitals, pediatrics departments of general hospitals, and pediatricians reported daily the numbers of cases of croup. A diagnosis of croup was based on symptoms of hoarseness and barking cough, inspiratory stridor, and dyspnea, and a sudden onset. The most important factors in croup etiology are parainfluenza viruses. The croup counts were modeled using Poisson regression with adjustments for weather, season, temperature,
humidity, and autoregressive lag. Statistically significant effects of both ambient particulate matter and NO₂ were found on the counts of respiratory illnesses. A relationship between short-term fluctuations in air pollution and short-term fluctuations in medical visits for croup symptoms was found in this study. The estimated relative risk was 1.28 with 95% confidence limits of 1.07 and 1.54 for an increase from 0.0053 to 0.0371 ppm (10 to 70 μg/m³) of NO₂. The NO₂ results may be confounded with effects of particle levels.

Rebmann et al. (1991) studied 875 cases of croup in Baden-Württemberg in relation to ambient NO₂ levels over a 2-year period. Statistical regression methods indicated weak but statistically significant influences of the daily ambient NO₂ mean on the occurrence of croup. Virologic testing was conducted on 205 cases that yielded positive results (including influenza A and B, paramyxovirus I-III, and RSV) in 34 cases.

14.3.2.4 Los Angeles Student Nurses Data

Hammer et al. (1974) reported a daily diary study of morbidity symptoms in student nurses in Los Angeles. Diaries on morbidity symptoms were collected weekly from October 1961 to June 1964. Initially, 110 student nurses (over 90% of the class) agreed to participate, but the class size decreased over the 3-year period of the study so that by the end of the third year, the cohort consisted of 30 student nurses. Schwartz and Zeger (1990) and Schwartz et al. (1988) reported later analyses of the data. They reexamined the original diaries from the study, which contained smoking and allergy histories as well as symptom reports. Ambient air pollution (NO₂, SO₂, carbon monoxide [CO], and photochemical oxidants) was measured at a monitoring location approximately 2.5 miles from the dormitory. Pack-years of cigarettes were predictive of the number of episodes of coughing and bringing up phlegm. A daily 1-h maximum NO₂ level of 0.091 ppm (170 μg/m³) was associated with increased risk of phlegm (odds ratio of 1.08, 95% confidence interval of 1.015 to 1.15) and sore throat (odds ratio of 1.26, 95% confidence interval of 1.18 to 1.35). Schwartz and Zeger (1990) note that although the use of only outdoor NO₂ measurements decreases sensitivity, the use of daily diaries should be more sensitive to detect effects of NO₂ than annual questionnaires. Smoking, allergies, temperature, other pollutants, and serial correlation were controlled for. No particulate measurements were available.
mean of daily 1-h maximum NO\textsubscript{2} levels over the study period was 0.13 ppm (245 \textmu g/m\textsuperscript{3}) with 25 and 75% levels of 0.06 and 0.17 ppm (113 and 320 \textmu g/m\textsuperscript{3}), respectively

14.3.2.5 Chestnut Ridge Study

In the Fall of 1980, Vedal et al. (1987) conducted a panel study on 351 children selected from the 1979 Chestnut Ridge study. Parents and children were instructed at the beginning of the school year in completing daily diaries of respiratory symptoms, which were used to define symptom outcomes. Lower respiratory illness was defined as wheeze, pain on breathing, or phlegm production. Of the 351 subjects selected for the 8 mo of follow-up, 128 participated in the completion of diaries. Three subgroups were established: one without respiratory symptoms, one with symptoms of persistent wheeze, and one with cough or phlegm production but without persistent wheeze. Nitrogen dioxide was measured at a single monitoring site in the study region. Maximum hourly levels for each 24-h period were used to reflect the daily pollutant level. During the period September 1980 to April 1981, the mean NO\textsubscript{2} maximum daily 1-h level was 40.5 \textmu g/m\textsuperscript{3} (0.021 ppm) with a range of 12 to 79 \textmu g/m\textsuperscript{3} (0.006 to 0.042 ppm). Regression models could not be fit for subjects who never had symptoms, thus only 55 subjects were included in the analysis of lower respiratory illness. Nitrogen dioxide levels were not predictive of any symptom outcome.

14.3.2.6 Finland Studies

Jaakkola et al. (1991) studied the effects of low-level air pollution in three Finland cities by comparing the frequency of upper respiratory infections over a 12-mo period in 1982 as reported by parents of children ages 14 through 18 mo (n = 679) and 6 years (n = 759). Pollutants studied included ambient levels of NO\textsubscript{2} with an annual mean of 15 \textmu g/m\textsuperscript{3} (0.008 ppm). Other pollutants monitored were SO\textsubscript{2}, hydrogen sulfide (H\textsubscript{2}S), and particulate matter (measured as \textmu g/m\textsuperscript{3}). Passive smoking and SES were taken into account. The authors report a significant association between the occurrence of upper respiratory infections and living in an air-polluted area for both age groups studied, both between and within cities. The adjusted odds ratio was 1.6 (95% confidence interval of 1.1 to 2.1) in the 6-year-old age group. The authors conclude that the combined effect of SO\textsubscript{2}, particulate matter, NO\textsubscript{2}, H\textsubscript{2}S, and other pollutants may be a contributing factor in the study results.
Ponka (1991) studied the effects of ambient air pollution and minimum temperature on the number of patients who had asthma attacks and who were admitted to hospital in Helsinki from 1987 to 1989. During the 3-year period, 4,209 hospitalizations for asthma occurred, an average of 3.84 admissions a day. The number of admissions increased during cold weather, ranging from −37.0 to +26.4 °C, with a 3-year mean of 4.7 °C. After standardization for minimum temperature, the multiple-regression analysis indicated that NO₂ and CO were significantly related to asthma admission. The annual NO₂ levels averaged 38.6 μg/m³ (0.02 ppm) for the 3-year period. During the period of high NO₂ (daily mean 45.8 μg/m³ [0.024 ppm]) levels, the mean number of all admissions was 29% greater than during the period of lower pollution (28.1 μg/m³ [0.015 ppm]). Indoor NO₂ levels or cooking fuel use were not discussed. The observed association of incidence of asthma attacks with relatively low levels of pollutants and cold weather accounted for an explanatory power of approximately 14% in the regression analysis. Other factors that may play a role in the incidence of asthma attacks were not discussed.

14.3.2.7 California Seventh-Day Adventist Study

In a California study, Euler et al. (1988) assessed the risk of chronic respiratory disease symptoms due to long-term exposure to ambient levels of TSP, oxidants, SO₂, and NO₂. Symptoms were ascertained using the National Heart, Lung, and Blood Institute questionnaire on 8,572 Southern California Seventh-Day Adventists (nonsmokers—25 years and older) who had lived 11 years or longer in their 1977 residential area. Tobacco smoke (active and passive) and occupational exposures were assessed by questionnaires, as were lifestyle characteristics relative to pollution exposure, such as time spent outside and residence history. For each of the 7,336 participants who responded and qualified for analysis, cumulative exposures to each pollutant were estimated using monthly residence zip code histories and interpolated exposures from state air monitoring stations.

Multiple logistic regression analyses were conducted for pollutants individually and together with eight covariables, including environmental tobacco smoke exposure at home and at work, past smoking, occupational exposure, sex, age, race, and education. Statistically significant associations with chronic respiratory symptoms were seen for (1) SO₂ (p = 0.03), relative risk of 1.18 for 13% of the study population with 500 h/year of
exposure above 0.04 ppm, (2) oxidants (p < 0.004) relative risk of 1.20 for 18% with 750 h/year above 0.1 ppm, and (3) TSP (p < 0.00001), relative risk of 1.22 for 25% with 750 h/year above 200 μg/m³. When these pollutant exposures were analyzed together, TSP was the only one showing statistical significance (p < 0.01). Nitrogen dioxide exposure levels in this population were not linked to chronic respiratory disease symptoms. Individuals working with smokers for 10 years had relative risks of 1.11 and those living with a smoker for 10 years had relative risks of 1.07.

14.3.2.8 Chattanooga Studies

Several studies were conducted in the greater Chattanooga area during the late 1960s and early 1970s. Although these studies were discussed in detail previously (U.S. Environmental Protection Agency, 1982a), there are at least two additional points that need to be made. First, there were many measurements made in the area by methods other than the Jacobs-Hochheiser method (e.g., chemiluminescence). Reevaluation of the Jacobs-Hochheiser method at a later time questioned its accuracy for use in the studies to estimate quantitative exposure-effect relationships. Second, much of the pollution may have been in the form of nitric acid (HNO₃), and possible health effects may be related to HNO₃ exposure rather than NO₂ itself. The source of pollution was a large trinitrotoluene (TNT) plant, located northeast of Chattanooga, which produced a substantial proportion of all TNT made in the United States during World War II and the Korean War. The plant was reopened in April 1966 to supply munitions for use in Vietnam. Annual averages of NO₂ reached 286 μg/m³ (0.152 ppm) near the arsenal (as measured by the Saltzman method), and nitrate fraction levels reached 4.1 μg/m³ at the downtown post office. It is likely that the elevated NO₂ levels were accompanied by elevated HNO₃ levels, although no direct measurements were made. The U.S. Environmental Protection Agency (1971) measured several factors related to ambient air pollution including corrosion of zinc, steel, and nylon. The corrosion levels in Chattanooga in 1967 and 1968 were among the highest in U.S. cities, and in the case of nylon, were 10 to 100 times the levels of most other cities. According to the report, the arsenal was known to emit acid gases. Additionally, Warner and Stevens (1973, 1975) give other evidence suggesting the presence of sulfuric acid and HNO₃. Thus, it is possible that any adverse health effects seen in Chattanooga during this time period were associated.
with combined exposure to HNO₃ and NO₂ rather than with NO₂ alone. However, no conclusion is possible because the health effects of HNO₃ are poorly understood (see Chapter 13).

Pearlman et al. (1971) reported the results of a respiratory disease survey conducted in the Chattanooga area in 1969. The study reported illness rates in children for the period June 1966 to June 1969. Higher rates of bronchitis in school-aged children were found in both the intermediate- and high-exposure areas, as compared with the low-exposure area. The results were not completely consistent with the exposure gradient because the rate of bronchitis in the intermediate area was just as high as in the high-pollution area.

Shy and co-workers (Shy, 1970, Shy et al., 1970a,b, 1973) studied the effects of community exposure to NO₂ in residential areas of Chattanooga on respiratory illness rates in families. The incidence of acute respiratory disease was assessed at 2-week intervals during the 1968-69 school year and the respiratory illness rates (adjusted for group differences in family size and composition) were reported to be significantly higher for each family segment (mothers, fathers, children) in the high-NO₂ exposure neighborhood than in the intermediate- and low-NO₂ areas. Although individual area pollution estimates are not available, one part of the high pollution area had an annual average NO₂ level of 286 µg/m³ (0.152 ppm). Areas more distant from the major NO₂ source (TNT plant) had lower levels.

Love et al. (1982) studied acute respiratory disease in the same area during the years 1972-73. Fathers, mothers, school children, and preschool children all showed significantly higher illness rates in the area designated as the high-pollution area during the beginning of 1972. There were almost no significant differences in illness rates during the periods September to December 1972 and January to April 1973. During the period January to June 1972, NO₂ levels (as measured by the continuous chemiluminescent method) ranged from 60.2 µg/m³ (0.032 ppm) in the high area to 28.9 µg/m³ (0.015 ppm) in the low area. However, by the second half of 1972, the exposures in all areas were quite comparable because of reduced emissions. Thus, the results of the study tend to confirm the effect of NO₂ or its by-products on acute respiratory disease.
14.3.2.9 Glendora, California, Study

In another California study, Detels et al (1979, 1981a,b) and Rokaw et al (1980) studied chronic respiratory disease symptoms and lung function in two areas of Los Angeles County. The low-exposure area was Lancaster, a city in the high desert country about 113 km from downtown Los Angeles, which was studied from November 1973 to October 1974. The high-exposure area was Glendora, an area in the Los Angeles basin, which was studied from April 1977 to March 1978. The aerometric exposures for Glendora were estimated from a station in Azusa, about 5 km away. Pollutants measured included total oxidant (ultraviolet absorption method), NOx (Saltzman method), CO (nondispersive infrared spectroscopy method), SO2 (conductimetric method), hydrocarbons (flame ionization detection method), and particles (high-volume TSP method). The 5-year averages were computed for those pollutants with sufficient data. Comparing Lancaster to Glendora, the NO2 levels were 0.033 versus 0.114 ppm, the total oxidant levels were 6.5 versus 11.6 ppm, and the hydrocarbons were 2.9 versus 4.8 ppm. Comparable differences existed for SO2, particles, and sulfate fraction of particles, but the data were only complete for the year 1977. The authors evaluated symptom prevalence of cough, sputum production, wheezing, and frequent chest illness. All symptoms except frequent chest illness showed significantly higher rates in Glendora for both sexes and all three smoking categories.

The two primary weaknesses of the study are (1) the two areas (Lancaster and Glendora) were measured at different times (1974 versus 1977), and (2) the areas are quite different with respect to climate, commuting patterns, altitude, SES, season, and general lifestyle. No specific analyses related to NO2 levels were discussed. The effects of smoking habits were carefully controlled and should not be considered as a serious confounder. The authors also did attempt to control for variability in measurement methods and technicians, and results of this are reported by Tashkin et al (1979).

14.4 STUDIES OF PULMONARY FUNCTION

Pulmonary function studies are part of a comprehensive investigation of the possible effects of any air pollutant. Measurements can be made in the field, they are noninvasive, and their reproducibility has been well documented. Age, height, gender, and presence of
respiratory symptoms are important determinants of lung function. In addition, changes in pulmonary function have been associated with environmental tobacco smoke (Hasselblad et al., 1981), with particulate matter in combination with SO₂ (Dockery et al., 1982, Dassen et al., 1986), and with other factors.

The rest of this section examines epidemiological studies relating NO₂ exposure to pulmonary function. The pulmonary function studies in this section are divided into indoor and outdoor subsections.

14.4.1 Indoor Studies

Several of the studies discussed earlier with regard to respiratory disease symptoms also included evaluations of pulmonary function. Ware et al. (1984), reporting on the Six City Study, described analyses of lung function values using multiple linear regression on the logarithm of the lung function measures. Covariates included sex, height, age, weight, smoking status of each parent, and educational attainment of the parents. Forced expiratory volume in 1 s (FEV₁) values were significantly lower for children of current smokers than for children of nonsmokers at both examinations and were highest for children of ex-smokers. Forced vital capacity (FVC) values were lower for children of nonsmokers than for children of current smokers at both examinations, but the difference was statistically significant only at the first examination. Both the increase in mean FVC and the decrease in mean FEV₁ among children of current smokers were linearly related to daily cigarette consumption. Exposure to gas stoves was associated with reductions of 0.7% in mean FEV₁ and 0.6% in mean FVC at the first examination (p < 0.01), and reductions of 0.3% at the second examination (not significant). The estimated effect of exposure to gas stoves was reduced by approximately 30% after adjustment for parental education. The authors state that the adjustment for parental education may be an over-adjustment, and may partially represent gas stove use because of association between parental education and type of stove. Hasabelnaby et al. (1989) provide estimation formulas for linear regression models that incorporate errors in exposure variables using this data set as an example.

Berkey et al. (1986) used the Six City Study data from children seen at two to five annual visits to evaluate factors affecting pulmonary function growth. Children whose mothers smoked one pack of cigarettes per day had levels of FEV₁ at age 8 years that were
approximately 0.81% lower than children of nonsmoking mothers (p < 0.0001) and had FEV₁ growth rates approximately 0.17% per year lower (p = 0.05). The same data provided no evidence for an effect of gas stove exposure on growth rate.

Neas et al. (1991), discussed earlier, also reported that indoor NO₂ levels were not significantly associated with a decline in children's pulmonary function levels on either of two examinations conducted prior to the indoor monitoring. The ratio between FEV₁ and FVC was actually increased in both boys and girls on both examinations.

Goldstein et al. (1988) reported preliminary data examining effects of acute exposure to NO₂ in inner-city apartments with gas cooking stoves on pulmonary function. Eleven asthmatic and 12 nonasthmatic women and children were monitored for 5 days with a portable continuous NO₂ monitoring instrument held at breathing level that provided 5-min average NO₂ levels. The average levels observed over the 48-h sampling period were over 100 µg/m³ (0.053 ppm) NO₂ in kitchens and over 70 µg/m³ (0.037 ppm) NO₂ in bedrooms, with peak levels significantly higher. Pulmonary function (FEV₁, FVC, functional expiratory volume at 25 to 75% of vital capacity [FEV₂₅₋₇₅%], and peak flow), as well as tracings of the entire flow curve, were monitored at several different points during the exposure. Although the data are limited, in this pilot study it seemed that at 5-min average NO₂ exposures below 0.3 ppm (564 µg/m³), FVC and peak expiratory flow (PEF) were as likely to be increased as decreased, whereas at exposure above 0.3 ppm, FVC and PEF mainly decreased for the adult asthmatic subjects.

Ekwo et al. (1983), discussed earlier, obtained pulmonary function measurements from 89 children whose parents did not smoke and 94 children whose parents smoked, and reported no differences in lung function associated with gas stove use in a cohort of Iowa children 6 to 12 years of age. Dijkstra et al. (1990) examined pulmonary functions in Dutch children in a study discussed earlier. Lung function was measured at the schools. There was a weak, negative association between maximal midexpiratory flow (MMEF) and exposure to NO₂. Forced expiratory volume in 1 s, PEF, and MMEF were all negatively associated with exposure to tobacco smoke. The authors concluded that the study failed to document clear associations between indoor exposure to NO₂ and lung function in Dutch children 6 to 12 years of age.
Lebowitz et al. (1985) studied a cluster sample of 117 middle-class households in Tucson, AZ. Symptom diaries and peak flows were obtained over a 2-year period. Outdoor sampling of O₃, TSP, CO, and NO₂ was measured in or near the clusters. Indoor sampling of O₃, TSP, respirable suspended particles, and CO was measured in a subsample of the homes. Additional information, such as the presence of a gas stove or smoking, also was obtained. The presence of a gas stove was used as a surrogate for indoor NOₓ exposure because it was not measured directly. The relationship of children's peak flow and gas stove use was of borderline significance (p = 0.066) for an analysis excluding TSP, and was not close to significance with TSP included in the analysis. For asthmatic subjects, gas stove use was significantly associated with peak flow decrements (p < 0.001). This was true across smoking groups, but the difference was greatest for smokers. Peak flow in adults was also related to gas stove use, but the level of significance was not given.

14.4.2 Outdoor Studies

Schwartz (1989) studied the effect of air pollution on lung function in U.S. children and youths aged 6 to 24 years. Forced expiratory volume, FEV₁, and peak flow measurements taken as part of the National Health and Nutrition Examination Survey II Study were examined after controlling for age, height, race, gender, body mass, cigarette smoking, and respiratory symptoms. Air pollution measurements were taken from all population-oriented monitors in the U.S. Environmental Protection Agency's (EPA's) SAROADS database. Each person was assigned the average value of each air pollutant for the 365 days preceding the spirogram. Highly significant negative regression coefficients were found for three pollutants (TSP, NO₂, and O₃) with the three lung function measurements. For an increase of NO₂ exposure of 0.015 ppm (28.3 μg/m³), an estimated decrease of about 0.045 L was seen in both FVC and FEV₁.

Vedal et al. (1987) conducted a panel study on 351 children selected from the 1979 Chestnut Ridge cross-sectional study of Pennsylvania elementary school-aged children (mean age = 9.5 years). Peak expiratory flow rate (PEFR) was measured daily in 144 children for 9 consecutive weeks and was regressed against daily maximum hourly ambient concentrations of NO₂, O₃, SO₂, and coefficient of haze. No air pollutant was strongly associated with level of PEFR. All pollutant levels were relatively low, daily maximum hourly NO₂ levels
ranged from 0.006 to 0.042 ppm (12 to 79 μg/m³). No indoor measurements were made, nor were any surrogates for indoor pollution included in the analysis.

As part of the Six City Studies, Dockery et al. (1989b) obtained pulmonary function data during the 1980 to 1981 school year. Respiratory illness and symptom data results were discussed for this study in the earlier studies of respiratory illness section. Only TSP concentration was consistently associated with estimated lower levels of pulmonary function. There was little evidence for an association between lower pulmonary function level and the annual mean concentration of NO₂ or any other pollutant.

In a study discussed earlier, Detels et al. (1979, 1981a,b) and Rokaw et al. (1980) studied lung function in adults in two areas of Los Angeles County—Lancaster, the low NO₂-exposure area, and Glendora, the high NO₂-exposure area. The authors found significantly lower peak flow values in Glendora when compared with Lancaster, and these differences were significant across all smoking categories and both sexes. The percent of subjects with FEV₁ or FVC below 50% of expected was also significantly higher in Glendora. Other lung function measurements showed less significant differences, but the trend was always toward lower values in Glendora. The primary weaknesses of the study were discussed earlier.

14.5 OUTCOMES RESULTING FROM OCCUPATIONAL EXPOSURES

Gamble et al. (1987) studied 232 workers in four diesel bus garages for the effects of NO₂ on acute respiratory illness and pulmonary function. Effects were assessed by an acute respiratory questionnaire and before- and after-shift spirometry. Measurements of NO₂ over the 6- to 7-h shift (using passive Palmes tube samplers) were made for each worker and were collected on the same day as the pulmonary function tests and questionnaires. Other irritant gases were measured and were well below federal standards. Mean NO₂ levels over a shift ranged from 0.56 (SD = 0.38) ppm NO₂ in the highest garage to 0.13 (SD = 0.06) ppm NO₂ in the lowest garage. Short-term NO₂ measurements indicated levels above 1 ppm as being common. The authors report that the prevalence of acute respiratory symptoms were elevated above expected in the high-exposure (>0.3 ppm) group only. No reduction in pulmonary function was associated with exposure.

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Gamble et al (1983) examined chronic respiratory effects in 259 sodium chloride miners. The Medical Research Council respiratory symptom questionnaire containing smoking history was administered by trained interviewers. A chest X-ray and spirometry were also conducted. Personal samples of NO₂ and respirable particles for jobs in each mine were used to estimate cumulative exposure. The cumulative mean exposure ranged from a low of 0.2 (SD = 0.1) ppm NO₂ to a high of 2.5 (SD = 1.3) ppm NO₂. Diesel emissions were the principal NO₂ source. The author reported that although cough was associated with age and smoking and dyspnea was associated with age, neither symptom was associated with indices of air pollution exposure (e.g., years worked, estimated cumulative NO₂ or respiratory particle exposure). Reduced pulmonary function showed no association with NO₂ exposure.

Robertson et al (1984) reported on a 4-year study of lung function in 560 British coal miners. Overall mean work shift average NO₂ levels at nine coal mine sites ranged from 0.02 to 0.06 ppm (38 to 113 μg/m³), and nitric oxide (NO) levels ranged from 0.13 to 1.19 ppm. No relationship was found between exposure and decline in FEV₁ or respiratory symptoms. Jacobsen et al (1988) conducted a more extensive investigation on nearly 20,000 miners at the same nine British coal mines to examine whether long-term exposure to low concentration of NO₂ and NO were associated with increased susceptibility to respiratory infections. The NOₓ source consisted of diesel emissions and blasting. Work-shift median levels were 0.2 ppm NO and 0.03 ppm NO₂. This complete and intensive study had problems with misclassification of exposure and outcome that are not uncommon when existing data are used for purposes that were not foreseen when the data were collected. The authors concluded that the long-term exposure to the levels above do not detectably increase the chance that miners will absent themselves from work because of a chest infection.

Douglas et al (1989) report data between 1955 and 1987 on 17 patients examined at the Mayo Clinic for silo-filler's disease shortly after exposure to silo gas (NO₂ levels ranged from 200 to 2,000 ppm). Health outcomes evaluated included hypoxemia, transient obstruction of the airways, and death. Meulenbelt and Sangster (1990) indicate that after a symptom-free episode immediately following exposure to NO₂, a severe respiratory failure can develop several hours later. The principal symptom, breathlessness, may become manifest 6 or more hours following exposure as a result of adult respiratory distress.

Other studies on high exposures (Lowry and Schuman, 1956, Grayson, 1956, Gregory et al., 1969, Yockey et al., 1980) are reviewed in U.S. Environmental Protection Agency (1982a). Levels above 300 ppm in occupational settings are likely to result in rapid death, whereas levels of 150 to 200 ppm may lead to death 2 to 3 weeks after exposure. Levels between 25 and 100 ppm are usually, but not always, followed by essentially complete recovery.

14.6 SYNTHESIS OF THE EVIDENCE

The weight of the evidence does not indicate that NO₂ exposure at levels reported in the studies reviewed here has any consistent effect on pulmonary function of a biologically significant magnitude. Many of the indoor studies, however, do suggest an increase in respiratory morbidity in children from exposure to NO₂ levels measured in these studies, although the effects reported did not reach statistical significance (p < 0.05) in the majority of the studies. The consistency of results across the indoor studies is examined and the evidence is synthesized in a quantitative analysis presented below.

In order to compare available studies on respiratory effects of NO₂, a common endpoint for a health outcome effect was defined, and then each indoor study was compared with this standard endpoint. The endpoint chosen was the presence of lower respiratory symptoms and illness in children aged 5 to 12 years. An attempt was made to include as many indoor studies as possible. The requirements for inclusion were (1) the health endpoint measured must be reasonably close to the standard endpoint, (2) significant exposure differences between subjects must exist and some estimate of exposure must be available, and (3) an odds ratio for a specified exposure estimate must have been calculated, or data must be presented so that an odds ratio can be calculated.

14.6.1 Health Outcome Measures

One major concern in attempting to interpret these studies is that the respiratory morbidity variables measured in the various studies may represent differing health outcomes.
that may have different mechanisms of causation related to NO₂ exposure estimates. For example, the origin of cough may be different than that of wheeze (i.e., the agents that cause them may be different). Various disease syndromes were evaluated in different studies: croup, bronchitis, bronchiolitis, asthma, and pneumonia. The instruments measuring health outcomes in the studies are also different. If the similarity between the outcome measures between and within the studies is not adequate, the potential interpretation of a quantitative analysis may be limited. Are the respiratory morbidity measures in these studies of children a relatively similar outcome measure in both a statistical sense and as a biological end point across the studies? This discussion considers this question by evaluating outcome measures used in the various NO₂ studies, similarities in outcome measures in lower respiratory illness studies in children, the use of questionnaires as instruments to measure lower respiratory morbidity, and, in general, measures of lower respiratory morbidity.

The studies in the quantitative analysis that follows used health outcome measures that provide an indication of the state of respiratory health of various samples of children aged 5 to 12 years old. The NO₂ studies utilized standard questionnaires to evaluate lower respiratory health in children. Diagnoses of specific respiratory diseases such as bronchiolitis or asthma were not made. The factor of importance here is that an attempt was made to measure some aspect of lower respiratory morbidity. Table 14-18 lists the health outcome measures for each study considered. Whereas specific measures such as colds going to chest (Melia et al., 1977), chest congestion, and phlegm with colds (Ekwo et al., 1983) are used to provide measures of lower respiratory morbidity, other measures use indexes, grouped responses, or combined indicators of lower respiratory morbidity, some of which include measures such as colds going to chest.

In the Melia et al. (1977, 1979, 1980, 1982a) studies, a self-administered questionnaire was completed by parents of children in the study. Questions were asked about each child’s respiratory disease episodes and symptoms during the previous 12 mo. The respiratory symptoms and diseases surveyed included asthma in the last year, wheeze, bronchitis in the last year, cough (night or day), and colds going to chest. Irwig et al. (1975) examined the association of these reported questions with objectively measured reduced peak flow rates in a sample of the children examined in 1973. Because the answers to the morbidity questions were related to the reduced adjusted mean peak flow rates (an indicator of decreased
<table>
<thead>
<tr>
<th>Reference</th>
<th>Health Outcome Used in Meta-Analysis</th>
<th>Method&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NO&lt;sub&gt;2&lt;/sub&gt; Exposure Measure Used in Analysis&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Age (years)</th>
<th>Sample Size</th>
<th>Where/When</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melia et al (1977)</td>
<td>Colds going to chest showed a prevalence of 26 8-19 8%</td>
<td>Symptoms during past 12 mo recalled by child's parent in completing respiratory symptoms questionnaire</td>
<td>Gas stove vs electric stove</td>
<td>6-11</td>
<td>5,658</td>
<td>28 Areas of England and Scotland (1973)</td>
</tr>
<tr>
<td>Melia et al (1979)</td>
<td>Responses to respiratory questions grouped into (a) none or (b) one or more symptoms or disease types Colds going to chest (26 4-19 6%) showed the highest prevalence, followed by wheeze (10 1-6 2%), cough, and episodes of asthma or bronchitis in last year</td>
<td>As above</td>
<td>Gas stove vs electric stove</td>
<td>5-10</td>
<td>4,827</td>
<td>27 areas of England and Scotland (1977)</td>
</tr>
<tr>
<td>Melia et al (1980)</td>
<td>Group response to respiratory questions as above</td>
<td>As above</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt; measured with Palmes tubes Gas stove homes only</td>
<td>6-7</td>
<td>103</td>
<td>Middlesborough, England (1978)</td>
</tr>
<tr>
<td>Florey et al (1979)</td>
<td>As above</td>
<td>As above</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt; measured with Palmes tubes Gas stove homes only</td>
<td>5-6</td>
<td>188</td>
<td>Middlesborough, England (1980)</td>
</tr>
<tr>
<td>Goldstein et al (1979)</td>
<td>As above</td>
<td>As above</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt; measured with Palmes tubes Gas stove homes only</td>
<td>5-6</td>
<td>188</td>
<td>Middlesborough, England (1980)</td>
</tr>
<tr>
<td>Melia et al (1982a)</td>
<td>As above</td>
<td>As above</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt; measured with Palmes tubes Gas stove homes only</td>
<td>5-6</td>
<td>188</td>
<td>Middlesborough, England (1980)</td>
</tr>
<tr>
<td>Ware et al (1984)</td>
<td>Lower respiratory illness index (index of respiratory health) indicating during past year the presence of (a) bronchitis, (b) respiratory illness that kept the child home 3 days or more, or (c) persistent cough for 3 mo of the year</td>
<td>Questionnaire (Ferris, 1978) completed by parent for symptoms during previous 12 mo</td>
<td>Gas vs electric</td>
<td>6-10</td>
<td>8,240</td>
<td>Six U.S. cities (1974-1979)</td>
</tr>
<tr>
<td>Reference</td>
<td>Health Outcome Used in Meta-Analysis</td>
<td>Methoda</td>
<td>NO₂ Exposure Measure Used in Analysis</td>
<td>Age (years)</td>
<td>Sample Size</td>
<td>Where/When</td>
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<tr>
<td>Neas et al (1990, 1991)</td>
<td>Combined indicator of one or more lower respiratory symptoms as defined. The highest prevalences were for chronic phlegm and wheeze. The other symptoms in the index are shortness of breath, chronic cough, and bronchitis. Chest illness reflects a restriction of the child's activities for 3 or more days.</td>
<td>Symptom questionnaire completed by parent for the year during which measurements of NO₂ were taken</td>
<td>NO₂ measured with Palmes tubes and electric stoves</td>
<td>7-11</td>
<td>1,286</td>
<td>Six U S cities (1983-1986)</td>
</tr>
<tr>
<td>Ekwo et al (1983)</td>
<td>Chest congestion and phlegm with colds</td>
<td>Questionnaire (ATS) completed by parent</td>
<td>Gas stove vs electric stove</td>
<td>6-12</td>
<td>1,138</td>
<td>Iowa City, Iowa</td>
</tr>
<tr>
<td>Dykstra et al (1990)</td>
<td>Respiratory illness combination variable of presence of one or more of cough, wheeze, or asthma</td>
<td>Questionnaire (WHO) completed by parent</td>
<td>NO₂ measured with Palmes tubes and electric appliances</td>
<td>6-12</td>
<td>775</td>
<td>Netherlands (1986)</td>
</tr>
<tr>
<td>Brunekreef et al (1987)</td>
<td>Respiratory illness</td>
<td>Telephone interview by nurse epidemiologist</td>
<td>Gas stove vs electric stove</td>
<td>&lt;12</td>
<td>176</td>
<td>Columbus, Ohio (1978)</td>
</tr>
</tbody>
</table>

aATS = American Thoracic Society
bWHO = World Health Organization
bNO₂ = Nitrogen dioxide
respiratory function), this suggested that the questions may be indicators of lower respiratory morbidity (whereas others such as earache or hospitalization for upper respiratory disease in the last 12 mo may not). Thus, the more subjective morbidity measurements by the questionnaires were supported to some extent by more direct objective lung function measurements. Melia et al. (1977) indicated that the highest prevalence was for colds going to the chest (approximately 25%), followed by wheeze (approximately 10%). Bronchitis and asthma episodes in the past year had respective prevalences of less than 6% and 3%. The 1977 studies showed very similar prevalences (Melia et al., 1979) to the 1973 data, with the prevalence of asthma and bronchitis episodes both under 4% and colds going to chest at approximately 25% in 1977.

For these Melia studies, two indicators, colds going to chest and wheeze, provide the major contribution to the combined indicator for lower respiratory health. Disease indicators such as asthma and bronchitis episodes in the past year, although measures of lower respiratory disease, may play a smaller role. The Melia et al. (1979) study provides data that allow the development of graphs of the marginal likelihood functions (see Figure 14-4) of the odds ratios for symptoms and diseases and any respiratory illness for the combined indicator of boys and girls. The odds ratio for colds to chest is 1.21 (SD of the log [OR] = 0.0675) and for any respiratory illness is 1.24 (SD of the log [OR] = 0.0703). This demonstrates the similarity of these two outcome measures. More specifically, it shows how colds going to the chest represent an important component of Melia’s respiratory index.

In the quantitative analysis, the outcome measure used is colds going to chest for the Melia et al. (1977) study and the lower respiratory index for the other Melia studies.

Other NO₂ studies use different indexes or combinations that include symptoms such as chronic phlegm and wheeze, persistent cough, respiratory illness, and asthma and bronchitis (Ware et al., 1984, Neas et al., 1990, 1991, Dijkstra et al., 1990, Keller et al., 1979b). These symptoms in the indexes are all indicators of involvement of the lower respiratory tract and, as such, form a measure of lower respiratory health. In most cases, chest colds and wheeze, or similar indicators, are the prominent symptoms in the index. Use of the same index in several of the NO₂ studies and similar indexes in the other NO₂ studies provides consistency in the outcome measurements of the studies.
Figure 14-4. For the Melia et al. (1979) study, a graph of the marginal likelihood function of the odds ratios for combined gender (boys and girls) of the respiratory illness outcome measures developed by EPA.

How do the outcome measures in the NO₂ studies compare with indicators of lower respiratory health status done in other studies? The studies of lower respiratory illness (unrelated to NO₂ exposures) discussed in Section 14.3 were based on visits to physicians. The signs and symptoms that were most common in those studies, wet cough and wheeze, are similar to the most common end points in the NO₂ studies, that is, colds going to chest and wheeze. Thus, to an extent, the NO₂ respiratory studies are providing a measure of lower respiratory health somewhat equivalent to these other studies. Specific pediatric diseases and agents such as bronchiolitis and RSV are investigated in these other studies and not in the NO₂ respiratory studies. The overall pattern and incidence of lower respiratory
illness are considered to be consistent in different geographic regions over time (Wright et al., 1989, Denny and Clyde, 1986, McConnochie et al., 1988)

Lower respiratory illness is an entity commonly defined by criteria for clinical diagnosis (Wright et al., 1989) Key studies (Monto et al., 1971, Taussig et al., 1989, Glezen et al., 1971, Gardner et al., 1984, and Maletzky et al., 1971) consider the classification of lower respiratory disease and surveillance methods Standardized and uniformly accepted clinical criteria have not been developed for the illnesses considered to make up lower respiratory illness, that is, for croup, tracheobronchitis, bronchiolitis, and pneumonia The extent to which the diagnostic criteria for lower respiratory illness were documented varies in these studies The characteristic signs and symptoms are wheeze, phlegm from chest, and deep cough These are basic respiratory symptoms indicative of lower respiratory involvement Samet et al. (1992) classified illnesses as lower respiratory tract illness if wet cough or wheeze occurred at any time during the illness exposure Bates et al. (1990) note that differentiation among "acute bronchitis", "acute bronchiolitis", and acute exacerbation of "asthma" is clinically difficult, therefore, it is unrealistic to expect finite and noncontroversial differentiation among these conditions

For the purpose of analysis of illness rates, children with lower respiratory morbidity may be considered to belong to a single population with a similar illness. Although different diseases are grouped together as lower respiratory illness (such as croup, bronchiolitis, and pneumonia), the lower respiratory illness syndrome designation (Monto et al., 1971) is a means of grouping these illnesses of similar pattern for analytic purposes It is relatively easy to classify illnesses on the basis of anatomic area of involvement Graham (1990) states that classification by anatomic site remains the preferred system for most physicians and is compatible with the International Classification of Diseases system Such a classification is often a good indication of the severity of illness

The respiratory questionnaires used in the NO2 studies determined the state of lower respiratory health in the subjects by tabulating responses to questions on respiratory symptoms such as cough and wheeze and on respiratory illnesses such as asthma and bronchitis Symptom reporting provides subjective evidence of respiratory infection and diseases such as bronchitis Diseases are characterized by groups of symptoms Disease end points such as asthma and bronchiolitis may have similar defining symptoms Lower
respiratory morbidity reflects a broader grouping, within which the ability to differentiate between the wheeze of asthma and wheeze related to other causes or infections in the pediatric age group may not be readily possible. Wright et al. (1989) note that wheeze is recorded as occurring for several diagnoses to include croup, bronchiolitis, bronchitis, and pneumonia. Questions of asthma in the past year may not represent a disease-defining measure as much as a measure of wheeze. Additionally, asthma may be related to the occurrence of infection as a precipitating cause, thus further confusing differences between symptoms and disease.

The NO₂ studies of lower respiratory health are implemented by use of standardized questionnaires filled out by the parent for illness and symptoms during the past 12 mo. The purpose of a respiratory symptom questionnaire is to compare prevalence of symptoms (Fairbarn et al., 1959) and to follow their rate of progression in populations. This facilitates the quantitative comparison between groups. This, however, contrasts fundamentally with the usual objective of clinical medicine, that is, a decision about an individual providing an accurate diagnosis of the patient's condition to determine the correct treatment or prognosis. In surveys of prevalence, valid comparisons can be made with less accurate data, providing that a sufficient population is studied and that inaccuracies of reporting are randomly distributed between the groups being compared. Standardized questionnaires permit meaningful comparisons of the results of prevalence surveys (Samet, 1978). Mitchell et al. (1976) note that the information obtained from the subjects in a respiratory health study that used a questionnaire are probably an accurate reflection of the lung disease experienced by the total population of these communities.

Childhood lower respiratory morbidity is characterized by a grouping of similar symptoms and diseases that reflect changes located anatomically in the lower respiratory tract. This characterization represents an indication of severity of the respiratory morbidity status of the children and is a multifaceted approach to respiratory health in a population living under natural conditions. Lower respiratory morbidity is the combination of different respiratory effects that have in common an evaluation of the morbidity status of the lower respiratory tract. The measure of effect on the lower respiratory tract varied among the studies; the indicators, however, are conventional symptom and illness outcomes. The symptoms are tabulated from similar standardized questionnaires (Ferris, 1978) and are
directed at eliciting the same basic data—an indication of the presence of illness or infection in the lower respiratory tract.

Although the use of identical health outcome measures would be most desirable, the level of similarity and common elements between the outcome measures in the NO₂ studies provide some confidence in their use in the quantitative analysis. However, the symptoms and illnesses combined are to some extent different and could indeed reflect different underlying processes. Thus, caution is necessary in interpreting the analysis. This concern is addressed further later in this section as part of the statistical aspects of the random-effects model.

14.6.2 Biologically Plausible Hypothesis

The human clinical and animal toxicological studies in Chapters 13 and 15 that examined NO₂ effects on aspects of the respiratory host defense system provide a biologically plausible hypothesis compatible with the relationship seen between respiratory symptoms and morbidity and NO₂ exposure in epidemiologic studies. However, research gaps in both animal toxicological and clinical studies exist, indicating the need for further research efforts. A brief discussion is presented here.

The lung is one of the sites of microbial infection. Although many types of microorganisms are implicated in respiratory infection, viruses represent a major cause of respiratory disease, particularly for infants and children. In a viral respiratory infection, viral replication produces injury and, thus, the signs and symptoms of respiratory illness (Douglas, 1986). The respiratory system has several defense mechanisms against inhaled infectious and chemical agents. Host defense mechanisms consist of nonspecific and specific components. The nonspecific aspects include mucociliary clearance and alveolar macrophages, whereas humoral and cellular immunity offer specific defenses. The immune system functions through a sequence of events, starting with the nonspecific components followed by responses by the specific components. The immune system is a principal factor in the host's interaction with infectious agents such as viruses and in the host's ability to contain and/or eradicate the establishment of infection. To some extent, an increase of reported respiratory symptoms in some epidemiology studies may be an indication of the ability of the respiratory host-defense mechanism to either overcome an infection or to limit
its severity. Nitrogen dioxide may affect the immune system in such a way that one or several aspects of the immune system do not function at a level sufficient to limit the extent or occurrence of infection. Nitrogen dioxide may to some degree influence respiratory symptom rates by direct toxic mechanisms. Meulenberg and Sangster (1990) note that NO₂ may cause direct epithelial damage that could increase susceptibility to infection.

The evidence from animal toxicological and human clinical studies of host defenses provide a rationale for investigating the relation between exposure to NO₂ and an increase in frequency and severity of respiratory symptoms and/or infections in humans. When microorganisms attack a lung that has been exposed to NO₂, host defense mechanisms altered by the NO₂ exposure may result in increased severity or rate of respiratory illness. Although the host defense system reacts both very specifically and generally to the challenge, the overall response in humans is expressed as a generalized demonstration of signs and symptoms that may be associated with a site such as the lower respiratory tract and also may be reported or objectively discerned as a general outcome such as a chest cold, cough, or an incident of asthma or bronchitis.

14.6.3 Publication Bias

Publication bias, also known as the "file drawer problem" (Rosenthal, 1979), is the result of the increased likelihood of publication of studies that have positive results, leading to a bias in the literature reviewed towards positive results. There are two factors that make this bias less likely for epidemiological studies. First, the studies are expensive, well publicized, and the results are usually published in order to give credit to the researchers involved. Second, many of the studies included in this section did not produce statistically significant findings, indicating that there was not a problem in publishing negative studies. However, some studies are necessarily excluded because they provide insufficient information. Although this can lead to bias, there is little that can be done to correct for this problem. This problem is not normally referred to as publication bias, but it is a similar problem.
14.6.4 Quantitative Analysis

In order to compare available indoor studies on respiratory effects of NO₂, a common end point for a health outcome effect was defined, and then each study was compared with this standard end point. The end point chosen was the presence of lower respiratory symptoms and illness in children aged 5 to 12 years. An assumption has been made that the relative odds of developing this lower respiratory morbidity outcome is similar across this age range as a function of NO₂ exposure, even though the actual rates may not be (This is a common assumption in many analyses).

An attempt was made to include as many indoor studies as possible. The requirements for inclusion were (1) the health end point measured must be reasonably close to the standard end point, (2) significant exposure differences between subjects must exist and some estimate of exposure must be available, and (3) an odds ratio for a specified exposure estimate must have been calculated, or data must be presented so that an odds ratio can be calculated. All studies that met the criteria for inclusion were included. Quality scores were not assigned to the studies. There is no evidence that the use of quality scores improves estimates (see Emerson et al., 1990). Separate analyses for studies that had specific features that might be considered in quality scores, such as measured NO₂ instead of surrogate estimates, are examined later as part of the analysis.

The term "exposure" is used to denote the pattern of concentrations of a pollutant in air through which an individual passes during a fixed period of time. The term "exposure estimate" will be used for any measure that estimates some function of that pattern, such as the arithmetic average. Exposure estimates include both personal monitors used for some fraction of the total exposure time as well as fixed monitors located in rooms known to be occupied by individuals for some fraction of the time. Some estimates may be based on averages from sites with similar exposure characteristics, such as presence of a gas stove. Thus, exposure estimates are defined as an estimate based on some data of the exposure of the group being studied. Such an estimate cannot perfectly characterize true exposure. See Section 7.3 for a more detailed discussion on exposure to NO₂.

The goal was to estimate the odds ratio corresponding to an increase in concentration level of 0.015 ppm (28.3 μg/m³) as an estimate of NO₂ exposure. Studies with NO₂ exposure measures used 1- to 2-week integrated indoor measurements by Palmes passive...
diffusion tubes that provide an estimate for chronic exposure. Studies that characterized NO₂ exposure by differences between gas stove and electric stove use estimated a value for this difference. Four studies measured NO₂ levels. Five studies estimated exposure to NO₂ based on the presence or absence of a gas stove as a surrogate for NO₂ measurements. Exposure measurement error related to use of a surrogate was discussed earlier. To use these studies in the meta-analysis, numerical values of exposure estimates must be determined. Three of the studies were conducted in the United States (Ware et al., 1984, Ekwo et al., 1983, and Keller et al., 1979a,b) and two in Great Britain (Meha et al., 1977, 1979).

Limited data are available from which to estimate NO₂ exposure values. Appropriate estimates ideally would be country specific, current with the studies in location and time, and derived from a representative sample that appropriately characterizes the exposure. The Neas et al. (1991) study may be an appropriate source to estimate a value for the three United States studies. This study had a large sample size, measured levels during two seasons, and was conducted in the same United States cities as Ware et al. (1984) was. Neas et al. (1991) reported a housewide average difference of 0.0173 ppm (32.5 μg/m³) NO₂ between homes with electric stoves and homes with gas stoves with pilot lights. In two British studies (Melia et al., 1980, 1982a,b) conducted by the same authors that conducted Melia et al. (1977, 1979), data on the difference in levels between homes with electric stoves and homes with gas stoves is provided. Melia et al. (1980, 1982a,b) provide data that indicate 0.0165 ppm (31.1 μg/m³) as an estimate of this difference in average NO₂ concentrations in bedrooms of homes in Britain.

The effects studied may be related to peak exposures, average exposures, or a combination of the two. To the extent that health effects depend on peak exposures rather than average exposures, the above exposure estimates introduce exposure measurement error. These studies cannot distinguish between the relative contributions of peak and average exposures and their relationship with the observed health effects. Additionally, a by-product of NO₂, HONO, may be a factor in observed effects, however, limited health and aerometric data are available that examine such possibilities.

The above factors are used when evaluating each study. The British studies provide several estimates of the subject odds ratio. Melia et al. (1977) studied children aged 6 to
11 years and developed an indicator of the presence of at least one of a group of symptoms including cough, colds going to the chest, and bronchitis. The symptom reported most often was "colds going to chest", which was used as an indicator of lower respiratory morbidity. This study did not measure NO₂ exposure, and so the assumption was made that the increase in NO₂ exposure from gas stove use in England was reasonably similar to that in the other British studies that measured NO₂, that is, an increase of 0.0165 ppm (31.1 μg/m³). The estimated odds ratio was 1.31, with 95% confidence limits of 1.16 and 1.48. After adjusting to a standard increase of 0.015 ppm (28.3 μg/m³), the odds ratio became 1.28 with 95% confidence limits of 1.14 and 1.43. No adjustment was made for parental smoking in this study.

The cross-sectional data reported by Melia et al. (1979) on children aged 5 to 10 years also was employed to estimate an odds ratio, although no exposure estimates were made. The presence or absence of a gas stove was used as a surrogate as in the Melia et al. (1977) study. The estimated odds ratio was 1.24, with 95% confidence limits of 1.09 and 1.42. After adjusting to a standard NO₂ increase of 0.015 ppm (28.3 μg/m³), the odds ratio became 1.22 with 95% confidence limits of 1.08 and 1.37.

Melia et al. (1980) studied children aged 6 to 7 years and measured bedroom NO₂ levels for the exposure estimate. This study applied the same combined health end point as the previous study. The estimated odds ratio for an NO₂ increase of 0.015 ppm (28.3 μg/m³) was 1.49 with 95% confidence limits of 1.04 and 2.14.

Melia et al. (1982a) studied children aged 5 to 6 years, measured NO₂ exposure in the bedroom, and also applied the same combined health end point. The 10th and 90th percentiles of the weekly measured concentrations were 0.009 and 0.065 ppm NO₂, respectively, in bedrooms (Melia et al., 1982b). The estimated odds ratio for an NO₂ increase of 0.015 ppm was 1.11, with 95% confidence limits of 0.84 and 1.46.

In the first Six City study cohort, Ware et al. (1984) reported an index of respiratory illness. Exposure to NO₂ was based on the presence or absence of a gas stove (0.0173 ppm [32.5 μg/m³]). The estimated odds ratio was 1.08 with 95% confidence limits of 0.97 and 1.19. After adjusting to a standard NO₂ increase of 0.015 ppm (28.3 μg/m³), the odds ratio became 1.07 with 95% confidence limits of 0.98 and 1.17.
A second cohort of subjects in the Six City study was initially reported on by Dockery et al. (1989a). This cohort of children aged 7 to 11 years was then reinterviewed after indoor NO$_2$ measurements were made, and the results of this analysis were reported by Neas et al. (1990, 1991). The 10th and 90th percentiles of the weekly measured concentrations were 0.008 and 0.033 ppm NO$_2$, respectively in bedrooms (Neas et al., 1991). The estimated odds ratio for an increase in the presence of any respiratory symptom resulting from an increase in NO$_2$ exposure of 0.015 ppm (28.3 μg/m$^3$) was 1.40, with 95% confidence limits of 1.14 and 1.72.

Ekwo et al. (1983) studied several respiratory illness end points from children surveyed at ages 6 to 12 years. No exposure measurements were obtained, and the exposure was based on the presence or absence of a gas stove (0.0173 ppm [32.5 μg/m$^3$]). None of the end points matched the end point of interest closely. The two most similar end points were hospitalization for chest illness before age 2 and chest congestion and phlegm with colds. The estimated odds ratio for hospitalization was 2.40. The estimated confidence limits for cough and phlegm with colds was 1.09, with 95% confidence limits of 0.82 and 1.45. This last symptom appears to be most similar to the end point of interest, and so it was included in the synthesis.

The data presented by Dijkstra et al. (1990) on the study in the Netherlands were analyzed and gave an estimated odds ratio of 0.94 for an increase of 0.015 ppm (28.3 μg/m$^3$) in NO$_2$ exposure. The 95% confidence limits were 0.70 and 1.27. The study had measured NO$_2$ exposure data, but the EPA analysis did not adjust for covariates because the covariates were not included in the tables that included NO$_2$ exposure.

Keller et al. (1979b) did not find any statistically significant changes in respiratory disease associated with gas stove use, but the unadjusted estimated odds ratio for lower respiratory illness was 0.72, with 95% confidence limits of 0.30 and 1.74. Assuming that the NO$_2$ exposure increase was 0.0173 ppm (32.5 μg/m$^3$), the odds ratio was adjusted to an exposure of 0.015 ppm (28.3 μg/m$^3$). This resulted in an odds ratio of 0.75 with 95% confidence limits of 0.35 and 1.62.

Three studies with sufficient information for analysis were excluded from the synthesis. The Berwick et al. (1989) analysis has been criticized for its lack of consistency across age groups, which may have resulted from the very small sample sizes. The Swiss study...
(Braun-Fahrlaender et al., 1989, 1992) examined end points that might not be considered as
similar to the other studies, such as upper respiratory disease, breathing difficulties, and
duration of various respiratory measures. The Melia et al. (1988, 1990) study did not
demonstrate significant estimated NO₂ exposure differences between the two groups
contrasted (0.0034 ppm [6.4 μg/m³]). These differences estimated in exposure were much
smaller than those seen for any other study of gas stove exposure. This may reflect use of
gas ranges without pilot lights and changes in cooking practices such as increased use of
microwave ranges (see Section 7-3). If the relative risk were adjusted for an increase of
0.015 ppm (28.3 μg/m³), the relative risk would be about 1.29, which is comparable to the
odds ratios seen in the other studies. Because the difference in exposure groups was so
small, requiring a very large adjustment, it was decided not to combine this study with the
other studies. For these reasons, the above studies were not included in the synthesis.
These studies, however, qualitatively support the results of the synthesis.

Graphs of the odds ratio from each indoor study included in the quantitative analysis
are depicted in Figure 14-5. Each curve can be given one of three interpretations: (1) the
normal approximation to the marginal likelihood of the logarithm of the odds ratio,
(2) a distribution such that the 2.5 percentile and the 97.5 percentile points of the distribution
are the 95% confidence limits of the estimated odds ratio, and (3) the posterior for the odds
ratio for a particular study given a flat prior on the log-odds ratio. The basic information for
each curve is provided in Table 14-19.

Synthesizing evidence (often referred to as meta-analysis) is not new, having been used
as early as 1904 (Pearson, 1904). Sacks et al. (1987) defines meta-analysis as a discipline
that critically reviews and statistically combines the results of previous research. Meta-
analyses are being used much more frequently now, as indicated by Mann (1990). For
example, the National Research Council (1986) combined evidence on the effect of
environmental tobacco smoke on lung cancer using Peto's method as described by Yusuf
et al. (1985). Also, several methods for combining clinical trials were discussed by Laird
and Mosteller (1990). The evidence to be combined in this section comes from
epidemiological studies and, as a result, some of the methods used for clinical trials are not
appropriate for this section.
Figure 14-5. U.S. Environmental Protection Agency meta-analysis of indoor epidemiologic studies of nitrogen dioxide exposure effects on respiratory disease in children 5 to 12 years old. Each curve can be treated as a likelihood function or posterior probability distribution. If treated as a likelihood function, the 95% confidence limits for the odds ratio can be calculated as those two points on the horizontal axis for which 95% of the area under the curve is contained between the two points. If treated as a posterior probability distribution, then the area under the curve between any two points is the probability that the odds ratio lies between those two points.

Two basic models are employed in order to combine evidence (Hasselblad et al, 1992). The first model assumes that each study estimates the same fixed, but unknown, parameter. Most methods of combining evidence make this assumption. One of the earliest attempts to combine data using a fixed-effects model was given by Birge (1932). His method weights the estimates inversely by their variances and produces a combined estimate and associated confidence limits. Other methods include the Mantel-Haenszel method (Mantel and Haenszel, 1959), which is used to combine contingency tables. Recently, Bayesian methods
TABLE 14-19. SUMMARY OF ODDS RATIOS FROM INDOOR STUDIES OF THE EFFECTS OF NITROGEN DIOXIDE INCREASED BY 0.015 ppm

<table>
<thead>
<tr>
<th>Authors</th>
<th>Estimated Odds Ratio</th>
<th>2.5 and 97.5 Percentiles (Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mella et al (1977)</td>
<td>1.28</td>
<td>1.14 to 1.43</td>
</tr>
<tr>
<td>Mella et al (1979)</td>
<td>1.22</td>
<td>1.08 to 1.37</td>
</tr>
<tr>
<td>Mella et al (1980)</td>
<td>1.49</td>
<td>1.04 to 2.14</td>
</tr>
<tr>
<td>Mella et al (1982a)</td>
<td>1.11</td>
<td>0.84 to 1.46</td>
</tr>
<tr>
<td>Ware et al (1984)</td>
<td>1.07</td>
<td>0.98 to 1.17</td>
</tr>
<tr>
<td>Neas et al (1991)</td>
<td>1.40</td>
<td>1.14 to 1.72</td>
</tr>
<tr>
<td>Eijkstra et al (1990)</td>
<td>1.09</td>
<td>0.82 to 1.45</td>
</tr>
<tr>
<td>Keller et al (1979b)</td>
<td>0.75</td>
<td>0.35 to 1.62</td>
</tr>
</tbody>
</table>

have been used to combine evidence, and methods particularly appropriate to these kinds of studies were described by Eddy (1989) and Eddy et al (1990a,b) Bayesian analyses require the choice of a prior distribution for the parameter of interest, which is often a noninformative prior. A noninformative prior is one that, prior to seeing the evidence, favors no value of the parameter over any other. The interesting fact about use of these methods is that, for the data sets considered in Table 14-19, the results of the computations were nearly identical. This is because the (marginal) likelihood for the odds ratio is closely approximated by a lognormal curve. The interpretations of these curves are different, as described earlier.

The second basic model assumes that the parameter of interest is not fixed, but is itself a random variable from a distribution. The value of this random variable is different for each study, but each study gives some information about the mean of the distribution. These models go by several names, including random-effects models, mixed models, two-stage models, or hierarchical models. The purpose of a random-effects model is to relax the assumption that each study is estimating exactly the same parameter. This idea is not new, having been discussed by Cochran (1937). For a discussion of the interpretation of random-effects models in clinical trials and several methods of estimating the parameters of these models, see DerSimonian and Laird (1986). If the studies being combined tend to estimate the same parameter, then the results using a random-effects model will approach the results using a fixed-effects model. On the other hand, if the studies are estimating very different
parameters, then the confidence limits will tend to be much broader than those obtained from a fixed-effects model.

The nine indoor studies described earlier (Tables 14-18 and 14-19) were combined using both kinds of models. The results using a fixed-effects model are labeled "fixed", and results of the random-effects model are labeled "random" (see Figure 14-5). Methods for estimating the parameters of a random-effects model were described by DerSimonian and Laird (1986) and Eddy et al. (1992). The results of the analyses are provided in Table 14-20.

**TABLE 14-20. U.S. ENVIRONMENTAL PROTECTION AGENCY COMBINED ANALYSES OF INDOOR STUDIES ON RESPIRATORY ILLNESS EFFECTS OF NITROGEN DIOXIDE INCREASED BY 0.015 ppm**

<table>
<thead>
<tr>
<th>Groupa</th>
<th>Number of Studies</th>
<th>Odds Ratio</th>
<th>Confidence Interval</th>
<th>Odds Ratio</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>9</td>
<td>1.17</td>
<td>1.11 to 1.23</td>
<td>1.18</td>
<td>1.08 to 1.28</td>
</tr>
<tr>
<td>United States</td>
<td>4</td>
<td>1.11</td>
<td>1.02 to 1.20</td>
<td>1.13</td>
<td>0.97 to 1.32</td>
</tr>
<tr>
<td>British</td>
<td>4</td>
<td>1.25</td>
<td>1.15 to 1.35</td>
<td>1.25</td>
<td>1.13 to 1.37</td>
</tr>
<tr>
<td>Measured NO₂</td>
<td>4</td>
<td>1.23</td>
<td>1.08 to 1.41</td>
<td>1.22</td>
<td>0.99 to 1.50</td>
</tr>
<tr>
<td>Gas stove surrogate</td>
<td>5</td>
<td>1.15</td>
<td>1.09 to 1.23</td>
<td>1.16</td>
<td>1.03 to 1.30</td>
</tr>
<tr>
<td>SES adjusted</td>
<td>3</td>
<td>1.27</td>
<td>1.17 to 1.37</td>
<td>1.27</td>
<td>1.15 to 1.41</td>
</tr>
<tr>
<td>SES not adjusted</td>
<td>6</td>
<td>1.07</td>
<td>1.00 to 1.16</td>
<td>1.08</td>
<td>0.97 to 1.21</td>
</tr>
<tr>
<td>Smoking adjusted</td>
<td>2</td>
<td>1.28</td>
<td>1.09 to 1.52</td>
<td>1.25</td>
<td>0.92 to 1.71</td>
</tr>
<tr>
<td>Smoking not adjusted</td>
<td>7</td>
<td>1.15</td>
<td>1.09 to 1.22</td>
<td>1.16</td>
<td>1.04 to 1.28</td>
</tr>
<tr>
<td>Gender adjusted</td>
<td>5</td>
<td>1.26</td>
<td>1.18 to 1.36</td>
<td>1.27</td>
<td>1.16 to 1.39</td>
</tr>
<tr>
<td>Gender not adjusted</td>
<td>4</td>
<td>1.05</td>
<td>0.97 to 1.14</td>
<td>1.05</td>
<td>0.96 to 1.15</td>
</tr>
</tbody>
</table>

aN₂O = Nitrogen dioxide  
SES = Socioeconomic status

The first line of Table 14-20 shows the results of combining all nine indoor studies using a fixed model. The estimated odds ratio is 1.17 and the 95% confidence limits are 1.11 and 1.23. The analysis was made assuming that the parameters were the same (homogeneous), and this can be tested. The chi-square test for homogeneity for the nine studies was 12.32 for 8 degrees of freedom, which has a p-value of 0.1375. Thus, there is some evidence that the parameters from each study are not identical. The estimates for the random-effects model (also shown on the first line of Table 14-20) are similar to the estimates for the fixed model, but the confidence limits are slightly broader. The conclusion.
from both models is the same, namely that the odds ratio is estimated to be about 1.2 with 95% confidence intervals ranging from about 1.1 to 1.3 (Hasselblad et al., 1992). Many researchers have suggested that the random-effects model is the more appropriate model because it does not assume that all studies estimate the same parameter. Furthermore, the random-effects estimates will approach the fixed-effects estimates when studies give similar estimates.

These studies include results from North America and Europe. Meta-analyses of studies from different countries are common. For example, Canner (1987), Littenberg (1988), and Jaeschke et al. (1990) all combined studies done in both North America and Europe and did not adjust for geographic differences. The indoor NO₂ studies were compared by country. Four of them were done in Great Britain (Melia studies), and four in the United States (Ware et al., Neas et al., Ekwo et al., Keller et al.). The British studies provide the highest estimated odds ratio (random-effects model), 1.25, the U.S. studies give a combined estimate of 1.13.

Four of the nine indoor studies used measured NO₂ values, whereas the other five did not. The use of a surrogate for exposure should tend to reduce the estimate of the effect (see Samet and Utell, 1990). The measured NO₂ studies gave an estimated odds ratio (random-effects model) of 1.22, whereas the others gave an estimate of 1.16, which is consistent with a measurement error effect.

Table 14-21 lists the important covariates considered in these nine studies and shows if the covariate was used in the study and the meta-analysis. Study design and exposure measurement source are also presented. The effect of having adjusted for various covariates can be seen in Table 14-20. In general, those studies that adjusted for a particular covariate found larger odds ratios as compared with those that did not.

Although there may be reasons to weight certain studies or groups of studies more heavily than others, the final conclusion has to be that there is an increase in the odds of respiratory disease of children exposed to NO₂, especially those of elementary school age. Subject to assumptions made for the combined analysis, the main conclusion from that analysis was that an increased risk of about 20% for respiratory symptoms and disease corresponded to each increase of 0.015 ppm (28.3 μg/m³) in estimated 2-week average NO₂ exposure, where mean weekly concentrations in bedrooms in studies reporting NO₂ levels.
TABLE 14-21. COVARIATE TREATMENT AND OTHER FACTORS IN SELECTED NITROGEN DIOXIDE EPIDEMIOLOGY STUDIES IN META-ANALYSIS

<table>
<thead>
<tr>
<th>Reference</th>
<th>Covariates&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Parental Exposure</th>
<th>Smoking</th>
<th>Gender</th>
<th>Design</th>
<th>Exposure Measurement Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melia et al (1977)</td>
<td>A</td>
<td>NM</td>
<td>A</td>
<td>A</td>
<td>Cross-sectional</td>
<td>Gas stove vs electric stove&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Melia et al (1979)</td>
<td>A</td>
<td>M</td>
<td>A</td>
<td>A</td>
<td>Cross-sectional</td>
<td>Gas stove vs electric stove&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Melia et al (1980)</td>
<td>M</td>
<td>M</td>
<td>A</td>
<td>A</td>
<td>Cross-sectional</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt; measured with Palmes tubes Gas stove homes only</td>
</tr>
<tr>
<td>Melia et al (1982a)</td>
<td>M</td>
<td>M</td>
<td>A</td>
<td>A</td>
<td>Cross-sectional</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt; measured with Palmes tubes Gas stove homes only</td>
</tr>
<tr>
<td>Ware et al (1984)</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>Cross-sectional</td>
<td>Gas stove vs electric stove&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neas et al (1991)</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>Cross-sectional</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt; measured with Palmes tubes Gas and electric stove homes</td>
</tr>
<tr>
<td>Ekwo et al (1983)</td>
<td>NM</td>
<td>A</td>
<td>M</td>
<td>M</td>
<td>Cross-sectional</td>
<td>Gas stove vs electric stove&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dijkstra et al (1990)</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>Cross-sectional</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt; measured with Palmes tubes NO&lt;sub&gt;2&lt;/sub&gt; emissions sources in homes</td>
</tr>
<tr>
<td>Keller et al (1979b)</td>
<td>M</td>
<td>NM</td>
<td>M</td>
<td>M</td>
<td>Prospective</td>
<td>Gas stove vs electric stove&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>SES = Socioeconomic status  
A = Covariate included in study and meta-analysis  
NM = Not measured in study  
M = Measured in study but data not available for meta-analysis  
<sup>b</sup>Estimate of exposure derived from assumption of gas stove versus electric stove levels in bedrooms in England from data in Melia et al (1980, 1982a,b) of approximately 0.0165 ppm  
<sup>c</sup>Estimate of exposure derived from assumption of gas stove with pilot light versus electric stove levels averaged in the home in the United States in Neas et al (1991) of approximately 0.0173 ppm

were predominately between 0.008 and 0.065 ppm NO<sub>2</sub> (Hasselblad et al., 1992) The studies using measured NO<sub>2</sub> give a slightly higher estimate of the odds ratio The estimates are not sensitive to the assumption that each study is estimating the same parameter as indicated by the random-effects model In fact, the finding of increased risk across a wide
variety of study conditions suggests that the effects seen are not an artifact of any one particular study.

These results are not sensitive to the inclusion or exclusion of any one study. It would have been possible to include the hospitalization results of Ekwo et al. (1983), the analysis of the Swiss study, or the Berwick et al. (1989) study. None of these studies would have made any real change in the estimated odds ratios or their 95% confidence limits.

Various researchers have conducted studies of infants less than 2 years of age (see Table 14-22). A major difference for this group of studies is that the health outcome measures are less uniform than the studies of older children. For purposes of comparability, a meta-analysis similar to the one for older children was calculated.

The seven studies of infants shown in Table 14-22 show mixed results. A test of homogeneity of the odds ratios gives a chi-squared value of 22.66 for 6 degrees of freedom, which has a p-value of 0.0009 that implies that the studies are not homogenous. The variation in results could be due to several factors, including different health outcome measures and other factors. Dockery et al. (1989a) note that the associations discussed in Ware et al. (1984) and Dockery et al. (1989a) must be viewed with caution because they compare recalled respiratory events early in the child's life. Because of the heterogeneity, the studies were combined using a random effects model. Subject to the assumptions made for the meta-analysis, the combined odds ratio for the increase in respiratory disease per increase of 0.015 ppm NO$_2$ was 1.09 with a 95% confidence interval of 0.95 to 1.26, where mean weekly concentrations in bedrooms were predominantly between 0.005 and 0.050 ppm NO$_2$ in studies reporting levels. Thus, although the overall combined estimate is positive, it clearly contains the no-effect value of 1.0 (i.e., is not statistically significant), and so we cannot conclude that the evidence suggests an effect in infants comparable to that seen in older children.

There is always the concern that the studies described in this document are not the complete list of studies, but contain primarily the positive studies because these are the studies most likely to get published. Alternatively, nonsignificant results may not be reported with sufficient quantitative detail to permit their inclusion. Both of these effects can be considered as "publication bias." There are two reasons to be less concerned with
<table>
<thead>
<tr>
<th>Reference</th>
<th>Estimated Odds Ratio</th>
<th>2.5 and 97.5 Percentiles</th>
<th>Health Outcome</th>
<th>NO₂ Exposure Estimate (ppm)</th>
<th>Age</th>
<th>Where/When</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melta et al. (1983)</td>
<td>0.63</td>
<td>0.36-1.10</td>
<td>Respiratory illness incidence</td>
<td>0.0165&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1 year</td>
<td>England (1978)</td>
</tr>
<tr>
<td>Ekwo et al. (1983)</td>
<td>2.4</td>
<td>1.06-3.74</td>
<td>Hospitalization for chest illness before age 2</td>
<td>0.0173&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2 years</td>
<td>Iowa</td>
</tr>
<tr>
<td>Ware et al. (1984)</td>
<td>1.11</td>
<td>0.97-1.27</td>
<td>Respiratory illness before age 2</td>
<td>0.0173&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;2 years</td>
<td>Six U.S. cities (1974-1979)</td>
</tr>
<tr>
<td>Ogston et al. (1985)</td>
<td>1.14</td>
<td>0.86-1.50</td>
<td>Respiratory illness incidence</td>
<td>0.0165&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1 year</td>
<td>Scotland (1980)</td>
</tr>
<tr>
<td>Dockery et al. (1989a)</td>
<td>1.15</td>
<td>0.96-1.37</td>
<td>Respiratory illness before age 2</td>
<td>0.015&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;2 years</td>
<td>Six U.S. Cities (1983-1986)</td>
</tr>
<tr>
<td>Margolis et al. (1992)</td>
<td>1.12</td>
<td>0.63-2.04</td>
<td>Persistent lower respiratory symptoms</td>
<td>0.0105&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;1 year</td>
<td>North Carolina (1986-1988)</td>
</tr>
<tr>
<td>Samet et al. (1993)</td>
<td>0.985&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.935-1.038&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Lower respiratory illness incidence</td>
<td>0.015&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&lt;18 mo</td>
<td>Albuquerque (1988-1990)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Estimate of exposure derived from assumption of gas stove versus electric stove levels in bedrooms in England from data in Melta et al. (1980, 1982) of approximately 0.0165 ppm

<sup>b</sup>Estimate of exposure derived from assumption of gas stove with pilot light versus electric stove levels averaged in the home in the United States in Neas et al. (1991) of approximately 0.0173 ppm

<sup>c</sup>Estimate of exposure derived from assumption of gas stove versus electric stove levels averaged in the home in United States in Neas et al. (1991) of approximately 0.015 ppm

<sup>d</sup>Estimate of exposure derived from assumption of gas stove versus electric stove levels averaged in the home in the Albuquerque study (Samet et al., 1993) of approximately 0.0105 ppm

<sup>e</sup>Computed from logistic regression coefficient derived from Samet et al. (1993)

<sup>f</sup>Exposure level used to convert logistic regression to an odds ratio
publication bias in this particular situation. First, epidemiological studies are very expensive and require the work of many individuals. The designs of studies are usually described to the scientific community before the results are even known. Second, most of the studies cited in this section were reported as negative studies by the authors themselves, indicating that there was no difficulty publishing negative results. However, some studies are necessarily excluded because they provide insufficient information. Although this can lead to bias, there is little that can be done to correct for this problem. This problem is not normally referred to as publication bias, but it is a similar problem.

14.6.5 Summary of Synthesis of Evidence

The evidence from individual studies of the effect of NO₂ on lower respiratory symptoms and disease is somewhat mixed. Most of the indoor studies used in the synthesis showed increased respiratory disease rates associated with increased exposure. A few of the individual studies were statistically significant. Combining the indoor studies giving quantitative estimates of effects tend to show increases of lower respiratory morbidity in children associated with long-term exposure to NO₂. Combining the indoor studies as if the end points were similar gives an estimated odds ratio of 1.2 (95% confidence limits of 1.1 and 1.3) for the effect per 0.015 ppm increase of NO₂ on lower respiratory morbidity (Hasselblad et al., 1992). This suggests that subject to assumptions made for the combined analysis, the main conclusion from that analysis was that an increase of about 20% in the odds of lower respiratory symptoms and disease corresponded to each increase of 0.015 ppm (28.3 μg/m³) in estimated 2-week average NO₂ exposure, where mean weekly concentrations in bedrooms in studies reporting NO₂ levels were predominately between 0.008 and 0.065 ppm NO₂ (Hasselblad et al., 1992). Thus, the combined evidence is supportive for the effects of estimated exposure to NO₂ on lower respiratory symptoms and disease in children aged 5 to 12 years.

In the individual indoor studies of infants 2 years of age and younger, no consistent relationship was found between estimates of NO₂ exposure and the prevalence of respiratory symptoms and disease. Based on a meta-analyses of these indoor infant studies, subject to the assumptions made for the meta-analysis, the combined odds ratio for the increase in respiratory disease per increase of 0.015 ppm NO₂ was 1.09 with a 95% confidence interval.
of 0.95 to 1.26, where mean weekly concentrations in bedrooms were predominately between 0.005 and 0.050 ppm NO$_2$ in studies reporting levels. Thus, although the overall combined estimate is positive, it clearly contains the no-effect value of 1.0, (i.e., is not statistically significant), and so we cannot conclude that the evidence suggests an effect in infants comparable to that seen in older children.

Several uncertainties need to be considered in interpreting the above studies and results of the EPA meta-analysis. Measurement error in exposure is potentially one of the most important methodological problems in epidemiological studies of NO$_2$. Thus, measured NO$_2$ concentrations are not exposure values per se, rather, estimating actual exposure requires knowledge of both pollutant levels and related human activity patterns. The effects studied may be related to peak exposures, average exposures, or a combination of the two. To the extent that health effects depend on peak exposures rather than average exposures, the exposure estimates used in the above studies and meta-analyses introduce exposure measurement error. These studies cannot distinguish between the relative contributions of peak and average exposures and their relationship with the observed health effects. Additionally, a by-product of NO$_2$, HONO, may be a factor in observed effects. However, only very limited health and aerometrical data are available that examine such possibilities. Also, although the level of similarity and common elements between the outcome measures in the NO$_2$ studies provide some confidence in their use in the quantitative analysis, the symptoms and illnesses combined are to some extent different and could indeed reflect different underlying processes. Thus, caution is necessary in interpreting the meta-analysis results.

14.7 CONCLUSIONS

Although there is evidence that suggests that increased estimated NO$_2$ exposure is associated with increased respiratory symptoms in children aged 5 to 12 years, the exposure estimated may be inadequate to determine a quantitative relationship between estimates of exposure and symptoms. The studies with measured NO$_2$ exposure did so only for periods of 1 to 2 weeks and reported the values as averages. None of the studies attempted to relate
the effects seen to the pattern of exposure, such as short-term peaks. Furthermore, the extrapolation to possible patterns of ambient exposure is difficult.

Several researchers studied a different population group that consisted of infants 2 years of age and younger. In the individual studies of infants 2 years of age and younger, no consistent relationship was found between estimates of NO₂ exposure and the prevalence of respiratory symptoms and disease. Based on a meta-analyses of these infant studies, the overall combined estimate is positive, however, it clearly contains the no-effect value of 1.0, (i.e., is not statistically significant); and so we cannot conclude that the evidence suggests an effect in infants.

Several studies attempted to relate some measure of indoor and outdoor NO₂ exposure to long-term changes in pulmonary function. These changes were marginally significant. No short-term studies had indoor exposures. Most studies did not find any effects, which is consistent with results from controlled human exposure studies (see Chapter 15). However, the basic conclusion is that there is insufficient epidemiological evidence to make any conclusion about the long- or short-term effects of NO₂ on pulmonary function.

14.8 SUMMARY

This chapter discusses the epidemiological evidence for the effects of NOₓ on human health. The major emphasis is on the effects of NO₂ because it is the NOₓ compound studied in most epidemiological studies and because it is the NOₓ compound currently of greatest concern from a public health perspective. The results from the various epidemiological studies of NO₂ exposure effects on human health outcomes are summarized in Appendix 14A.

The studies considered in this chapter were evaluated for several key factors, including (1) measurement error in exposure, (2) misclassification of the health outcome, (3) adjustment for covariates, (4) selection bias, (5) internal consistency, and (6) plausibility of the effect based on other evidence. The health outcome should be an outcome for which there is good reason to suspect that NO₂ exposure has an effect. Two health outcome measures are generally considered lung function measurements and respiratory illness. Each study is reviewed with special attention given to the factors just discussed.

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studies that address these factors more appropriately provide a stronger basis for the conclusions that they draw.

Respiratory illness and factors that affect its rate and/or severity are important public health concerns. This chapter discussed epidemiological findings relating NO₂ exposure to respiratory illness. This effect is of public health importance because of the potential for exposure to NO₂ and because childhood respiratory illness is common (Samet et al., 1983, Samet and Utell, 1990). This takes on added importance because recurrent childhood respiratory illness may be a risk factor for later increased susceptibility to lung damage (Glezen, 1989).

Animal toxicological studies in Chapter 13 suggest that NO₂ exposure can impair components of the respiratory host defense system. The observed increase in respiratory symptoms and disease among children in epidemiologic studies of NO₂ exposure may be the result of an impaired respiratory host defense system. The biological plausibility of this hypothesis is supported by the animal toxicology data, but the hypothesis requires further testing.

Several of the indoor epidemiological studies gave some evidence that repeated NO₂ exposure increases respiratory illness in children, although many were not statistically significant. Melia et al. (1977) first reported on a survey of children in randomly selected areas of England and Scotland using the presence of a gas stove as a measure of NO₂ exposure. A reanalysis of those data yields an estimated odds ratio of 1.31 for the presence of respiratory symptoms. The cross-sectional study of Melia et al. (1979) also found that the presence of a gas stove was associated with increased risk of respiratory disease. The odds ratio was 1.24 with 95% confidence limits of 1.09 and 1.42. Melia et al. (1980) described the results of a third study of respiratory symptoms in children aged 6 to 7 years in northern England. Multiple logistic regression analysis of the data presented by Melia et al. (1980) showed a significant increase in symptoms as a function of bedroom NO₂ levels. Melia et al. (1982a) reported on a fourth study of children in England. Multiple logistic regression analysis of these data was not statistically significant, although the symptoms were positively related to NO₂ exposure. The analysis by Hasselblad et al. (1992) suggests that an increase of 0.015 ppm (28.3 μg/m³) in bedroom NO₂ levels yields an 11% increase in the odds of respiratory illness.
The analysis of the Six City studies by Ware et al. (1984) estimated an unadjusted odds ratio of 1.08 (95% confidence limits of 0.97 and 1.19) for a lower respiratory illness index associated with gas stove use. Other indicators such as bronchitis, cough, and wheeze did not show any increased incidence. Neas et al. (1990, 1991) analyzed a different Six City Study cohort enrolled later, used a different symptom questionnaire, and made indoor NO₂ measurements for all subjects. They found increased respiratory disease and gave an estimated odds ratio of 1.40 (95% confidence limits of 1.14 and 1.72) at an exposure of 0.015 ppm (28.3 μg/m³).

Ekwo et al. (1983) studied respiratory symptoms in relation to gas stove use in Iowa City, IA. Gas stove use provided an odds ratio of 2.4 for hospitalization for chest illness before age 2, and 1.1 for chest congestion and phlegm with colds. Dijkstra et al. (1990) studied the effect of indoor factors on respiratory health in children in the Netherlands. A logistic regression analysis (Hasselblad et al., 1992) yielded an odds ratio of 0.94 with 95% confidence limits of 0.66 and 1.33, thus showing no evidence of an increase in respiratory disease with increasing NO₂ exposure. Keller et al. (1979b) did not find any statistically significant changes in respiratory disease associated with gas stove use, the unadjusted estimated odds ratio for respiratory illness was 0.72, with 95% confidence limits of 0.30 and 1.74.

Samet et al. (1993) report preliminary results of a prospective cohort study of respiratory illness during the first 18 mo of life in relationship to estimates of NO₂ exposure in Albuquerque, NM. The findings indicated that in a population of healthy infants, no significant association between NO₂ exposure estimate and respiratory illness were found.

Other studies did not provide sufficient information to derive any quantitative estimates of the effect of NO₂ or gas stove use on respiratory disease. Several other studies contain information about the effects of NO₂ on respiratory illness, but most of the studies either used very different health end points or did not provide quantitative estimates of the effects. In Melia et al. (1983), infants under 1 year of age were examined. No relation was found between type of fuel used for cooking and the prevalence of respiratory symptoms. Ogston et al. (1985) studied respiratory disease in 1-year-olds in the Tayside region of northern Scotland. The presence of a gas stove yielded an increase in upper respiratory illness. Schenker et al. (1983) studied children in Chestnut Ridge, PA, but did not report any
quantitative data of any relationship between daily respiratory symptoms and NO₂ levels. Braun-Fahrlaender et al. (1992) found indoor NO₂ levels predictive of duration of respiratory disease episodes. The study of Berwick (1987) showed increased relative risk of respiratory disease in some age groups, but not in others. Dekker et al. (1991) reported an increase in asthma in children aged 5 to 8 years (n = 60) in relation to the presence of a gas stove in Canadian homes. Hedberg et al. (1989) and Smith et al. (1992) reported respiratory symptoms in relation to NO₂ exposures greater than 15 ppm (2,800 μg/m³) in skating rinks.

Several studies examined the relationship between estimates of ambient NO₂ levels and respiratory health measures. Dockery et al. (1989b) examined relationships between various respiratory symptoms and ambient NO₂ levels. Braun-Fahrlaender et al. (1992) reported associations between outdoor long-term measures of NO₂ and duration of respiratory episodes. Schwartz et al. (1991) showed a relationship between short-term fluctuations in air pollution and short-term fluctuations in medical visits for croup symptoms. Rebmann et al. (1991) reported a relationship between croup with positive virologic testing and NO₂ levels.

Several of the indoor studies suggest an increase in respiratory symptoms in children aged 5 to 12 years from estimated exposure to NO₂. The associations in the majority of the studies do not reach statistical significance. The consistency of these studies was examined and the evidence was synthesized in a quantitative analysis. The studies described used different indicators to study health endpoints. In order to compare these indoor studies, a standard endpoint was defined, and then each study was compared with this standard endpoint. The endpoint chosen was the presence of lower respiratory symptoms and disease in children aged 5 to 12 years. It was assumed that the relative odds of developing lower respiratory symptoms and disease are similar across this age range as a function of NO₂ exposure, even though the actual rates may not be. (This is a common assumption in many analyses.) The goal was to estimate the odds ratio corresponding to each increase of 0.015 ppm (28.3 μg/m³) in NO₂ exposure (Hasselblad et al., 1992).

An attempt was made to include as many indoor studies as possible. The requirements for inclusion were (1) the health endpoint measured must be reasonably close to the standard endpoint, (2) exposure differences between subjects must exist and some estimate of exposure must be available, and (3) an odds ratio for a specified exposure estimate must have
been calculated, or data must be presented so that it can be calculated (Hasselblad et al., 1992)

Two models for combining evidence were employed (Hasselblad et al., 1992) The first was a fixed-effects model, which assumed that every study was estimating the same parameter. The second basic model assumed that the parameter of interest was not fixed, but was itself a random variable from a distribution. This kind of model is designated by several names, including random-effects models or hierarchical models. The purpose of a random-effects model is to relax the assumption that each study is estimating exactly the same parameter. DerSimonian and Laird (1986) discuss the random-effects model. Many researchers have suggested that the random-effects model is the more appropriate model because it does not assume that all studies estimate the same parameter. Furthermore, the random-effects estimates will approach the fixed-effects estimates when studies give similar estimates.

Subject to assumptions made for the combined analysis, the main conclusion from that analysis was that an increased risk of about 20% for respiratory symptoms and disease corresponded to each increase of 0.015 ppm (28.3 μg/m³) in estimated 2-week average NO₂ exposure, where mean weekly concentrations in bedrooms in studies reporting NO₂ levels were predominately between 0.008 and 0.065 ppm NO₂ (Hasselblad et al., 1992). The measured NO₂ studies gave an estimated odds ratio (random-effects model) of 1.22, whereas the others yield an estimate of 1.16, which is consistent with a measurement error effect. The effect of having adjusted for covariates such as SES, smoking, and gender was that those studies that adjusted for a particular covariate found larger odds ratios as compared with those that did not.

Several uncertainties need to be considered in interpreting the above studies and results of the EPA meta-analysis. Measurement error in exposure is potentially one of the most important methodological problems in epidemiological studies of NO₂. Thus measured NO₂ concentrations are not exposure values per se, rather, estimating actual exposure requires knowledge of both pollutant levels and related human activity patterns. The effects studied may be related to peak exposures, average exposures, or a combination of the two. To the extent that health effects depend on peak exposures rather than average exposures, the exposure estimates used in the above studies and meta-analyses introduce exposure...
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Several researchers studied a different population group that consisted of infants 2 years of age and younger. In the individual studies of infants 2 years of age and younger, no consistent relationship was found between estimates of NO₂ exposure and the prevalence of respiratory symptoms and disease. Based on a meta-analyses of these infant studies, the overall combined estimate is positive, however, it clearly contains the no-effect value of 1.0, (i.e., is not statistically significant), and so we cannot conclude that the evidence suggests an effect in infants.

The Harvard Six City study (Ware et al., 1984, Berkey et al., 1986, Neas et al., 1991) attempted to relate some measure of indoor and outdoor NO₂ exposure to long-term changes in pulmonary function. These changes were marginally significant. Most studies did not find any effects, which is consistent with controlled human exposure study data (see Chapter 15). However, the basic conclusion is that there is insufficient epidemiological evidence to make any conclusion about the long- or short-term effects of NO₂ on pulmonary function.
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APPENDIX 14A.

SUMMARY OF EPIDEMIOLOGICAL STUDIES OF NITROGEN DIOXIDE HEALTH EFFECTS
### TABLE 14A-1. SUMMARY OF EPIDEMIOLOGICAL STUDIES OF NITROGEN DIOXIDE EFFECTS ON RESPIRATORY ILLNESS

<table>
<thead>
<tr>
<th>Study</th>
<th>NO₂ Exposure</th>
<th>Effects Seen</th>
<th>Discussion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children aged 6-11 years with and without gas stoves in the house in 28 areas of England and Scotland</td>
<td>Gas stove use</td>
<td>Respiratory symptoms (bronchitis, cough, wheeze) higher with gas stoves present</td>
<td>Indoor NO₂ not measured at time of study</td>
<td>Melia et al (1977)</td>
</tr>
<tr>
<td>4,827 Children aged 5-10 years in a second British Cohort</td>
<td>Gas stove use</td>
<td>Respiratory symptoms higher in children with gas stoves, Smoking in home, and use of pilot lights examined</td>
<td>Result significant for boys but not girls</td>
<td>Melia et al (1979)</td>
</tr>
<tr>
<td>808 Children aged 6-7 years in Middlesborough, England</td>
<td>NO₂, 7-318 μg/m³ (0.004-0.169 ppm) in bedroom with gas stoves, 6-70 μg/m³ (0.004-0.0371 ppm) without gas stoves</td>
<td>Incidence of respiratory illness higher in homes with gas stoves (p = 0.10)</td>
<td>Marginally significant results Subset data with gas stove only</td>
<td>Melia et al (1980) Goldstein et al (1979) and Florey et al (1979)</td>
</tr>
<tr>
<td>Children aged 5-6 years in Middlesborough, England</td>
<td>NO₂ levels ranged from 16-530 μg/m³ (0.009-0.281 ppm)</td>
<td>Slight trend for increase in respiratory disease rates</td>
<td>Results not even marginally significant</td>
<td>Melia et al (1982)</td>
</tr>
<tr>
<td>Six City study of 9,000 grade-school children in the United States</td>
<td>7-49 μg/m³ (0.004-0.026 ppm) estimate of total personal exposure gas stove use used as a surrogate</td>
<td>Marginally significant association of gas stove use with respiratory illness rate in children under 2 years</td>
<td>Differences marginally significant Incomplete indoor exposure</td>
<td>Spender et al (1980) Ware et al (1984)</td>
</tr>
<tr>
<td>Six City study of 6,273 children aged 7-11 years in the United States</td>
<td>Measured indoor exposure average 17.4 ppb higher in homes with gas stoves and pilot lights</td>
<td>Increases in individual symptoms of 5-29%, combined symptom odds ratio of 1.47</td>
<td>Larger increase in combined symptoms than in individual symptoms</td>
<td>Dockery et al (1989a) Neas et al (1990, 1991)</td>
</tr>
<tr>
<td>Respiratory illness study of 1,565 infants in the Tayside region of Scotland</td>
<td>Gas stove use</td>
<td>Increase of 14% in respiratory disease in homes with gas stove use</td>
<td>Indoor NO₂ not measured at time of study Results not statistically significant</td>
<td>Ogston et al (1985)</td>
</tr>
</tbody>
</table>
### TABLE 14A-1 (cont’d). SUMMARY OF EPIDEMIOLOGICAL STUDIES OF NITROGEN DIOXIDE EFFECTS ON RESPIRATORY ILLNESS

<table>
<thead>
<tr>
<th>Study</th>
<th>NO₂ Exposure</th>
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<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory symptom study of 1,355 children aged 6-12 years in the Iowa City Schools</td>
<td>Gas stove use</td>
<td>Increase in chest congestion of 10% and increase of hospitalization of more than 100%</td>
<td>Only hospitalization was statistically significant End points different from other studies No indoor air measurements</td>
<td>Ekwo et al (1983)</td>
</tr>
<tr>
<td>Respiratory symptom study of 6- to 9-year-old children in the southeastern region of the Netherlands</td>
<td>Weekly average NO₂ ranged from 22-42 µg/m³ (0.012-0.022 ppm) Gas appliance</td>
<td>No evidence of any increase in respiratory disease</td>
<td>Wide confidence intervals for effects measured</td>
<td>Dijkstra et al (1990)</td>
</tr>
<tr>
<td>Respiratory illness study of children under age 12 years in Columbus, OH</td>
<td>Annual average NO₂ ranged from 38-94 µg/m³ (0.020-0.050 ppm)</td>
<td>No difference found in children's respiratory illness</td>
<td>Adjustments for previous illness may have lost any effect Analysis model different from other studies</td>
<td>Keller et al (1979a,b)</td>
</tr>
<tr>
<td>Prospective cohort study of respiratory infection during the first 18 mo of life in relationship to NO₂ exposure in Albuquerque, NM</td>
<td>Personal exposure estimate based on Palmes tube and activity data</td>
<td>Careful standardized ascertainment of illness, assessment of potential confounding factors</td>
<td>No significant associations found between NO₂ exposure estimates and respiratory illness in infants</td>
<td>Samet et al (1993, 1992), Samet and Spengler (1989)</td>
</tr>
<tr>
<td>Respiratory symptom study of children aged 0-5 years in four areas of Switzerland</td>
<td>Outdoor mean NO₂ levels ranged from 25-52 µg/m³ Indoor levels ranged from 11-34 µg/m³</td>
<td>Increase symptom scores in children exposed to outdoor NO₂ levels of 30 µg/m³ (0.0159 ppm)</td>
<td>End points different from other studies Effect of indoor NO₂ marginally significant</td>
<td>Braun-Fahrlaender et al (1989)</td>
</tr>
<tr>
<td>Twelve-week study of lower and upper respiratory symptoms in women and children</td>
<td>Outdoor NO₂ levels ranged from 9-19 µg/m³ Indoor NO₂ ranged from 6-91 µg/m³ (0.003-0.048 ppm)</td>
<td>Children under age 7 years with exposure to more than 30 µg/m³ (0.0159 ppm) had 2.17 times the lower respiratory illness</td>
<td>Inconsistent results by age group, possibly due to very small sample sizes available</td>
<td>Berwick et al (1984, 1987, 1989) Berwick (1987) Leaderer et al (1986)</td>
</tr>
<tr>
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<tr>
<td>Study of 5,561 adults aged 25-39 years living in Lancaster and Glendora, CA</td>
<td>Annual means over the 5-year period in Lancaster and Glendora (Azuza station) averaged 3.2 and 11.4 ppm, respectively</td>
<td>Decreased FEV₁ and FVC and increased cough, phlegm, and wheezing found in Glendora when compared to Lancaster</td>
<td>Areas measured at different times, other differences impossible to adjust for</td>
<td>Detels et al (1981a,b)</td>
</tr>
<tr>
<td>Respiratory disease study of approximately 2,800 adults and children for three different 6-mo periods in Chattanooga, TN</td>
<td>Annual averages of daily NO₂ levels ranged from 11-4 ppm in 1972, and from 22-40 ppm in 1973</td>
<td>Increased respiratory illness in all age and sex groups found in 1972, but not found in 1973</td>
<td>No indoor measurements NO₂ measured by Jacobs-Hochhesser method for some periods</td>
<td>Love et al (1982)</td>
</tr>
<tr>
<td>Study of respiratory symptoms in four French cities</td>
<td>NO₂, 12-16 µg/m³ (0.006-0.008 ppm) daily means, NO, 7-140 µg/m³ daily means</td>
<td>No relationship of respiratory symptoms with NO₂</td>
<td>No indoor measurements Low NO₂ exposure</td>
<td>PAARC (1982a,b)</td>
</tr>
<tr>
<td>Respiratory symptom and pulmonary function study of 4th-6th graders in Akron, OH</td>
<td>NO₂ averaged 54 µg/m³ (0.029 ppm) in polluted area, 377 µg/m³ (0.019 ppm) in cleaner area</td>
<td>Higher rates of acute respiratory disease in polluted area</td>
<td>No indoor measurements Small differences seen Relatively low NO₂ exposure</td>
<td>Mostardi et al (1981a)</td>
</tr>
<tr>
<td>Study of asthma emergency room visits and hospitalizations for children at the Children's Hospital of Los Angeles</td>
<td>Monthly average NO₂ levels ranged from 12-18 ppm, and NO levels ranged from 13-59 ppm</td>
<td>Increased asthma emergency room visits and hospitalizations correlated significantly with NO and NO₂, as well as coefficients of haze and hydrocarbons</td>
<td>No indoor measurements Pollutants could not be separated</td>
<td>Richards et al (1981)</td>
</tr>
<tr>
<td>Study of acute illnesses in elderly patients in long-term care</td>
<td>Mean value of nitrogen oxides indoors was 0.062 ppm and outdoors was 0.055 ppm</td>
<td>Respiratory diseases correlated with NO₂</td>
<td>No adjustment for covarates Details of pollution monitoring not given</td>
<td>Loewenstein et al (1985)</td>
</tr>
<tr>
<td>Study</td>
<td>NO₂ Exposure</td>
<td>Effects Seen</td>
<td>Discussion</td>
<td>References</td>
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<tr>
<td>Study of COPD rates in COPD and normal subjects in Helsinki, Finland</td>
<td>NO₂, 5-70 µg/m³ (0.003-0.0371 ppm)</td>
<td>COPD correlated with NO₂</td>
<td>No indoor measurements</td>
<td>Pershagen et al (1984)</td>
</tr>
<tr>
<td>Los Angeles Student Nurse Data originally collected daily from 1962-64, reanalyzed by Schwartz</td>
<td>Daily outdoor NO₂ exposure averaged 0.13 ppm over a 3-year period Sites located within 2.5 m of the subject</td>
<td>NO₂ exposure related to phlegm, sore throat, and eye irritation</td>
<td>No indoor NO₂ exposure measurements, and only one outdoor station</td>
<td>Schwartz et al (1988), Schwartz and Zeger (1990)</td>
</tr>
<tr>
<td>Respiratory disease study of 4,071 children aged 5-14 years in Pennsylvania</td>
<td>Presence of gas stove used as a surrogate for NO₂ exposure</td>
<td>No significant association between use of gas stove and any symptom or illness variable</td>
<td>No indoor NO₂ exposure</td>
<td>Schenker et al (1983)</td>
</tr>
<tr>
<td>The data reported by Love et al. 1982 were analyzed for short-term effects</td>
<td>Daily NO₂ levels were split into three categories using the cut-offs of 75 and 150 µg/m³ (0.04-0.08 ppm)</td>
<td>No consistent short-term effect of NO₂ on acute respiratory disease was found</td>
<td>No indoor measurements</td>
<td>Harrington and Krupnick (1985)</td>
</tr>
<tr>
<td>Study of influenza cases over a 2-mo period in Sofia, Bulgaria</td>
<td>NO₂ had means of 21 and 37 µg/m³ (0.014-0.45 ppm) for the two epidemics</td>
<td>NO₂ related to NO₂ Two-day lag in illnesses</td>
<td>No indoor measurements</td>
<td>Kalpazanov et al (1976)</td>
</tr>
<tr>
<td>Acute symptom study of 35 asthmatics in Barcelona, Spain</td>
<td>Mean daily maximum hourly NO₂ levels ranged from 271-846 µg/m³ (0.14-0.45 ppm) for two weeks in December 1985</td>
<td>Symptoms in asthmatics not related to NO₂ levels Second study relates symptoms to soybean dust</td>
<td>Effects related to soybean dust dust in definitive study</td>
<td>Anto et al (1986)</td>
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</table>
### TABLE 14A-1 (cont’d). SUMMARY OF EPIDEMIOLOGICAL STUDIES OF NITROGEN DIOXIDE EFFECTS ON RESPIRATORY ILLNESS\textsuperscript{a}

<table>
<thead>
<tr>
<th>Study</th>
<th>NO\textsubscript{2} Exposure</th>
<th>Effects Seen</th>
<th>Discussion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study of asthma in 20 children seen in two Japanese hospitals</td>
<td>Quarterly mean NO\textsubscript{2} levels ranged from 3.0-5.7 ppm</td>
<td>No relationship found between frequency of attacks and NO\textsubscript{2} levels</td>
<td>No indoor measurements Attack rates are generally longest when NO\textsubscript{2} is highest</td>
<td>Watanabe et al (1984)</td>
</tr>
<tr>
<td>Ecological study attempting to relate nitrates and oxides of nitrogen to mortality</td>
<td>County-wide NO\textsubscript{x} estimates</td>
<td>Two million death certificates examined</td>
<td>Both positive and negative coefficients found in the multiple regression, which were almost always not statistically significant</td>
<td>Mendelsohn and Orcutt (1979)</td>
</tr>
<tr>
<td>Ecological analysis of mortality rates and air pollution</td>
<td>NO\textsubscript{2} data from the National Air Sampling Network</td>
<td>Mortality rate associated with various disease end points</td>
<td>Quality and applicability of both the mortality and monitoring data preclude considering the results as anything but speculative</td>
<td>Hickey et al (1970)</td>
</tr>
<tr>
<td>Study of variations in daily mortality in relation to daily air pollution in several U S cities from 1962-65</td>
<td>Nonspecific NO\textsubscript{x} levels</td>
<td>Multiple regression analysis showed significant negative association between winter mortality in New York City and daily NO\textsubscript{x} concentration but no association in summer In contrast, the data in Los Angeles were positively related in the winter but not in the summer</td>
<td>The results suggest that other factors are causing the relationship</td>
<td>Lebowitz (1971)</td>
</tr>
<tr>
<td>A study of the prevalence of respiratory symptoms in school children in two Israeli cities with different NO\textsubscript{2} levels</td>
<td>Maximum NO\textsubscript{x} levels in the cities of 62 and 23 µg/m\textsuperscript{3}, respectively</td>
<td>Various respiratory symptom rates were higher in Ashdod, the more heavily polluted city Confounding factors controlled</td>
<td>Effects related to both SO\textsubscript{2} and NO\textsubscript{x}</td>
<td>Goren and Hellmann (1988)</td>
</tr>
<tr>
<td>Study</td>
<td>NO₂ Exposure</td>
<td>Effects Seen</td>
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</tr>
<tr>
<td>Case-control study of 231 children in the Netherlands for respiratory symptoms vs NO₂ levels with different NO₂ levels</td>
<td>Indoor NO₂ levels</td>
<td>Respiratory symptoms determined by questionnaire and school health survey data</td>
<td>No difference in levels between case and controls</td>
<td>Hoek et al (1984)</td>
</tr>
<tr>
<td>Study of changes in respiratory symptom rates in relation to NO₂ levels in a rural district of Japan</td>
<td>NO₂ measurements from 1974-79</td>
<td>Significant correlation between daily maximum NO₂ and subacute phlegm, cough, and wheezing for prick skin test (house dust extract)—positive children</td>
<td>Similar results for SO₂ during this period The fact that SO₂ and NO₂ were correlated and that no covariates were included in the analysis means the study can only be considered suggestive</td>
<td>Kagamimori et al (1986)</td>
</tr>
<tr>
<td>Study of respiratory illness in 2,900 school children vs levels of NO₂ in Oska Prefecture, Japan</td>
<td>Annual outdoor NO₂ levels</td>
<td>Children attending schools in the highest polluted area had the highest rates of bronchial asthma and recurring respiratory infections</td>
<td>No indoor NO₂ data NO₂ levels analyzed in relation to distance between children’s homes and distance from highways</td>
<td>Nagira et al (1981)</td>
</tr>
<tr>
<td>Study of incidence of mediastinal and subcutaneous emphysema in young bronchial asthma patients in relation to NO₂ levels in Japan</td>
<td>Daily average outdoor NO₂ levels</td>
<td>Report correlation between NO₂ levels and occurrence at mediastinal and subcutaneous emphysema</td>
<td>No indoor NO₂ data Other factors that cause mediastinal and subcutaneous emphysema not evaluated</td>
<td>Odajima and Baba (1987)</td>
</tr>
<tr>
<td>Study of respiratory symptoms in over 90,000 school children in Japan in relation to NO₂ levels</td>
<td>Outdoor mean NO₂ value 3 years prior to the survey</td>
<td>Asthma-like symptoms was higher in district with the highest NO₂ concentration</td>
<td>No indoor NO₂ data, confounding with other pollutants Confounding due to smoking and socioeconomic factors</td>
<td>Tsunetoshi et al (1987)</td>
</tr>
<tr>
<td>Study</td>
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<tr>
<td>Study of respiratory symptoms in the district of Pisa, Italy, using Italian Natural Research Council Questionnaire on 3,729 adult subjects</td>
<td>Gas stove use</td>
<td>Higher prevalence rate for respiratory symptoms for those using tank gas for cooking</td>
<td>No NO₂ measurements</td>
<td>Viegli et al (1992, 1990)</td>
</tr>
<tr>
<td>Study respiratory infection in children and adults and absenteeism in Helsinki, Finland</td>
<td>Annual average of NO₂ was 47 μg/m³ (0.025 ppm)</td>
<td>The level of NO₂ has little effect on the frequency of disease. The effect of low temperature is highly significant</td>
<td>No indoor NO₂ measurements. Possible confounding with temperature</td>
<td>Ponka (1990)</td>
</tr>
<tr>
<td>Study of respiratory illnesses in children and mothers in Hong Kong</td>
<td>Mean levels of NO₂ estimated by personal samplers</td>
<td>Among 312 mothers, statistically significant allergic rhinitis for NO₂ levels of 22.6 vs 19.0 ppb, whereas among the children there was no statistically significant difference in presence vs. absence of the respiratory symptoms by NO₂ levels</td>
<td>Difference in mean levels of NO₂ for children for different symptoms were very small, ranging from 0.00-1.79 ppb (0.3-4 μg/m³)</td>
<td>Koo et al (1990)</td>
</tr>
<tr>
<td>Study of croup incidence and NO₂ levels in Bochum, Germany</td>
<td>Outdoor NO₂ levels, daily averages</td>
<td>Positive correlation between NO₂ levels and incidence of croup as determined by hospital admissions</td>
<td>No indoor NO₂ data. No discussion of confounding factors</td>
<td>Severen and Mietens (1987)</td>
</tr>
<tr>
<td>Study of croup incidence and NO₂ levels in Mannheim and Darmstadt, Germany</td>
<td>Outdoor NO₂ levels, annual and monthly average</td>
<td>Monthly NO₂ average in Mannheim but not in Darmstadt showed linear correlation with monthly cases of croup</td>
<td>No indoor NO₂ data. No discussion of confounding factors</td>
<td>Wemmer (1984)</td>
</tr>
</tbody>
</table>
### TABLE 14A-1 (cont’d). SUMMARY OF EPIDEMIOLOGICAL STUDIES OF NITROGEN DIOXIDE EFFECTS ON RESPIRATORY ILLNESS

<table>
<thead>
<tr>
<th>Study</th>
<th>NO\textsubscript{2} Exposure</th>
<th>Effects Seen</th>
<th>Discussion</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Study of croup incidence and NO\textsubscript{2} levels in Mannheim and Darmstadt, Germany</td>
<td>Outdoor NO\textsubscript{2} levels, annual and monthly average</td>
<td>Monthly NO\textsubscript{2} average in Mannheim, but not in Darmstadt, showed linear correlation with monthly cases of croup</td>
<td>No indoor NO\textsubscript{2} data, no discussion of confounding factors</td>
<td>Wemmer (1984)</td>
</tr>
<tr>
<td>Study on the influence of various factors to include NO\textsubscript{2} on the incidence of croup in Innsbruck, Austria</td>
<td>NO\textsubscript{2} levels were measured by a chemoluminescence detector at a measuring station in the inner city area</td>
<td>Pseudocroup has a multifactor pathogenesis Rapid changes in air pollutants (especially NO and NO\textsubscript{2}) are followed by increased occurrence of croup</td>
<td>No indoor NO\textsubscript{2} data, weather conditions cannot be eliminated as an important factor whether directly or indirectly</td>
<td>Guggenbichler et al (1990)</td>
</tr>
<tr>
<td>A pilot study of 130 third graders of NO\textsubscript{2} levels and hematological, clinical and immunological end points in Rostock, Germany</td>
<td>Indoor monitors for NO\textsubscript{2} and other pollutants</td>
<td>Selected results of questionnaires and smoking history</td>
<td>Limited findings presented in the preliminary study</td>
<td>Thielebeule and Huelse (1989)</td>
</tr>
<tr>
<td>Study of NO\textsubscript{2} exposure and respiratory disease function and symptoms in Japan</td>
<td>Indoor NO\textsubscript{2} measurements, urine hydroxyproline measurements</td>
<td>Respiratory disease questionnaires (American Thoracic Society), pulmonary function tests in 1,000 3-year-old children</td>
<td>Prevalence rate of respiratory symptoms higher near roadways with heavy traffic and higher pollutant levels</td>
<td>Ono et al (1990), Tomnaga and Ono (1985)</td>
</tr>
<tr>
<td>Study of acute illness symptoms and pulmonary function in New York in relation to NO\textsubscript{2} exposure</td>
<td>Measures of personal exposure to NO\textsubscript{2}</td>
<td>Asthmatic and nonasthmatic women and children are evaluated for PFT and respiratory symptoms</td>
<td>Preliminary data</td>
<td>Goldstein et al (1987)</td>
</tr>
<tr>
<td>Study</td>
<td>NO₂ Exposure</td>
<td>Effects Seen</td>
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</tr>
<tr>
<td>Study of chronic respiratory disease symptoms in 8,572 Southern California Seventh-Day Adventists</td>
<td>Hours of SO₂ exposure above 4 pphm, oxidants above 10 pphm, and TSP above 200 μg/m³ NO₂ exposure levels were not linked to health effects</td>
<td>Relative risks of about 1 2 for the pollutants listed</td>
<td>No relationship with measured NO₂.</td>
<td>Euler et al (1988)</td>
</tr>
<tr>
<td>Study of 875 cases of croup in Baden-Wurttemberg in relation to NO₂ exposure in a 2-year prospective study</td>
<td>Daily ambient levels</td>
<td>Statistical regression methods indicate weak but statistically significant influences of the daily NO₂ mean on the occurrence of croup</td>
<td>Air pollution considered a weak factor, whereas essential conditions for croup are individual and familiar disposition and virus infection</td>
<td>Rebmann et al (1991)</td>
</tr>
<tr>
<td>Study of asthma attack incidence over a 3-year period in Finland in relation to pollutant levels</td>
<td>Ambient NO₂ levels averaged 38 6 μg/m³ over the 3-year period</td>
<td>During the high NO₂ (mean 45 8 μg/m³) levels, the mean number of all admissions was 29% greater than during lower levels (28 1 μg/m³)</td>
<td>Indoor NO₂ level and cooking fuel used were not considered Minimum temperature was associated with asthma admissions</td>
<td>Ponka (1991)</td>
</tr>
<tr>
<td>Study of upper respiratory infections in children in Finland in relation to NO₂ and other pollutants</td>
<td>Ambient NO₂ levels averaged 15 μg/m³</td>
<td>A significant association of upper respiratory infections and air pollution in the two age groups studied</td>
<td>Passive smoking and SES evaluated Indoor NO₂ levels and cooking fuels not considered</td>
<td>Jaakkola et al (1991)</td>
</tr>
<tr>
<td>Study of prevalence of asthma in children in relation to NO₂ levels in Germany</td>
<td>Indoor and outdoor NO₂ data taken into account.</td>
<td>Stoves used as a heating device had a 4 8-fold relative risk for asthma compared to other heating</td>
<td>704 children, 7 16 years old, took part in a standardized interview and exam Passive smoking was examined</td>
<td>Kuehr et al (1991)</td>
</tr>
<tr>
<td>Study</td>
<td>NO$_2$ Exposure</td>
<td>Effects Seen</td>
<td>Discussion</td>
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<tr>
<td>Panel study of 128 children whose parents completed a daily diary of respiratory symptoms in Chestnut Ridge region of western Pennsylvania in relation to NO$_2$ levels</td>
<td>Maximum hourly levels for each 24-h period averaged 40.5 µg/m$^3$ with a range of 12.7 µg/m$^3$</td>
<td>Ambient NO$_2$ levels were not predictive of any symptom outcome measures</td>
<td>The subgroup analysis had 55 subjects. No indoor levels or use of gas stoves were noted</td>
<td>Vedel et al (1987)</td>
</tr>
<tr>
<td>Longitudinal study of exacerbation of asthma measured by wheezing occurrence in relation to NO$_2$ exposure in approximately 24 asthmatic school children</td>
<td>Maximum daily ambient levels with average levels of 75 and 169 µg/m$^3$ in the &quot;medium&quot; and &quot;high&quot; exposure categories. Yearly average levels were 2.0 and 0.4 µg/m$^3$, respectively</td>
<td>NO$_2$ levels were not associated with the occurrence of symptoms in this group of asthmatics for the exposures that occurred</td>
<td>No indoor exposure data. The model adjusted for the effect of the presence of symptom on the previous day</td>
<td>Henry et al (1991)</td>
</tr>
<tr>
<td>Study of the prevalence of persistent respiratory symptoms in 393 infants in North Carolina</td>
<td>Gas stove cooking or electric stove use</td>
<td>Relative risk of 1.12 (95% confidence interval of 0.63 to 2.04)</td>
<td>Approximately 41 infants lived in homes with the environmental risk factor of gas cooking</td>
<td>Margolis et al (1992)</td>
</tr>
<tr>
<td>Study</td>
<td>NO₂ Exposure</td>
<td>Effects Seen</td>
<td>Discussion</td>
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<tr>
<td>Six City study of 9,000 grade school children</td>
<td>7-49 µg/m³ (0.004-0.026 ppm) estimate of total personal exposure gas stove use used as a surrogate</td>
<td>FEV₁ and FVC decreases ranged from 0-2 to 6%, depending on examination and adjustment</td>
<td>Differences marginally significant, incomplete indoor exposure</td>
<td>Speizer et al (1980)</td>
</tr>
<tr>
<td>Six City study analyzed for effects on pulmonary function growth</td>
<td>7-49 µg/m³ (0.006-0.04 ppm) estimate of total personal exposure gas stove use used as a surrogate</td>
<td>No effect of gas stove use on growth of pulmonary</td>
<td>Differences marginally significant, incomplete indoor exposure</td>
<td>Berkey et al (1986)</td>
</tr>
<tr>
<td>Lung function panel study of 351 children at the Chestnut Ridge Elementary School</td>
<td>NO₂ ranged from 12-79 µg/m³ (0.006-0.04 ppm)</td>
<td>Peak flow not affected by pollutant levels</td>
<td>No indoor pollutant measurements made, outdoor levels were low</td>
<td>Vedral et al (1987)</td>
</tr>
<tr>
<td>Lung function study of 117 middle-class households in Tucson, AZ</td>
<td>Gas stove use</td>
<td>Peak flow was marginally related to gas stove use (p = 0.066)</td>
<td>No indoor NO₂ measurements made</td>
<td>Lebowitz et al (1985)</td>
</tr>
<tr>
<td>Study of traffic policemen in urban Boston with nearby suburban areas</td>
<td>75-103 µg/m³ (0.04-0.055 ppm) annual mean daily concentrations</td>
<td>No differences in various pulmonary function tests</td>
<td>No indoor measurements, small sample sizes</td>
<td>Speizer and Ferris (1973a,b)</td>
</tr>
<tr>
<td>Study of FEV₀ 75 in school children in Chattanooga, TN</td>
<td>Ambient annual NO₂ levels as high as 286 µg/m³ (0 15 ppm) (Saltzman method)</td>
<td>Suggestion of lower FEV₀ 75 means in high NO₂ area</td>
<td>No indoor measurements, much of the NO₂ measured by Jacob-Hochheiser method, no strong differences found</td>
<td>Shy et al (1970a,b)</td>
</tr>
<tr>
<td>Study of nonsmoking adults in Los Angeles and San Diego</td>
<td>43-96 µg/m³ (0.023-0.051 ppm) annual mean daily concentrations 113-118 µg/m³ 90th percentile</td>
<td>No difference in several ventilatory measurements including spirometry and flow volume curves</td>
<td>No indoor exposures, complicated outdoor exposures</td>
<td>Cohen et al (1972)</td>
</tr>
</tbody>
</table>
### Table 14A-2 (cont'd). Summary of Epidemiological Studies of Nitrogen Dioxide Effects on Pulmonary Function

<table>
<thead>
<tr>
<th>Study</th>
<th>NO₂ Exposure</th>
<th>Effects Seen</th>
<th>Discussion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung function study of 12 asthmatic children living in the Sunair Home in California</td>
<td>Maximum hourly value of 22 pphm</td>
<td>Morning peak flow was reduced, but afternoon peak flow was not</td>
<td>Effects of other pollutants could not be separated. Seasonal factors not adjusted for. No indoor pollutant measurements</td>
<td>Richards et al (1981)</td>
</tr>
<tr>
<td>Office workers in Los Angeles and San Francisco</td>
<td>65-130 μg/m³ (0.034-0.07 ppm) median NO₂, 110-250 90th percentile</td>
<td>No difference in most pulmonary tests</td>
<td>No indoor measurements. Small sample size</td>
<td>Linn et al (1976)</td>
</tr>
<tr>
<td>Respiratory symptom and pulmonary function study of 4th through 6th graders in Akron, OH</td>
<td>NO₂ averaged 54 μg/m³ (0.029 ppm) in polluted area</td>
<td>Small decrease seen in ratio of FEV₁ to FVC</td>
<td>No indoor measurements. Small differences seen. Relatively low NO₂ exposure</td>
<td>Mostardi et al (1981a,b)</td>
</tr>
<tr>
<td>Study of pulmonary function in French cities</td>
<td>NO₂, 12-16 μg/m³ (0.006-0.008 ppm) daily means, NO, 7-140 μg/m³ daily means</td>
<td>No decrease in pulmonary function related to NO₂ exposure</td>
<td>No indoor measurements. Low NO₂ exposure</td>
<td>PAARC (1982a,b)</td>
</tr>
<tr>
<td>Study of 5,561 adults aged 25-39 years living in Lancaster and Glendora, CA</td>
<td>Annual means over the 5-year period in Lancaster and Glendora (Aziza station) averaged 3.2 and 11.4 ppm, respectively</td>
<td>Decreased FEV₁ and FVC and increased cough, phlegm, and wheezing found in Glendora when compared to Lancaster</td>
<td>Areas measured at different times. Other differences between areas impossible to adjust for</td>
<td>Detels et al (1981a,b)</td>
</tr>
<tr>
<td>Japanese study of school children in Japan, aged 11 years</td>
<td>40-360 μg/m³ (0.02-0.19 ppm) 1-h values at time of testing</td>
<td>Correlation of peak flow and 25% and 50% FVC with NO₂, NO, SO₂, and TSP</td>
<td>Correlations not adjusted for other pollutants</td>
<td>Kagawa and Toyama (1975)</td>
</tr>
</tbody>
</table>
TABLE 14A-2 (cont’d). SUMMARY OF EPIDEMIOLOGICAL STUDIES OF NITROGEN DIOXIDE EFFECTS ON PULMONARY FUNCTION

<table>
<thead>
<tr>
<th>Study</th>
<th>NO₂ Exposure</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Case control study of 213 nonsmoking women in the Tecumseh Community Health study evaluated FEV values vs various cooking fuels</td>
<td>Cooking fuels, duration of exposure to cooking fuels, and exhaust fan use</td>
<td>Association of low FEV and gas cooking was marginally significant (p = 0.07)</td>
<td>No indoor NO₂ data Small sample size reduces power, especially the limited number of gas stoves in sample</td>
<td>Jones et al (1983)</td>
</tr>
<tr>
<td>Study of 561 nonsmoking white adult residents of Maryland evaluating the effects of gas cooking on pulmonary function</td>
<td>Gas stove exposure</td>
<td>The use of gas cooking associated with a significantly greater percentage with impaired ventilatory function as measured both by FEV₁ &lt; 80% predicted and by FEV₁/FVC &lt; 70%</td>
<td>Adjustments for SES and control for smoking</td>
<td>Helsing et al (1982)</td>
</tr>
<tr>
<td>National Health and Nutrition Examination Survey II lung function data coupled with EPA’s SAROAD aerometric data Lung function measurements included FVC, FEV₁, and PEF</td>
<td>NO₂ exposures ranged from less than 0.01-0.08 ppm Individual estimates based on average of all stations within 10 mi of census tract</td>
<td>Highly significant regression coefficients showing a decrease in FVC, FEV₁, and PEF with increasing NO₂ exposure</td>
<td>No indoor NO₂ exposure measurements Outdoor estimates over a wide area</td>
<td>Schwartz (1989)</td>
</tr>
<tr>
<td>In a sample of over 16,000 children, PFT were compared to gas stove use</td>
<td>Gas stove use</td>
<td>Marginally significant decrease (p = 0.052) in lung function in girls 9-13 years of age PFT numbers were adjusted for parental smoking habits</td>
<td>No indoor measurements</td>
<td>Hasselblad et al (1981)</td>
</tr>
<tr>
<td>Study</td>
<td>NO₂ Exposure</td>
<td>Effects Seen</td>
<td>Discussion</td>
<td>References</td>
</tr>
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<td>---------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
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<td>------------------------------</td>
</tr>
<tr>
<td>A study of PFT in women in relation to NO₂ levels in the Netherlands</td>
<td>Weekly personal exposure estimates ranged from 15-300 µg/m³ (0.008-0.159 ppm)</td>
<td>Statistically significant negative associations between various pulmonary function measures and NO₂ exposure in nonsmoking women</td>
<td>No association with longitudinal pulmonary function decline</td>
<td>Fischer et al (1985, 1986) Remijn et al (1985)</td>
</tr>
<tr>
<td>A study of the effect of domestic air pollution on respiratory function in a group of housewives in Lebanon, CT</td>
<td>Gas stove use</td>
<td>Some decreases observed in lung function in homes with gas stoves</td>
<td>Sample sizes too small to give meaningful information</td>
<td>Hosein and Corey (1986)</td>
</tr>
<tr>
<td>Study of lung function and respiratory function in relation to homes with coal or gas cooking fuels</td>
<td>NO₂ levels of over 400 µg/m³ (0.21 ppm) in kitchens</td>
<td>Lung function measures in women were reduced by elevated levels of pollutants No respiratory symptoms were related to gas</td>
<td>Method of analysis and adjustments for covariate were not given</td>
<td>Liu and Wang (1987)</td>
</tr>
<tr>
<td>Study of children’s pulmonary function in relation to NO₂ exposure in the Netherlands</td>
<td>NO₂ levels measured by Palmes tubes in homes, mean concentration ranged from 23-72 µg/m³ (0.012-0.038 ppm) NO₂</td>
<td>PFTs were evaluated in over 800 children aged 6-12 years No relationship was found The power of the study to detect small effects on lung function was considered adequate</td>
<td>Exposure measure may not have captured short-term peak concentrations</td>
<td>Brunekreef et al (1990)</td>
</tr>
<tr>
<td>Study of NO₂ exposure on pulmonary function and symptoms in Japan</td>
<td>Indoor NO₂ measurements, urine hydroxyproline measurements</td>
<td>Respiratory disease questionnaires, PFTs in 1,000 3-year-old children</td>
<td>Ongoing study expected completion in 1989-90 with analysis at a later date</td>
<td>Tomimana and Ono (1985)</td>
</tr>
</tbody>
</table>
**TABLE 14A-2 (cont’d). SUMMARY OF EPIDEMIOLOGICAL STUDIES OF NITROGEN DIOXIDE EFFECTS ON PULMONARY FUNCTION**

<table>
<thead>
<tr>
<th>Study</th>
<th>NO₂ Exposure</th>
<th>Effects Seen</th>
<th>Discussion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study of children's bronchial reactivity in relation to NO₂ exposure in Switzerland</td>
<td>NO₂ levels measured by passive samplers</td>
<td>Bronchial reaction to carbachol measured in 312 school children in two exposure groups</td>
<td>Urban NO₂ levels of 36 2 μg/m³ vs 26 2 μg/m³ in rural area A statistical difference seen in bronchial reactivity in healthy children but not in asthmatics Effects of anti-asthmatic therapy in atopic children could not be determined</td>
<td>Gschwend-Eigenmann et al (1989)</td>
</tr>
<tr>
<td>Study of oxides of sulfur, NO₂, hydrocarbons, and particulate exposure on pulmonary function in two cities in Southern California</td>
<td>Outdoor daily peak hourly levels for NO₂ range from 0.03-0.11 ppm</td>
<td>Chronic exposure to the pollutant mix results in less rapid growth of lung function in children and a greater rate of deterioration in adulthood</td>
<td>The proportion of participants retested in the study was low (45-47%) due to migration out of the study area The impact of this may have been minimal through Differences between communities in age and height could have biased the results</td>
<td>Detels et al (1991)</td>
</tr>
</tbody>
</table>

*Abbreviations*  
NO₂ = Nitrogen dioxide, SO₂ = Sulfur dioxide, TSP = Total suspended particulate, FEV = Forced expiratory volume, FVC = Forced vital capacity, NO = Nitric oxide, COPD = Chronic obstructive pulmonary disease, NOₓ = Nitrogen oxides, PFT = Pulmonary function test, SES = Socioeconomic status, PEF = Peak expiratory flow
15. CONTROLLED HUMAN EXPOSURE STUDIES OF NITROGEN OXIDES

15.1 INTRODUCTION

This chapter discusses the effects of nitrogen oxides (NO\textsubscript{x}) on human volunteers exposed under controlled conditions. The NO\textsubscript{x} species of primary concern is nitrogen dioxide (NO\textsubscript{2}). Nitric oxide (NO) and nitrates have also been evaluated in controlled human exposures, and nitric acid (HNO\textsubscript{3}) effects have only recently been studied. The 1982 Air Quality Criteria for Oxides of Nitrogen Document (U.S. Environmental Protection Agency, 1982a) presents a comprehensive review of studies conducted up to about 1980. The present chapter focuses mainly on summaries and critiques of studies conducted since then, but also includes some discussion of the earlier literature as well.

Controlled human exposure studies of NO\textsubscript{2} deal with relatively brief, experimental exposures to higher concentrations compared to the annual arithmetic mean standard (0.053 ppm). One of the purposes in reviewing these studies is to evaluate the data base for short-term (typically <4 h) NO\textsubscript{2}-related health effects, thus, consideration of the time course of responses and the pattern of exposure in controlled human exposure studies is important.

Because of the widespread occurrence of NO\textsubscript{2} in both outdoor and indoor environments, there are major concerns regarding potential impacts of NO\textsubscript{2} exposure on human health, particularly with regard to effects on the lung. Dosimetry modeling and animal histological studies indicate that NO\textsubscript{2}’s impact should be primarily seen in the small airways and gas exchange regions of the lung, thus, tests that specifically evaluate responses in this region are of particular interest in evaluating the effects of NO\textsubscript{2}. In assessing NO\textsubscript{2} lung effects for criteria development purposes, a number of important questions need to be addressed, as posed in the following list of critical questions. Some of these questions are of a "generic" nature, applying to many ambient air pollutants, and others are specific to NO\textsubscript{2} inhalation. Several of the questions may be answered only partially by controlled human exposure studies and are addressed further by animal toxicological studies and/or epidemiological studies discussed in Chapters 13 and 14, respectively.
Nitrogen Dioxide Exposure and Human Health  Critical Questions

1. Does short-term NO₂ exposure cause acute changes in lung function, increased respiratory symptoms, or increased airways responsiveness in normal, healthy subjects at levels that may be expected in the ambient (or indoor) environment? Are these effects reproducible? If so, what is the possibility that such acute responses may contribute to chronic changes in lung function, promote the development of respiratory disease, cause acceleration of normal age-related declines in lung function, or aggravate existing respiratory disease?

2. Are there groups within the population at special risk for NO₂ health effects (i.e., groups comprised of persons who exhibit greater responses to NO₂ exposure than the average healthy subject)? Groups hypothesized as likely to be at special risk include young children, adolescents, elderly subjects, and patients of all ages with asthma, chronic obstructive lung disease, or other lung diseases. If there are subject groups who are more responsive, can they be identified prospectively (i.e., without exposing them to NO₂ first)?

3. Does NO₂ cause an inflammatory response in the lungs of healthy individuals or patients with lung disease? Specifically, does NO₂ exposure cause increased capillary permeability, increased local blood flow, extravasation of fluid or influx of leukocytes—especially neutrophils and eosinophils—into the interstitium and the airways, secretion of pro-inflammatory mediators, mast cell degranulation, or epithelial desquamation?

4. Does NO₂ exposure cause increased responses of the lung (including airways responsiveness, lung function, inflammation, cell damage, etc.) to (a) other pollutants such as ozone (O₃), sulfur dioxide (SO₂), or acid aerosols, (b) bronchoconstrictors such as histamine or methacholine, (c) other agents such as cold-dry air or exercise, or (d) specific antigenic substances?

5. Does NO₂ exposure alter (a) respiratory tract host defenses, (b) airway epithelial permeability or mucociliary clearance, or (c) local or systemic immune response to infection? As a consequence of NO₂ impacts on host defense system components, is the killing or removal of microorganisms impaired by NO₂ exposure? Also, are inflammatory responses or tissue injury caused by microorganisms worsened by coincident NO₂ exposure?

6. What is the time course of response to acute exposures? Are there both immediate and delayed responses? Do responses increase or decrease with increased exposure duration? What is the time course of response to repeated exposures? Do responses increase or decrease with increased frequency of exposure?
Controlled human exposure studies serve as an important source of inhalation toxicology data, particularly for the criteria pollutants such as NO\textsubscript{x}. Methodological and experimental design considerations for controlled human exposure studies have recently been reviewed (Folinsbee, 1988). These studies are typically conducted on volunteers who have been informed of the possible risks of such studies and who have given their "informed consent" to participate. The subject group that most often participates in such studies are young adult males with no history of respiratory disease, allergies, smoking, and no contraindication to exercise. In addition to young men, participants of either gender and of different racial groups have included children, adolescents, elderly persons, and adults. Other subject groups that have been specifically studied include healthy subjects with allergies, asthmatics, smokers, patients with chronic obstructive pulmonary disease (COPD), or otherwise healthy persons with upper respiratory infections. These latter subject groups may be considered potentially "sensitive subjects", especially asthmatics, COPD patients, children, and the elderly. For individuals with existing lung disease and/or hyperresponsive airways, special consideration of the potential impact of pollutant exposure is required. These individuals, including the healthy elderly population, often have limited pulmonary reserves and, therefore, a given insult has a greater physiological/pathological consequence (increased airways resistance, restriction of lung volume). Children are of special concern because their lungs are still growing and developing and, hence, the possibility of a long-term impact on lung health may be greater than for the mature adult lung.

Controlled exposures, by definition, occur in a laboratory setting. The most "natural" mode of exposure is unencumbered breathing within an exposure chamber. Other modes of exposure include facemask, hood, or mouthpiece exposures. A controlled exposure implies that the environmental variables such as the concentration of the pollutant, temperature, and humidity are monitored and maintained at some specified level. In addition, the duration of the exposure and amount of activity during the exposure are closely regulated. The activity level is closely correlated with the volume of air breathed into the lung. In order to simulate an outdoor exposure where the subject is active, many exposure studies include some form of controlled exercise. However, exercise alone may have some important confounding effects, particularly in the case of exercise-induced bronchoconstriction in asthmatics. Exercise alone may induce significant decrements in spirometric variables or significant increments in...
airway resistance. Exercise-induced bronchoconstriction is followed by a refractory period of several hours during which asthmatics are less susceptible to bronchoconstriction (Edmunds et al., 1978). This period of refractoriness could alter the subject's responsiveness to NO₂ or other inhaled substances. The major external determinants of the exposure "dose" of a pollutant are the concentration of pollutant, the duration of the exposure, and the volume of air breathed (specifically, the route, depth, and frequency of breathing) during the exposure. Further information is, of course, necessary to determine the actual dose delivered to the various "target" regions of the respiratory tract (i.e., total respiratory uptake), as is presented in Chapter 13. Many of these considerations have been discussed in greater detail by Folinsbee (1988).

In human exposure studies, the methods used for assessment of effects primarily involve "noninvasive" procedures. Various pulmonary function tests such as spirometric measures of lung volumes, measures of resistance of lung or nasal airways, ventilation volume (volume of air inhaled into the lung per minute), breathing pattern (frequency and depth of breathing), and numerous other "breathing" tests have been utilized (Bouhuys, 1974). These tests provide useful information about some of the basic physiological functions of the lung. Certain tests provide information primarily about large airway function, these include (a) dynamic spirometry tests (e.g., forced expiratory tests such as forced expiratory volume in 1 s [FEV₁], maximal and partial flow-volume curves [including those using gases of different densities such as helium], peak flow measurements, etc.), and (b) specific airway resistance/conductance measurements (SRₐw, SGₐw). The reader should refer to the glossary for more specific descriptions of various tests. These "standard pulmonary function" tests are relatively simple to administer, provide a good overall index of lung function, and have a relatively low coefficient of variation (CV), the CV is about 3% for FEV₁ and about 10 to 20% for SRₐw. However, because NO₂ deposits primarily in peripheral airways, many of the above tests may not provide the necessary information to fully evaluate the effects of NO₂. Other tests purported to provide evidence of small airway function include multiple breath nitrogen washout tests, closing volume tests, aerosol deposition/distribution tests, density dependence of flow-volume curves (using gases of different densities such as helium), and frequency dependence of dynamic compliance, but
none are used routinely and use of these procedures to assess "small airways function" is not widely accepted.

Somewhat more invasive procedures have also been more utilized in recent years to determine human responses to air pollutant exposures, including pharmacologic airway inhalation challenge tests, measurements of pulmonary clearance of inhaled aerosols, bronchoalveolar lavage, nasal lavage, and arterial blood gas measurements.

Airway inhalation challenge tests are used to evaluate the "responsiveness" of a subject's airways to inhaled materials. Airway responsiveness may change as a result of alterations in a disease state, such as inflammation associated with asthma or viral respiratory infection, or as a result of damage to the airway caused by disease or insult from inhaled toxic or allergenic materials. Thus, one of the problems in evaluating changes in airway responsiveness with respect to inhalation of air pollutants is that the baseline responsiveness can be changed by other factors not associated with pollutant exposure. In order to test for the degree of airway responsiveness, a chemical that causes constriction of the airways (such as histamine, carbachol, or methacholine) is typically used. Other challenge tests involve the use of allergenic substances, exercise, hypertonic saline, or cold-dry air. Responses are usually measured by evaluating changes in airway resistance or spirometry after each dose of the challenge is administered. Usually, the test will proceed until some target effect level is achieved (e.g., doubling of airway resistance) and the airway responsiveness is then characterized by the dose required to achieve that level. The procedures for administering and interpreting inhalation challenges are discussed in detail elsewhere (Cropp et al., 1980, Cropp, 1979, Chait et al., 1975, Fish and Kelly, 1979, O'Byrne et al., 1982).

Asthmatics as a group are significantly more responsive than healthy normal subjects to a variety of airway challenges. The differences in airway responsiveness may span several orders of magnitude (at least 100-fold) between normals and asthmatics (O'Connor et al., 1987). Nevertheless, there is considerable overlap between the more responsive healthy subjects and the less responsive (histamine) asthmatics (Pattemore et al., 1990). Airway responsiveness to methacholine appears to be somewhat better than airway responsiveness to histamine at differentiating normals and asthmatics, although responses to these two bronchoconstrictors are well correlated (r = 0.70) (Chatham et al., 1982). Unfortunately, because of the number of different provocative agents used in the airway challenges and the
variety of methodologies used to administer challenges, it is difficult to compare responses between laboratories in a quantitative manner. Thus, it is not useful to suggest standard ranges of responsiveness for normals and asthmatics.

Tests of pulmonary clearance of inhaled aerosols are used to assess the efficiency of the mucociliary clearance mechanism and to estimate pulmonary epithelial permeability. Typically, a radioactively labeled test aerosol, of a specific size range that will deposit in the lung region of interest, is deposited in the lung by inhalation. External detectors are then used to assess the amount of remaining test aerosol at various times after the initial deposition of the aerosol. This methodology is discussed in Clarke and Pavia (1980) and Raabe (1982) and in Section 13.2.2.1. A particular application of clearance of radiolabeled aerosols is for the estimation of epithelial permeability, typically using technetium-labeled diethylene triamine penta-acetate or pentetate. This methodology is discussed in Nolop et al. (1987).

In the past several years, bronchoalveolar lavage techniques have been used in clinical exposure studies of several different pollutants. In this procedure, a fiberoptic bronchoscope is passed into the airways and wedged in a subsegmental bronchus, where sterile buffered saline is used to wash free cells and airway secretions from the segment (Reynolds, 1987). The resulting lavage fluid may be analyzed for various chemical mediators or reaction products, numbers and types of cells, and the functions of some lung cell types. Another less invasive procedure, known as nasal lavage, may be used to obtain nasal secretions and cells (Graham et al., 1988).

There are a number of limitations of controlled human exposure or "clinical" studies. Many experimental animal models are derived from genetically pure strains, thus reducing the expected variability in biological response. Because of their heterogeneity, humans are expected to display a wider range of response to a variety of physiological and pathological stimuli. This variability and the small sample numbers limit the extent to which the data can be generalized to the population as a whole or to certain defined segments of the population (e.g., asthmatics). The small sample size may limit the interpretation of the study, especially when the results are negative (i.e., the null hypothesis is not rejected). One cannot have great confidence in a study that finds no effect of a treatment (in this case NO₂ exposure) if the sample size (i.e., the number of subjects) is too small to statistically detect an effect, if
This must be kept in mind in interpreting the results of human exposure studies with a small number of subjects. Investigators have reported a wide variation in responsiveness of asthmatics to NO₂, which may be partially attributed to intrinsic variation in response as well as variation in exposure variables. In addition, place of residence, season of the year, and indoor home environment may all be determinants of the asthmatic's response to NO₂. Controlled human exposure studies are ethically limited to acute or subchronic fully reversible functional and/or symptomatic responses. This may in many cases limit the magnitude of expected responses and, hence, the statistical significance of responses in studies with small numbers of subjects. Exposures seldom last longer than 1 to 2 weeks for up to 8 h per day. These data, therefore, are primarily useful in evaluation of short-term, NO₂-induced health effects.

True simulation of ambient conditions, given the number of potential pollutants and the variety of possible combinations, is not a realistic goal for controlled human exposure studies. For example, the typical temporal pattern of ambient concentrations is seldom duplicated in controlled exposure studies. However, simple mixtures of two or three pollutants can be evaluated to determine the potential for either additive or synergistic effects. Further discussion of the design considerations for human clinical studies are presented by Bates et al. (1970), Hackney et al. (1975a), and Folinsbee (1988) and were the subject of a symposium proceedings (Frank et al., 1985). Because controlled exposure studies of humans deal exclusively with acute or subchronic exposures, the applicability of these data is limited to short-term exposure effects and of limited usefulness in the evaluation of the effects of chronic NO₂ exposure.

More than 25 additional studies on the effects of NO₂ on healthy, normal subjects have become available since the 1982 Air Quality Criteria for Oxides of Nitrogen Document (U.S. Environmental Protection Agency, 1982a). Several new studies of the effects of NO₂ on individuals with pulmonary disease (asthma and COPD) have been published, helping to alleviate a critical information deficit of the earlier review (U.S. Environmental Protection Agency, 1982a,b). Although more than 10 new reports have been published, the data base concerning NO₂ effects in sensitive subjects still requires concentration-response studies in moderately sensitive asthmatics, information concerning the inflammatory response to NO₂ inhalation, further examination of the effects of NO₂ on infectivity in humans, and further
evaluation of patients with COPD. Only one study is available on pulmonary epithelial permeability or mucociliary clearance effects of NO₂ in humans.

One of the more important observations in studies of NO₂-exposed animals is that NO₂ exposure is associated with increased susceptibility to viral and bacterial infections due to impairment of host-defense mechanisms (see Section 13.2.1). Also, epidemiology studies clearly suggest a link between NO₂ exposure and increased rates of respiratory illness, especially in children (Section 14.3.1). These studies have provided a basis for several recent investigations of human immune host defenses after NO₂ exposure. Studies have utilized both in vitro exposure of cultured human cells (e.g., macrophages) and in vivo exposures of human subjects.

This chapter opens with discussion of effects on healthy adults of controlled human exposures to NO₂. Recently published reports generally support prior conclusions regarding the effects of NO₂ exposure on healthy young adults. Several of the newer studies examined the specific effect of NO₂ on cardiopulmonary function in normal adults (see Section 15.2). The NO₂ concentrations ranged from 0.2 to 4.0 ppm. In another group of studies, the effects of pollutant mixtures or ambient air, of which NO₂ was a component, were examined. These studies are summarized in Section 15.2.3.

Studies examining the specific effects of NO₂ in normal subjects support conclusions earlier reached in the Air Quality Criteria for Oxides of Nitrogen Document (U.S. Environmental Protection Agency, 1982a), in that they consistently demonstrated the absence of effect of NO₂ on lung function at concentrations between 0.3 and 0.6 ppm (Adams et al., 1987; Drechsler-Parks et al., 1987, Drechsler-Parks, 1987, Kagawa, 1986).

Four studies (Avol et al., 1983, 1985a, 1987, Linn et al., 1980a) have also been published in which NO₂ was a component of an ambient oxidant air mixture. The effects of ambient air exposures, if any, were attributed to O₃. There was no apparent influence of the very low (0.04 to 0.07 ppm) concentrations of NO₂ present in the ambient air. In addition, several controlled exposure studies used pollutant mixtures containing NO₂ in concentrations from 0.16 to 5.0 ppm (Kagawa, 1983a,b, Folinsbee et al., 1981, Islam and Ulmer, 1979a,b, Kagawa and Tsuru, 1979, Kagawa, 1986, Klemman et al., 1985, Linn et al., 1980b, Toyama et al., 1981, Von Nieding et al., 1979, Stacy et al., 1983, Drechsler-Parks et al., 1987).
At concentrations less than 1.0 ppm, these studies demonstrated no obvious effects of NO₂ in pollutant mixtures, which contained O₃, SO₂, and/or particles in addition to NO₂.

After the discussion of healthy subjects, NO₂ effects on sensitive subjects, including asthmatics and patients with COPD, are presented (Section 15.3). Several studies examining the effects of NO₂ concentrations in the range of 0.1 to 0.5 ppm on spirometry in asthmatic subjects suggest possible small changes in lung function (Bauer et al., 1986b, Roger et al., 1985, Koenig et al., 1987a,b, Avol et al., 1986). However, these changes were absent at higher NO₂ concentrations (Avol et al., 1986, Bylin et al., 1985, Linn et al., 1985b, 1986), thus failing to suggest a concentration-response relationship. Studies examining patients with COPD indicated pulmonary function changes with brief exposure to high concentrations (5 to 8 ppm for 5 min) or with more prolonged exposure to lower concentrations (0.3 ppm for 3.75 h).

Since a change in airway responsiveness appears to be one of the most sensitive indicators of response to NO₂ exposure, Section 15.4 discusses in more detail the effects of NO₂ exposure on airways responsiveness in both healthy and asthmatic subjects. Airway responsiveness has been shown to increase in healthy subjects after exposure to NO₂ concentrations in excess of 1.0 ppm. Evaluation in Section 15.4 of studies of NO₂ exposure effects on airways responsiveness in asthmatics indicates in some cases increased airway responsiveness to a variety of provocative mediators at exposure levels of 0.2 to 0.3 ppm NO₂, however, the occurrence of these responses appears to be influenced by the exposure protocol, particularly whether or not the exposure includes exercise.

Section 15.5 next reviews the effects of NO₂ and HNO₃ exposure on blood, urine, and bronchoalveolar lavage fluid (BAL) biochemistry. This is followed by discussion of an important array of studies examining the effects of exposure to NO₂ or HNO₃ exposure on human pulmonary host defense responses. These studies (Section 15.6) examine the roles that NO₂ exposure may play in potentiating susceptibility to respiratory infections. Next, Section 15.7 examines the effects of nitrates on human lung function. Finally, Section 15.8 presents conclusions and a summary of the chapter.
15.2 EFFECTS OF NITROGEN OXIDES IN HEALTHY SUBJECTS

Early studies indicated that the effect of NO\textsubscript{2} on airway resistance was noted at concentrations above 1.5 ppm in healthy volunteers (Abe, 1967, Von Nieding et al., 1970, 1973a). Other studies have indicated no significant lung function effects of NO\textsubscript{2} in healthy normal subjects at concentrations below 1.0 ppm (Folinsbee et al., 1978, Hackney et al., 1978, Beil and Ulmer, 1976, Kerr et al., 1979). This section presents a discussion on these and other studies, as well as studies on NO and NO\textsubscript{2} mixtures.

15.2.1 Lung Function Effects of Nitrogen Dioxide

This section is divided according to the concentrations of NO\textsubscript{2} used in the study. The first subsection deals with the effects of exposure to greater than 1.0 ppm, the second with the effects of exposure to less than 1.0 ppm. Studies dealing with NO\textsubscript{2} exposure in healthy subjects are summarized in Table 15-1 (The details of the exposure conditions, number of subjects, ventilation levels, temperature, relative humidity, and other experimental information are presented in Table 15-1. Occasional reference to this information is made in the text when necessary, but the reader should refer to the table for these details.)

15.2.1.1 Concentrations Above 1.0 ppm

The effects of NO\textsubscript{2} levels greater than 1.0 ppm have been examined in several laboratories. In two studies, Von Nieding and Wagner (1977) and Von Nieding et al. (1979) studied 11 males exposed to 5.0 ppm NO\textsubscript{2} for 2 h while performing light, intermittent exercise. Airway resistance increased from 1.51 to 2.41 cm H\textsubscript{2}O/L/s after 2 h of exposure. There was also an apparent decrease of arterial oxygen partial pressure (PaO\textsubscript{2}) from 90 to 82 torr. (These samples were taken from "arterialized venous" blood drawn from the ear lobe.) The statistical analysis of this data is impacted slightly due to an adjustment in the PaO\textsubscript{2} data prior to testing for significance. Von Nieding and Wagner (1977) state "To increase the power of the tests PaO\textsubscript{2}-differences \leq 5 \text{ mm Hg} and R\textsubscript{T}-increases \leq 0.5 \text{ cm H}_{2}\text{O}/\text{L/s} were regarded as zero." This transformation would increase the likelihood of finding a significant effect.

In a subsequent synopsis of several studies, Von Nieding et al. (1980) discussed the results of two experimental exposures that were previously published (in German). In the
<table>
<thead>
<tr>
<th>Reference</th>
<th>NO\textsubscript{2} (ppm)</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Vent (L/min)</th>
<th>Temp (°C)</th>
<th>Relative Humidity (Percent)</th>
<th>Number and Gender of Subjects</th>
<th>Subject Characteristics</th>
<th>Notes on Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abe (1967)</td>
<td>40-50</td>
<td>10</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Bag exposure technique Airway resistance increased 30 min after end of exposure No change in spirometry</td>
</tr>
<tr>
<td>Adams et al (1987)</td>
<td>60</td>
<td>60</td>
<td>70</td>
<td>21-25</td>
<td>45-60</td>
<td>20 M</td>
<td>Normal</td>
<td>No effect of NO\textsubscript{2} on spirometry or airway resistance</td>
<td></td>
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<tr>
<td>Bed and Ulmer (1976)</td>
<td>10</td>
<td>120</td>
<td>--</td>
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<td>--</td>
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<td>Responsiveness to acetylcholine challenge Increased after 7.5 ppm (120 min) and 5.0 ppm (14 h) Resistance increased after all but the 1.0-ppm exposure</td>
</tr>
<tr>
<td>Boushey et al (1988) (part 2)</td>
<td>06</td>
<td>120/day for 4 days</td>
<td>60</td>
<td>21-0</td>
<td>56</td>
<td>4 M/1 F</td>
<td>Healthy, NS, 21-36 years, FEV\textsubscript{1}/FVC% range 73-83% &quot;normal&quot; methacholine responsiveness</td>
<td>No effects of repeated NO\textsubscript{2} exposure on respiratory function (SRaw, FVC, FEV\textsubscript{1}) or symptoms Slight increase in circulating (venous) lymphocytes 1.792 ± 544\textsuperscript{3} mm\textsuperscript{3} (post-NO\textsubscript{2}) vs 1.598 ± 549/mm\textsuperscript{3} (baseline) No change in BAL lymphocytes except an increase in natural killer cells 7.2 ± 3.1% (post-NO\textsubscript{2}) vs 4.2 ± 2.4% (baseline) No change observed in IL-1 or TNF</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>NO₂ (ppm)</td>
<td>Exposure Duration (min)</td>
<td>Exercise Duration (min)</td>
<td>Exercise Vent (L/min)</td>
<td>Temp (°C)</td>
<td>Relative Humidity (Percent)</td>
<td>Number and Gender of Subjects</td>
<td>Subject Characteristics</td>
<td>Notes on Effects</td>
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<td>---------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Bylin et al (1985)</td>
<td>0.0</td>
<td>20</td>
<td>--</td>
<td>--</td>
<td>22</td>
<td>35</td>
<td>5 M/4 F</td>
<td>20-36 years, NS</td>
<td>Suggestion of change in SRₐw in normals. SRₐw tended to increase at 0.25 ppm and tended to decrease at 0.50 ppm. Analysis of variance indicates no significance. No effects on bronchial reactivity. Median odor threshold 0.04 ppm.</td>
</tr>
<tr>
<td>Chaney et al (1981)</td>
<td>0.2</td>
<td>120</td>
<td>0</td>
<td>--</td>
<td>22</td>
<td>40</td>
<td>19 M (15 controls)</td>
<td>Young adult, normal, normal</td>
<td>Increase in blood glutathione levels after NO₂ exposure.</td>
</tr>
<tr>
<td>Devlin et al (1992)</td>
<td>2.0</td>
<td>240</td>
<td>120</td>
<td>50</td>
<td>22</td>
<td>40</td>
<td>10</td>
<td>Healthy NS</td>
<td>Increased bronchial PMNs and decreased macrophage phagocytosis.</td>
</tr>
<tr>
<td>Drechsler-Parks et al (1987)</td>
<td>0.60</td>
<td>120</td>
<td>60</td>
<td>25</td>
<td>24</td>
<td>54</td>
<td>8 M/8 F</td>
<td>51-76 years</td>
<td>No statistically significant changes in lung function due to NO₂ exposure.</td>
</tr>
<tr>
<td>Drechsler-Parks (1987)</td>
<td>0.60</td>
<td>120</td>
<td>60</td>
<td>25</td>
<td>24</td>
<td>55</td>
<td>8 M/8 F</td>
<td>18-26 years, NS</td>
<td>No significant changes in spirometry attributable to NO₂.</td>
</tr>
<tr>
<td>Folinsbee et al (1978)</td>
<td>0.62</td>
<td>120</td>
<td>15</td>
<td>33</td>
<td>25</td>
<td>45</td>
<td>5 M</td>
<td>Healthy</td>
<td>No significant pulmonary function responses attributed to NO₂ exposure.</td>
</tr>
<tr>
<td>Reference</td>
<td>NO₂ (ppm)</td>
<td>Exposure Duration (min)</td>
<td>Exercise Duration (min)</td>
<td>Exercise Vent (L/min)</td>
<td>Temp (°C)</td>
<td>Relative Humidity (Percent)</td>
<td>Number and Gender of Subjects</td>
<td>Subject Characteristics</td>
<td>Notes on Effects</td>
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<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Frampton et al (1989a)</td>
<td>0.6</td>
<td>180</td>
<td>60</td>
<td>~40</td>
<td>22</td>
<td>30</td>
<td>7 M/2 F</td>
<td>Healthy, NS</td>
<td>No change in spirometry, R₉₀, or carbachol reactivity</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>135</td>
<td>60</td>
<td>(6 × 10)</td>
<td>22</td>
<td>30</td>
<td>11 M/4F</td>
<td>Nonreactive (carbachol)</td>
<td>No change in cell recovery or differential counts</td>
</tr>
<tr>
<td></td>
<td>with 2.0 spikes</td>
<td>(3 × 15)</td>
<td>(6 × 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible decrease in macrophage inactivation of respiratory virus in vitro</td>
<td>Possible decrease in macrophage inactivation of respiratory virus in vitro</td>
</tr>
<tr>
<td>Frampton et al (1989b)</td>
<td>0.6</td>
<td>180</td>
<td>60</td>
<td>39</td>
<td>22.0</td>
<td>30</td>
<td>6 M/2 F</td>
<td>30 ± 14 years</td>
<td>Total NO₂ uptake (1) 3.4 mg</td>
</tr>
<tr>
<td></td>
<td>VAR (0.05)</td>
<td>180</td>
<td>60</td>
<td>43</td>
<td>22.0</td>
<td>30</td>
<td>11 M/4 F</td>
<td>25 ± 12 years</td>
<td>(2) 5.6 mg, (3) ~3.3 mg</td>
</tr>
<tr>
<td></td>
<td>(20 ppm) with 3 × 15</td>
<td>180</td>
<td>60</td>
<td>~40</td>
<td>22.0</td>
<td>30</td>
<td>5 M/3 F</td>
<td>32 ± 16 years</td>
<td>(4) 81 mg BAL fluid analysis showed no significant effect on total protein or albumin</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>180</td>
<td>60</td>
<td>39</td>
<td>22.0</td>
<td>30</td>
<td>12 M/3 F</td>
<td>23 ± 0.7 years</td>
<td>There was an apparent increase in alpha-2-macroglobulin 3.5 h after exposure to 0.60 ppm (Group 1) but not after the other protocols</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>180</td>
<td>60</td>
<td>39</td>
<td>22.0</td>
<td>30</td>
<td>Healthy, NS</td>
<td>No changes in percentage of lymphocytes or neutrophils</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Authors concluded that NO₂ at these concentrations neither altered epithelial permeability nor caused inflammatory cell influx</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 15-1 (cont’d). RESPONSES OF HEALTHY SUBJECTS TO NITROGEN DIOXIDE EXPOSURE

<table>
<thead>
<tr>
<th>Reference</th>
<th>NO₂ (ppm)</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Vent (L/min)</th>
<th>Temp (°C)</th>
<th>Relative Humidity (Percent)</th>
<th>Number of Subjects</th>
<th>Gender of Subjects</th>
<th>Subject Characteristics</th>
<th>Notes on Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frampton et al (1991)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>See</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Groups 1, 2, and 4 above</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frampton et al (1992)</td>
<td>20</td>
<td>360</td>
<td>Intermittent</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>12</td>
<td>Healthy, NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomgs et al (1989)</td>
<td>10</td>
<td>120/day, 200</td>
<td>--</td>
<td>--</td>
<td>22</td>
<td>60</td>
<td>21</td>
<td>Healthy, NS, seronegative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(see Kulle and Clements, 1988)</td>
<td>0</td>
<td>3 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

There were no changes in airway mechanics (FVC, FEV₁, SGₑₑₑₑₑₑ) Responsiveness to carbachol was significantly increased after 1.5 ppm NO₂ (Group 4) but not after the other exposures (Groups 1 and 2) Degree of baseline responsiveness to carbachol was not related to response after 1.5 ppm

Immediate and 18-h post-BAL increase in PMNs

Overall trend for a slight decrement in FEV₁ with NO₂ exposure (≤1%) Methacholine response decreased with consecutive tests, but not as a result of NO₂ exposure or infection status Study conducted over 3-year period NO₂ did not significantly increase viral infectivity, although a trend was observed This study had a low power to detect small differences in infection rate
### TABLE 15-1 (cont’d). RESPONSES OF HEALTHY SUBJECTS TO NITROGEN DIOXIDE EXPOSURE

<table>
<thead>
<tr>
<th>Reference</th>
<th>NO₂ (ppm)</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Vent (L/min)</th>
<th>Temp (°C)</th>
<th>Relative Humidity (Percent)</th>
<th>Number of Subjects</th>
<th>Gender of Subjects</th>
<th>Subject Characteristics</th>
<th>Notes on Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hackney et al (1978)</td>
<td>10</td>
<td>120 (2 consecutive days)</td>
<td>60</td>
<td>Light</td>
<td>31</td>
<td>35</td>
<td>16</td>
<td>Healthy</td>
<td>Air-NO₂-NO₂ fixed exposure sequence</td>
<td>A 1.5% decrease in FVC after second day of NO₂ exposure Not clear that the decreased FVC is an NO₂ effect or an order effect No other effects</td>
</tr>
<tr>
<td>Hazucha et al (1982, 1983)</td>
<td>1</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>21</td>
<td>40</td>
<td>15 M</td>
<td>23-39 years, NS</td>
<td>No symptoms, no odor detection, no effect on SRₜₐw</td>
<td>No change in α1-protease inhibitor after NO₂ exposure</td>
</tr>
<tr>
<td>Johnson et al (1990)</td>
<td>See Frampton et al (1989b) Groups 2 and 4</td>
<td>180 Intermittent</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>3 M/5 F</td>
<td>Healthy</td>
<td>No responses No symptoms, no pulmonary function effects Suggested individual changes in SGₜₐw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joerres et al (1992)</td>
<td>10</td>
<td>120</td>
<td>60</td>
<td>50W</td>
<td>27-29</td>
<td>50-60</td>
<td>6 M</td>
<td>19-24 years No symptoms, no pulmonary function effects Suggested individual changes in SGₜₐw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kagawa and Tsuru (1979)</td>
<td>0 15</td>
<td>120</td>
<td>60</td>
<td>50W</td>
<td>28</td>
<td>55</td>
<td>8 M</td>
<td>19-24 years</td>
<td>Suggested change in density dependance of expired flow</td>
<td></td>
</tr>
<tr>
<td>Kagawa (1982)</td>
<td>1 0 ppm NO</td>
<td>120</td>
<td>60</td>
<td>50W</td>
<td>28</td>
<td>55</td>
<td>8 M</td>
<td>19-24 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>NO₂ (ppm)</td>
<td>Exposure Duration (min)</td>
<td>Exercise Duration (min)</td>
<td>Exercise Vent (L/min)</td>
<td>Temp (°C)</td>
<td>Relative Humidity (Percent)</td>
<td>Number and Gender of Subjects</td>
<td>Subject Characteristics</td>
<td>Notes on Effects</td>
<td></td>
</tr>
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<td>-------------------------------</td>
<td>---------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Kagawa (1986)</td>
<td>0.30</td>
<td>120</td>
<td>60</td>
<td>50W</td>
<td>28</td>
<td>50-60</td>
<td>6</td>
<td>19-25 years</td>
<td>No effect on SGₐ, other NO₂ mixtures studied but effect of NO₂ cannot be ascertained</td>
<td></td>
</tr>
<tr>
<td>Kerr et al. (1979)</td>
<td>0.5</td>
<td>120</td>
<td>15</td>
<td>Light/moderate</td>
<td>24</td>
<td>45</td>
<td>10</td>
<td>Healthy, three ex-smokers in group</td>
<td>Decreased quasistatic compliance Nonrandom exposure sequence air-NO₂ No change in spirometry or resistance Apparent compliance change may be due to exposure order</td>
<td></td>
</tr>
<tr>
<td>Kim et al. (1991)</td>
<td>0.18</td>
<td>30</td>
<td>L 10</td>
<td>L ≈ 25</td>
<td>22</td>
<td>45</td>
<td>9M</td>
<td>18-23 years, &quot;collegiate athletes&quot;</td>
<td>No change in lung function</td>
<td></td>
</tr>
<tr>
<td>Koenig et al. (1985)</td>
<td>0.12</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>22</td>
<td>75+</td>
<td>4 M/6 F</td>
<td>13-18 years</td>
<td>No effects on lung function</td>
<td></td>
</tr>
<tr>
<td>Koenig et al. (1987a,b)</td>
<td>0.12</td>
<td>40</td>
<td>10</td>
<td>32.5</td>
<td>22</td>
<td>75</td>
<td>3 M/7 F</td>
<td>14-19 years</td>
<td>No effects of either 0.12 or 0.18 ppm NO₂ on Rₜ or spirometry</td>
<td></td>
</tr>
<tr>
<td>Kulle (1982)</td>
<td>0.50</td>
<td>120</td>
<td>15</td>
<td>--</td>
<td>24</td>
<td>45</td>
<td>10</td>
<td>Normal adults</td>
<td>Decreased static lung compliance</td>
<td></td>
</tr>
<tr>
<td>Linn and Hackney (1983) and Linn et al. (1985b)</td>
<td>4.0</td>
<td>75</td>
<td>L 15</td>
<td>L 20-29</td>
<td>21</td>
<td>50</td>
<td>16 M/9 F</td>
<td>18-45 years, NS</td>
<td>No change in SRₐ, associated with NO₂ Small but significant decrease in blood pressure, some mild increase in symptoms</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 15-1 (cont'd). RESPONSES OF HEALTHY SUBJECTS TO NITROGEN DIOXIDE EXPOSURE

<table>
<thead>
<tr>
<th>Reference</th>
<th>NO₂ (ppm)</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (mm)</th>
<th>Exercise Vent (L/min)</th>
<th>Temp (°C)</th>
<th>Relative Humidity (Percent)</th>
<th>Number and Gender of Subjects</th>
<th>Subject Characteristics</th>
<th>Notes on Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohsemm (1987b)</td>
<td>2.0</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>21</td>
<td>50</td>
<td>8 M/3 F</td>
<td>18-36 years, NS</td>
<td>Vitamin C blocked NO₂-induced increase in airway reactivity to methacholine</td>
</tr>
<tr>
<td>Mohsemm (1988)</td>
<td>2.0</td>
<td>120</td>
<td>--</td>
<td>--</td>
<td>21</td>
<td>50</td>
<td>13 M/5 F</td>
<td>Normal, NS, 18-33 years</td>
<td>No symptoms, no lung function changes Increased methacholine reactivity</td>
</tr>
<tr>
<td>Mohsemm and Gee (1987)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 years, NS</td>
<td>45% decrease in α-1-protease inhibitor in BAL fluid</td>
</tr>
<tr>
<td>Morrow and Utell (1989)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muelenaer et al (1987)</td>
<td></td>
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<td></td>
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<tr>
<td>Reference</td>
<td>NO₂ (ppm)</td>
<td>Exposure Duration (min)</td>
<td>Exercise Duration (min)</td>
<td>Exercise Vent (L/min)</td>
<td>Temp (°C)</td>
<td>Relative Humidity (Percent)</td>
<td>Number and Gender of Subjects</td>
<td>Subject Characteristics</td>
<td>Notes on Effects</td>
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</tr>
<tr>
<td>Rehn et al (1982)</td>
<td>0</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>22-27</td>
<td>25-45</td>
<td>Healthy, young M</td>
<td>Possible small increase in $R_{aw}$ at 0.27 ppm</td>
<td>No change in nasal or tracheobronchial clearance</td>
</tr>
<tr>
<td>Sackner et al (1980)</td>
<td>0.1</td>
<td>240</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>6</td>
<td>Normal adults</td>
<td>No effects of NO₂ in resting adults</td>
</tr>
<tr>
<td>Sandstroem et al (1989)</td>
<td>2.25</td>
<td>20</td>
<td>20</td>
<td>~35</td>
<td>--</td>
<td>--</td>
<td>8</td>
<td>Healthy, NS</td>
<td>Increased levels of mast cells in BAL fluid at all concentrations</td>
</tr>
<tr>
<td>Sandstroem et al (1990a)</td>
<td>4.0</td>
<td>20 min(?) alternate days for 12 days</td>
<td>20(?)</td>
<td>~35(?)</td>
<td>--</td>
<td>--</td>
<td>8</td>
<td>Healthy, NS</td>
<td>Total cell counts were reduced ( R_{aw} )</td>
</tr>
<tr>
<td>Stacy et al (1983)</td>
<td>0.5</td>
<td>240</td>
<td>30</td>
<td>55</td>
<td>30</td>
<td>60</td>
<td>10 M</td>
<td>26-4 years</td>
<td>No significant effects on spirometry or ( R_{aw} )</td>
</tr>
<tr>
<td>Smeglin et al (1985)</td>
<td>0.30</td>
<td>240</td>
<td>30 min</td>
<td>~30</td>
<td>--</td>
<td>--</td>
<td>20</td>
<td>21-48 years, NS</td>
<td>No change in lung function or airway reactivity</td>
</tr>
<tr>
<td>Suzuki and Ishikawa (1965)</td>
<td>0.7-2.0</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased resistance 10 min after exposure</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>NO$_2$ (ppm)</td>
<td>Exposure Duration (min)</td>
<td>Exercise Duration (min)</td>
<td>Exercise Vent (L/min)</td>
<td>Temp ($^\circ$C)</td>
<td>Relative Humidity (Percent)</td>
<td>Number and Gender of Subjects</td>
<td>Subject Characteristics</td>
<td>Notes on Effects</td>
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<td>----------------------------------</td>
</tr>
<tr>
<td>Toyama et al (1981)</td>
<td>0.7</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>5</td>
<td></td>
<td>No effects on airway conductance or responsiveness</td>
</tr>
<tr>
<td>Von Nieding et al (1973a)</td>
<td>5.0</td>
<td>15</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>16</td>
<td>Healthy</td>
<td>Decreased DLCO 18%</td>
</tr>
<tr>
<td>Von Nieding et al (1977)</td>
<td>5.0</td>
<td>120</td>
<td>Intermittent</td>
<td>Light</td>
<td>22</td>
<td>55</td>
<td>11 M</td>
<td>Healthy</td>
<td>Increased resistance 60% Remained elevated for 60 min Possible decrease in PaO$_2$</td>
</tr>
<tr>
<td>Von Nieding et al (1979)</td>
<td>5.0</td>
<td>120 (4 × 15)</td>
<td>60</td>
<td>220</td>
<td>22</td>
<td>55</td>
<td>11 M</td>
<td>Healthy</td>
<td>Resistance increased 60% Remained elevated 60 min after exposure Possible decrease in earlobe PO$_2$</td>
</tr>
</tbody>
</table>

*Abbreviations*

NO$_2$ = Nitrogen dioxide  
M = Male  
F = Female  
S = Smoker  
NS = Nonsmoker  
FEV$_1$ = Forced expiratory volume in 1 s  
FVC = Forced vital capacity  
SR$_{aw}$ = Specific airway resistance  
W = Watts  
L = Light  
H = Heavy  
RT = Total respiratory resistance  
DLCO = Diffusing capacity for carbon monoxide  
PaO$_2$ = Arterial partial pressure of oxygen  
PO$_2$ = Partial pressure of oxygen  
BAL = Bronchoalveolar lavage  
IL-1 = Interleukin-1  
TNF = Tumor necrosis factor  
PMNs = Polymorphonuclear leukocytes  
R$_{aw}$ = Airway resistance  
VAR = Variable  
SG$_{aw}$ = Specific airway conductance  

first study, 14 normal patients were exposed to 5 to 8 ppm NO\(_2\) for up to 5 min on 4 separate days. The airway resistance (R\(_{aw}\)) increased by an average of 0.58 cm H\(_2\)O/L/s (range 0.39 to 1.03 for the individual four-exposure mean) after the NO\(_2\) exposures. It was noted that there were no differences in response between smokers and nonsmokers.

Bell and Ulmer (1976) studied the effects of 2-h exposures to 0.0, 1.0 (n = 8), 2.5 (n = 8), 5.0, and 7.5 ppm NO\(_2\) in 16 healthy resting subjects. An additional group of 8 healthy resting subjects were exposed for 14 h to 5.0 ppm NO\(_2\) for 2 consecutive days. They found a small, significant increase in total respiratory resistance (R\(_T\)) after exposure to 2.5 ppm NO\(_2\) or greater. The main response, no more than 1 cm H\(_2\)O/L/s above a baseline of 2.6 cm H\(_2\)O/L/s, occurred during the first 30 min of exposure and the response was not appreciably increased by raising the NO\(_2\) concentration to 5.0 or 7.5 ppm NO\(_2\). The increase in R\(_T\) following NO\(_2\) exposure was related to the baseline airway responsiveness to acetylcholine. Airway responsiveness to acetylcholine was increased after exposure to 7.5 ppm for 2 h or to 5.0 ppm for 14 h, but not after the 2-h exposures to 5.0 ppm or less. The pattern of response in the 14-h exposure indicated an initial increase in resistance during the first 30 min (≈30%), a slight decline in resistance over the subsequent 90 min, and then a modest further increase over the next 14 h (a total increase of ≈60%). Resistance returned to baseline during the subsequent 10 h and this response pattern was repeated on the second exposure day. The 1982 Air Quality Criteria for Nitrogen Oxides Document cited this study as indicating that responses were "clearly demonstrated to occur in healthy adults with single 2-h exposures to NO\(_2\) ranging from 4,700 to 14,000 μg/m\(^3\) (2.5 to 7.5 ppm)."

Linn et al. (1985b) exposed 25 healthy, nonsmoking subjects (9 female, 16 male) for 75 min to 4.0 ppm of NO\(_2\) or purified air. Subjects were exposed to each condition twice, for a total of four exposures. The authors reported that approximately 11 μg/m\(^3\) of particulate nitrate was present during NO\(_2\) exposures. During the exposures, subjects performed 15 min of light (25 L/min) and 15 min of heavy (50 L/min) exercise. There were no significant effects of NO\(_2\) on R\(_{aw}\) or symptoms. Although heart rate and skin conductance were similarly unaffected, there was a slight but statistically significant reduction in systolic blood pressure associated with NO\(_2\) exposure. Although inhalation of NO\(_2\) can result in increased blood levels of nitrite and nitrate ion, the mechanism for this small change in systolic pressure has not been established. Blood pressure readings were
obtained using an automated procedure while the subject was seated quietly in the body plethysmograph.

Mohsenin (1987b) studied the effects of 1-h resting exposure to 2.0 ppm NO₂ on 11 normal subjects to determine the effect of ascorbic acid administration prior to NO₂ exposure. The author hypothesized that the antioxidant properties of ascorbic acid would modify the effect of NO₂. There were a total of four exposures. In the first set of clean air and NO₂ exposures, the subjects received a placebo for 3 days prior to the exposures. In the second air/NO₂ exposure pair, the subjects received vitamin C. In both cases, the order of the NO₂ and air exposures were randomized. The blood ascorbic acid levels were increased from 0.76 mg/dL after placebo to 1.90 mg/dL after vitamin C supplementation. Neither plethysmography nor spirometry tests indicated a significant effect of NO₂ in these subjects under placebo or vitamin C conditions. There was a significant increase in airway responsiveness to methacholine (bromide) after NO₂ exposure. Responsiveness to methacholine was quantified by the dose required to reduce SGₐw by 40% (PD40), this corresponds to a 67% increase in SRₐw. (A 50% decrease in SGₐw corresponds to a doubling of SRₐw.) After the two air exposures, PD40 averaged about 64 mg/mL, but was reduced to 53 mg/mL after NO₂ exposure and placebo treatment. When the subjects were given ascorbic acid prior to exposure, methacholine responsiveness after NO₂ exposure was unchanged. Ascorbic acid pretreatment apparently blocked the airway responsiveness increase, which had previously been observed with NO₂ exposure, although it had no effect on baseline methacholine responsiveness. However, ascorbic acid has previously been shown to cause a decrease in methacholine responsiveness in both normals and asthmatics (Mohsenin et al., 1983, Ogilvy et al., 1981). Thus, it is unclear whether ascorbic acid blocks the effect of NO₂ on airways responsiveness or whether there was a direct effect of ascorbate on methacholine responsiveness subsequent to vitamin C supplementation.

Mohsenin (1988) studied the response of 18 normal adults exposed to 2 ppm NO₂ for 1 h at rest. There were no symptoms, no changes in lung volume, no change in flow-volume characteristics on either full or partial expiratory flow-volume (PEFV) curves, and no change in SGₐw. However, airway responsiveness to methacholine was increased following exposure in 13 of 18 subjects and decreased in only 2 of the 18 (p = 0.003) subjects. The dose of
methacholine needed to cause a 40% reduction in SGaw was 101 ± 44 mg/mL after air and 81 ± 45 mg/mL after NO2.

Kulle and Clements (1988) studied the effects of NO2 exposure on infectivity of live attenuated influenza A/Korea/reassortment virus in healthy nonsmoking adults (see Goings et al., 1989). Independent control and exposure groups were exposed to clean air for 1 day and then either clean air or NO2 (1, 2, or 3 ppm) for the next 3 consecutive days. Included in this evaluation were measurements of respiratory symptoms, lung function, and airway reactivity to methacholine in the 2- and 3-ppm studies. There were no significant changes in respiratory or other symptoms as a result of a 3-ppm NO2 exposure. The only apparently significant changes in spirometry were observed in the control group, who showed slightly less decrease in forced vital capacity (FVC) or forced expiratory flow at 25 to 75% of vital capacity (VC) (FEF25-75%) during the last of four consecutive clean air exposures. Airway responsiveness to methacholine was measured following exposure to 2 and 3 ppm. The clean air control groups showed a small significant decrease in airway responsiveness on the second, third, and fourth days, but airway responsiveness remained unchanged in the NO2-exposed subjects. Influenza virus infection did not alter airway responsiveness in either air or NO2 exposure groups. Reactivity returned to control at 2 and 4 weeks after the exposure series. The infectivity portion of this study is discussed in Section 15.4.

Frampton et al. (1991) studied a group of 39 healthy nonsmokers exposed for 3 h to either 0.60 ppm (n = 9), 1.5 ppm (n = 15), or a variable concentration protocol where three 15 min "peaks" of 2.0 ppm were added to a background level of 0.05 ppm (See Frampton et al., 1989b, in Section 15.4.2 for details). There were no direct effects on lung function (FVC, FEV1, SGaw) after any of these exposures. However, there was a small statistically significant increase in FVC and FEV1 response to carbachol challenge after the 1.5 ppm exposure, indicating an increase in airway responsiveness. There was no increase in airway responsiveness after the 0.6 ppm or the peaks protocol. However, one subject had a 20% greater drop in FEV1 after the peaks NO2 exposure than after the air exposure. This observation suggests the possibility that some subjects may be affected by NO2 to a much greater extent than others.
15.2.1.2 Concentrations Below 1.0 ppm

In NO$_2$ exposure studies conducted at concentrations below 1.0 ppm, the findings have been generally negative. Although some authors have indicated occasional findings, there does not appear to be a consistent pattern of response at these low NO$_2$ concentrations that would be indicative of short-term health effects.

Kagawa and Tsuru (1979) studied six healthy men exposed to 0.15 ppm NO$_2$ for 2 h while performing light, intermittent exercise. There were no symptoms reported during NO$_2$ exposure. Although the authors suggested that there might be some responses to NO$_2$ exposure, the overall pattern of response does not support the conclusion that changes in lung function were induced by NO$_2$. These authors reported "significance" for individual subjects, although the precise technique for making this judgment is unclear. Two mean differences were reportedly "significant" (multiple t-test unadjusted for multiple comparisons), a 0.5% decrease in VC and a 16% decrease in the ratio of FEF$_{75\%}$(He)/FEF$_{75\%}$(air). However, nonsignificant responses of greater magnitude were observed under other exposure conditions (e.g., air control). It appears that these "significant" observations may only be chance occurrences out of nearly 100 t-tests, 6 of which showed "significance." Furthermore, a temporary 0.5% decrease in VC is of little, if any, physiological significance.

Subsequently, Kagawa (1983a) reported the results of exposing an additional seven subjects to 0.15 ppm NO$_2$ (also to other pollutants, separately and in combination) for 2 h with light, intermittent exercise. Using the same protocol and exposure conditions as those of Kagawa and Tsuru (1979) in this new data set, no statistically significant mean changes were found to be associated with NO$_2$ exposure in any of the plethysmographic or spirometric tests.

Toyama and colleagues (1981) exposed five healthy subjects (two were smokers, three were investigators) to 0.7 ppm NO$_2$ for 60 min while at rest. They observed no responses to this NO$_2$ exposure that altered airway conductance or flow-volume tests.

Kulle (1982) presented a reanalysis of the data previously published by Kerr et al. (1979). In this study, 10 normal, 13 asthmatic, and 7 chronic bronchitic subjects were exposed to 0.5 ppm NO$_2$ for 2 h, several of the subjects were smokers (3 normals, 3 asthmatics, and 5 bronchitics). There were no significant effects of NO$_2$ exposure on...
pulmonary function, but it was unclear whether the change in quasistatic compliance was due to NO₂ exposure or was a statistical artifact, as the original authors (Kerr et al., 1979) suggested. Rather than compare the data across the postexposure measurements obtained with clean air and NO₂, respectively, in the reanalysis (Kulle, 1982), a difference score (post - pre) was determined for each condition and the differences were tested for significance. All subjects perceived the odor of NO₂ upon entering the exposure chamber. The author reported a significant increase in the normal subjects in the Phase IV of the single breath nitrogen washout test. However, the data suggest that the difference was probably due to a reduced preexposure value on the NO₂ exposure day, an effect that could not be attributed to NO₂. Quasistatic lung compliance was decreased after NO₂ exposure in the normal group. The absence of a change in dynamic compliance suggests that the original authors (Kerr et al., 1979) may have been correct in concluding that "significance" was probably due to chance alone. No other spirometric or plethysmographic measurements were significantly altered by NO₂ exposure. With the exception of the apparently artifactual change in closing volume, no new conclusions can be drawn from the reanalysis of these data.

Stacy et al. (1983), as part of a large multipollutant exposure study, exposed a group of 10 men to 0.5 ppm NO₂ for 4 h, including two 15-min periods of moderately heavy exercise. None of the plethysmographic or spirometric tests showed a significant effect of NO₂. The experimental design of this study was complex, having a total of 20 treatment cells. The data were analyzed by both a multivariate analysis of variance with an adjusted p value (α level) of 0.0026 and by individual t-tests with a less conservative p value of 0.05. Neither analysis indicated significant effects of NO₂.

Hazucha et al. (1982, 1983) studied a group of 15 healthy adult males exposed to either air or 0.1 ppm NO₂ for 1 h. Control measurements were performed on the day before and the day after the exposure. The subjects did not detect the odor of NO₂, nor was there an increase of symptoms related to the NO₂ exposure. There were no effects of NO₂ on spirometry, airway resistance (SRₐₐ or Rₐ), or methacholine responsiveness.

Rehn et al. (1982) reported a small (17%) increase in SRₐₐ after exposure of eight healthy men to 0.27 ppm (500 μg/m³) for 1 h. However, no response was seen at 1.06 ppm.
This was reported in a technical paper (in Swedish) and has not yet been published in a peer-reviewed journal.

Byln et al. (1985) exposed eight normal subjects to 230, 460, and 910 μg/m³ (0.12, 0.24, and 0.48 ppm) for 20 min. An analysis of variance did not reveal any significant effects of NO₂ on changes in SRaw, but specific comparisons indicated a significant 11% increase in SRaw at 0.24 ppm and a 9% decrease in SRaw at 0.48 ppm. Even though statistically significant, such small changes (±15%) in airway resistance are well within the normal variation of 10 to 20% (Pelzer and Thomson, 1966, Skoogh, 1973). Histamine bronchial responsiveness was tested after the 0.48-ppm exposure, but there were no changes.

Koenig et al. (1987a) exposed normal subjects to (1) 0.12 ppm NO₂ for two 30-min periods at rest, (2) 0.12 ppm for 30 min at rest plus 10 min with exercise, and (3) 0.18 ppm for 30 min at rest and 10 min during exercise. For the at-rest 0.12-ppm NO₂ exposures, there were no significant changes in lung function, symptoms, or arterial oxygen saturation. Nor were there significant NO₂ effects on lung function with the mild exercise exposures to 0.12 and 0.18 ppm NO₂.

Morrow and Utell (1989) studied both young (20 to 48) healthy subjects and elderly (49 to 69) healthy subjects exposed to 0.3 ppm NO₂ for 3.75 h. The young subjects performed a total of 30 min of moderate exercise during exposure and the older subjects exercised for 21 min. There were no differences between air exposure and NO₂ exposure for symptom responses, changes in lung function, or in airway responsiveness to carbachol in either young or older subjects.

The effects of 0.60-ppm NO₂ exposures on young men and women during 1 h of continuous heavy exercise were studied by Adams et al. (1987). There were no significant effects of NO₂ exposure on airway resistance, symptoms, spirometry, or exercise responses.

Kim et al. (1991) studied nine athletes exposed to filtered air, 0.18, and 0.30 ppm NO₂ for 30 min while exercising (running and walking). Sixteen minutes were spent running at a ventilation of about 72 L/min, 10 of the remaining 14 min were spent walking. Overall ventilation is estimated to have averaged about 50 L/min. There were no significant changes in respiratory symptoms, FEV₁, R₉, peak expiratory flow rate, or ventilation (V₅₀%VC) as a result of NO₂ exposure in this group of athletic male subjects.
In another study, young (18 to 26) and older (51 to 76) men and women were exposed to 0.60 ppm NO\textsubscript{2} by Drechsler-Parks et al. (1987) and Drechsler-Parks (1987). Subjects performed light, intermittent exercise during the 2-h exposures. There were no effects on spirometry or symptoms. None of the individual pre–post exposure differences in NO\textsubscript{2} (as compared to air) for FVC or FEV\textsubscript{1} were outside of the normal range (i.e., there were no individual subjects who appeared reactive to NO\textsubscript{2}).

15.2.1.3 Respiratory Symptom and Sensory Effects of Nitrogen Dioxide Exposure

Several studies reported in the previous section also examined symptomatic responses of subjects exposed to NO\textsubscript{2}. None of the studies of NO\textsubscript{2} exposure in normal subjects, including exposure for as long as 75 min to 4.0 ppm NO\textsubscript{2} resulted in a significant increase in respiratory symptoms. Sensory effects were examined in at least two studies (Byln et al., 1985, Hazucha et al., 1983). The subjects in the study of Hazucha et al. (1983) were unable to detect the odor of 0.1 ppm NO\textsubscript{2}. Byln et al. (1985) reported an odor threshold of 0.04 ppm for normals and 0.08 ppm for asthmatics.

15.2.1.4 Mucociliary Clearance After Nitrogen Dioxide Exposure

Rehn et al. (1982) examined the effects of NO\textsubscript{2} exposure on mucociliary clearance in both the nose and lung. Nasal clearance was determined using the rate of saccharin transport from nares to oropharynx. Tracheobronchial clearance was determined by monitoring the rate of disappearance of radiolabeled Teflon aerosol. After a 1-h exposure to either 0.27 or 1.06 ppm (500 or 2,000 \( \mu \text{g/m}^3 \)) NO\textsubscript{2}, there were no changes in either nasal or tracheobronchial clearance rates.

15.2.2 Effects of Nitric Oxide

In addition to NO\textsubscript{2}, Kagawa (1982) examined the effects of 1 ppm NO exposure for 2 h in eight normal subjects. The data were analyzed by multiple t-tests using individual data. All changes were referenced to the mean baseline (i.e., mean of the preexposure measurement for the air and the NO exposure) value rather than the corresponding measurement time from the clean air exposure. Three "significant" individual changes in SG_{aw} were reported at 1 h of exposure: one increase and two decreases. After 2 h of
exposure, there were three decreases and one increase, and in the postexposure period, there were two increases and two decreases, all reported to be "significant". These statistical analyses may not be the most appropriate Analysis of the mean data using similar procedures (i.e., multiple t-test referenced to the mean baseline level) produced only one significant change an 11% decrease in the ratio $H e V_{50/air} V_{50}$ Given that this effect (1) occurred only at one of the three measurement points for the NO exposure, (2) was one of 98 paired t-tests, and (3) was significant at only the $p < 0.05$ level, it is reasonable to suggest that the effect may have occurred by chance.

A study of the effects of a mixture of NO$_2$ (0.3 ppm) and NO (0.6 ppm) was recently reported by Kagawa (1990). Exposures lasted 120 min and included mild (50 W) intermittent exercise. There were no significant changes in pulmonary function (airway conductance $[G_{aw}]$, $V_{50\%VC}$, slope of alveolar nitrogen concentration), symptoms, or airway responsiveness to acetylcholine.

Von Nieding et al. (1973b) exposed healthy subjects and smokers to 15 to 39 ppm NO for 15 min. Total respiratory resistance increased significantly ($\approx$ 10 to 12%) after exposure to $\geq 20$ ppm NO. Diffusing capacity was not changed, but a small decrease (7 to 8 torr) in $PaO_2$ was noted.

### 15.2.3 Effects of Nitrogen Dioxide Gas or Gas/Aerosol Mixtures on Lung Function in Normal Subjects

Several studies of NO$_2$-containing pollutant mixtures were previously discussed in the Air Quality Criteria for Oxides of Nitrogen Document (U.S. Environmental Protection Agency, 1982a). The general finding of these studies was that NO$_2$ did not enhance the effects caused by other oxidants, notably O$_3$ (Hackney et al., 1975a,b,c, Von Nieding et al., 1977, Horvath and Folinsbee, 1979 [preliminary report of Folinsbee et al., 1981], Suzuki and Ishikawa, 1965). On the other hand, Abe (1967) studied NO$_2$-SO$_2$ mixtures (4 to 5 ppm) and reported that their effects were additive, with both gases causing bronchoconstriction. Independently, the effect of SO$_2$ was immediate and short-lasting, whereas the effect of NO$_2$ was delayed and more persistent. The effect of the NO$_2$-SO$_2$ mixture was intermediate between the two responses. Other reports suggesting possible interactions of NO$_2$, principally with some type of particle, included Schlipkoeter and
Brockhaus (1963) and Nakamura (1964, cited in U.S. Environmental Protection Agency, 1982a.) These studies are reviewed extensively in Air Quality Criteria for Oxides of Nitrogen Document (U.S. Environmental Protection Agency, 1982a)

Table 15-2 summarizes studies of healthy subjects exposed to NO2-containing pollutant mixtures. The pollutant mixture of greatest interest, in terms of research effort, has been the combination of NO2 and O3 (Adams et al., 1987, Drechsler-Parks, 1987, Drechsler-Parks et al., 1989, Folinsbee et al., 1981, Kagawa and Tsuru, 1979, Kagawa, 1983a,b, Toyama et al., 1981) Also reported were several studies of NO2-SO2 combination exposures (Kagawa, 1983a,b, Kleinman et al., 1985, Linn et al., 1980a) Other pollutant mixtures containing NO2 were also studied (Kagawa, 1986, Islam and Ulmer, 1979a,b, Stacy et al., 1983; Kleinman et al., 1985)

Several recent studies evaluated the effects of O3 and NO2 in combination. Three studies investigated the effects of 0.5 to 0.6 ppm NO2 in combination with 0.3 to 0.5 ppm O3 (Adams et al., 1987, Folinsbee et al., 1981, Drechsler-Parks, 1987) In these studies, NO2 alone had no effect on measured health endpoints and the significant effects of O3 on lung function were not altered by the presence of NO2. Kagawa and Tsuru (1979) and Kagawa (1983a) reported conflicting results. In their first study, it was suggested that the effects of NO2 and O3 were "more than additive." However, in the second study, they reported no significant enhancement of the effect of O3 by the mixture of other pollutants (All pollutants—NO2, O3, and SO2—were at a concentration of 0.15 ppm). All of the above studies included exercise during the 1- to 2-h exposures. Toyama et al. (1981) also studied subjects exposed to NO2-O3 mixtures (0.5 ppm of each gas). Because the concentration of each gas was 0.7 ppm when the exposures were to a single pollutant, it is impossible to determine if there was an additive effect. The above studies taken as a whole suggest strongly that there is no interaction between NO2 and O3 that would result in enhancement of acute O3-induced changes in lung function in normal subjects following short duration exposures at NO2 concentrations less than 1 ppm. However, this conclusion is not necessarily applicable to other measured endpoints.

Hazucha et al. (1992) studied the effects of exposure to NO2 followed by exposure to O3 in a group of healthy nonsmokers. Subjects were exposed to 0.60 ppm NO2 and then, 3 h later, were exposed to 0.3 ppm O3. Mild intermittent exercise was performed during the
**TABLE 15-2. EXPOSURE OF HEALTHY SUBJECTS TO NITROGEN DIOXIDE MIXTURES**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Exposure Concentration</th>
<th>Exposure Duration</th>
<th>Exercise Duration</th>
<th>Exercise Vent</th>
<th>Temp (°C)</th>
<th>Relative Humidity (%)</th>
<th>Number and Gender of Subjects</th>
<th>Subject Characteristics</th>
<th>Notes on Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adams et al (1987)</td>
<td>0.60 NO₂ + 0.30 O₃</td>
<td>60</td>
<td>60</td>
<td>70</td>
<td>21-25</td>
<td>45-60</td>
<td>20 M/20 F</td>
<td>Healthy young adults</td>
<td>No additional effect of NO₂ over and above effect of O₃</td>
</tr>
<tr>
<td>Avol et al (1983)</td>
<td>0.05 NO₂ (Amb)</td>
<td>60</td>
<td>60</td>
<td>56</td>
<td>32</td>
<td>43</td>
<td>42 M/8 F</td>
<td>Healthy young adult cyclists</td>
<td>No apparent effect over and above that of O₃ alone</td>
</tr>
<tr>
<td>Avol et al (1985a),</td>
<td>0.04 NO₂ (Amb)</td>
<td>60</td>
<td>60</td>
<td>22.4</td>
<td>32.7</td>
<td>43</td>
<td>33 M/33 F</td>
<td>Children, 8-11 years</td>
<td>No effects of ambient air exposures</td>
</tr>
<tr>
<td>Avol et al (1985b)</td>
<td>0.055 NO₂ (Amb)</td>
<td>60</td>
<td>60</td>
<td>32</td>
<td>32</td>
<td>45</td>
<td>46 M/13 F</td>
<td>Adolescents, 12-15 years</td>
<td>Ambient air exposure effect attributed to O₃</td>
</tr>
<tr>
<td>Drechsler-Parks (1987)</td>
<td>0.60 NO₂ + 0.45 O₃</td>
<td>120</td>
<td>60</td>
<td>25</td>
<td>24</td>
<td>55</td>
<td>8 M/8 F</td>
<td>18-26 years, NS</td>
<td>No significant changes attributable to NO₂</td>
</tr>
<tr>
<td>Folmsbee et al (1981)</td>
<td>0.50 NO₂ + 0.5 O₃</td>
<td>120</td>
<td>30</td>
<td>40</td>
<td>25</td>
<td>45</td>
<td>10 M</td>
<td>Young adults, NS</td>
<td>FEV₁, decreased by 8-14% No differences between O₃ + NO₂ and O₃ alone</td>
</tr>
<tr>
<td>Hackney et al (1975b)</td>
<td>(a) 0.50 O₃ + 0.29 NO₂</td>
<td>240</td>
<td>120</td>
<td>~20</td>
<td>31</td>
<td>35</td>
<td>4</td>
<td>Healthy</td>
<td>With each group, minimal alterations in pulmonary function caused by O₃ exposure Effects were not increased by addition of NO₂ or NO₂ and CO to test atmospheres</td>
</tr>
<tr>
<td>Reference</td>
<td>Exposure Concentration (ppm)</td>
<td>Exposure Duration (min)</td>
<td>Exercise Duration (min)</td>
<td>Exercise Vent (L/min)</td>
<td>Temp (°C)</td>
<td>Relative Humidity (Percent)</td>
<td>Number and Gender of Subjects</td>
<td>Subject Characteristics</td>
<td>Notes on Effects</td>
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<tr>
<td>Hackney et al (1975c)</td>
<td>(a) 0 25 O$_3$ + 0 29 NO$_2$</td>
<td>120</td>
<td>60</td>
<td>~20</td>
<td>31</td>
<td>35</td>
<td>7</td>
<td>Healthy</td>
<td>Little or no change in pulmonary function found with O$_3$ alone. Addition of NO$_2$ or of NO$_2$ and CO did not noticeably increase the effect. Seven subjects included, some believed to be unusually reactive to respiratory irritants.</td>
</tr>
<tr>
<td></td>
<td>(b) 0 25 O$_3$ + 0 29 NO$_2$ + 30 00 CO</td>
<td>(2 consecutive days of exposure)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazucha et al (1992)</td>
<td>0 60 NO$_2$ followed by 0 30 O$_3$</td>
<td>120</td>
<td>60</td>
<td>40</td>
<td>22</td>
<td>40</td>
<td>15 M</td>
<td>Healthy, NS</td>
<td>NO$_2$-O$_3$ sequence increased effect of O$_3$ on airway responsiveness.</td>
</tr>
<tr>
<td>Islam and Ulmer (1979a)</td>
<td>5 0 NO$_2$ + 0 1 O$_3$ + 5 0 SO$_2$</td>
<td>120</td>
<td>60</td>
<td>?</td>
<td>22</td>
<td>60</td>
<td>8 M</td>
<td>&lt;30 years</td>
<td>FVC (-5%), FEV$_1$ (-11 7%), decreased with exercise exposure to this mixture in &lt;30 years group.</td>
</tr>
<tr>
<td>Islam and Ulmer (1979b)</td>
<td>0 16 NO$_2$ 0 34 SO$_2$ 0 08 O$_3$</td>
<td>480</td>
<td>0</td>
<td>--</td>
<td>22</td>
<td>60</td>
<td>15</td>
<td>16-26 years</td>
<td>No change in FVC, acetylcholine airway reactivity.</td>
</tr>
<tr>
<td>Kagawa (1983b)</td>
<td>0 15 NO$_2$ 0 15 O$_3$ 0 15 SO$_2$</td>
<td>120</td>
<td>60</td>
<td>~25</td>
<td>--</td>
<td>--</td>
<td>7 M</td>
<td>19-23 years</td>
<td>No significant enhancement of the effects of O$_3$ and SO$_2$ by presence of NO$_2$.</td>
</tr>
<tr>
<td>Reference</td>
<td>Exposure Concentration (ppm)</td>
<td>Exposure Duration (min)</td>
<td>Exercise Duration (min)</td>
<td>Exercise Vent (L/min)</td>
<td>Temp (°C)</td>
<td>Relative Humidity (Percent)</td>
<td>Number and Gender of Subjects</td>
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</tr>
<tr>
<td>Kagawa (1986)</td>
<td>0.30 NO₂ + 0.30 O₃ + 200 μg/m³ H₂SO₄</td>
<td>120</td>
<td>20</td>
<td>~25</td>
<td>28-29</td>
<td>50-60</td>
<td>6 Japanese men (some smokers)</td>
<td>Possible small decrease in SGₐw</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15 NO₂ + 0.15 O₃ + 200 μg/m³ H₂SO₄</td>
<td>120</td>
<td>60</td>
<td>~25</td>
<td>28-29</td>
<td>59-60</td>
<td>3 Japanese men (some smokers)</td>
<td>Possible small decrease in SGₐw</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15 NO₂ + 0.15 O₃ + 0.15 SO₂ + 200 μg/m³ H₂SO₄</td>
<td>135</td>
<td>60</td>
<td>~20</td>
<td>20</td>
<td>85</td>
<td>11 M/9 F</td>
<td>No effects on function, possible symptom responses NO₂ effects not discernible from mixture</td>
<td></td>
</tr>
<tr>
<td>Kleinman et al (1985)</td>
<td>0.50 NO₂ + 0.5 SO₂ + 20 μg/m³ ZnSO₄ (NH₄)₂SO₄ + 330 μg/m³ NaCl</td>
<td>120</td>
<td>60</td>
<td>~20</td>
<td>33</td>
<td>32</td>
<td>14 M/20 F</td>
<td>Small decreases in FVC, FEV₁, in ambient air mostly attributable to O₃ No association of NO₂ levels with lung function change</td>
<td></td>
</tr>
<tr>
<td>Linn et al (1980b)</td>
<td>0.07 Ambient and other pollutants</td>
<td>120</td>
<td>60</td>
<td>~20</td>
<td>32</td>
<td></td>
<td>29 years</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 15-2 (cont'd). EXPOSURE OF HEALTHY SUBJECTS TO NITROGEN DIOXIDE MIXTURES

<table>
<thead>
<tr>
<th>Reference</th>
<th>Exposure Concentration ppm</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Vent (L/min)</th>
<th>Temp (°C)</th>
<th>Humidity (Percent)</th>
<th>Number and Gender of Subjects</th>
<th>Subject Characteristics</th>
<th>Notes on Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lunn et al (1980a)</td>
<td>0.50 NO₂ + 0.50 SO₂</td>
<td>120</td>
<td>60</td>
<td>~20</td>
<td>31</td>
<td>40</td>
<td>10 M/14 F</td>
<td></td>
<td>No significant effect on lung function in normals. Trend for a slight decrease in FVC after combined exposure.</td>
</tr>
<tr>
<td>Von Nieding et al (1979)</td>
<td>5.0 SO₂ + 0.1 O₃</td>
<td>120</td>
<td>60</td>
<td>~20 (70W)</td>
<td>22</td>
<td>55</td>
<td>23-38 years, two atopic</td>
<td></td>
<td>R&lt;sub&gt;T&lt;/sub&gt; increased from 1.5 to 2.4 (p &lt; 0.01), questionable decrease in PaO₂ (8 torr).</td>
</tr>
<tr>
<td></td>
<td>0.1 NO₂ + 0.3 SO₂</td>
<td>120</td>
<td>60</td>
<td>~20</td>
<td>22</td>
<td>55</td>
<td>23-38 years, two atopic</td>
<td></td>
<td>No effects at all.</td>
</tr>
</tbody>
</table>

<sup>a</sup>Abbreviations

NO₂ = Nitrogen dioxide
O₃ = Ozone
M = Male
F = Female
Amb = Ambient
NS = Nonsmoker
FEV₁ = Forced expiratory volume in 1 s
CO = Carbon monoxide
SO₂ = Sulfur dioxide

FVC = Forced vital capacity
H₂SO₄ = Sulfuric acid
SG₈₅ = Specific airway conductance
ZnSO₄(NH₄)₂SO₄ = Zinc ammonium sulfate
NaCl = Sodium chloride
S = Active smoker
W = Watts
R<sub>T</sub> = Total respiratory resistance
PaO₂ = Arterial partial pressure of oxygen
exposures  Nitrogen dioxide alone caused no significant lung function responses, but there was a slightly greater decrease in \( \text{FEV}_1 \) after the \( \text{NO}_2-\text{O}_3 \) sequence than after the \( \text{air}-\text{O}_3 \) sequence. Methacholine airway responsiveness was significantly increased with the \( \text{NO}_2-\text{O}_3 \) sequence compared to an \( \text{air}-\text{O}_3 \) sequence, indicating that prior \( \text{NO}_2 \) exposure enhanced the airway responsiveness increase typically found with \( \text{O}_3 \) exposure.

Linn et al. (1980a) exposed a group of normal subjects to a mixture of 0.5 ppm \( \text{NO}_2 \) and 0.5 ppm \( \text{SO}_2 \) for a 2-h period, during which light, intermittent exercise was performed. There were no lung function (spirometry, closing volume, \( R_T \)) responses. There was an overall increase in symptoms due to the \( \text{NO}_2-\text{SO}_2 \) exposure, but no significant increase in any specific symptom category. Using the same 2-h intermittent, light exercise exposure protocol, Klemman et al. (1985) examined the effects of similar \( \text{NO}_2-\text{SO}_2 \) levels in combination with sodium chloride (NaCl) aerosol (330 \( \mu \text{g/m}^3 \)) and zinc ammonium sulfate (20 \( \mu \text{g/m}^3 \)). They found a slight increase in symptoms in the aerosol plus gas exposure compared to the aerosol alone, suggesting that the mixture was slightly more irritating. There were no pulmonary function effects (spirometry, closing volume, \( R_T \)) of this exposure regimen.

Kagawa (1983a,b) reported results of \( \text{SO}_2-\text{NO}_2 \) (0.15 ppm each) exposures in normal subjects for 2 h with light, intermittent exercise. Unfortunately, the analysis of differences (using repeated t-tests) is confusing. If a reasonable alpha level (\( < 0.01 \)) is used to determine significance (based on the large number of comparisons), then there were no statistically significant changes in \( G_{aw} \) in response to the \( \text{NO}_2-\text{SO}_2 \) mixture.

Islam and Ulmer (1979a) examined the effects of a mixture of 5 ppm \( \text{NO}_2 \), 5 ppm \( \text{SO}_2 \), and 0.1 ppm \( \text{O}_3 \) on a group of 24 healthy subjects divided into three groups according to age. There were two series of 2-h exposures—one at rest, the other including exercise. In subjects <30 years, \( R_{aw} \) increased 48\%, \( \text{FVC} \) decreased 5\%, and \( \text{FEV}_1 \) decreased 11.7\%, but \( \text{PaO}_2 \) (determined from ear lobe blood samples) was unchanged. Similar effects occurred in the older subjects, but the changes were of a smaller magnitude. Arterial oxygen partial pressure fell (6.8 and 8.3 torr) during the exposures in the older subjects, but it also decreased (4.5 and 4.3 torr) during the control exposures. The methods of data analysis were not presented in the paper so that the statistical significance of the observed changes cannot be evaluated. Furthermore, the additivity of effects due to the different pollutants cannot be determined.
Islam and Ulmer (1979b) also studied 15 healthy subjects exposed to 0.34 ppm SO₂, 0.16 ppm NO₂, and 0.08 ppm O₃ for 8 h at rest on four successive days. This mixture did not cause any changes in lung function, blood gases, or blood chemistry.

Studies using several gas and/or aerosol mixtures were conducted by Stacy et al. (1983) and Kagawa (1986). Stacy et al. (1983) exposed healthy, young males to mixtures of NO₂ (0.5 ppm) and aerosols of sulfuric acid (H₂SO₄, 100 µg/m³), ammonium sulfate (133 µg/m³), ammonium bisulfate (116 µg/m³), or ammonium nitrate (NH₄NO₃, 80 µg/m³). There were no effects of any of the pollutant mixtures on spirometry, plethysmography, or symptoms. Kagawa (1986) studied the effects of several NO₂ mixtures: (A) NO₂ (0.30 ppm), O₃ (0.30 ppm), and H₂SO₄ (200 µg/m³), (B) NO₂ (0.15 ppm), O₃ (0.15 ppm), and H₂SO₄ (200 µg/m³), or (C) NO₂ (0.15 ppm), O₃ (0.15 ppm), SO₂ (0.15 ppm), and H₂SO₄ (200 µg/m³). Exposure A included 20 min of exercise (total) and exposures B and C included 60 min of exercise over 2 h. Symptoms were attributed to O₃ exposure. Small, possibly significant decreases (≤10%) in Gaw were observed after exposure to mixtures A and B. A possible decrease in FEV₁ (unknown magnitude) was observed after exposure C. The differences observed with these mixtures were no different than responses observed with O₃ alone, indicating no enhanced response due to the presence of NO₂ in the mixture.

Several reports of exposure to NO₂-containing ambient air mixtures have been published by the Rancho Los Amigos group (Linn et al., 1980b, Linn and Hackney, 1983, Avol et al., 1983, Avol et al., 1985a, Avol et al., 1987). The mean NO₂ level in the ambient air (from the Los Angeles Air Basin) ranged from 0.04 to 0.07 ppm during the approximately 2-h exposure periods. These studies were conducted during the summer smog seasons of 1978-84, but were not specifically designed to test for the effects of NO₂ on lung function. In the Linn et al. (1980b) study, there was no association between NO₂ levels and symptom or lung function effects either in normal or asthmatic subjects. In the Linn et al. (1982) study, the O₃ levels were only 0.03 ppm, and there were no significant effects associated with ambient exposure in normal or asthmatic subjects. There was a relationship between O₃ concentration and change in FEV₁ in the Avol et al. (1983) report. There were few differences between the responses of asthmatics and normal subjects in these studies and no apparent influence of NO₂ level. Ambient air exposure of adolescents (Avol et al., 1985a) was associated with decreased FEV₁ in male and female adolescents, with a
somewhat larger response in female subjects (−7.5%) than for males (−3.4%), but the responses tended to be associated with the O₃ levels. A similar study (Avol et al., 1987) was conducted with exercising children, who showed a trend to larger pulmonary function decrements with increasing O₃ levels. Although these ambient air exposure studies were not designed to test for the interaction of NO₂ with other pollutants, even though Los Angeles has the highest NO₂ levels in the United States, they do illustrate that the lung function effects of ambient air exposure (in Los Angeles) appear to be primarily accounted for by the presence of O₃.

There has been one study of pulmonary effects of nitric acid (HNO₃) vapor followed by exposure to O₃ (Ans et al., 1991b). Ten healthy men, previously determined to be sensitive to O₃ (≥10% ΔFEV₁ after exposure to 0 20 ppm O₃ for 3 h), were exposed on different days for 2 h to either air, distilled water fog, or fog containing 500 μg/m³ (≈200 ppb) of HNO₃. One hour after each of these three randomly ordered exposures, the subjects were exposed to 0 20 ppm O₃ for 3 h. During exposure, subjects exercised for 50 min/h at a ventilation of ≈40 L/min. The decrease in FEV₁ after the O₃ exposure tended to be slightly larger after the air preexposure (−26%) than with either of the fog exposures (−17% and −18%, respectively). Neither SRaw, methacholine responsiveness, nor symptoms were different among exposure conditions. Nitric acid vapor or water fog alone did not induce any symptoms or changes in pulmonary function. The results of this study do not support the hypothesis that HNO₃ exposure will increase responses to O₃.

There appears to be no obvious synergistic or more than additive interactions between NO₂ and other pollutant gases or particles that have been evaluated to date. Pulmonary function responses to O₃, for example, do not appear to be increased by the addition of low levels (<0.6 ppm) of NO₂. The variety of physiologic end points used to evaluate combination exposures have, however, been limited primarily to spirometry and plethysmography.

15.2.4 Summary

In the studies summarized in this section, several observations indicated that, at concentrations in excess of 2.0 ppm, there were functional changes in the lungs of healthy normal volunteers that could be attributed to NO₂ exposure. Increases in Raw were reported...
at concentrations of 5 to 8 ppm from both short (5-mm) and longer (1- to 2-h) exposures
(Von Nieding et al., 1979, Von Nieding and Wagner, 1977, Von Nieding et al., 1980, Islam
and Ulmer, 1979a,b) At slightly lower concentrations (2 to 4 ppm), no changes were seen
in resistance or spirometry (Linn et al., 1985b, Mohsenin, 1987b, 1988) However,
Mohsenin reported an increase in airway responsiveness to methacholine (bromide) after
exposure to 2 ppm, Frampton et al. (1991) reported increased carbachol response after
exposure to 1.5 ppm, and Hazucha et al. (1992) reported augmentation of ozone-induced
airways hyperresponsiveness by prior NO\textsubscript{2} (0 6 ppm) exposure None of the seven studies of
exposure to less than 1.0 ppm in normal subjects demonstrated clear responses to NO\textsubscript{2}
There were several instances of isolated observations indicating statistical significance, but
there was no consistent pattern of response For example, Kagawa and Tsuru (1979)
reported small changes in VC at 0.15 ppm, but did not verify this observation in two
subsequent studies (Kagawa, 1983a,b, 1986), even at 0.30 ppm Furthermore, other studies
(Folinsbee et al., 1978, Toyama et al., 1981, Stacy et al., 1983, Adams et al., 1987, and
Drechsler-Parks et al., 1987), conducted at higher concentrations (0.5 to 0.7 ppm), found no
evidence of lung function effects

15.3 THE EFFECTS OF NITROGEN OXIDE EXPOSURE IN
SENSITIVE SUBJECTS

Certain groups within the population may be more responsive to the effects of NO\textsubscript{2}
exposure, including persons with respiratory disease, children, the elderly, and other
individuals not readily identified as members of a specific group The reasons for paying
special attention to these groups is that potential for NO\textsubscript{2}-induced responses or exacerbation
of disease may be much higher than in healthy young adults Studies on other NO\textsubscript{x} gases
and NO\textsubscript{x} mixtures are also discussed

The airways of asthmatic subjects may be hyperresponsive to a variety of inhaled
materials, including pollens, cold-dry air, allergens, and air pollutants Asthmatics have the
potential to be among the most susceptible members of the population with regard to
respiratory responses to NO\textsubscript{2} (Section 15.3.1) On the average, asthmatics are much more
sensitive to inhaled bronchoconstrictors such as histamine, methacholine, or carbachol The
potential addition of an NO2-induced increase in airway response to the already heightened responsiveness to other substances raises the possibility of exacerbation of this pulmonary disease by NO2. One of the potential mechanisms by which NO2 could affect asthmatics is via a change in airways responsiveness. This is discussed in Section 15.4.

Other potentially susceptible groups include patients with COPD, these subjects are discussed in Section 15.3.2. A major concern with COPD patients is the absence of an adequate pulmonary reserve. Any alteration in lung function in these patients can potentially cause serious problems.

15.3.1 The Effects of Nitrogen Dioxide on Asthmatics

An important issue in the evaluation of human clinical exposure studies involving asthmatics is the variability in response between, and even within, laboratories. In the absence of significant differences in the exposure protocol or exposure dose, an explanation frequently invoked to explain the differences in response is that the characteristics or severity of the disease may differ from one subject group to another.

The Expert Panel Report from the National Asthma Education Program (National Heart Lung and Blood Institute, National Institutes of Health, 1991) has recently defined asthma in the following manner:

Asthma is a lung disease with the following characteristics: (1) airway obstruction that is reversible (but not completely so in some patients) either spontaneously or with treatment, (2) airway inflammation, and (3) increased airway responsiveness to a variety of stimuli.

According to the National Institutes of Health (1991), about 10 million people, or 4% of the population of the United States, have asthma. The prevalence is higher among African Americans, older (8 to 11 years) children, and urban residents (Schwartz et al., 1990). There is a broad range of severity of asthma ranging from mild to severe (see Table 15-3). Common symptoms include cough, wheezing, shortness of breath, chest tightness, and sputum production. A positive response (skin test) to common inhalant allergens is a typical feature of asthma. Asthma is characterized by an exaggerated bronchoconstrictor response to
### TABLE 15-3. CLASSIFICATION OF ASTHMA BY SEVERITY OF DISEASEa

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A: Pretreatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of exacerbations</td>
<td>Exacerbations of cough and wheezing no more often than 1-2 times/week</td>
<td>Exacerbation of cough and wheezing on a more frequent basis than 1-2 times/week</td>
<td>Virtually daily wheezing Exacerbations frequent, often severe Tendency to have sudden severe exacerbations Urgent visits to hospital emergency departments or doctor’s office &gt;3 times/year Hospitalization &gt;2 times/year, perhaps with respiratory insufficiency or, rarely, respiratory failure and history of intubation May have had cough syncope or hypoxic seizures</td>
</tr>
<tr>
<td>Frequency of symptoms</td>
<td>Few clinical signs or symptoms of asthma between exacerbations</td>
<td>Cough and low grade wheezing between acute exacerbations often present</td>
<td>Continuous albeit low-grade cough and wheezing almost always present</td>
</tr>
<tr>
<td>Degree of exercise tolerance</td>
<td>Good exercise tolerance but may not tolerate vigorous exercise, especially prolonged running</td>
<td>Exercise tolerance diminished</td>
<td>Very poor exercise tolerance with marked limitation of activity</td>
</tr>
<tr>
<td>Frequency of nocturnal asthma</td>
<td>Symptoms of nocturnal asthma occur no more often than 1-2 times/month</td>
<td>Symptoms of nocturnal asthma present 2-3 times/week</td>
<td>Considerable, almost nightly sleep interruption due to asthma Chest tight in early morning</td>
</tr>
<tr>
<td>School or work attendance</td>
<td>Good school or work attendance</td>
<td>School or work attendance may be affected</td>
<td>Poor school or work attendance</td>
</tr>
<tr>
<td>Pulmonary function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Peak Expiratory Flow Rate (PEFR)</td>
<td>PEFR &gt; 80% predicted Variabilityb &lt;20%</td>
<td>PEFR 60-80% predicted Variability 20-30%</td>
<td>PEFR &lt; 60% predicted Variability &gt; 30%</td>
</tr>
<tr>
<td>• Spirometry</td>
<td>Minimal or no evidence of airway obstruction on spirometry Normal expiratory flow volume curve, lung volumes not increased Usually a &gt;15% response to acute aerosol bronchodilator administration, even though baseline near normal</td>
<td>Signs of airway obstruction on spirometry are evident Flow volume curve shows reduced expiratory flow at low lung volumes Lung volumes often increased Usually a &gt;15% response to acute aerosol bronchodilator administration</td>
<td>Substantial degree of airway obstruction on spirometry Flow volume curve shows marked concavity Spirometry may not be normalized even with high dose steroids May have substantial increase in lung volumes and marked unevenness of ventilation Incomplete reversibility to acute aerosol bronchodilator administration</td>
</tr>
<tr>
<td>• Methacholine sensitivity</td>
<td>Methacholine PC20 &gt; 20 mg/mL c</td>
<td>Methacholine PC20 between 2 and 20 mg/mL</td>
<td>Methacholine PC20 &lt; 2 mg/mL</td>
</tr>
<tr>
<td><strong>B: After optimal treatment is established</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response to and duration of therapy</td>
<td>Exacerbations respond to bronchodilators without the use of systemic corticosteroids in 12-24 h Regular drug therapy not usually required except for short periods of time</td>
<td>Periodic use of bronchodilators required during exacerbations for a week or more Systemic steroids usually required for exacerbations as well Continuous around-the-clock drug therapy required Regular use of anti-inflammatory agents may be required for prolonged periods of time</td>
<td>Requires continuous, multiple around-the-clock drug therapy including daily corticosteroids, either aerosol or systemic, often in high doses</td>
</tr>
</tbody>
</table>

*Characteristics are general, because asthma is highly variable, these characteristics may overlap However, an individual may switch into different categories over time

bVariability means the difference either between a morning and evening measure or among morning peak flow measurements each day for a week

cAlthough the degree of methacholine/histamine sensitivity generally correlates with severity of symptoms and medication requirements, there are exceptions

Source: National Institutes of Health (1991)
many physical changes (e.g., cold or dry air, exercise) and chemical and pharmacologic agents (e.g., histamine or methacholine). Asthma is typically associated with airway inflammation and epithelial injury (National Institutes of Health, 1991, Beasley et al., 1989, Laitinen et al., 1985, Wardlaw et al., 1988).

In addition to basic anthropometric information such as age, height, weight, gender, and race, other information may be useful in characterizing asthmatics. In order to evaluate differences between subject populations from one study to another, useful information might include baseline lung function, frequency of asthma episodes, nonspecific bronchial responsiveness, reversibility of bronchoconstriction, types of medication and frequency of use, specific serum immunoglobulin E (IgE) levels, skin test responses, response to exercise challenge, duration of disease, and factors that precipitate or aggravate the disease.

In many cases of chamber exposures of asthmatics, the exposures are accompanied by moderate exercise. The potential for an increase in airway resistance or decline in lung volumes or forced expiratory flow caused by exercise alone is an important covariate in these studies. Exercise, even of moderate intensity, can induce some increase in airway resistance, even in clean air at normal room temperature and relative humidity (RH) (i.e., at 20 °C, 50% RH). In order to determine the true effect of an air pollutant in exercising asthmatics, the response to exercise must be considered. Accordingly, in all studies summarized in this section, a control exposure to clean air was performed, including exercise when appropriate.

Asthmatics who participate in controlled human exposure studies typically have mild allergic asthma. In many cases, these individuals can go without medication altogether or can discontinue medication for brief periods of time if exposures are conducted outside their normal allergy season.

Controlled human exposure studies that evaluated respiratory effects of NO₂ exposure of asthmatics are summarized below in two tables—one that describes characteristics of the asthmatic subjects tested (Table 15–4), and another one that presents the exposure conditions used and observed responses to NO₂ (Table 15-5).

Symptomatic effects were observed in asthmatics exposed to 0.5 ppm for 2 h in a study reported by Kerr et al. (1979). However, only four of the subjects reported symptoms of respiratory discomfort, and the authors concluded that "The symptoms reported were minimal, did not correlate with functional changes, and are of doubtful significance."
TABLE 15-4. CHARACTERISTICS OF ASTHMATIC SUBJECTS EXPOSED TO NITROGEN DIOXIDE

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number</th>
<th>Age (years)</th>
<th>FEV/LFVC</th>
<th>SRaw (L*cmH2O/L/s)</th>
<th>IgE Elevated</th>
<th>Reversibility</th>
<th>Allergy</th>
<th>Medications</th>
<th>Medication Withholding</th>
<th>Airway Reactivity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmed et al (1983b)</td>
<td>20 M/34 F</td>
<td>18-39</td>
<td>58-97</td>
<td>3 4-20 1</td>
<td>--</td>
<td>--</td>
<td>10/20</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>MET HIST EIB COLD</td>
</tr>
<tr>
<td>Ahmed et al (1983a)</td>
<td>9</td>
<td>20-51</td>
<td>53-96</td>
<td>43-14 9</td>
<td>--</td>
<td>--</td>
<td>Ragweed</td>
<td>--</td>
<td>24-48 h</td>
<td>Ragweed antigen</td>
<td><em>History of bronchial asthma</em></td>
</tr>
<tr>
<td>Bauer et al (1986a)</td>
<td>15</td>
<td>20-45</td>
<td>49-83</td>
<td>439</td>
<td>--</td>
<td>--</td>
<td>13 IB</td>
<td>7 OB</td>
<td>12 h IB</td>
<td>48 h AH</td>
<td>COLD Mild asymptomatic</td>
</tr>
<tr>
<td>Byhn et al (1988)</td>
<td>8 M/12 F</td>
<td>17-56</td>
<td>--</td>
<td>3 76 ± 0 33</td>
<td>Some</td>
<td>Y 12/20</td>
<td>2 IB, OB</td>
<td>12 h OB</td>
<td>8 h IB</td>
<td>HIST</td>
<td>Very mild asymptomatic</td>
</tr>
<tr>
<td>Hazucha et al (1983, 1982)</td>
<td>15 M</td>
<td>21-46</td>
<td>--</td>
<td>6 73</td>
<td>History</td>
<td>--</td>
<td>None daily</td>
<td>48 h</td>
<td>MET</td>
<td>Mild or inactive disease</td>
<td></td>
</tr>
<tr>
<td>Joerres and Magnusson</td>
<td>10 M/4 F</td>
<td>20-55</td>
<td>38-83</td>
<td>2 8-18 2</td>
<td>--</td>
<td>--</td>
<td>12/14</td>
<td>11/14 IB</td>
<td>10 h IB</td>
<td>Histamune SO2</td>
<td>&quot;Mild asthma&quot;, all subjects asymptomatic except one (FEV1/FVC ratio 38%)</td>
</tr>
<tr>
<td>Kerr et al (1979) also Kuile (1982)</td>
<td>9 M/4 F</td>
<td>19-50</td>
<td>≈71</td>
<td>≈74</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>3 Smokers</td>
</tr>
<tr>
<td>Reference</td>
<td>Number</td>
<td>Age (years)</td>
<td>FEV₁/FVC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>SR&lt;sub&gt;aw&lt;/sub&gt;</td>
<td>IgE&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Reversibility&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Allergy&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Medications&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Medication Withholding&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Airway Reactivity&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Notes</td>
</tr>
<tr>
<td>-------------------------</td>
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</tr>
<tr>
<td>Klemman et al (1983)</td>
<td>12 M/19 F</td>
<td>18-55</td>
<td>74</td>
<td>2.62</td>
<td>-</td>
<td>-</td>
<td>16/31</td>
<td>8 None</td>
<td>4 h MET</td>
<td>Wide range of clinical severity</td>
<td></td>
</tr>
<tr>
<td>Joeres and Magnussen (1991)</td>
<td>9 M/2F</td>
<td>18-55</td>
<td>67-96</td>
<td>3.9-10.7</td>
<td>7/11</td>
<td>6 None</td>
<td>8 h</td>
<td>MET</td>
<td>MILD asymptomatic asthmatics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koenig et al (1985)</td>
<td>14 M/6 F</td>
<td>12-18</td>
<td>FEV₁ 1.95-4.10</td>
<td>Rt = 4.57</td>
<td>Y</td>
<td>Y</td>
<td>8 IB</td>
<td>8 h</td>
<td>MET</td>
<td>Asymptomatic extrinsic allergic asthmatics</td>
<td></td>
</tr>
<tr>
<td>Koenig et al (1987a,b)</td>
<td>7 M/3 F</td>
<td>12-18</td>
<td>2.3-4.2</td>
<td>Rt = 5.07</td>
<td>Y</td>
<td>Y</td>
<td>7 IB</td>
<td>4 h</td>
<td>MET</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koenig et al (1989a)</td>
<td>6 M/3 F</td>
<td>12-18</td>
<td>2.4-5.4</td>
<td>FEV₁</td>
<td>Y</td>
<td>Y</td>
<td>6 IB</td>
<td>4 h</td>
<td>EIB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koenig et al (1989b)</td>
<td>5 M/4 F</td>
<td>12-17</td>
<td>2.24-4.0</td>
<td>-</td>
<td>9/9</td>
<td>-</td>
<td>5 IB</td>
<td>4 h</td>
<td>MET</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunn et al (1980a)</td>
<td>33 M/12 F</td>
<td>2.32 (FEV₁)</td>
<td>3.45</td>
<td>Rt = 3.45</td>
<td>-</td>
<td>Y</td>
<td>4 h</td>
<td>Physicians diagnosed asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunn and Hackney (1984)</td>
<td>12 M/11 F</td>
<td>18-34</td>
<td>67-100</td>
<td>5.48 ± 2.33</td>
<td>-</td>
<td>-</td>
<td>4 h</td>
<td>Physicians diagnosed asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunn et al (1985b)</td>
<td>12 M/11 F</td>
<td>18-34</td>
<td>67-100</td>
<td>5.48 ± 2.33</td>
<td>-</td>
<td>-</td>
<td>4 h</td>
<td>SO₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunn et al (1986)</td>
<td>15 M/6 F</td>
<td>20-34</td>
<td>3.16 (FEV₁)</td>
<td>4.21</td>
<td>13/21</td>
<td>-</td>
<td>10 IB</td>
<td>4 h</td>
<td>COLD</td>
<td>Mild asthmatics</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Characteristics of asthmatic subjects exposed to nitrogen dioxide.
### TABLE 15-4 (cont’d). CHARACTERISTICS OF ASTHMATIC SUBJECTS EXPOSED TO NITROGEN DIOXIDE

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number</th>
<th>Age (years)</th>
<th>FEV₁/FVC&lt;sup&gt;b&lt;/sup&gt; %</th>
<th>SR&lt;sub&gt;aw&lt;/sub&gt; (L·cmH₂O/L·s)</th>
<th>IgE&lt;sup&gt;c&lt;/sup&gt; Elevated</th>
<th>Reversibility&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Allergy&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Medication&lt;sup&gt;f&lt;/sup&gt;</th>
<th>Medication Withholding&lt;sup&gt;g&lt;/sup&gt;</th>
<th>Airway Reactivity&lt;sup&gt;h&lt;/sup&gt;</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohsenin (1987b)</td>
<td>10</td>
<td>22-44</td>
<td>60-93</td>
<td>3.8-13 2</td>
<td>–</td>
<td>Y</td>
<td>–</td>
<td>No steroids</td>
<td>24 h</td>
<td>MET</td>
<td>Mild asthma</td>
</tr>
<tr>
<td>Morrow and Utell (1989)</td>
<td>10 M/10 F</td>
<td>19-54</td>
<td>42-86</td>
<td>(50)</td>
<td>–</td>
<td>Y</td>
<td>14/20</td>
<td>4 IB</td>
<td>5 OB</td>
<td>CARB</td>
<td>Duration asthma</td>
</tr>
<tr>
<td>Orehek et al (1976)</td>
<td>13 M/7 F</td>
<td>15-44</td>
<td>6.8 ± 0.6</td>
<td>–</td>
<td>–</td>
<td>16/20</td>
<td>No steroids</td>
<td>24 h</td>
<td>CARB</td>
<td>Grass Pollen</td>
<td>1 smoker, 13 mild/7 moderate</td>
</tr>
<tr>
<td>Orehek et al (1981)</td>
<td>6 M/1 F</td>
<td>31 1</td>
<td>5.4 ± 1.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Grass Pollen</td>
<td>3 asthmatics, 4 allergic</td>
</tr>
<tr>
<td>Roger et al (1990)</td>
<td>A 13 M</td>
<td>19-35</td>
<td>55-81</td>
<td>2.6-13 5</td>
<td>80-2,040</td>
<td>Y</td>
<td>12/13</td>
<td>IB, OB</td>
<td>48 h, OB</td>
<td>MET</td>
<td>Mild asthma, no cromolyn, no steroids</td>
</tr>
<tr>
<td></td>
<td>B 21</td>
<td>19-30</td>
<td>59-85</td>
<td>3.2-10 6</td>
<td>38-2,040</td>
<td>Y</td>
<td>18/21</td>
<td>IB, OB</td>
<td>12 h, IB</td>
<td>MET</td>
<td>No respiratory illness within 4 weeks</td>
</tr>
<tr>
<td>Rubenstein et al (1990)</td>
<td>5 M/4 F</td>
<td>23-34</td>
<td>51-85</td>
<td>4.8-12 0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>9 IB</td>
<td>8 h, IB</td>
<td>MET</td>
<td>No respiratory illness within 4 weeks</td>
</tr>
</tbody>
</table>

<sup>a</sup> Abbreviations

- FEV<sub>1</sub> = Forced expiratory volume in 1 s
- FVC = Forced vital capacity
- SR<sub>aw</sub> = Specific airway resistance
- IgE = Immunoglobulin E
- M = Male
- F = Female
- MET = Methacholine
- HIST = Histamine
- EIB = Exercise-induced bronchospasm
- COLD = Cold air
- OB = Oral theophylline

<sup>b</sup> Ratio of FEV<sub>1</sub>/FVC in percent

<sup>c</sup> Elevation of IgE levels above those reported for the normal population

<sup>d</sup> Reversibility of bronchoconstriction tested with bronchodilator

<sup>e</sup> Allergy—number of subjects with one or more known allergies—by history or skin testing—Y indicates that subjects were classified as "allergic asthmatics"

<sup>f</sup> Standard medications and number of subjects using them (see list), none indicates no regular use of medication during the study period

<sup>g</sup> Period for which specific types of medication were withheld prior to the study (in hours)

<sup>h</sup> Airway reactivity was tested using one or more standard methods (see list)
<table>
<thead>
<tr>
<th>Reference</th>
<th>NO₂ (ppm)</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Ventilation (L/min)</th>
<th>Temp (°C)</th>
<th>Relative Humidity (Percent)</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmed et al (1983b)</td>
<td>0.1</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>No significant effect on SGₐw and FEV₁, variable effect on carbachol reactivity No information on controlled exposure</td>
</tr>
<tr>
<td>Ahmed et al (1983a)</td>
<td>0.1</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>No effect of NO₂ on FEV₁, SGₐw, or bronchial reactivity to ragweed antigen, either immediately or 24-h postexposure</td>
</tr>
<tr>
<td>Avol et al (1988)</td>
<td>0.3</td>
<td>120</td>
<td>60</td>
<td>40</td>
<td>22</td>
<td>46</td>
<td>Exercise-related increases in symptoms Possible NO₂ related decrease in FEV₁ and PEFR Increased cold air response after 0.3 ppm</td>
</tr>
<tr>
<td>Avol et al (1989)</td>
<td>0.6</td>
<td>120</td>
<td>60</td>
<td>41</td>
<td>--</td>
<td>--</td>
<td>More consistent increases in SRₐw at 0.6 ppm, but not significantly different from air and 0.3 ppm</td>
</tr>
<tr>
<td>Bauer et al (1986a)</td>
<td>0.3</td>
<td>30</td>
<td>10</td>
<td>30</td>
<td>20</td>
<td>9-14</td>
<td>After 60 min of exposure, FEV, FVC, and PEFR were significantly reduced (-3.4, -4.0, and -5.6%, respectively) No change in airways responsiveness to cold air challenge SRₐw increased 17% after NO₂ exposure After 180 min of exposure, the responses had returned to baseline levels</td>
</tr>
<tr>
<td>Bylin et al (1985)</td>
<td>0.12</td>
<td>20</td>
<td>--</td>
<td>--</td>
<td>22</td>
<td>35</td>
<td>Resting 20-min exposures produced no effects Slight excess decrease in FEV₁ and PEFR in NO₂ plus exercise above that caused by exercise alone PEFR -16% (air), -28% (NO₂), FEV₁ -5.5% (air), -9.3% (NO₂) Significantly increased response to cold air after NO₂ exposure</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>20</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>No significant change in SRₐw at any NO₂ levels Histamine reactivity tended to increase</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>20</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>No significant effect on SGₐw and FEV₁, variable effect on carbachol reactivity No information on controlled exposure</td>
</tr>
</tbody>
</table>
## TABLE 15-5 (cont’d). EXPOSURE CONDITIONS AND RESPONSES IN ASTHMATICS EXPOSED TO NITROGEN DIOXIDE

<table>
<thead>
<tr>
<th>Reference</th>
<th>NO₂ (ppm)</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Ventilation (L/min)</th>
<th>Temp (°C)</th>
<th>Relative Humidity (Percent)</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bylin et al (1988)</td>
<td>0 14</td>
<td>30</td>
<td>--</td>
<td>--</td>
<td>25 9</td>
<td>43</td>
<td>Overall trend for SRₐw to decline during exposure period, not related to NO₂ concentration. Histamine bronchial reactivity tended to increase after 0 14 and 0 27 ppm NO₂ exposure.</td>
</tr>
<tr>
<td></td>
<td>0 27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazucha et al (1982)</td>
<td>0 10</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>21 40</td>
<td></td>
<td>No significant changes associated with NO₂ exposure.</td>
</tr>
<tr>
<td>Hazucha et al (1983)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joerres and Magnussen (1990)</td>
<td>0 25</td>
<td>30</td>
<td>--</td>
<td>--</td>
<td>24 50</td>
<td></td>
<td>After resting breathing of 0 25 ppm NO₂, responsiveness to inhaled SO₂ was increased. No effect of NO₂ alone on SRₐw.</td>
</tr>
<tr>
<td>Kleinman et al (1983)</td>
<td>0 20</td>
<td>120</td>
<td>60</td>
<td>≈20</td>
<td>22 50</td>
<td></td>
<td>No effects on spirometry or airway resistance. Airway reactivity to methacholine results variable—tended to increase with exposure.</td>
</tr>
<tr>
<td>Koenig et al (1985)</td>
<td>0 12</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>22 75</td>
<td></td>
<td>No significant responses in pulmonary function due to NO₂. Increased symptoms after NO₂ exposures. (Same as Koenig et al., 1985) No change in FEV₁, Rₜ increased 10 4% (NS), 3% decrease in FEV₁ (p &lt; 0.06).</td>
</tr>
<tr>
<td>Koenig et al (1987a,b)</td>
<td>I 0 12</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>22 75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>II 0 12</td>
<td>40</td>
<td>10</td>
<td>33</td>
<td>22 75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III 0 18</td>
<td>40</td>
<td>10</td>
<td>39</td>
<td>22 75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koenig et al (1989b)</td>
<td>50 ppb HNO₃</td>
<td>40</td>
<td>10</td>
<td>≈25-30</td>
<td>25 65</td>
<td></td>
<td>FEV₁ decreased 4.4% after HNO₃ and 1.7% after HNO₃ air exposure. Rₜ increased 22.5% after HNO₃ and 7.4% after air exposure.</td>
</tr>
<tr>
<td>Koenig et al (1989a)</td>
<td>57 ppb HNO₃</td>
<td>45</td>
<td>30</td>
<td>25-30</td>
<td>22 65</td>
<td></td>
<td>FEV₁ decreased 3.3% after NO₂ exposure and 1.7% after air exposure (difference NS). Reduction of oral ammonia did not increase response (~1.7%).</td>
</tr>
<tr>
<td>Kulle (1982) (same as Kerr et al, 1979)</td>
<td>0 50</td>
<td>120</td>
<td>15</td>
<td>--</td>
<td>24 45</td>
<td></td>
<td>Increased respiratory symptoms in 4/13 subjects. Increased static lung compliance. Impossible to determine amount of effect due to NO₂.</td>
</tr>
<tr>
<td>Joerres and Magnussen (1991)</td>
<td>0 25</td>
<td>30</td>
<td>10</td>
<td>30</td>
<td>26 20</td>
<td></td>
<td>Mouthpiece exposure system. No changes in methacholine responsiveness were observed after NO₂ exposure in these mild asthmatics.</td>
</tr>
</tbody>
</table>
TABLE 15-5 (cont’d). EXPOSURE CONDITIONS AND RESPONSES IN ASTHMATICS EXPOSED TO NITROGEN DIOXIDE

<table>
<thead>
<tr>
<th>Reference</th>
<th>NO₂ (ppm)</th>
<th>Exposure Duration (mm)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Ventilation (L/min)</th>
<th>Temp (°C)</th>
<th>Relative Humidity (Percent)</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lunn et al (1980a)</td>
<td>0.5 + 0.3 ppm SO₂</td>
<td>120</td>
<td>60</td>
<td>≈20</td>
<td>31</td>
<td>40</td>
<td>No significant effect on spirometry or Rₜ</td>
</tr>
<tr>
<td>Lunn and Hackney (1984)</td>
<td>4.0</td>
<td>75</td>
<td>a 15</td>
<td>a 25</td>
<td>21</td>
<td>50</td>
<td>No NO₂ effects on SRₐw, symptoms, heart rate, or skin conductance Small decrease in systolic blood pressure</td>
</tr>
<tr>
<td>Lunn et al (1985b)</td>
<td>0.3</td>
<td>60</td>
<td>30</td>
<td>41</td>
<td>22</td>
<td>50</td>
<td>No effect of NO₂ Exercise-related increase in SRₐw under all conditions</td>
</tr>
<tr>
<td>Mohsenin (1987b)</td>
<td>0.5</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>21</td>
<td>50</td>
<td>No change in symptoms Significant group mean increase in responsiveness to methacholine after NO₂ exposure No other function changes</td>
</tr>
<tr>
<td>Morrow and Utell (1989)</td>
<td>0.30</td>
<td>225</td>
<td>30</td>
<td>30-40</td>
<td>21 0</td>
<td>40</td>
<td>Group findings indicated no significant responses No change in lung function, symptoms, or carbachol reactivity Subjects previously studied (Bauer et al, 1986a) showed possible responses to NO₂ New subject subgroup showed significantly greater response in air exposures</td>
</tr>
<tr>
<td>Orehek et al (1976)</td>
<td>0.11 (n = 20)</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>13/20 subjects had enhanced responses to carbachol after 0.11 ppm NO₂</td>
</tr>
<tr>
<td></td>
<td>0.26 (n = 4)</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1/4 subjects had enhanced responses to carbachol after 0.26 ppm NO₂</td>
</tr>
</tbody>
</table>

References:
- LlIDl et al (1980a)
- LlIDl and Hackney (1984)
- LlIDl et al (1985b)
- LlIDl et al (1986)
- Mohsenin (1987b)
- Orehek et al (1976)
### TABLE 15-5 (cont'd). EXPOSURE CONDITIONS AND RESPONSES IN ASTHMATICS EXPOSED TO NITROGEN DIOXIDE b

<table>
<thead>
<tr>
<th>Reference</th>
<th>NO₂ (ppm)</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Ventilation (L/min)</th>
<th>Temp (°C)</th>
<th>Relative Humidity (Percent)</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orehek et al (1981)</td>
<td>0 11</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>No change in SR_{aw}, or in responsiveness to grass pollen in 3 allergic asthmatics and 4 allergic subjects</td>
</tr>
<tr>
<td></td>
<td>(0.07-0.16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roger et al (1990)</td>
<td>A 0 3</td>
<td>110</td>
<td>60</td>
<td>42</td>
<td>20</td>
<td>40</td>
<td>FEV₁ decreased 11% in NO₂ but only 7% in air, after first 10 min of exercise. Smaller changes later in exposure</td>
</tr>
<tr>
<td></td>
<td>B 0 15</td>
<td>75</td>
<td>30</td>
<td>42</td>
<td>20</td>
<td>40</td>
<td>No increase in airway reactivity to methacholine 2 h after exposure. No change in FEV₁ or SR_{aw} as a result of NO₂ exposure</td>
</tr>
<tr>
<td></td>
<td>0 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubinstein et al (1990)</td>
<td>0 30</td>
<td>30</td>
<td>20</td>
<td>3*</td>
<td>22</td>
<td>55</td>
<td>No changes in SR_{aw}, FVC, FEV₁, SBN₂ or symptoms after NO₂ exposure. NO₂ exposure did not increase airways responsiveness to SO₂</td>
</tr>
</tbody>
</table>

*Abbreviations*

- NO₂ = Nitrogen dioxide
- SR_{aw} = Specific airway conductance
- FEV₁ = Forced expiratory volume in 1 s
- PEFR = Peak expiratory flow
- SR_{aw} = Specific airway resistance
- FVC = Forced vital capacity
- R_T = Total respiratory resistance
- NS = Not significant
- HNO₃ = Nitric acid
- SO₂ = Sulfur dioxide
- SBN₂ = Single breath nitrogen washout
Avol et al. (1988) studied a group of 59 moderate-to-severe asthmatics exposed to clean air, 0.3 ppm, and 0.6 ppm NO₂ for 2 h while performing moderate (minute ventilation $[V_e] = 41$ L/min), intermittent ($6 \times 10$ min) exercise. Each subject was exposed once each to clean air, 0.30 ppm, and 0.6 ppm. There were significant changes in $SR_{aw}$ and FEV₁ as a function of exposure duration for all exposure conditions, but there was no significant effect of NO₂ exposure on these measures of pulmonary function. Cold air bronchial reactivity (assessed by the decrease in FEV₁ after breathing cold-dry air) was measured 1 h postexposure and then again the following day. There was a significant interaction between response and time of testing (i.e., 1 h postexposure and 24 h postexposure), suggesting a slightly increased response after exposure to 0.30 ppm, but not after 0.6 ppm. There were no respiratory symptom responses attributable to NO₂ exposure. A post hoc analysis of a subgroup of subjects with the most abnormal lung function (i.e., FEV₁/FVC ratios $<0.65$) revealed no statistically significant effects of NO₂. In addition to the controlled exposures, 36 subjects also were exposed to ambient air containing 0.09 ppm NO₂ and low levels of other pollutants. Neither lung function, cold-air reactivity, nor symptom responses were significantly different in ambient air than in clean air.

Bauer et al. (1986a) reported a statistically significant spirometric response to NO₂ in a group of 15 asthmatics exposed to 0.3 ppm NO₂ by mouthpiece for 20 min at rest followed by 10 min of exercise (30 L/min). These subjects were characterized as having "mild obstructive lung disease (asthma)". All subjects had elevated response to cold air bronchoprovocation. Nitrogen dioxide deposition studies indicated that 72% (at rest) and 87% (during exercise) of the inhaled NO₂ was deposited within the respiratory tract. According to the authors, the measurements of NO₂ deposition were in general agreement with the model predictions of Miller et al. (1982) (see also Section 13.2.1). After NO₂ exposure, 9 of 15 asthmatics had a decrease in FEV₁ relative to their postexercise FEV₁ in clean air. The postexercise FEV₁ was 4.1% lower after NO₂ (mean = 2,788 mL) than after air (mean = 2,906 mL) exposure, the pre- to postexercise difference on the NO₂ day (10.1%) and the pre- to post-NO₂ minus the pre- to post-air (i.e., delta-delta) differences (6%) were significant using a paired t-test. These differences were no longer present by 60 min after the exposure. Maximum expiratory flow at 60% total lung capacity (TLC) (PEFV curve) was also decreased more after NO₂ exposure than after air exposure. Changes
Airway responsiveness to cold air in this study was determined as follows. At each ventilation rate of cold air breathing, the respiratory heat exchange (RHE) was calculated. From the relationship of the log RHE versus the percentage decrease in FEV\(_1\), the RHE, which caused a 10% decrease in FEV\(_1\), was linearly interpolated and is referred to as PD\(_{10}\)RHE (provocative dose in RHE units needed to decrease FEV\(_1\) by 10%). Of the 12 subjects for whom the PD\(_{10}\)RHE could be determined, 9 showed an increased response to cold air after the NO\(_2\) exposure. The average PD\(_{10}\)RHE decreased from 0.83 kcal/min after air exposure to 0.54 kcal/min after NO\(_2\) exposure.

One of the factors that may have led to the demonstration of increased response after exposure to a low concentration of NO\(_2\) in this group of asthmatics could be the fact that a mouthpiece exposure system containing relatively dry air (RH of 9 to 14% at 20 °C) was used, and that there was possibly some interaction between the NO\(_2\) effect and airway drying. It is well known that breathing dry (cold) air will induce bronchoconstriction in asthmatics, and that the effect of SO\(_2\) on asthmatics is exacerbated by cold-dry air breathed via the mouth (U S Environmental Protection Agency, 1986, SO\(_2\) document addendum). Concern over this possible confounding effect is tempered by the fact that Bauer et al. (1986a) controlled for the airway drying effect by exposing subjects to clean air at the same temperature and RH. However, if the formation of HNO\(_3\) or nitrous acid is potentially involved in the observed responses, the air chemistry could be strongly influenced by RH (Sequestration of HNO\(_3\) on surfaces is increased with increased ambient water vapor content).

Eight asthmatics exposed to 0.0, 0.1, 0.25, and 0.5 ppm NO\(_2\) for 20 min were studied by Bylm et al. (1985). Exposures were conducted in a body plethysmograph and the range of concentrations was +18% to −26% of the target concentration. Changes in \(S_{R_{aw}}\) during the four exposures averaged +3%, +9%, −2%, and −14%, respectively, the CV for the \(S_{R_{aw}}\) measurements was 19% for these subjects. A three-way analysis of variance revealed no significant differences in \(S_{R_{aw}}\) due to NO\(_2\) exposure. There was a tendency for the pre- to postexposure difference for thoracic gas volume (TGV) to be larger for the NO\(_2\) exposures (9 to 10%). However, the absolute volume of TGV was at most 3 to 4% lower than at comparable times in other NO\(_2\) exposures and only 2% less than the air exposure. The
significance of this difference was in the higher preexposure values for the 0 1- and 0 5-ppm NO₂ exposures, such an effect, if real, should not be attributed to NO₂. There were no significant changes in tidal volume or respiratory rate, which would have been suggestive of an irritant response. At the highest concentration tested (0 5 ppm), histamine bronchial responsiveness was also evaluated after exposure. The authors reported a significant increase in histamine responsiveness due to NO₂ exposure. Significance was evaluated by a sign test (p < 0 04, responsiveness increased in five subjects and was unchanged in three). However, this finding should be interpreted cautiously because the sham (air) exposure histamine challenge had to be discontinued in two subjects, one of whom was later classified as having increased responsiveness. Five of the eight asthmatics had several months previously been hyperreactive to histamine but were not at the time of the NO₂ exposures. This paper suggested possible increased histamine reactivity after 0 50 ppm NO₂ exposure of asthmatics but no direct effect of NO₂ on Raw at concentrations up to 0 5 ppm for 20 min.

Bylin et al. (1988) also reported the effects of 260, 510, and 1,000 μg/m³ (0 14, 0 27, 0 53 ppm, respectively) on a group of 20 mild asthmatics. There were no significant changes in SRaw, although there was a general trend for SRaw to fall throughout the period of exposure regardless of the pollutant level. There was, however, a significant increase (p = 0 03) in airway responsiveness to histamine after 30-min exposure to the middle concentration (i.e., 510 μg/m³), but not at the lowest and highest concentration (see note below). The absence of a concentration-related increase in responsiveness, and the fact that the significance of these findings is based on repeated application of a nonparametric pair comparison test using an alpha level (p value) not adjusted for multiple comparisons, suggests that these results should be interpreted with caution. This observation contrasts with the earlier observation (Bylin et al., 1985) that suggested a possible increased responsiveness after exposure to 910 μg/m³. (Note, however, the discussion above regarding the statistical approach used in the 1985 study.) The raw data presented in the paper were subjected to reanalysis (data available on request to EPA) using a Friedman nonparametric analogue of an F test, which is probably more appropriate for these data than a series of Wilcoxon matched pairs signed rank tests. The Friedman test showed no difference across treatment groups (i.e., there was no statistically significant increase in histamine responsiveness as a result of NO₂ exposure).
In a study that was an important precedent to a number of studies, Orehek et al. (1976) studied the effects of low levels of NO₂ exposure on the bronchial sensitivity of mild asthmatic patients to carbachol, a bronchoconstricting agent. Exposures took place in an airtight room. Nitrogen dioxide concentration started at 246 μg/m³ (0.13 ppm) and declined to 169 μg/m³ (0.09 ppm) over 60 min, the average concentration was 210 μg/m³ (0.11 ppm). Changes in $SR_{aw}$ from pre- to postexposure were measured and an airway challenge to carbachol was used to assess postexposure airway responsiveness. Following NO₂ exposure, increases in $SR_{aw}$ were observed in only 3 of 20 asthmatic test subjects. For all 20 of the asthmatic subjects, dose-response curves were developed for changes in $SR_{aw}$ as a result of the subjects inhaling carbachol. These response curves were compared after a 1-h exposure to either clean air or NO₂. Nitrogen dioxide exposure was associated with increased airway responsiveness to carbachol in 13 of 20 subjects. The mean dose of carbachol producing a twofold (100%) increase in $SR_{aw}$ in the 13 most sensitive subjects (i.e., responders) was significantly decreased from 0.66 mg to 0.36 mg as a result of NO₂ exposure. Seven of the asthmatic subjects (nonresponders) showed neither an increase in $SR_{aw}$ in response to the exposure to NO₂ alone nor an enhanced effect of NO₂ on carbachol-induced bronchoconstriction.

The results of this study are of interest because they are suggestive of possible bronchoconstrictive responses being produced in some asthmatics by very low concentrations of NO₂. The criticism of this reported change was that the comparisons of $SR_{aw}$ were made in subjects who were selected, not at the time of NO₂ exposure, but after the fact, following the carbachol exposure. For example, the mean of measurements of $SR_{aw}$ in the 13 responders to the carbachol treatment was significantly higher after the NO₂ exposure than it had been prior to exposure. An important criticism of this study (discussed by Hazucha et al., 1983) is that, in addition to the retrospective stratification of subjects into responders and nonresponders, other statistical methods may have been more appropriate than the selected group of paired t-tests used in this study.

Orehek et al. (1981) also studied seven allergic subjects, three of whom had asthma, who were exposed to 0.11 ppm NO₂ for 1 h. The major hypothesis to be tested was that NO₂ may alter bronchial responsiveness to an inhaled allergen (grass pollen). Studies were conducted outside the typical pollen season. The exposure technique was somewhat primitive.
in that NO\textsubscript{2} was added to an exposure room with a starting concentration of 0.16 ppm, which was allowed to decay during the exposure to a concentration of 0.07 ppm. There was no change in R\textsubscript{aw} or symptoms as a result of the NO\textsubscript{2} exposure. There was also no difference in the R\textsubscript{aw} response to allergen challenge in these subjects (i.e., NO\textsubscript{2} did not act synergistically with allergen challenge). Furthermore, there was no difference between the responses of the three asthmatics and the other four subjects.

Hazucha et al. (1982, 1983) published two reports that contain complementary data from a study in which the Orehek protocol was repeated. In contrast to the report of Orehek et al. (1976), Hazucha et al. (1982, 1983) found no statistically significant change in airway reactivity to methacholine (another bronchoconstricting agent) in a group of 20 methacholine-reactive mild asthmatics following a 1-h resting exposure to 0.1 ppm NO\textsubscript{2}. A small (8%) increase in SR\textsubscript{aw} (p = 0.23) was observed after NO\textsubscript{2} exposure. Three of the 15 subjects had a greater than 20% decrease in the dose of methacholine required to double SR\textsubscript{aw} (PD\textsubscript{100}). However, at least three of the subjects had a change of similar magnitude in the opposite direction, judging from the graphical presentation of the methacholine dose-response curves. Respiratory system resistance measured by the forced oscillation method was not changed by NO\textsubscript{2} exposure. Hazucha et al. (1983) suggested that the difference in the conclusions regarding statistical significance reached by Orehek et al. (1976), despite similar findings, was because "the statistical approach used by Orehek was not appropriate." Hazucha et al. (1983) discussed the factors that led to their conclusions that, had they analyzed their data in a similar manner to Orehek et al. (1976), the findings would have been comparable.

The hypothesis that NO\textsubscript{2} exposure may cause airway hyperresponsiveness was also examined by Kleinman et al. (1983), who employed a different experimental design than Orehek et al. (1976) and Hazucha et al. (1983). They studied 31 mild-to-moderate asthmatics who were exposed to 0.2 ppm NO\textsubscript{2} for 2 h while performing light, intermittent exercise. There were no significant effects of NO\textsubscript{2} exposure on forced expired spirometry. Total R\textsubscript{T} (forced oscillation) tended to increase (9%) after NO\textsubscript{2} exposure, but the difference was not significant (p = 0.11). Symptom responses tended to be slightly higher after air exposures. A number of different methods were used to evaluate the methacholine challenge data. The general tendency was for greater responsiveness to methacholine after NO\textsubscript{2} exposure. The determination of the dose that would cause a 10% decrease in FEV\textsubscript{1} (D10)
was the most "conventional" approach (see O'Connor et al., 1987, for a discussion of techniques) to assessing methacholine responsiveness. In the 21 subjects in which this dose could be ascertained, D10 was $8.6 \pm 16.2 \mu g$ on the air day and $3.0 \pm 6.2 \mu g$ on the NO2 days ($p < 0.05$ by t-test and Wilcoxon test). Thus, it appears that the results of this study suggest a possible increase in airway responsiveness after a 2-h exposure to 0 20 ppm NO2.

Koenig et al. (1985) have studied the effects of a 1-h resting exposure of asthmatic adolescents to 0 12 ppm NO2. There were no "consistent significant changes in pulmonary functional parameters" after NO2 exposure. Although symptom data were not presented, the authors indicated that subjects had more symptoms after NO2 exposure but that the trend was not significant.

Subsequent studies by Koenig et al. (1987a,b) of mouthpiece exposures to 0 12 ppm NO2, which incorporated exercise (30-min rest followed by 10-min exercise), indicated increases in $R_T$ and decreases in FEV1 after both air and NO2 exposure. These changes were apparently due to exercise alone ($R_T$ increased 8.1% with air and 10.4% with NO2, postexercise FEV1 was decreased 7.4% with air and 4.1% with NO2). In the final phase of the study, subjects were exposed to 0 18 ppm NO2 using the same exercise protocol. In this case, no differences in $R_T$ were seen and FEV1 decreases were 1.3 and 3.3% for air and NO2, respectively. Thus, difference ($p = 0.06$) may indicate a possible response trend. There were no differences in symptoms between exposure conditions in either the 0 12- or 0 18-ppm NO2 exercise exposure studies.

Morrow and Utell (1989) studied a group of 20 asthmatics exposed to 0 30 ppm NO2 for 3.75 h. The exposure included three 10-min periods of moderate exercise. There were no statistically significant group changes in symptoms, spirometry, plethysmography, or airway reactivity to carbachol as a result of the NO2 exposure. Some of the subjects (n = 7) had participated in the Bauer et al. (1986a) study. The 13 remaining (new) subjects were judged to have more severe asthma than the "repeaters." Although the repeaters tended to have responses that were similar to those in the previous study (larger FEV1 decrements in NO2 than in air), the new subjects had significantly greater FEV1 decrements during the air exposures.

Linn et al. (1985b) and Linn and Hackney (1984) exposed a group of 23 mild asthmatics to 4 ppm NO2. Subjects completed a total of four exposures (two each to NO2...
Exposures lasted for 75 min and included two 15-min exercise periods separated by a 25-min rest period. The first exercise was light (25 L/min) and the second was heavy (49 L/min). All subjects were responsive to inhaling 0.75 ppm SO₂ during exercise. Mean baseline preexposure $S_{raw}$ measurements varied from 5.48 and 5.59 on the air exposure days to 6.14 and 6.44 on the NO₂ exposure days, although it is unlikely that the slightly higher baseline values on the NO₂ exposure days affected the subjects' responses. Airway resistance increased after exercise and more so after the heavy (57.2%) than after the light (17.6%) exercise (percentages represent mean values collapsed across all exposure conditions). There was no significant difference in lung function that could be attributed to NO₂, if anything, $S_{raw}$ tended to be slightly lower with the NO₂ exposures. Other physiological tests, such as skin conductance and heart rate, were not different between exposure conditions. As with the group of normal subjects studied under similar conditions, these asthmatics had a slightly, but significantly, lower systolic blood pressure towards the end of the NO₂ exposure. The authors suggested the possibility that NO₂ deposited in the respiratory tract may form a vasoactive substance such as an organic or inorganic nitrate. Nitrate formation after NO₂ inhalation has been observed in animal studies (Postlethwait and Mustafa, 1981). However, measurements of blood levels of nitrate were not performed by Linn et al. (1985b). Both symptoms and state-trait anxiety scores were evaluated during and after exposure, there were no significant variations that could be attributed to NO₂ exposure.

It is difficult to explain the differences between this group of asthmatics exposed to 4 ppm for 75 min (with exercise) compared to the group exposed to 0.30 ppm for 30 min with exercise studied by Bauer et al. (1986a). The subjects of Bauer et al. were exposed to NO₂ in dry air through a mouthpiece, which could have caused some "drying" of the upper airways. This would not be a factor in the Linn et al. (1985b) study, where a chamber exposure was used. Another possible explanation is that the asthmatics studied by Linn et al. were accustomed to NO₂ exposure because of their place of residence (although the ambient levels in Los Angeles are, of course, much lower than 4 ppm). However, the indoor environment can be an important avenue of NO₂ exposure, but is not known for either group. Secondly, the asthmatics in the Linn et al. study, although reactive to SO₂, tended to have milder disease, none used regular asthma medications and all but three subjects had an
FEV\textsubscript{1}/FVC ratio in excess of 75\%  All of the subjects in the Bauer et al. study used some form of bronchodilator (oral or inhaled) and 9 of 15 subjects had a baseline FEV\textsubscript{1}/FVC ratio less than 75\%  It is not clear whether the effects of NO\textsubscript{2} could have been confounded by exposure to an ambient aeroallergen  Although subjects in the Linn et al. study were exposed in March, a time when outdoor pollen aeroallergens would have tended to be minimal for the several preceding months, winter is the peak season for fungal aeroallergens (Street and Hamburger, 1976, McLean et al., 1991)  Also, increased bronchial reactivity to cold air was an important finding in the Bauer et al. study, but it was not measured in the Linn et al. study

Further studies were conducted by Linn et al. (1986) on 21 (mild to mild) asthmatics exposed to 0, 0.30, 1.0, and 3.0 ppm NO\textsubscript{2} for 1 h  The exposures included intermittent, moderate exercise ($V_E = 41$ L/min)  This group was characterized as "clinically mild extrinsic (allergic)" asthmatics who required infrequent, if any, medication  As in the previous study with 4.0 ppm NO\textsubscript{2} exposures, there were no significant effects of NO\textsubscript{2} on spirometry, $SR_{aw}$, or symptoms  Furthermore, there was no significant effect on airway reactivity as measured by cold-air challenge (see Section 15.4)  In order to examine the suggestion that the severity of response to NO\textsubscript{2} may be related to the clinical severity of asthma, the authors selected three subjects whom they characterized as having more severe illness.  There was no indication that the responses of these subjects were related to NO\textsubscript{2} exposure, although they experienced markedly larger changes in resistance than other milder asthmatics under all exposure conditions  Heart rate or minute ventilation did not vary significantly with NO\textsubscript{2} exposure  The previously observed decrease in systolic pressure, associated with 4.0 ppm NO\textsubscript{2} exposure, was not examined in these subjects

Mohsenin (1987a) studied 10 mild asthmatics exposed to 0.5 ppm NO\textsubscript{2} for 1 h at rest in an environmental chamber  There were no changes in symptoms, spirometry, or plethysmography that could be attributed to NO\textsubscript{2} exposure  The response to methacholine (bromide) was evaluated with partial expiratory flow at 40\% VC ($PEF_{40\% VC}$), rather than changes in $SR_{aw}$ or FEV\textsubscript{1}, to test for "small airway abnormality" without the influence of prior deep breaths  There was a significant increase in airway responsiveness to methacholine after the NO\textsubscript{2} exposure  The dose of methacholine required to decrease $PEF_{40\% VC}$ by 40\% was 9.2 ± 15 after air and 4.6 ± 8.2 after NO\textsubscript{2} ($p = 0.042$)
Roger et al (1990) reported the results of NO\textsubscript{2} exposure in mild asthmatics. The first was a pilot study of 12 mild asthmatics exposed to 0.30 ppm for 110 min, including three 10-min periods of exercise. After the first 10 min of exercise in NO\textsubscript{2}, they found an 11% decrement in FEV\textsubscript{1}, which was significantly larger than the 7% decrease seen after the clean air exposure. These differences between air and NO\textsubscript{2} exposure persisted for the remainder of the exposure period, although the overall responses were progressively less with successive periods of exercise, as is common with exercise-induced asthma when the exercise stimulus is intermittent.

A concentration-response study was subsequently conducted (Roger et al, 1990) with 21 mild asthmatics, including 6 subjects from the pilot study, who were exposed to 0.0, 0.15, 0.30, and 0.60 ppm NO\textsubscript{2}. The 75-min exposures included three 10-min exercise periods. In contrast to the pilot study, there were no differences in response between the air and NO\textsubscript{2} exposure at any exposure concentration or time during the exposure. Bronchial reactivity to methacholine, tested 2 h after the exposures, was similar for air and NO\textsubscript{2} exposures. There were no significant differences in symptom scores across the four exposure conditions. The authors were unable to specifically identify factors that could have caused the difference in response between the pilot study and the larger, more comprehensive concentration-response study. They suggested that the pilot study asthmatics may have had more reactive airways, based on their poorer baseline lung function and greater airway responsiveness to methacholine compared to the subjects in the concentration-response study. Furthermore, the studies were conducted during different seasons, which may account for some of the variability in response.

Rasmussen et al (1990) presented a preliminary report of a concentration-response study of healthy asthmatic subjects exposed to 0.1, 0.2, and 0.8 ppm NO\textsubscript{2}. Exposures lasted 120 min and included 10 min of exercise. There were no significant changes in lung function (SR\textsub{aw}, FEV\textsub{1}) or airway responsiveness to histamine resulting from NO\textsubscript{2} exposure at any concentration in either normal or asthmatic subjects. Also assessed were acoustic rhinometry, nasal mucociliary clearance, and alveolar epithelial permeability, these results were not reported.

A series of abstracts have been presented by investigators from Mt Sinai Medical Center in Miami (Sackner et al, 1980, Ahmed et al, 1983a,b), these reports have not
appeared in the peer-reviewed literature but are available as technical reports (Ahmed et al., 1983a,b). The latter report presents data that are qualitatively similar to Orehek et al. (1981) and Hazucha et al. (1983) in that some subjects (13 out of 20) showed increased airways responsiveness to carbachol after NO$_2$ exposure and some (7 out of 20) did not. Even with the post hoc separation of subjects into "reactive" and "nonreactive" groups, the increase in airway responsiveness in the reactive group ($n = 13$) was not statistically significant. There were no significant changes in lung function. Adequate characterization of the exposure conditions was not presented. The former report (Ahmed et al., 1983a) dealt with effects of NO$_2$ on nine ragweed-sensitive asthmatics. There were no group mean changes in $G_{aw}$ or $FEV_1$ after NO$_2$ exposure. There was also no change in bronchial responsiveness to a ragweed antigen inhalation challenge either immediately or 24 h after exposure to 0.1 ppm NO$_2$.

The effects of prior NO$_2$ exposure on SO$_2$-induced bronchoconstriction has been examined in two studies (Jorres and Magnussen, 1990, Rubinstein et al., 1990). Jorres and Magnussen (1990) exposed 14 mild-to-moderate asthmatic subjects to 0.25 ppm NO$_2$ for 30 min while breathing through a mouthpiece at rest. There were no changes in $SR_{aw}$ as a result of the exposure. After the exposure, airways responsiveness to SO$_2$ was assessed by isocapnic hyperventilation of 0.75 ppm SO$_2$ using stepwise increases in ventilation, the initial level was 15 L/min with subsequent increases to 30, 45, 60 L/min, and so forth. After each 3-min period of hyperventilation, $SR_{aw}$ was determined. The ventilation of SO$_2$ required to produce a 100% increase in $SR_{aw}$ ($PV_{100}SR_{aw}(SO_2)$) was estimated using interpolation of ventilation versus $SR_{aw}$ (dose-response) curves. The $PV_{100}SR_{aw}(SO_2)$ was significantly reduced after NO$_2$ exposure compared to after filtered air exposure, suggesting that the airways were more responsive to SO$_2$ as a result of the prior NO$_2$ exposure.

Rubinstein et al. (1990) exposed nine asthmatics to 0.30 ppm NO$_2$ for 30 min (including 20-min light exercise). There were no significant effects of NO$_2$ exposure on lung function (single breath nitrogen washout, $SR_{aw}$, FVC, FEV$_1$) or respiratory symptoms, although a slight increase in $SR_{aw}$ was observed as a result of exercise. An SO$_2$-bronchoprovocation test was administered after exercise, but using a different technique than Jörres and Magnussen (1990). Increasing amounts of SO$_2$ were administered by successive doubling of the SO$_2$ concentration (0.25, 0.5, 1.0, 2.0, 4.0 ppm) at a constant, isocapnic
ventilation of 20 L/min, maintained for 4 min Specific airway resistance was measured after each step increase in SO₂ concentration. The concentration of SO₂ required to increase SRaw by 8 units (PD₈₅SO₂) was interpolated from a dose-response curve of SO₂ concentration versus SRaw. The PD₈₅SO₂ was 1 25 ± 0 70 ppm after air exposure and 1 31 ± 0 75 after NO₂ exposure, indicating no mean change in responsiveness to SO₂. Only one subject showed a tendency toward increased responsiveness to SO₂ after NO₂ exposure (see also Section 15.4).

The contrasting findings in these two studies is somewhat puzzling because the subjects of Rubinstein et al. (1990) were exposed to a higher NO₂ concentration and exercised during exposure. However, Jorres and Magnussen's subjects appeared to have had slightly more severe asthma and were somewhat older. The modest increase in SRaw induced by exercise in the Rubinstein et al. study may have interfered with the response to SO₂ (i.e., the subjects may have been in a refractory state). Finally, the different method of administering the SO₂ bronchoprovocation test (i.e., increased VE at constant SO₂ vs. increasing SO₂ at constant VE) may produce a different response because hyperventilation alone could contribute to the increase in SRaw (Deal et al., 1979, Eschenbacher and Sheppard, 1985). Thus, although similar, the two SO₂ challenges are not necessarily comparable.

15.3.1.1 Effects of Nitric Acid Vapor on Asthmatics

Koenig and associates (1988, 1989a,b) have recently reported preliminary results of a study of adolescent asthmatics exposed to HNO₃ vapor. In the first report (Koenig et al., 1988), subjects were exposed to 50 and 100 ppb HNO₃ and to 50 ppb HNO₃ plus 68 µg/m³ H₂SO₄. The average FEV₁ decreased following exposure (30-min rest followed by 10-min mild exercise) under all three conditions, although there were no significant differences among the responses to these exposures.

Koenig et al. (1989a) reported the responses of adolescent asthmatics to a 40-min exposure to 50 ppb (2 µM/m³) HNO₃ vapor exposure via a mouthpiece exposure system. In this study, after 30 min of rest and 10 min of exercise while breathing HNO₃ vapor, there was a 4 4% decrease in FEV₁ compared to 1 8% decrease after air breathing. A 22 5% increase in total respiratory resistance was also observed after HNO₃, compared to a 7 5% increase after air.
In further studies by Koenig et al. (1989b), subjects were exposed to air and 57 ± 16 ppb HNO₃ twice, once without and once with a preliminary gargle of lemonade, intended to reduce oral ammonia (NH₃) levels. During the 45-min exposure, subjects exercised twice for 15 min at a ventilation of about 25 L/min. Baseline oral NH₃ of 318 ± 84 ppb was reduced to 113 ± 98 ppb after lemonade gargle. There were small, but not statistically significant, decrements in FEV₁ after all exposures, -3.3% after HNO₃ alone and -1.7% after both air and HNO₃ plus lemonade. Similar trends (-9.4%, HNO₃, -5.5%, HNO₃ plus lemonade, -5.1%, air) were observed for V₅₀%VC. The data did not support the hypothesis that reduction of oral ammonia (by using a lemonade gargle) would increase the response to HNO₃ because HNO₃, in the absence of ammonia, would not be converted to NH₄NO₃ in the upper airway. Nevertheless, the authors made the interesting suggestion that, in mixtures of HNO₃ vapor and H₂SO₄ aerosol, gaseous NH₃ may react more rapidly with the gaseous HNO₃ than with the aerosol, thus reducing the potential neutralization of H₂SO₄. It should be emphasized that this is speculation based on the physicochemical properties of HNO₃ vapor and H₂SO₄ aerosol and is not supported by experimental observations. A complete report of these studies is not currently available (i.e., as of October 1992).

15.3.2 Effects of Nitrogen Dioxide on Patients with Chronic Obstructive Lung Disease

Patients with COPD represent an important potentially sensitive population group. Some of these patients have airways hyperresponsiveness to physical and chemical stimuli. In addition, because of their already compromised lung function, they have much less reserve than people with normal lung function. The poor distribution of ventilation in patients with COPD may lead to a greater delivery of NO₂ to the segment of the lung that is well ventilated, thus resulting in a greater regional tissue dose. Tables 15-6 and 15-7 summarize these studies.

In a review of their studies, Von Nieding and Wagner (1979) summarized previously reported findings. The main observations were that Rₐₑₑ increased in chronic bronchitics exposed to 2.0 ppm NO₂ or greater and that, after exposure to 4 to 5 ppm NO₂, PaO₂ was decreased and the alveolar-arterial oxygen gradient was widened.
<table>
<thead>
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<th>Reference</th>
<th>Number and Gender of Subjects</th>
<th>Age (Percent)</th>
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<th>Diagnosis</th>
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<th>Medication Withholding</th>
<th>Airway Reactivity</th>
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<td>7 M/7 F</td>
<td>24-53</td>
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<td>CBR</td>
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<td>--</td>
<td>--</td>
<td>8 mild</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12 moderate</td>
</tr>
<tr>
<td>Von Nieding and Wagner (1979)</td>
<td>116 M/3 F</td>
<td>25-74</td>
<td>--</td>
<td>Y</td>
<td>Chronic nonspecific lung disease</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 M/7 F</td>
<td></td>
<td>R$_T$ = 5 93</td>
<td></td>
<td>CBR</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Chronic nonspecific lung disease</td>
</tr>
<tr>
<td>Von Nieding et al (1973a)</td>
<td>84 M</td>
<td>30-72</td>
<td>--</td>
<td>3 5-10</td>
<td>COPD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations*

- FEV$_1$ = Forced expiratory volume in 1s
- FVC = Forced vital capacity
- SR$_{aw}$ = Specific airway resistance
- SG$_{aw}$ = Specific airway conductance
- CBR = Chronic bronchitis
- M = Male
- F = Female
- EM = Emphysema
- AS = Asthma
- IB = Inhaled $\beta$ agonist
- OB = Oral bronchodilator (theophylline)
- ST = Oral corticosteroid
- COPD = Chronic obstructive pulmonary disease
- Total respiratory resistance
### TABLE 15-7. EXPOSURE CONDITIONS AND RESPONSES IN COPD PATIENTS EXPOSED TO NITROGEN DIOXIDE

<table>
<thead>
<tr>
<th>Reference</th>
<th>Conc (ppm)</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Ventilation (L/min)</th>
<th>Temp (°C)</th>
<th>Relative Humidity (Percent)</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kerr et al (1979)</td>
<td>0.5</td>
<td>120</td>
<td>15</td>
<td>25</td>
<td>24</td>
<td>45</td>
<td>No effects in bronchitics alone Possible decrease in quasistatic compliance</td>
</tr>
<tr>
<td>Lmn et al (1985a)</td>
<td>0.5</td>
<td>60</td>
<td>30</td>
<td>16</td>
<td>22.5</td>
<td>49</td>
<td>No change in FVC, FEV₁, etc., at any NO₂ level SRaw tended to increase after first exercise period Possible decrease in peak flow at 2 ppm No symptom changes No change in SaO₂</td>
</tr>
<tr>
<td>Morrow and Utell (1989)</td>
<td>0.30</td>
<td>225</td>
<td>21</td>
<td>25</td>
<td>21</td>
<td>40</td>
<td>Total NO₂ inhaled dose 1 215 mg Decrease of 9.6% in FVC after exposure 5.2% decline in FEV₁ significant after ≈4-h exposure</td>
</tr>
<tr>
<td>Von Nieding and Wagner (1979)</td>
<td>1-8</td>
<td>5-60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>At 4-5 ppm for 15 min, PaO₂ decreased (arterialized capillary blood) Raw increased with exposure to 1.6 ppm or greater</td>
</tr>
<tr>
<td>Von Nieding et al (1973a)</td>
<td>(a) 1-5</td>
<td>30 breaths (15 min)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Increase in Raw related to NO₂ concentration No effect on Raw below 1.5 ppm</td>
</tr>
<tr>
<td></td>
<td>(b) 5</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Changes in PO₂ of earlobe capillary blood Change occurred in first 15 min, effect did not increase with further exposure</td>
</tr>
<tr>
<td>Von Nieding et al (1971, 1970)</td>
<td>0.5-5</td>
<td>15</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Decrease in earlobe blood PO₂ at 4.0 ppm and above Increased Raw at concentrations of 1.6 ppm and above</td>
</tr>
</tbody>
</table>

*Abbreviations*

FVC = Forced vital capacity
FEV₁ = Forced expiratory volume in 1 s
NO₂ = Nitrogen dioxide
SRaw = Specific airway resistance
SaO₂ = Arterial oxygen saturation
PaO₂ = Arterial partial pressure of oxygen
Raw = Airway resistance
PO₂ = Partial pressure of oxygen
The results of two NO₂ exposure studies were discussed in Von Nieding et al (1980). In the first study, 14 healthy and 14 bronchitic patients were exposed to 5 to 8 ppm NO₂ for up to 5 min on 4 separate days. The mean increase in Raw was 1.07 cm H₂O/L/s. Except for three subjects with an increase in Rₐₑₐₙ of greater than 2.0 cm H₂O/L/s, the responses of the bronchitics were similar to the healthy subjects.

In the second study (Von Nieding et al, 1980), 30 healthy subjects and 40 bronchitic subjects were exposed to 5 ppm NO₂ for 5 min. The subjects were divided into clusters according to their preexposure Rₐₑₐₙs, which ranged from less than 1 cm H₂O/L/s to greater than 4.0 cm H₂O/L/s. There was a tendency for the response to NO₂ to be greater in the subjects with the highest baseline Rₐₑₐₙ. In subjects with baseline Rₐₑₐₙ > 4.0 cm H₂O/L/s, the increase in Rₐₑₐₙ averaged just less than 1.5 cm H₂O/L/s, the increase was less than 0.5 cm H₂O/L/s in subjects with baseline Rₐₑₐₙ < 1 cm H₂O/L/s. Percentage changes ranged from approximately 25 to 50%. Unfortunately, this synopsis does not provide a more comprehensive review of the data.

More recently, Linn and co-workers (1985a) studied a diverse group of 22 COPD patients, including men and women with emphysema and chronic bronchitis, exposed, while exercising intermittently, for 1 h to 0.5, 1.0, and 2.0 ppm NO₂. In agreement with the previous Von Nieding and Wagner (1979) study, no changes in arterial oxygenation (ear oximetry measurements of hemoglobin saturation) were observed. Also, no changes in lung function (spirometry, plethysmography) were observed that could be attributed to NO₂. The only exception was the tendency (not statistically significant) for peak flow to be slightly lower (about 5%) during the 2.0-ppm exposures. No increase in symptoms was reported.

Morrow and Utell (1989) examined the responses of 20 patients with COPD who were exposed to 0.3 ppm NO₂ for 3.75 h, during which time they performed mild exercise for three 7-min periods. Forced vital capacity (FVC) showed progressive and significant decreases during and following NO₂ exposure, with the largest change (−9.6%) occurring after 3.75 h of exposure. Smaller decrements were seen in FEV₁ (−5.2%) at the end of exposure. There was no effect on SGₑₑₑₑ or diffusing capacity as a result of NO₂ exposure. The differences between subjects with more severe disease (FEV₁ < 60% predicted) and those with milder disease (FEV₁ ≥ 60% predicted) in terms of their relative responses to NO₂ were generally not significant, except for a possible slightly greater decrease in FEV₁ in
the milder COPD group. When the COPD patients were compared with healthy, elderly nonsmokers, there was an apparently significant difference between the two groups in their response to NO2, that is, the COPD patients showed a decrement, but the healthy nonsmokers showed an improvement in FEV1. There were also apparent differences in NO2 response between healthy, elderly smokers and healthy, elderly nonsmokers. These exploratory post hoc analyses generate interesting hypotheses, but they do not explain whether the COPD patients responded to NO2 because of their current or previous smoking habit or because of some predisposition to NO2 effects caused by their lung disease. The reasons for the marked difference in response between these subjects and those of Linn et al. (1985a) are unclear. Possible explanations include differences in ambient concentrations due to the place of residence (Los Angeles vs Rochester, but see discussion in Section 15.3.1 regarding this factor) and, more importantly, duration of exposure (4 h vs 1 h). However, the higher concentrations used in the Linn et al. (1985a) study could be expected to produce greater effects in equally reactive subjects. Differences in the severity of COPD could be related to the differences in response, although the subjects of Linn et al. had similar or worse lung function than the subjects of Morrow and Utell. Note also that the mild COPD subjects of Morrow and Utell had greater responses.

15.3.3 Summary

Although findings in asthmatics have been mixed, the pulmonary function responses to NO2, within the ambient range, are relatively small when compared to SO2 exposure (see Table 15-8 and Figures 15-1 and 15-2). Several studies (Bauer et al., 1986b, Roger et al., 1985; Koenig et al., 1987a,b, Avol et al., 1986) suggest possible small changes in spirometry or plethysmography at concentrations in the range of 0.1 to 0.5 ppm. However, the absence of changes at higher NO2 concentrations, failing to suggest a concentration-response relationship (Avol et al., 1986, Bylin et al., 1985, Linn et al., 1985b, Linn et al., 1986), is problematic. Patients with COPD experience pulmonary function changes with brief exposure to high concentrations (5 to 8 ppm for 5 min) or with more prolonged exposure to lower concentrations (0.3 ppm for 3.75 h). In both asthmatic and COPD populations, there remain several unanswered questions regarding the interaction of disease state and exposure variables.
## TABLE 15-8. AIRWAY RESISTANCE AND FORCED EXPIRATORY VOLUME IN ONE SECOND CHANGES IN ASTHMATICS EXPOSED TO NITROGEN DIOXIDE

<table>
<thead>
<tr>
<th>Reference</th>
<th>NO₂</th>
<th>Air</th>
<th>NO₂</th>
<th>Percent Δ FEV₁</th>
<th>Percent Δ SRₘₜ or SGₘₜ or ΔRₜ̅</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avol et al (1988)</td>
<td>0 3</td>
<td>-10</td>
<td>-11</td>
<td></td>
<td>+34</td>
<td>40 5</td>
</tr>
<tr>
<td></td>
<td>0 6</td>
<td>-11</td>
<td>-12</td>
<td></td>
<td>+34</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ΔSRₘₜ is mean for last two measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Shows the directional change in response</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Air (10 FEV₁ s decreased, 5 increased) NO₂ (all FEV₁ s decreased)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bauer et al (1986a)</td>
<td>0 3</td>
<td>-4 1</td>
<td>-10 1</td>
<td>NA</td>
<td>NA</td>
<td>Air (10-5+), NO₂ (15-)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[(−7 0)]</td>
<td></td>
<td>[Excludes two anomalous subjects ]</td>
</tr>
<tr>
<td>Bylin et al (1985)</td>
<td>0 12</td>
<td></td>
<td></td>
<td>+3 0</td>
<td>+4 1</td>
<td>Data given for 20 min of exposure</td>
</tr>
<tr>
<td></td>
<td>0 25</td>
<td></td>
<td></td>
<td>+3 0</td>
<td>-3 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 50</td>
<td></td>
<td></td>
<td>+3 0</td>
<td>-23 0</td>
<td></td>
</tr>
<tr>
<td>Bylin et al (1988)</td>
<td>0 14</td>
<td></td>
<td></td>
<td>-12</td>
<td>-7 5</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>0 27</td>
<td></td>
<td></td>
<td>-12</td>
<td>-8 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 53</td>
<td></td>
<td></td>
<td>-12</td>
<td>-13 5</td>
<td></td>
</tr>
<tr>
<td>Hazucha et al (1982, 1983)</td>
<td>0 1</td>
<td></td>
<td></td>
<td>-1 9</td>
<td>+6 6</td>
<td>NC</td>
</tr>
<tr>
<td>Kleinman et al (1983)</td>
<td>0 2</td>
<td>-3 3</td>
<td>-2 61</td>
<td>Rₜ = +1</td>
<td>+9</td>
<td>Greater %ΔFEV₁ due to higher baseline on NO₂ day</td>
</tr>
<tr>
<td>Koeng et al (1985)</td>
<td>0 12</td>
<td></td>
<td></td>
<td>-3 0</td>
<td>Rₜ = -5 3</td>
<td>-4 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rₜ = -5 3</td>
<td>-4 4</td>
<td></td>
</tr>
<tr>
<td>Koenig et al (1987a,b)</td>
<td>0 12</td>
<td>-6 3</td>
<td>-6 1</td>
<td>Rₜ = -4 3</td>
<td>+10 3</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>0 18</td>
<td>-1 3</td>
<td>-3 3</td>
<td>Rₜ = +5 1</td>
<td>+11 6</td>
<td></td>
</tr>
<tr>
<td>Linn et al (1985b)</td>
<td>4 ppm</td>
<td></td>
<td></td>
<td></td>
<td>+37</td>
<td>+15</td>
</tr>
<tr>
<td></td>
<td>(twice)</td>
<td></td>
<td></td>
<td></td>
<td>+17</td>
<td>+22</td>
</tr>
<tr>
<td>Reference</td>
<td>NO₂</td>
<td>Air Δ FEV₁</td>
<td>NO₂</td>
<td>Percent Δ SRAW or SGaw or ΔRₜ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>-----</td>
<td>------------</td>
<td>-----</td>
<td>-------------------------------------</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunn et al (1986)</td>
<td>0 3</td>
<td>-2.5</td>
<td>-0.3</td>
<td>+63</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 0</td>
<td></td>
<td>-1.9</td>
<td>+35</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 0</td>
<td></td>
<td>+0.6</td>
<td>+29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roger et al (1990)</td>
<td>0 3</td>
<td>-6</td>
<td>-12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>-3.5</td>
<td>-3.9</td>
<td>+52</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>-1.7</td>
<td></td>
<td>+54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>-3.7</td>
<td></td>
<td>+55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations*

FEV₁ = Forced expiratory volume in 1 s  
SRAW = Specific airway resistance  
SGaw = Specific airway conductance  
Rₜ = Total respiratory resistance  
NA = Not available  
NC = No comment  

*Change after 3rd exercise period*
Figure 15-1. Percent change (post-air vs. post-NO₂) in FEV₁ vs. NO₂ dose in ppm × L in asthmatics.

Table: Data

<table>
<thead>
<tr>
<th>Study</th>
<th>Symbol</th>
<th>ppm × L</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avo et al., 1988</td>
<td>A</td>
<td>972</td>
<td>-1.2</td>
</tr>
<tr>
<td>Avo et al., 1989</td>
<td>A'</td>
<td>1,944</td>
<td>+0.9</td>
</tr>
<tr>
<td>Bauer et al., 1988</td>
<td>B</td>
<td>162</td>
<td>-4.1</td>
</tr>
<tr>
<td>Koeng et al., 1987a,b</td>
<td>K</td>
<td>135</td>
<td>-0.3</td>
</tr>
<tr>
<td>Lmn et al., 1986</td>
<td>L</td>
<td>477</td>
<td>+0.3</td>
</tr>
<tr>
<td>Bauer et al., 1988</td>
<td>L</td>
<td>1,950</td>
<td>+1.3</td>
</tr>
<tr>
<td>Mohsenin 1987a</td>
<td>M</td>
<td>4,770</td>
<td>+2.3</td>
</tr>
<tr>
<td>Roger et al., 1990</td>
<td>R</td>
<td>1,050</td>
<td>+0.1</td>
</tr>
</tbody>
</table>

*Statistically significant

*Figure 15-2. Percent change ([post-NO₂ - post-air]/post-air) in resistance (Rₐw, SRₐw, or Rₜ) vs. NO₂ dose in ppm × L in asthmatics.
15.4 EFFECTS OF NITROGEN DIOXIDE EXPOSURE ON AIRWAY RESPONSIVENESS

Physiological changes in the airways induced by a variety of inhaled substances have been used to assess the "responsiveness" or "reactivity" of the airways. Comparing results across studies is difficult because of the variety of types of airway challenges, the variety of methods used to administer the tests, the different physiological end points used to quantify the responses, differences in waiting period after the exposure to determine reactivity, and whether or not the exposure involved exercise. A variety of stimuli have been used to challenge the airways, including (1) chemical mediators such as histamine, methacholine, carbachol, or hypertonic saline, (2) physical methods such as exercise or isocapnic hyperpnea with cold air; (3) other pollutants such as SO₂, or (4) specific antigenic substances such as ragweed or grass pollen. In this section, the effects of NO₂ on measures of airway responsiveness are discussed. A more detailed discussion of overall aspects of most of the studies, including exposure conditions and measurement of other variables, are presented in Sections 15.2 and 15.3 and the tables associated with those sections.

Despite the absence of bronchial or airway hyperresponsiveness in some asthmatics and its presence in some nonasthmatics (Pattemore et al., 1990), there is a correlation between increased asthma symptoms or increased medication usage and increased airway responsiveness (Britton et al., 1988). Alterations in airway responsiveness may also occur as a result of repeated challenges with methacholine (Beckett et al., 1992), histamine (Hamielec et al., 1988), hypertonic saline (Belcher et al., 1987), exercise (Stearns et al., 1981), or, in some cases, by interaction between two different challenges. Either histamine (Hamielec et al., 1988) or hypertonic saline challenge (Belcher et al., 1987) administered before an exercise challenge can reduce the airway response to exercise. Prior exercise-induced bronchoconstriction can reduce responsiveness to hypertonic saline (Belcher et al., 1987), but not, apparently, to histamine (Belcher et al., 1987, Hamielec et al., 1988). Thus, although the responses to a number of airway challenges may be correlated (Chatham et al., 1982), they cannot be considered equivalent.
15.4.1 Healthy Subjects

In a small number of recent studies, the effects of NO₂ on airway responsiveness in healthy, normal subjects have been reported. Airway responsiveness has been shown to increase in normal subjects after exposure to NO₂ concentrations in excess of 1.0 ppm. Bell and Ulmer (1976) found increased responsiveness to acetylcholine in subjects exposed to either 7.5 ppm for 2 h or to 5.0 ppm for 14 h. Mohsenin (1987b, 1988) found increased responsiveness to methacholine after 1-h exposures to 2.0 ppm, and Frampton et al. (1991) reported increased carbachol responsiveness after 3-h exposures to 1.5 ppm. Mohsenin (1987b) also reported that the increased airway responsiveness post-NO₂ exposure could be blocked by elevation of serum ascorbate levels through vitamin C pretreatment (see Section 15.2.1.1). In contrast, using subjects exposed to 0.1 ppm at rest, Hazucha et al. (1982) and Ahmed et al. (1983b) found no significant change in airway responsiveness to cholinergic agonists (methacholine and carbachol, respectively). A 20-min exposure to 0.48 ppm similarly had no significant effect on airway responsiveness to histamine (Bylin et al., 1985). Kulle and Clements (1988) examined airway responsiveness after 2-h exposures to 2.0 and 3.0 ppm NO₂ on 3 consecutive days. Nasal inoculation with influenza virus occurred on the second day of NO₂ exposure. Although there was a significant trend for airway responsiveness to decline in one group of subjects exposed to clean air, there was no trend for airway responsiveness to increase after exposure to either NO₂ concentration. Responses were not altered after virus inoculation. Thus, in three studies, NO₂ concentrations of at least 1.5 ppm NO₂ inhaled over at least 60 min were associated with increased responsiveness to either cholinergic or histaminergic agonists. The mechanism for this increase in responsiveness needs to be established before the clinical implications of this finding can be ascertained.

15.4.2 Asthmatic Subjects

A change in airway responsiveness appears to be one of the more sensitive indicators of response to NO₂ exposure in asthmatics. The findings from the various studies reported here are summarized in Table 15-9. See Section 15.3.1 for a more detailed description of the exposure and measurement methodology.
### TABLE 15-9. CHANGES IN AIRWAY RESPONSIVENESS ASSOCIATED WITH NITROGEN DIOXIDE EXPOSURE

<table>
<thead>
<tr>
<th>Number</th>
<th>NO$_2$ (ppm)</th>
<th>Duration of Exp (mn)</th>
<th>Challenge Type</th>
<th>End Point</th>
<th>Time Postexp (mn)</th>
<th>Exercise</th>
<th>Change in AR$^b$</th>
<th>Average PD ± SD$^c$</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Air NO$_2$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ASTHMATICS**

- **Ahmed et al (1983b)** 20 0 1 60 CARB SG$_{aw}$ - N 13 7 6.04 2.67
- **Ahmed et al (1983a)** 19 0 1 60 RAG SG$_{aw}$ - N 10 8 8.97 ± 24 7 3.36 ± 4.63
- **Hazucha et al (1983)** 15 0 1 60 METH SR$_{aw}$ 20 N 6 7 1.9 ± 0 4 2.0 ± 1 0
- **Orehk et al (1976)** 20 0 1 60 CARB SR$_{aw}$ IM N 14 3 0.56 0.36
- **Rasmussen et al (1990)** 20 0 1 120 METH FEV$_1$ IM Y - - (AIR-NO$_2$ = 0.00)
- **Orehk et al (1981)** 7 0 11 60 GRASS SR$_{aw}$ IM N - - 1.2 ± 0.3 1.3 ± 0.3

**Bylm et al (1988)**

- 20 0 14 30 HIST SR$_{aw}$ 25 N 14 6 - -

**Roger et al (1990)**

- 19 0 15 80 METH SR$_{aw}$ 60 Y 10 7 3.3 ± 0.7 3.1 ± 0.7

**Kleinman et al (1983)**

- 31 0 20 120 METH FEV$_1$ IM Y 20 7 8.6 ± 16 3.0 ± 6.2

**Rasmussen et al (1990)**

- 20 0 20 120 METH FEV$_1$ IM Y - - (AIR-NO$_2$ = 0.02)

**Joerres and Magnussen (1990)**

- 14 0 25 30 SO$_2$ SR$_{aw}$ 27 N 11 2 46.5 ± 5.1 3.7 ± 3.5

**Joerres and Magnussen (1991)**

- 11 0 25 30 METH SR$_{aw}$ 60 Y 7 4 0.41 ± 1.6 0.41 ± 1.6

**Bylm et al (1988)**

- 20 0 27 30 HIST SR$_{aw}$ 25 N 14 6 - -


- 37 0 30 120 COLD FEV$_1$ 60 Y 11 16 -8.4 ± 11 -10.7 ± 12 Delta FEV$_1$

**Avol et al (1989)**

- 34 0 30 30 COLD FEV$_1$ 60 Y 12 21 -5.3 ± 12 -4.7 ± 13 Delta FEV$_1$
<table>
<thead>
<tr>
<th>Author et al.</th>
<th>Number</th>
<th>NO₂ (ppm)</th>
<th>Duration of Exp (min)</th>
<th>Challenge Type</th>
<th>End Point</th>
<th>Time Postexp (min)</th>
<th>Exercise</th>
<th>Change in AR</th>
<th>Average PD ± SD</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bauer et al (1986a)</td>
<td>12</td>
<td>0 30</td>
<td>30</td>
<td>COLD</td>
<td>FEV₁</td>
<td>60</td>
<td>Y</td>
<td>3</td>
<td>0.83 ± 0.42</td>
<td>0.54 ± 0.33 PD₁₀RHE²⁴</td>
</tr>
<tr>
<td>Lunn et al (1986)</td>
<td>21</td>
<td>0 30</td>
<td>120</td>
<td>COLD</td>
<td>FEV₁</td>
<td>IM</td>
<td>Y</td>
<td>-</td>
<td>-11 ± 4</td>
<td>-12 ± 1 Delta FEV₁</td>
</tr>
<tr>
<td>Morrow and Utell (1989)</td>
<td>20</td>
<td>0 30</td>
<td>225</td>
<td>CARB</td>
<td>SGₐₑ</td>
<td>-</td>
<td>Y</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Roger et al (1990)</td>
<td>19</td>
<td>0 30</td>
<td>80</td>
<td>METH</td>
<td>SRₐₑ</td>
<td>60</td>
<td>Y</td>
<td>9</td>
<td>3.3 ± 0.7</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>Rubinstein et al (1990)</td>
<td>9</td>
<td>0 30</td>
<td>30</td>
<td>SO₂</td>
<td>SRₐₑ</td>
<td>60</td>
<td>Y</td>
<td>4</td>
<td>1.3 ± 0.7</td>
<td>1.3 ± 0.8</td>
</tr>
<tr>
<td>Bylin et al (1985)</td>
<td>8</td>
<td>0 48</td>
<td>20</td>
<td>HIST</td>
<td>SRₐₑ</td>
<td>20</td>
<td>N</td>
<td>5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mohsenin (1987a)</td>
<td>10</td>
<td>0 50</td>
<td>60</td>
<td>METH</td>
<td>SGₐₑ</td>
<td>IM</td>
<td>N</td>
<td>7</td>
<td>9.2 ± 1.5</td>
<td>4.6 ± 8.0</td>
</tr>
<tr>
<td>Avol et al (1988)</td>
<td>37</td>
<td>0 60</td>
<td>120</td>
<td>COLD</td>
<td>FEV₁</td>
<td>60</td>
<td>Y</td>
<td>13</td>
<td>-8.4 ± 1.1</td>
<td>-10.4 ± 1.4 Delta FEV₁</td>
</tr>
<tr>
<td>Roger et al (1990)</td>
<td>19</td>
<td>0 60</td>
<td>80</td>
<td>METH</td>
<td>SRₐₑ</td>
<td>60</td>
<td>Y</td>
<td>11</td>
<td>3.3 ± 0.7</td>
<td>3.7 ± 1.1</td>
</tr>
<tr>
<td>Rasmussen et al (1990)</td>
<td>20</td>
<td>0 80</td>
<td>120</td>
<td>METH</td>
<td>FEV₁</td>
<td>IM</td>
<td>Y</td>
<td>-</td>
<td>(AIR-NO₂ = -0.06)</td>
<td></td>
</tr>
<tr>
<td>Lunn et al (1986)</td>
<td>21</td>
<td>1 00</td>
<td>120</td>
<td>COLD</td>
<td>FEV₁</td>
<td>IM</td>
<td>Y</td>
<td>-</td>
<td>-11 ± 4</td>
<td>-11 ± 2 Delta FEV₁</td>
</tr>
</tbody>
</table>

**HEALTHY SUBJECTS**

<table>
<thead>
<tr>
<th>Author et al.</th>
<th>Number</th>
<th>NO₂ (ppm)</th>
<th>Duration of Exp (min)</th>
<th>Challenge Type</th>
<th>End Point</th>
<th>Time Postexp (min)</th>
<th>Exercise</th>
<th>Change in AR</th>
<th>Average PD ± SD</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmed et al (1983b)</td>
<td>20</td>
<td>0 1</td>
<td>60</td>
<td>CARB</td>
<td>SGₐₑ</td>
<td>-</td>
<td>N</td>
<td>10</td>
<td>20.7</td>
<td>19.6</td>
</tr>
<tr>
<td>Hazucha et al (1983)</td>
<td>15</td>
<td>0 1</td>
<td>60</td>
<td>METH</td>
<td>SRₐₑ</td>
<td>20</td>
<td>N</td>
<td>6</td>
<td>16.2 ± 2.7</td>
<td>18.3 ± 3.0</td>
</tr>
<tr>
<td>Bylin et al (1985)</td>
<td>8</td>
<td>0 48</td>
<td>20</td>
<td>HIST</td>
<td>SRₐₑ</td>
<td>20</td>
<td>N</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Frampton et al (1991)</td>
<td>15</td>
<td>1 5</td>
<td>180</td>
<td>CARB</td>
<td>FEV₁</td>
<td>30</td>
<td>Y</td>
<td>11</td>
<td>-4.8 ± 1</td>
<td>-7.5 ± 1 Delta FEV₁</td>
</tr>
</tbody>
</table>
### TABLE 15-9 (cont’d). CHANGES IN AIRWAY RESPONSIVENESS ASSOCIATED WITH NITROGEN DIOXIDE EXPOSURE

<table>
<thead>
<tr>
<th>Number</th>
<th>NO₂ (ppm)</th>
<th>Duration of Exp (min)</th>
<th>Challenge Type</th>
<th>End Point</th>
<th>Time Postexp (min)</th>
<th>Change in AR b</th>
<th>Average PD ± SD c</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kulle and Clements (1988)</td>
<td>21</td>
<td>20</td>
<td>120</td>
<td>METH</td>
<td>FEV₁</td>
<td>IM</td>
<td>N</td>
<td>-10 ⁷ e</td>
</tr>
<tr>
<td>Kulle and Clements (1988)</td>
<td>21</td>
<td>30</td>
<td>120</td>
<td>METH</td>
<td>FEV₁</td>
<td>IM</td>
<td>N</td>
<td>-7 0</td>
</tr>
<tr>
<td>Mohsenin (1988)</td>
<td>18</td>
<td>20</td>
<td>60</td>
<td>METH</td>
<td>SGₐₘ</td>
<td>IM</td>
<td>N</td>
<td>12</td>
</tr>
<tr>
<td>Bell and Ulmer (1976)</td>
<td>16</td>
<td>25</td>
<td>120</td>
<td>ACH</td>
<td>Rₜ</td>
<td>IM</td>
<td>N</td>
<td>0 8</td>
</tr>
<tr>
<td>Bell and Ulmer (1976)</td>
<td>16</td>
<td>50</td>
<td>120</td>
<td>ACH</td>
<td>Rₜ</td>
<td>IM</td>
<td>N</td>
<td>0 9</td>
</tr>
<tr>
<td>Bell and Ulmer (1976)</td>
<td>16</td>
<td>75</td>
<td>120</td>
<td>ACH</td>
<td>Rₜ</td>
<td>IM</td>
<td>N</td>
<td>0 7</td>
</tr>
</tbody>
</table>

**Abbreviations**

- NO₂ = Nitrogen dioxide
- FEV₁ = Forced expiratory volume in 1 s
- CARB = Carbachol
- Y = Included exercise
- SGₐₘ = Specific airway conductance
- GRASS = Grass pollen
- N = Rest
- HIST = Histamine
- RAG = Ragweed
- SO₂ = Sulfur dioxide
- METH = Methacholine
- COLD = Cold-dry air
- SRₐₘ = Specific airway resistance
- ACH = Acetylcholine
- IM = Immediately after exposure
- RT = Total respiratory resistance

b Change in AR. + = increased AR after NO₂ compared to air. - = decreased AR after NO₂ compared to air.
c PD ± SD = Mean ± Standard deviation (SD) of provocative dose (PD) (±SEM = standard error of mean) See individual papers for calculation of PD.
d PD70RHE = Respiratory heat exchange (loss) for 10% drop in FEV₁.
e Separate control and exposure groups. First day of three consecutive daily exposures.
There have been several studies of NO$_2$-exposed asthmatics in which the airway responsiveness was evaluated using cholinergic agonists (carbachol, acetylcholine, methacholine). Subjects were exposed to 0.1 to 0.2 ppm NO$_2$ in five such studies. Of these, both Hazucha et al. (1983) and Roger et al. (1990) found no significant change in group mean response to methacholine challenge. Ahmed et al. (1983b) reported a trend for airway responsiveness to carbachol to increase after a 1-h exposure to 0.1 ppm NO$_2$, but the trend was not significant ($p = 0.07$). Nevertheless, some subjects appeared to be more responsive than others. Orehek et al. (1976) reported that 13 of 20 subjects exposed to 0.1 ppm NO$_2$ experienced an increased airway responsiveness to carbachol. In these 13 subjects, the mean PD$_{100}$ decreased from 0.66 to 0.36 mg. However, in the seven "nonresponders", the PD$_{100}$ of 0.36 mg remained unchanged. A number of questions have been raised about the analytical approach used in this study, and these are discussed in more detail in Section 15.3.1. Klemman et al. (1983) also evaluated airway responsiveness to methacholine after a 2-h exposure to 0.2 ppm NO$_2$. The dose of methacholine required to cause a 10% drop in FEV$_1$ decreased from 8.6 to 3.0 µg. As a group, these studies appear to suggest that some individuals, if not a subgroup of asthmatics, may experience increased airway responsiveness after NO$_2$ exposure.

It might be anticipated, when there is a trend for a response at a low concentration, that exposure to increased concentrations would tend to confirm the trend by producing a less equivocal response. Mohsenin (1987a) found a significant decrease in the dose of methacholine required to produce a 40% decrease in flow at 40% of VC on a partial flow volume curve, the PD$_{40}$ decreased from 9.2 after air to 4.6 after exposure to 0.5 NO$_2$ for 1 h. On the other hand, at both 0.3 and 0.6 ppm for 110 min, Roger et al. (1990) found no difference in airway responsiveness to methacholine. Morrow and Utell (1989) also found no change in airway responsiveness to carbachol after a 3.75-h exposure to 0.3 ppm. These differences cannot be explained either on the basis of NO$_2$ concentration or total NO$_2$ dose because the total dose in the Mohsenin (1987a) study was lower than either of the other two studies.

Histamine airway challenges have been used in three studies following NO$_2$ exposure. Two studies by Bylin et al. (1985, 1988), at NO$_2$ concentrations ranging from 0.14 to 0.53 ppm, suggest possible increased responsiveness to histamine after a 20- to 30-min
resting NO$_2$ exposure. In the first study, 5 of 8 subjects showed an increase in response after a 0.48-ppm exposure, and in the second study, 14 of 20 subjects showed an increase in response after a 0.27-ppm exposure. However, the second, larger study ($n = 20$) did not confirm the observations (at 0.53 ppm) of the first study and a somewhat more conservative statistical approach (Friedman nonparametric test) failed to confirm the significance of these findings. In a preliminary report, Rasmussen et al. (1990) examined the effects of 3-h exposures to 0.1, 0.2, and 0.8 ppm NO$_2$ on airway responsiveness to histamine. They found no significant group mean change in airway responsiveness. Again, these results are suggestive that some asthmatics may experience increased airway responsiveness after NO$_2$ exposure, but the inconsistent nature of the findings from study to study and the absence of a dose-response relationship is problematic.

Bauer et al. (1986a), Linn et al. (1986), and Avol et al. (1988, 1989) have examined the effects of NO$_2$ exposure on airway responsiveness to cold air inhalation. Bauer et al. (1986a) found an increase in cold air airway responsiveness after a 30-min exposure to 0.30 ppm NO$_2$. The airway responsiveness was expressed as the quantity of respiratory heat loss required to produce a 10% drop in FEV$_1$, which averaged 0.83 kcal/min after air exposure and 0.54 kcal/min ($p < 0.05$) after NO$_2$ exposure, indicating an increase in airway responsiveness. Linn et al. (1986) found no change in airway responsiveness to cold air after 1-h exposures to 0.3, 1.0, or 3.0 ppm NO$_2$ in a group of 21 asthmatics. Avol et al. (1988) found a trend for a group mean increase in airway responsiveness to cold air after 0.3 ppm, but not after 0.60 ppm, NO$_2$ exposure, this increased response was observed in only 11 of the 29 subjects at 0.30 ppm. In a study of young asthmatics, also exposed to 0.30 ppm NO$_2$ for 1 h, Avol et al. (1989) found no mean change in cold air airway responsiveness. Indeed, only 12 of 33 subjects demonstrated a change in cold air airway responsiveness in the direction indicative of increased responsiveness. Again, these cannot be explained on the basis of NO$_2$ concentration or total NO$_2$ exposure dose because both were lower in the Bauer et al. (1986a) study, where a significant change in the airway responsiveness was observed. Comparison of the studies in Table 15-9 and 15-4 indicates that the Bauer et al. (1986a) study was shorter, included less exercise, and utilized a mouthpiece exposure system. Additional discussion is presented in Section 15.3.1.
Airway responsiveness to SO₂ has been evaluated after 30 min of exposure to 0.25 to 0.30 ppm NO₂ in two studies. Jorres and Magnussen (1990) found an increased airway responsiveness to SO₂ after a resting exposure to 0.25 ppm NO₂, but Rubinstein et al. (1990) found no change in airway responsiveness to SO₂ after a 0.30-ppm NO₂ exposure of similar duration that included 20 min of exercise. The SO₂ challenges were administered by different techniques, Jorres and Magnussen used a series of increasing levels of ventilation at a constant SO₂ concentration, whereas Rubinstein et al. (1990) used increasing concentrations of SO₂ at a constant ventilation.

The effects of NO₂ on airway responsiveness to a specific antigen have been examined in only two studies. Ahmed et al. (1983a) reported no increase in airway responsiveness to ragweed antigen in a group of allergic asthmatics following 60 min of exposure to 0.1 ppm NO₂. Orehek et al. (1981) found no change in airway responsiveness to grass pollen in a group of allergic subjects (including three asthmatics) after a 60-min exposure to 0.11 ppm NO₂.

From the studies for which individual data were readily available, the number of subjects whose airway responsiveness increased and whose airway responsiveness decreased is listed in Table 15-9. Tabulation of data from this table provided information regarding the direction of the change (i.e., increase or decrease) in airway responsiveness following NO₂ exposure. One of the problems in this kind of analysis is that it is often difficult to distinguish between a negative and a no-change situation (i.e., it is less likely that airway responsiveness would decrease from its baseline level than increase).

Of the 105 subjects exposed to <0.20 ppm, the overall data indicated 67 subjects with increased airway responsiveness and 38 with decreased airway responsiveness. Similar ratios were observed for exercise and rest exposures. For the studies of exposure to 0.20 to 0.30 ppm, using all types of challenges, airway responsiveness increased in 96 subjects and decreased in 73. For studies involving exercise during the exposure, airway responsiveness increased in 71 and decreased in 65. However, both studies involving resting exposure showed significant increases in airway responsiveness, whereas only two of nine studies using exercise exposures were significant. In the resting studies, airway responsiveness increased in 25 subjects and decreased in 8 subjects. These studies also were of shorter duration (~30 min) than many of the exercise studies. Airway responsiveness increased in
29 and decreased in 13 subjects in studies of 30-min duration, whereas there were 41 increases and 53 decreases in exposures lasting 60 min or longer. At concentrations greater than 0.30 ppm, the overall total indicated 48 increases and 33 decreases in airway responsiveness. For resting studies, 24 subjects had increased airway responsiveness and only 9 showed decreased airway responsiveness (5 did not change), whereas 23 increased and 24 decreased in the exercise studies. These data are summarized in Table 15-10.

The studies in which the change in airway responsiveness was assessed after NO$_2$ exposure are presented in three concentration ranges in Table 15-10 and are divided according to whether or not exercise was involved in the exposure. The data are presented as the fraction of the total number of subjects with increased airway responsiveness. The increase in airway responsiveness does not appear to be associated with any particular type of airway challenge. The overall percentage of increased airway responsiveness in NO$_2$-exposed subjects was 59%. This is accounted for almost entirely by the resting studies, with an overall percentage of 69% (p < 0.01) (106 increased and 48 decreased), because, in the exercising studies, responses were about equally balanced between increased and decreased responsiveness (104 increased and 96 decreased). There was a trend (p < 0.05) for a slightly larger percentage (~75%) of subjects to have increased airway responsiveness after NO$_2$ exposure when the exposure is performed both under resting conditions and at concentrations above 0.20 ppm. In fact, of the six studies reporting a significant response (Kleinman et al., 1983, Bauer et al., 1986a, Bylin et al., 1988, Jorres and Magnussen, 1990, Mohsenin, 1987a, Bylin et al., 1985), four were resting exposures and, in four, the exposure duration was 30 min or less.

The implication of this trend is unclear because the brief duration and low ventilation during exposure indicate that the NO$_2$ exposure dose in these studies is relatively low. If this trend is real, some interesting hypotheses could be generated. Is it possible that exercise during exposure somehow interferes with the mechanism causing increased airway responsiveness? It is known, for example, that repeated exercise induces a refractory state such that the subject is less sensitive to exercise-induced bronchoconstriction (Edmunds et al., 1978, Ben-Dov et al., 1982). In many cases of NO$_2$ exposures involving exercise, repeated bouts of exercise were performed during exposure, which could possibly have made the subjects refractory to the effects of NO$_2$. During exercise, the responsiveness to
<table>
<thead>
<tr>
<th>Nitrogen Dioxide Concentration ([NO₂]) (ppm)</th>
<th>All Exposures</th>
<th>Exposures with Exercise</th>
<th>Exposure at Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASTHMATICS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 &lt; [NO₂] &lt; 0.20</td>
<td>0.64 (105)b</td>
<td>0.59 (17)</td>
<td>0.65 (88)b</td>
</tr>
<tr>
<td>0.20 ≤ [NO₂] ≤ 0.30</td>
<td>0.57 (169)</td>
<td>0.52 (136)</td>
<td>0.76 (33)b</td>
</tr>
<tr>
<td>0.30 &lt; [NO₂]</td>
<td>0.59 (81)</td>
<td>0.49 (48)</td>
<td>0.73 (33)c</td>
</tr>
<tr>
<td>All [NO₂]</td>
<td>0.59 (355)b</td>
<td>0.52 (201)</td>
<td>0.69 (154)b</td>
</tr>
<tr>
<td><strong>HEALTHY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[NO₂] &lt; 1.0</td>
<td>0.47 (36)</td>
<td></td>
<td>0.47 (36)</td>
</tr>
<tr>
<td>[NO₂] &gt; 1.0</td>
<td>0.79 (29)b</td>
<td>0.73 (15)</td>
<td>0.86 (14)c</td>
</tr>
</tbody>
</table>

*Data are fraction of subjects indicating an increase in airways responsiveness above the value for clean air. Numbers in parenthesis indicate actual number of subjects in each category. Total number = 354.

 bp < 0.01 two-tailed sign test

 cp < 0.05 two-tailed sign test
methacholine is reduced substantially (Inman et al., 1990) and exercise causes a more rapid reversal of methacholine-induced bronchoconstriction than occurs at rest (Freedman et al., 1988). On the other hand, is there a biphasic response of NO2 causing increased airway responsiveness at low exposure doses (for example, causing mast cell degranulation, vis à vis Sandstroem et al. [1990a]), with a reversal of this response occurring at higher exposure doses, possibly through a direct relaxing effect on airway smooth muscle? For example, nitrites formed in the lungs of NO2-exposed animals (Postlethwait and Mustafa, 1981) may have a direct relaxing effect on smooth muscle, including bronchial smooth muscle.

In healthy subjects, an increase in airway responsiveness clearly occurs at higher NO2 exposure concentrations (Beil and Ulmer, 1976, Frampton et al., 1991, Mohsenm, 1988). In the normal subjects at all concentrations, there were 37 airway responsiveness increases and 23 airway responsiveness decreases. At greater than 1.0 ppm, there were 23 increases and 6 decreases, that is, a ratio of 0.79 (p < 0.01).

15.5 EFFECTS OF NITROGEN DIOXIDE OR NITRIC ACID EXPOSURE ON BLOOD, URINE, AND BRONCHOALVEOLAR LAVAGE FLUID BIOCHEMISTRY

The effects of NO2 on the constituents of bronchoalveolar lavage (BAL) fluid, blood, and urine have been examined, both in vivo and in vitro. The general purpose of these studies has been to examine mechanisms of pulmonary effects or to determine NO2-induced alterations in body fluids that could potentially result in systemic effects. Investigations have been aimed at determining the effects of NO2 on levels of serum enzymes and antioxidants, as well as direct effects on red blood cells and hemoglobin. Studies of the effects of NO2 on airway lining fluids have focused on changes in alpha1-antitrypsin levels. Potential effects of NO2 on collagen metabolism have been investigated by examining urinary excretion of collagen metabolites.

15.5.1 Biochemical Effects in Blood

Chaney et al. (1981) examined the effects of 0.20 ppm NO2 on various blood parameters in 19 healthy subjects exposed for 2 h while exercising intermittently. A control
group of 15 subjects was exposed to clean air. They observed a significant increase in glutathione (GSH) levels after exposure. None of the other blood parameters (red blood cell GSH reductase, 2,3-diphosphoglycerate, methemoglobin, vitamin E, immunoglobulin, and complement C3) were changed significantly. The significance of the response reported in this study appears to be the result of a difference between the control group and the exposure group in general (different subjects were used in each group). The changes in GSH were small and were within the normal range, with the average baseline level of GSH being approximately 38.5 mg/dL. The postexposure average of the air group was 36.4 mg/dL ± 1.35 (standard error of the mean [SEM]) and of the NO₂ groups was 40.3 ± 1.19 (SEM) mg/dL. The authors suggested that the increased level of GSH may be in response to oxidation of hemoglobin to methemoglobin by NO₂. However, Gohl et al. (1988) have recently demonstrated substantial decreases in GSH levels during prolonged submaximal exercise, which was followed by elevated GSH levels in the postexercise period. GSH levels varied from 0.15 mM during exercise to 0.6 mM 3 days postexercise, varying about a baseline level of approximately 0.4 mM. It is not clear to what extent the observations of Chaney et al. (1981) may have been confounded by this exercise effect.

It should be noted that Posin et al. (1978) found no association between NO₂ exposure (1 ppm for 2.5 h) and GSH levels, although there were apparent changes in blood biochemistry including increased levels of GSH reductase. However, it is not clear from the Posin et al. (1978) study that any of the observed "effects" can be attributed to NO₂ exposure, there was no concentration-response relationship, effects were not reproducible from concentration to concentration, and similar effects were seen with clean air exposures.

In vitro exposure of human blood to high levels of NO₂ (6 and 45 ppm) resulted in methemoglobin formation (Chiodi et al., 1983). However, Borland et al. (1985) were unable to demonstrate increased methemoglobin levels in smokers exposed to high NO levels from cigarette smoke. Methemoglobin is also formed during in vitro exposure to NO (1,000 ppm) (Chiodi and Mohler, 1985). These observations appear to have no relevance to the potential effects of ambient NO₂.
15.5.2 Bronchoalveolar Lavage Fluid Biochemistry

Mohsenin and Gee (1987) have reported that subjects exposed to 3 to 4 ppm NO₂ for 3 h had a 45% decrease in the activity of alpha-1-protease inhibitor (α₁PI) the major lung protease inhibitor of the enzyme elastase. These levels were measured in BAL fluid obtained 3.5 to 4 h after exposure. Alpha-1-protease inhibitor is "important in protecting the lung from proteolytic damage, particularly from the elastase of neutrophils." The mean elastase inhibitory capacity decreased from 95 ± 12% in the air group to 55% in the NO₂-exposed group. (Due to analytical impurities in the standard, the 95% inhibition measured in the air-exposed group was presumed equivalent to 100%, thus the 45% difference.) The authors noted that even a 50% reduction in α₁PI activity is not associated with an increased risk of emphysema (Kabiraj et al., 1982). However, reduction in protease inhibition could result in connective tissue damage and could conceivably be important in individuals with an α₁-antitrypsin deficiency.

Johnson et al. (1990) also examined the response of α₁PI to in vivo NO₂ exposure in a group of 24 healthy nonsmokers. The subjects were exposed to either 1.5 ppm NO₂ for 3 h or to a variable concentration consisting of a baseline level of 0.05 ppm NO₂ with three 15-min "peaks" of 20 ppm. Details of the exposure protocol and subject characteristics are provided in Frampton et al. (1989b) (discussed in Table 15-1, Section 15.2). Bronchoalveolar lavage was performed 3.5 h after exposure and the fluid was frozen for subsequent analysis. The functional activity of α₁PI was taken to be the elastase inhibitory activity corrected for the concentration of α₁PI determined by immunoassay. Neither the levels of α₁PI, as determined by immunoreactivity, nor its functional activity were significantly changed by NO₂ exposure.

The different findings by Johnson et al. (1990) and Mohsenin and Gee (1987) with regard to α₁PI activity may be accounted for by the considerably larger (about two- to threefold) exposure levels in the latter study. Furthermore, different methods were used to handle the BAL fluid and to quantify α₁PI concentrations in the two studies. As discussed by Mohsenin and Gee (1987), there appears to be a large range of α₁PI activity that is compatible with lung health, and there is broad range of activity of α₁PI in relation to its concentration. The importance of small changes in α₁PI is not clearly established, and
therefore, the usefulness of changes in $\alpha_1$PI activity as a marker of NO$_2$ exposure will require additional research

15.5.3 Urine Biochemistry

Muelenaer et al (1987) studied normal males exposed to 0.6 ppm NO$_2$ for 4 h/day on three consecutive days to examine the possibility that NO$_2$ exposure caused diffuse pulmonary injury. They used hydroxyproline excretion as a marker of increased collagen catabolism or connective tissue injury. Subjects had no residential NO$_2$ exposure, no allergies or infections that might have produced inflammatory responses, and were minimally exposed to environmental tobacco smoke. Despite controlling for these potentially confounding variables, the authors observed no significant changes in hydroxyproline excretion as a result of NO$_2$ exposure, either immediately or for up to 9 days after exposure.

15.6 EFFECTS OF NITROGEN DIOXIDE OR NITRIC ACID VAPOR EXPOSURE ON HUMAN PULMONARY HOST DEFENSE RESPONSES

From the epidemiological (Chapter 14) and animal toxicology (Section 13.2.2.1) literature, it is clear that there is considerable concern regarding the role NO$_2$ exposure may play in potentiating susceptibility to both bacterial and viral infections. Important host defenses that may be affected by NO$_2$ exposure include the mucociliary clearance system, alveolar macrophages (e.g., altered viral inactivation), and humoral and cell-mediated immune responses (e.g., changes in antibodies and changes in cell populations and their activities in the lung). The effects of NO$_2$ exposure on viral infectivity have been studied in human volunteers. The effects of NO$_2$ on macrophage functions have been examined using macrophages from NO$_2$-exposed subjects or macrophages exposed to NO$_2$ in vitro. The effects of NO$_2$ on mucociliary clearance in humans are discussed in Section 15.2.1.4.

Kulle and Clements (1988) and Goungs et al (1989) (two reports of the same study) examined the effect of NO$_2$ exposure on the infectivity rate of live attenuated influenza A/Korea/reassortment virus in healthy, nonsmoking adults exposed to NO$_2$. Seven separate groups were exposed to either clean air ($n = 23, 21, 21$) or to NO$_2$ at 1.0 ($n = 22$),
2.0 (n = 21, 22), or 3.0 ppm (n = 22). The exposures consisted of 1 preliminary day of clean air exposure and then 3 consecutive days of the treatment (i.e., either NO\textsubscript{2} or clean air). The virus was administered intranasally after the second exposure day (i.e., the third of the four days). Infectivity was defined as evidence of virus recovery or a rise in either nasal wash or serum antibody titers after virus inoculation. Infectivity rates in the three clean air groups were 65, 71, and 71%, respectively, and were 77, 57, 91, and 91% in the 3.0, 2.0, 2.0, and 1.0 ppm NO\textsubscript{2} exposure groups, respectively. Although the rates of infection were elevated after NO\textsubscript{2} exposure in three of four NO\textsubscript{2}-exposed groups, these changes were not significant. The investigators and an expert review committee (Kulle and Clements, 1988) concluded that the results of the study were inconclusive rather than negative, thus implying that the hypothesis that NO\textsubscript{2} exposure may alter the frequency or severity of viral infections was neither confirmed nor denied by the results of this study.

Goings et al. (1989) have further elaborated on the results of the above study. They made the point that the experimental design had a low power to detect a 20% difference in infection rate of influenza A/Korea (i.e., 71% vs. 91%) and, thus, the lack of statistical significance is not unexpected. At least 70 subjects would be required to detect such a difference using this virus, which has a relatively high rate of infectivity. Finally, the influenza A/Korea/reassortment virus is not likely to infect the lower respiratory tract where most of the NO\textsubscript{2} deposition occurs. There is also the possibility that the results may have been confounded by an influenza epidemic, which occurred concurrently with this study, although caused by a different but related virus. The epidemic occurred between Year 1 and Year 2.

Frampton et al. (1989a) studied two groups of normal subjects exposed to NO\textsubscript{2} under two different protocols that had the same concentration \times time (C \times T) product. One group was exposed continuously for 3 h to 0.60 ppm and the other was exposed to a background level of 0.05 ppm with three "spikes" of 2.0 ppm for 15 min each. The C \times T product for each of these two protocols was the same. The major aims of this study were to test the hypothesis that the ability of alveolar macrophages to inactivate influenza virus was reduced by NO\textsubscript{2} exposure, and to examine the possibility that a series of peak exposures would cause more impairment than a constant concentration (see also Section 13.2.2.1). Healthy, normal nonsmokers with no history of airway hyperresponsiveness or of recent upper respiratory
Infection were exposed to both air and NO\textsubscript{2} in random sequence. Exposures included six 10-min exercise periods, coinciding with the "spikes" in the second protocol. There were no significant effects of these exposures on spirometry or plethysmography under either protocol. Alveolar macrophages obtained by BAL were tested in vitro for their ability to inactivate influenza (A/AA/Marton/43 H1N1) virus and for the in vitro production of Interleukin-1 (IL-1) by virus-exposed macrophages. Interleukin-1 is an important proinflammatory protein produced by macrophages that performs a number of functions, including induction of fibroblast proliferation and activation of lymphocytes, and is chemotactic for monocytes during the immune response to infection. There were no differences in total cell recovery, viability, or differential cell counts between air- and NO\textsubscript{2}-exposed samples for either protocol. There was a trend ($p < 0.07$) for less effective inactivation of virus by macrophages obtained from subjects exposed continuously to 0.60 ppm NO\textsubscript{2}. This trend was due to the responses of only four of the nine subjects. The macrophages harvested from these four subjects also showed an increase in IL-1 production not seen in macrophages from the other subjects. No effects of virus inactivation were seen in the subjects exposed to the 2.0-ppm spikes. Although the results of this study were not statistically significant, the study had relatively low power to detect an effect. The findings are provocative and suggest that further work is necessary to test the hypothesis that NO\textsubscript{2} may influence host defense mechanisms in humans.

Frampton et al. (1989b) also analyzed the protein content of BAL fluid obtained from NO\textsubscript{2}-exposed subjects at either 3.5 or 18 h postexposure. Three different exposure protocols were used: 3-h exposure to 0.60 ppm or 1.5 ppm NO\textsubscript{2} or a 3-h variable concentration exposure where three 15-min "peaks" of 2.0 ppm were superimposed on a background of 0.05 ppm NO\textsubscript{2}. Exposures included 10 min of exercise during each half-hour of exposure. There were no significant changes in pulmonary function or respiratory symptoms observed after NO\textsubscript{2} exposure. Airway reactivity, assessed by carbachol inhalation, was increased after the 1.5 ppm NO\textsubscript{2} exposure (Frampton et al., 1991). Two groups of subjects were exposed to 0.60 ppm so that BAL could be obtained either at 3.5 or 18 h postexposure. Analysis of BAL fluid obtained 3.5 h after a 0.60-ppm exposure indicated an increase in alpha-2-macroglobulin ($\alpha_2$-M), a regulatory protein that has antiprotease activity and immunoregulatory effects. The observed increase in $\alpha_2$-M appears to be transient (no change
was seen at 18 h postexposure) and was not observed at a higher NO\textsubscript{2} concentration (15 ppm). Further information appears to be necessary to establish the implications of this finding.

The effects of NO\textsubscript{2} on macrophage function in NO\textsubscript{2}-exposed animals is discussed in Section 13.2.1. Nitrogen dioxide-induced changes have been noted in macrophages harvested from animals exposed to NO\textsubscript{2} concentrations less than 10 ppm. These studies are discussed in detail in the animal toxicology chapter (Chapter 13).

The effect of \textit{in vitro} exposure to NO\textsubscript{2} on alveolar macrophages harvested by BAL was examined by Pinkston et al. (1988). Fifteen healthy adults underwent BAL to provide macrophages for culture. After an 18-h incubation, the cells were exposed to 5, 10, or 15 ppm NO\textsubscript{2} or 5\% carbon dioxide as a control for an additional 3 h. Following the exposure, some of the cells were incubated for an additional 24 h, and cell-free supernatants were then obtained for analysis of neutrophil chemotactic factor (NCF). Other macrophage cultures were incubated for 24 h with influenza virus and the supernatant was then obtained for analysis of IL-1. There were no changes in macrophage viability, determined by trypan blue exclusion, in cells exposed to any of the three NO\textsubscript{2} concentrations. There were no changes in release of NCF in any of the NO\textsubscript{2}-exposed cell cultures. Furthermore, NO\textsubscript{2} exposure did not impair the ability of cells to release NCF after stimulation with activated zymosan. Nitrogen dioxide exposure did not stimulate release of IL-1 from exposed macrophages. Influenza virus stimulated the release of IL-1, but there were no significant differences between NO\textsubscript{2}-exposed and air-exposed macrophage cultures. Therefore, NO\textsubscript{2} exposure triggered neither the release of NCF, which would attract neutrophils to the airways, nor the release of IL-1, which activates lymphocytes (among other functions). Equally important, NO\textsubscript{2} exposure did not impair the ability of macrophages to produce either IL-1 or NCF in response to conventional stimuli.

Sandstroem et al. (1989) exposed a group of 18 healthy nonsmokers to 2.25, 4.0, and/or 5.5 ppm (n = 8 in each concentration group) for 20 min of moderate exercise ($\dot{V}_E = 35$ L/min) in an exposure chamber. Bronchoalveolar lavage was performed at least 3 weeks before and 24 h after each exposure. Increased levels of mast cells in BAL fluid were observed after all NO\textsubscript{2} exposures. Increased levels of lymphocytes were observed only at the two higher concentrations.
In order to determine the time course of this response, Sandstroem et al. (1990a) exposed 32 subjects to 4 ppm NO\textsubscript{2} for 20 min, including 15 min of mild exercise, and then performed BAL at 4, 8, 24, or 72 h postexposure (in four different groups of eight subjects). Increased levels of mast cells and lymphocytes were observed at 4, 8, and 24 h, but not at 72 h postexposure. There was no change in macrophage numbers nor in albumin concentration in BAL fluid. Eosinophils, neutrophils, and epithelial cell counts were not altered as a result of NO\textsubscript{2} exposure. Unpleasant odor and mild nasopharyngeal irritation were typical symptoms. There were no changes in spirometry. The observation of increased numbers of mast cells appears to be unique to this study, although other investigators (Frampton et al., 1989a,b) may not have looked for changes in mast cell numbers. The authors considered the increased numbers of mast cells and lymphocytes to represent a nonspecific inflammatory response.

Rasmussen et al. (1992) studied 14 healthy nonsmoking adult subjects exposed to 2.3 ppm NO\textsubscript{2} and to clean air for 5 h with a 1-week interval between exposure. Indications of a decrease in alveolar permeability were observed after the NO\textsubscript{2} exposure. The results support the assumption that a delayed response is a feature of the human response to NO\textsubscript{2} and stresses the importance of an extended period of observation in future NO\textsubscript{2} exposure studies.

Three recent studies examined the effects of multihour exposures to 1 to 2 ppm NO\textsubscript{2} on lavaged cells and mediators. Devlin et al. (1992) studied healthy subjects exposed to 2.0 ppm NO\textsubscript{2} for 4 h with alternating 15 min periods of rest and moderate exercise. One of the main findings after NO\textsubscript{2} exposure was a threefold increase in polymorphonuclear leukocytes (PMNs) in the first lavage sample representing predominantly bronchial cells and fluid. In addition, macrophages recovered from the predominantly alveolar fraction showed a 42% decrease in ability to phagocytose Candida albicans and a 72% decrease in release of superoxide anion. Frampton et al. (1992) exposed exercising subjects to 2.0 ppm NO\textsubscript{2} for 6 h. Bronchoalveolar lavage was performed either immediately or 18 h postexposure. There was a modest increase in lavage fluid PMN levels (<twofold increase) but no change in lymphocytes. Alveolar macrophage production of superoxide anion was not altered in these subjects. These two studies suggest that NO\textsubscript{2} exposure may induce a mild bronchial inflammation and may also lead to impaired macrophage function. Jorres et al. (1992)
examined both healthy and asthmatic subjects exposed to 1 ppm NO\textsubscript{2} for 3 h, but observed no changes in cells or mediators in BAL fluid or in the appearance of bronchial mucosal biopsies after this exposure. Neither macrophage function nor a specific bronchial washing were examined in this study.

Boushey et al. (1988) studied five healthy volunteers exposed to 0.60 ppm NO\textsubscript{2} on 4 days over a 6-day period. Exposures lasted 2 h each and included alternating 15-min periods of rest and exercise ($V_E \approx 30$ to $40$ L/min). On the final (fourth) day of NO\textsubscript{2} exposure, venous blood samples were obtained and a BAL was performed. Baseline BAL and pulmonary function data were obtained on a separate occasion. There were no effects of repeated NO\textsubscript{2} exposure on pulmonary function ($SR_{aw}$, FVC, FEV$_1$) or respiratory symptoms. Following the fourth day of NO\textsubscript{2} exposure, a slight increase in circulating (venous blood) lymphocytes was observed ($1792 \pm 544$/mm$^3$ post-NO\textsubscript{2} vs $1598 \pm 549$/mm$^3$ baseline). The only change observed in BAL cells was an apparent increase ($p < 0.04$) in natural killer (NK) cells from $4.2 \pm 2.4\%$ (baseline) to $7.2 \pm 3.1\%$ (post-NO\textsubscript{2}). The authors expressed reservations that the apparent increase in NK cells may have been an artifact of the cell separation process. Interleukin-1 and tumor necrosis factor levels in BAL fluid were not detectable. Tumor necrosis factor is another proinflammatory protein that, among other activities, promotes adherence of PMNs to endothelial cells and enhances their phagocytic activity.

Sandstroem et al. (1990b) studied a group of eight healthy nonsmokers exposed to 4.0 ppm NO\textsubscript{2} for 20 min/day (moderate exercise, $V_E \approx 35$ L/min) on alternate days over a 12-day period (seven exposures total). Bronchoalveolar lavage was performed 2 weeks before the first exposure and 24 h after the last exposure. The first 20 mL of BAL fluid was treated separately and presumed to represent primarily bronchial cells and secretions. After NO\textsubscript{2} exposure, there was a reduction in numbers of macrophages in the bronchoalveolar portion, although on a per cell basis, alveolar macrophage phagocytic activity was increased. There were decreased numbers of mast cells in the bronchial portion of the lavage fluid. In addition, there were reduced numbers of T-suppressor, B lymphocyte, and NK cells in the alveolar portion of the BAL fluid compared to the baseline lavage. These observations contrast with those seen by Sandstrom et al. (1989) after single NO\textsubscript{2} exposures, suggesting some alteration in bronchial and alveolar cell populations after repeated NO\textsubscript{2} exposure.
most obvious difference between Sandstrom et al (1990b) and Boushey et al (1988) is the higher NO\textsubscript{2} concentration and the longer duration of the former study. Further work is necessary to confirm these observations, to determine the time course of response to repeated exposure, and to determine the NO\textsubscript{2} exposure dose necessary to invoke modification of bronchoalveolar cell populations.

The effects of HNO\textsubscript{3} vapor exposure (in vivo) have been examined in two recent studies. Becker et al (1992) exposed nine healthy subjects to 200 μg/m\textsuperscript{3} (80 ppb) of HNO\textsubscript{3} vapor for 120 min, including 100 min of moderate exercise (V\textsubscript{E} = 42 L/min). Bronchoalveolar lavage performed 18 h postexposure indicated an increased phagocytic activity of macrophages harvested from the HNO\textsubscript{3}-exposed lung. Alveolar macrophages also showed increased resistance to infection with respiratory syncytial virus. Compared to air exposure, there were no increases in inflammatory mediators (such as prostaglandin E\textsubscript{2}, leukotriene B\textsubscript{4}, C\textsubscript{3}a, or neutrophils) or in cell damage indicators such as lactate dehydrogenase (LDH) or lavage fluid protein. The absence of markers of tissue damage (LDH) or permeability (lavage fluid protein), suggest that, under these exposure conditions, HNO\textsubscript{3} did not cause frank tissue damage.

Aris et al (1991a) exposed 10 healthy subjects to 500 μg/m\textsuperscript{3} HNO\textsubscript{3} vapor for 4 h, including moderate exercise. Lavage fluid was obtained from both bronchial as well as bronchoalveolar washings, and bronchial biopsy specimens were obtained. No change in LDH levels or lavage protein were observed as a result of HNO\textsubscript{3} exposure. These investigators found no differences in differential cell counts in the lavage fluid of both bronchial and bronchoalveolar washings. They also exposed a different group of subjects to 500 μg/m\textsuperscript{3} HNO\textsubscript{3} plus 0 20 ppm O\textsubscript{3} and found no potentiation of the O\textsubscript{3}-induced inflammatory response by the addition of HNO\textsubscript{3} vapor to the exposure. Their data suggest that, at these concentrations, HNO\textsubscript{3} does not cause tissue injury, nor does HNO\textsubscript{3} alter the inflammatory response typical of O\textsubscript{3} exposure.

15.7 EFFECTS OF NITRATES ON HUMAN LUNG FUNCTION

Five studies have been conducted on human exposure to nitrate aerosols since 1979 (see Table 15-11). These studies have been discussed in the Acid Aerosol Issues Paper (U.S.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Nitrate Species and Conc (µg/m³)</th>
<th>Exposure Duration (min)</th>
<th>Exercise Duration (min)</th>
<th>Exercise Ventilation (L/min)</th>
<th>Temp (°C)</th>
<th>Humidity (Percent)</th>
<th>Number of Subjects</th>
<th>Subject Char</th>
<th>Aerosol MMAD</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klemman et al (1980)</td>
<td>NH₄NO₃ 200</td>
<td>120</td>
<td>60</td>
<td>≈ 20</td>
<td>31</td>
<td>40</td>
<td>20</td>
<td>Normal</td>
<td>1 1</td>
<td>No significant changes in normals or asthmatics except possible decrease in Rₜ. No symptom effects</td>
</tr>
<tr>
<td>Sackner et al (1979)</td>
<td>NaNO₃ 10,100,100</td>
<td>10</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>5</td>
<td>Normal</td>
<td>0 2</td>
<td>No changes</td>
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<td></td>
<td></td>
<td>Asthma</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>0 55</td>
<td>No effects</td>
</tr>
<tr>
<td>Stacy et al (1983)</td>
<td>80 (NH₄NO₃)</td>
<td>240</td>
<td>30</td>
<td>55</td>
<td>30</td>
<td>60</td>
<td>12</td>
<td>Normal</td>
<td>0 46</td>
<td>No effects</td>
</tr>
<tr>
<td></td>
<td>80 (NH₄NO₃) +0 5 ppm NO₂</td>
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<td></td>
<td>Asthma</td>
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<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>0 49</td>
<td>No symptoms SGₑw decreased 17% and max 40% TLC decreased 12% after nitrate, within 2 days of onset of illness. Similar effects 1 week later, but not 3 weeks later</td>
</tr>
</tbody>
</table>

**Abbreviations**

- MMAD = Mass median aerodynamic diameter
- NaNO₃ = Sodium nitrate
- NH₄NO₃ = Ammonium nitrate
- SGₑw = Specific airway conductance
- max40%TLC = Maximum expiratory flow at 40% of TLC on a PEFV curve
- Rₜ = Total respiratory resistance
Environmental Protection Agency, 1989) The only obvious effect was a decrease in $G_{aw}$ and in PEFV curves in normal subjects with influenza exposed to 7,000 $\mu$g/m$^3$ of sodium nitrate (NaNO$_3$) aerosol. This is probably three orders of magnitude (i.e., approximately 1,000 times) above the nitrate concentration that could exist in the ambient air. These studies indicate that, at least as far as lung function is concerned, there is no present concern for adverse effects from current ambient levels of nitrate aerosols.

Sackner et al. (1979) studied a diverse group of normal and asthmatic subjects exposed to concentrations reaching 1,000 $\mu$g/m$^3$ of NaNO$_3$ for 10 min at rest. There were no significant effects on an extensive battery of pulmonary function tests.

Utell et al. (1979) studied both normal and asthmatic volunteers exposed to 7,000 $\mu$g/m$^3$ of 0.46 $\mu$m NaNO$_3$ aerosol for 16 min via mouthpiece. The major health effect end points measured in their study included $R_{aw}$, both full and PEFV curves, airway reactivity to carbachol, and aerosol deposition. Aerosol deposition as a percentage of inhaled aerosol averaged about 50% for normals and about 56% for asthmatics, the group differences were not significant. The exposure to NaNO$_3$ aerosol was indistinguishable from the control NaCl exposure in normals. Similarly, there were no effects of NaNO$_3$ exposure on asthmatics.

Utell et al. (1980) subsequently studied 11 subjects with influenza exposed to the same NaNO$_3$ regimen as above. The subjects were initially exposed at the time of illness and then were reexposed 1, 3, and 6 weeks later. Aerosol deposition ranged from 45 to 50% over the four exposure sessions. All subjects had cough and fever, and 10 of 11 subjects had viral or immunologic evidence of acute influenza. Baseline measurements of FVC and FEV$_1$ were within normal limits and did not change throughout the 6-week period. There were small but significant decreases in $G_{aw}$ following NaNO$_3$ inhalation, but not after NaCl exposure. This difference was present during acute illness and 1 week later, but was not seen at 3 and 6 weeks after illness. The decrease in $SG_{aw}$ seen on the initial exposure was accompanied by a decrease in partial expiratory flow at 40% TLC, this was also observed at the 1-week follow-up exposure. This study suggests that the presence of an acute viral respiratory tract infection may render humans more susceptible to the acute effects of nitrate aerosols. Nevertheless, the concentration of nitrates used in this exposure study exceed maximum ambient levels by more than 100-fold.
In addition to NaNO₃ aerosols, ammonium nitrate (NH₄NO₃) exposure has been studied by Kleinman and associates (1980). Twenty normal and 19 asthmatic subjects were exposed to a nominal 200 μg/m³ of 1.1 μm NH₄NO₃ aerosol. The 2-h exposures included mild, intermittent exercise and were conducted under warm conditions (31 °C, 40% RH). There were no significant physiologically meaningful effects of the NH₄NO₃ exposure in either subject group.

Stacy et al. (1983) also studied the effects of 80 μg/m³ of NH₄NO₃ in a group of healthy male adults. As in the Kleinman et al. (1980) study, there were no changes in lung function or symptoms.

15.8 CONCLUSIONS AND DISCUSSION

At the beginning of this chapter, a series of questions were posed concerning the potential biological responses to NO₂ exposure in humans. Some of these questions can be answered in part using the data presented in this section, others will clearly require additional research.

Symptoms associated with NO₂ exposure in healthy subjects have been limited to detection of the odor of NO₂, in some cases at surprisingly low concentrations, less than 0.1 ppm (Bylin et al., 1985). Few of the studies examined in this review noted a significant increase in respiratory symptoms. Sandstrom et al. (1990a) noted mild nasopharyngeal irritation after exposure to 4 ppm for 20 min.

Nitrogen dioxide exposure at sufficiently high concentrations produces changes in lung function in healthy subjects. A number of investigators have reported increased airway resistance after exposure to NO₂ concentrations exceeding 2.5 ppm (Beil and Ulmer, 1976, Von Nieding et al., 1979, Von Nieding and Wagner, 1977, Von Nieding et al., 1980). However, at concentrations of NO₂ between 2 and 4 ppm, some investigators have not observed any NO₂-induced changes in airway resistance or spirometry (Linn et al., 1985b, Mohsenin, 1987b, Mohsenin, 1988, Sandstroem et al., 1990a). At NO₂ exposure concentrations below 1.0 ppm, there is little if any convincing evidence of change in lung volumes, flow-volume characteristics of the lung, or airways resistance in healthy subjects. Nitrogen dioxide is believed to have its primary effect on small airways. However, routine
Spirometry and airway resistance measurements are not sensitive indicators of small airways function. Thus, the absence of change in these physiological indicators of large airways function at low NO₂ concentrations should not be viewed as evidence that NO₂ has no effects on lung function. Further developments will be necessary to permit sensitive, reproducible, noninvasive evaluation of small airways, the primary site of NO₂ deposition in the lung.

Nitrogen dioxide exposure does result in increased airway responsiveness in normal subjects exposed to concentrations in excess of 1.0 ppm. Mohsenin (1987b) and Frampton et al. (1991) reported an increase in airway responsiveness after exposure to 2.0 and 1.5 ppm, respectively. Repeated bouts of airway inflammation could promote deleterious long-term changes in the lung, such as loss of elasticity and acceleration of age-related changes in lung function. However, the development of such responses is only speculative, given the present level of scientific evidence.

Potentially sensitive subjects in the population include children, older adults, patients with asthma or COPD, or individuals who may be unusually sensitive to NO₂ for other reasons. There are insufficient data on children, adolescents, or older adults, either healthy or with asthma, to determine their NO₂ responsiveness relative to healthy young adults.

At the concentrations that may fall within the ambient range (e.g., <1.0 ppm), the effects of NO₂ on lung function (i.e., spirometry, airway resistance) in asthmatics have tended to be small. For example, Bauer et al. (1986a) observed a 4 to 6% decline in FEV₁ in asthmatics exposed to 0.3 ppm NO₂ for 30 min. Koenig et al. (1988) reported a 4% decrease in FVC, but no significant change in other spirometry variables, after exposure of adolescent asthmatics to 0.30 ppm NO₂. On the other hand, several other investigators (Avol et al., 1988, Bylin et al., 1985, Hazucha et al., 1982, 1983, Kleinman et al., 1983, Koenig et al., 1985, Linn et al., 1985b, 1986, Mohsenin, 1987a, Roger et al., 1990) have not found any significant changes in spirometry or airway resistance of asthmatics exposed to concentrations <1.0 ppm. Again, spirometry and airway resistance are not sensitive measures of small airways function, where NO₂ is known to be primarily deposited.

A second important category of sensitive subjects includes patients with COPD, who have shown increased airway resistance after brief exposures to greater than 1.6 ppm NO₂ (Von Nieding et al., 1970, 1971, 1973a) (see Table 15-6). In addition, during a longer (4-h) exposure, Morrow and Utell (1989) reported decreased (approx 5%) FVC in COPD patients.
exposed to 0.30 ppm. Other investigators (Linn et al., 1985a, Kerr et al., 1979) did not find responses in COPD patients even with exposures to levels as high as 2.0 ppm. It appears that brief acute exposure to relatively high concentrations of NO₂ (>2 ppm) will cause bronchoconstriction in some COPD patients and that these responses may also be observed with longer exposures to lower concentrations.

An unresolved issue with the current data base is the existence of NO₂-induced pulmonary responses in asthmatics that have been reported at low but not at high NO₂ exposures. Although small functional responses have been observed in studies from various laboratories, effects are not consistently present and demonstrating reproducibility of responses has been difficult, even within the same laboratory. Furthermore, all responses to NO₂ that have been observed in asthmatics have occurred at concentrations between 0.2 and 0.5 ppm. Changes in lung function or airway reactivity have not been seen even at much higher concentrations (i.e., up to 4 ppm). There is, at present, no plausible explanation for this apparent lack of a concentration-response relationship. There is a possibility that a portion of the variability in response to NO₂ may be attributed to differences in the severity of asthma. This is a complex issue and has not been studied adequately at this time.

In patients with chronic obstructive lung disease, Bauer et al. (1987) and Morrow and Utell (1989) have observed decreased lung function (FVC, FEV₁) after exposure to 0.30 ppm for 4 h, but Linn et al. (1985a) and Von Nieding and Wagner (1979) found no effects in COPD patients from short duration exposures below 2.0 ppm. It appears that further work will be necessary to provide enough information to estimate the concentration-response relationships for NO₂ exposure of asthmatics and COPD patients, who appear to be the sensitive subpopulations.

In several studies of asthmatics exposed to NO₂, airway responsiveness to a variety of agents has been demonstrated. However, in many other studies using similar experimental exposures, there was no significant change in airway responsiveness. In order to evaluate this apparent dilemma, a meta-analysis was utilized as described in Section 15.4. Without regard to the type of airway challenge, NO₂ concentration, exposure duration, or other variables, the overall trend was for airway responsiveness to increase (59% of 354 subjects increased). This trend was somewhat more convincing for exposures conducted under nonexercising conditions (69% of 154 subjects increased), indeed, the excess positive
responses were almost entirely accounted for by exposures conducted under resting conditions. The implications of this overall trend are unclear and will require further investigations to verify if there is an interaction with exercise-induced changes in lung function that may possibly obscure changes in airway responsiveness due to NO₂ exposure. Increased airway responsiveness could potentially lead to temporary exacerbation of asthma, possibly leading to increased medication usage or even increased hospital admissions. The lowest observed effect level for this response appears to be in the 0.2- to 0.3-ppm range.

Several recent studies have examined the possibility that NO₂ could induce a pulmonary inflammatory response and/or alter immune system host defenses. These studies typically include collection of cells and airways fluids from the lung using BAL. In contrast to O₃ exposure, NO₂ does not, at the concentrations studied, induce an increase in BAL levels of neutrophils, or eosinophils, the typical markers of inflammation following O₃ exposure. However, Devlin et al. (1992) have reported increased PMNs in bronchial washings. Sandstroem et al. (1990a) have observed an increase in mast cells and lymphocytes in BAL fluid, which they attribute to a nonspecific inflammatory response. Boushey et al. (1988) have reported an increase in natural killer lymphocytes in BAL fluid. Macrophage numbers have not been increased by NO₂ exposure, nor did their ability to kill virus appear to have been altered by exposure, although Frampton et al. (1989a) suggested that, in some subjects, macrophage responses may have been impaired. Rasmussen et al. (1992) observed indications of a decrease in alveolar permeability after exposure to 2.3 ppm NO₂ for 5 h. Mucociliary clearance was not altered after NO₂ exposure in the one study in which it was measured (Rehn et al., 1982). Nitrogen dioxide was found to cause a reduction in alpha-1-antiprotease activity in one study (Mohsenin and Gee, 1987), but not in another (Johnson et al., 1990). Following NO₂ exposure, Frampton et al. (1989b) found an increase in alpha-2-macroglobulin, a molecule that has immunoregulatory as well as antiprotease activity. Immunological responses to NO₂ exposure are just beginning to be elucidated and additional research will be required to determine whether these responses have any implications for epidemiologically determined associations between NO₂ exposure and increased respiratory tract infections.

The effects of repeated NO₂ exposure have been examined in two studies (Sandstroem et al., 1990b, Boushey et al., 1988). Boushey et al. (1988) reported only a slight increase
(12%) in circulating lymphocytes and a possible increase in natural killer lymphocytes after four 2-h exposures to 0.60 ppm. There were no detectable changes in inflammatory mediators. Sandstroem et al. (1990b), on the other hand, found decreased numbers of mast cells, macrophages, and lymphocytes in the BAL fluid. Despite the decreased numbers, the phagocytic activity of alveolar macrophages was enhanced. These observations suggest that host defense responses are different after repeated exposure than after a single acute exposure. More research appears to be necessary to confirm and expand these observations because of the important potential connection between altered host defense responses and increased respiratory infectivity.

In healthy adults, a variety of mixtures of other pollutants with NO\textsubscript{2} have been examined, primarily using spirometry and airway resistance measurements as end points. In general, NO\textsubscript{2} does not cause significant exacerbation of responses to other pollutants, such as O\textsubscript{3}, SO\textsubscript{2}, or particulate matter. In other words, there is no more than an additive response when NO\textsubscript{2} is included in the pollutant mixture. However, further investigation of NO\textsubscript{2} mixtures appears warranted using other biological markers, including measures of epithelial permeability, clearance, airway responsiveness, airway inflammation, and measures that are sensitive to changes in small airways function. In asthmatics, there is a tendency for increased responsiveness to cold air, methacholine, carbachol, and histamine after NO\textsubscript{2} exposure (see previous discussion). In one study, asthmatics were also more responsive to SO\textsubscript{2} after a previous exposure to NO\textsubscript{2} (Jorres and Magnussen, 1990). In addition to interactions with other pollutants, NO\textsubscript{2} exposure could potentially enhance (or inhibit) responses to other substances, particularly airborne antigens. In two studies (Ahmed et al., 1983a, Orehek et al., 1981), the response to grass pollen inhalation was examined in sensitive subjects after exposure to 0.1 ppm NO\textsubscript{2}, but no significant difference in the response after air and NO\textsubscript{2} exposures was observed. Given the increase in responsiveness to nonantigenic substances such as methacholine, histamine, SO\textsubscript{2}, or cold air discussed previously, it may be worthwhile to reexamine this hypothesis using higher NO\textsubscript{2} concentrations or more prolonged exposures.

Responses to other NO\textsubscript{x} species have also been studied. Nitric oxide does not appear to cause any lung function effects at low concentrations (<1.0 ppm) either alone (Kagawa, 1982) or combined with NO\textsubscript{2} (Kagawa, 1990). Von Nieding et al. (1973b) reported...
increased airways resistance in subjects exposed to excessively high concentrations of NO (>20 ppm) Responses to HNO₃ vapor have been studied in adolescent asthmatics (Koenig et al., 1989a,b) and in healthy adults (Aris et al., 1991b, Becker et al., 1992) Further investigation is needed to examine the responses to HNO₃ vapor Nitrates (e.g., sodium nitrate) have not been found to cause any deleterious effects (Utell et al., 1979, 1980, Kleiman et al., 1980, Stacy et al., 1983) at levels that might be expected in the atmosphere

The following conclusions may be drawn from the studies discussed here

1 Nitrogen dioxide causes decrements in lung function, particularly increased airway resistance in healthy subjects at concentrations exceeding 2.0 ppm for 2 h

2 Nitrogen dioxide exposure results in increased airway responsiveness in healthy, nonsmoking subjects exposed to concentrations exceeding 1.0 ppm for exposure durations of 1 h or longer

3 Nitrogen dioxide exposure at levels above 1.5 ppm may alter numbers and types of inflammatory cells in the distal airways or alveoli, but these responses depend upon exposure concentration, duration, and frequency. Nitrogen dioxide may alter function of cells within the lung and production of mediators that may be important in lung host defenses

4 Nitrogen dioxide exposure of asthmatics causes, in some subjects, increased airway responsiveness to a variety of provocative mediators, including cholinergic and histaminergic chemicals, SO₂, and cold air. However, the presence of these responses appears to be influenced by the exposure protocol, particularly whether or not the exposure includes exercise

5 Modest decrements in spirometric measures of lung function (3 to 8%) may occur in some asthmatics and COPD patients under certain NO₂ exposure conditions

6 Nitric acid levels in the range of 50 to 200 ppb may cause some pulmonary function responses in adolescent asthmatics, but not in healthy adults. Other commonly occurring NOₓ species do not appear to cause any pulmonary function responses at concentrations expected in the ambient environment, even at higher levels than in worst-case scenarios. However, not all nitrogen oxides acid species have been studied sufficiently

7 No association between lung function responses and respiratory symptom responses were observed. Furthermore, there is little evidence of a concentration-response relationship for changes in lung function, airway responsiveness, or symptoms at the NO₂ levels that are reviewed here
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16. HEALTH EFFECTS ASSOCIATED WITH EXPOSURE TO NITROGEN DIOXIDE

16.1 INTRODUCTION

This chapter concisely summarizes and integrates key information and conclusions from preceding chapters into a coherent framework or perspective upon which to base interpretations concerning human health risks posed by ambient or near-ambient levels of nitrogen dioxide (NO₂) in the United States. Toward this end, the chapter is organized into several sections, each of which discusses one or more major components of an overall health risk evaluation: (1) qualitative and quantitative characterization of key health effects of NO₂ and their biological bases, (2) identification of population groups potentially at enhanced risk for health effects associated with NO₂ exposure, (3) ambient and indoor NO₂ levels and related exposure aspects, and (4) a summary of NO₂ concentration-health effect relationships.

16.2 KEY HEALTH EFFECTS OF NITROGEN DIOXIDE

This section concisely discusses two key types of health effects that are of most concern at ambient or near-ambient concentrations of NO₂: (1) increases in airway responsiveness of asthmatic individuals after short-term exposures, and (2) increased occurrence of respiratory illness among children associated with longer term exposures to NO₂. A third category of NO₂ effects, emphysema, is also discussed but appears to be only of major concern with exposures to much higher than ambient levels of NO₂.

16.2.1 Airway Responsiveness in Asthmatics and Short-Term (One- to Three-Hour) Exposure to Nitrogen Dioxide

Asthmatics have airway hyperresponsiveness to a variety of chemical and physical stimuli and are considered to be one of the most NO₂-responsive groups in the population. The physiological end point that, to date, appears to be the most sensitive indicator of response to NO₂ in asthmatics is a change in airway responsiveness. Airway inhalation challenge tests are used to evaluate the "responsiveness" of a subject's airways to inhaled
To test for the degree of airway responsiveness, a pharmacologically active chemical (such as histamine, methacholine, or carbachol) that causes constriction of the airways is used. Responses are usually measured by evaluating changes in airway resistance or spirometry after each dose of the challenge is administered. Airway hyperresponsiveness is an abnormal degree of airway narrowing, caused primarily by airway smooth muscle shortening in response to nonspecific stimuli. An extensive discussion of such responses is presented in Chapter 15.

The Expert Panel Report from the U.S. National Asthma Education Program (National Institutes of Health, 1991) has recently defined asthma:

Asthma is a lung disease with the following characteristics: (1) airway obstruction that is reversible (but not completely so in some patients) either spontaneously or with treatment, (2) airway inflammation, and (3) increased airway responsiveness to a variety of stimuli.

About 10 million people in the United States, or 4% of the population, have asthma (National Institutes of Health, 1991). The prevalence is higher among African Americans, older (8 to 11 years) children, and urban residents (Schwartz et al., 1990). There is a broad range of severity of asthma, ranging from mild to severe. Common symptoms include cough, wheezing, shortness of breath, chest tightness, and sputum production. A positive response (skin test) to common inhalant allergens is a typical feature of asthma. Asthma is also associated with airway inflammation and epithelial injury (National Institutes of Health, 1991, Beasley et al., 1989, Lattinen et al., 1985, Wardlaw et al., 1988). Asthma is further characterized by an exaggerated bronchoconstrictor response to many physical changes (e.g., cold or dry air, exercise) and to chemical/pharmacologic agents (e.g., histamine or methacholine). The differences in airway responsiveness may span several orders of magnitude (at least 100-fold) between normal and asthmatic individuals (O’Connor et al., 1987). Despite the absence of airway hyperresponsiveness in some asthmatics and the presence of airway hyperresponsiveness in some nonasthmatics (Pattemore et al., 1990), there is a correlation between increased asthma symptoms or increased medication usage and increased airway responsiveness (Britton et al., 1988).

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At concentrations below 1.0 ppm NO₂, there is little (if any) convincing evidence of lung function decrements or changes in airway responsiveness in healthy individuals. There is, however, some evidence that acute exposure to NO₂ may cause an increase in airway responsiveness in asthmatics. This response has been observed only at relatively low NO₂ concentrations, mostly within the range of 0.20 to 0.30 ppm NO₂, which is of concern within the ambient environment. Analysis of data on asthmatics experimentally exposed to NO₂ in studies using various challenges produced airway responsiveness increases in 96 subjects and decreases in 73 subjects (Folinsbee, 1992). In the concentration range between 0.20 and 0.30 ppm, the excess increase in airway responsiveness was attributable to subjects exposed to NO₂ at rest. Because NO₂ does not appear to cause airway inflammation at these levels (although modest responses occur at higher [≥1.5-ppm] concentrations) and the increase in airway responsiveness appears to be fully reversible, the implications of the observed increase in responsiveness are unclear. Although it is conceivable that increased nonspecific airway responsiveness caused by NO₂ could lead to increased responses to a specific antigen, there is presently no plausible evidence to support this hypothesis. On the other hand, it is possible that persistence of airway hyperresponsiveness may be associated with an accelerated rate of decline in pulmonary function with age (O'Connor et al., 1987).

An unresolved issue with the current data base is the existence of NO₂-induced pulmonary function changes in asthmatics that have been reported at low, but not at high, NO₂ concentrations. Although small changes in spirometry or airway resistance have been observed in studies from various laboratories, effects are not consistently present and demonstrating reproducibility of responses has been difficult, even within the same laboratory. Furthermore, most responses to NO₂ that have been observed in asthmatics have occurred at concentrations between 0.2 and 0.5 ppm. Changes in lung function or airway responsiveness have not been observed even at much higher concentrations (i.e., up to 4 ppm). There is, at present, no plausible explanation for this apparent lack of a concentration-response relationship for both airway responsiveness and pulmonary function changes.

In summary, controlled human exposure studies are limited to acute, fully reversible functional and/or symptomatic responses. Although it is clear that some asthmatics are more susceptible than nonasthmatics to NO₂, the observed effects do not follow a
concentration-response relationship Therefore, the findings do not provide clear quantitative conclusions about the health effects of short-term exposure to NO₂

16.2.2 Respiratory Morbidity in Children Associated with Exposure to Nitrogen Dioxide

The effects of NO₂ on respiratory illness and the factors determining occurrence and severity are important public health concerns because of the potential for exposure to NO₂ and because childhood respiratory illness is very common (Samet et al., 1983, Samet and Utell, 1990). This takes on added importance because recurrent childhood respiratory illness may be a risk factor for later susceptibility to lung damage (Glezen, 1989, Samet et al., 1983, Gold et al., 1989).

The discussion of epidemiological findings in Chapter 14 indicates that the combined evidence is supportive of an effect of estimated exposure to NO₂ on respiratory symptoms and disease in children aged 5 to 12 years as indicated by the EPA meta-analysis of nine selected indoor studies presented in Chapter 14. However, in the individual studies of infants 2 years of age and younger, no consistent relationship was found between estimates of NO₂ exposure and the prevalence of respiratory symptoms and disease. Based on a meta-analyses of these infant studies, the overall combined estimate is positive, however, it clearly contains the no-effect value of 1.0, (i.e., is not statistically significant), and so we cannot conclude that the evidence suggests an effect in infants.

Several uncertainties need to be considered in interpreting the subject indoor air studies and results of the U.S. Environmental Protection Agency (EPA) meta-analysis. Measurement error in exposure is potentially one of the most important methodological problems in epidemiological studies of NO₂ (as discussed in more detail in Chapter 14). Thus measured NO₂ concentrations are not exposure values per se, rather, estimating actual exposure requires knowledge of both pollutant levels and related human activity patterns. The effects studied may be related to peak exposures, average exposures, or a combination of the two. To the extent that health effects depend on peak exposures rather than average exposures, the exposure estimates used in the above studies and meta-analyses introduce exposure measurement error. These studies cannot distinguish between the relative contributions of peak and average exposures and their relationship with the observed health
effects. Additionally, a by-product of NO\textsubscript{2}, nitrous acid (HONO), may be a factor in observed effects, however, only very limited health and aerometric data are available that examine such possibilities. Also, although the level of similarity and common elements between the outcome measures in the NO\textsubscript{2} studies provide some confidence in their use in the quantitative analysis, the symptoms and illnesses combined are to some extent different and could indeed reflect different underlying processes. Thus, caution is necessary in interpreting the meta-analysis results.

Although there is evidence that suggests that increased estimated NO\textsubscript{2} exposure is associated with increased respiratory symptoms in children aged 5 to 12 years, the exposure estimates may be inadequate to determine a quantitative relationship between estimated exposure and symptoms. The studies that measured NO\textsubscript{2} exposure did so only for periods of 1 to 2 weeks and reported the values as averages. None of the studies attempted to relate the effects seen to the pattern of exposure, such as short-term peaks. Furthermore, the extrapolation to possible patterns of ambient exposure is difficult.

As for possible mechanisms underlying NO\textsubscript{2}-induction of respiratory morbidity effects of the types observed to be increased in the above epidemiological studies, animal studies discussed in Chapter 13 show that NO\textsubscript{2} exposure can (1) impair components of the respiratory host defense system and (2) increase susceptibility to respiratory infection. Increases in respiratory symptoms and disease among children observed in epidemiologic studies of NO\textsubscript{2} exposure may, therefore, reflect increased susceptibility to respiratory infection due to NO\textsubscript{2} impacts on respiratory defenses. The animal toxicology data, as discussed below, provide a biologically plausible basis for hypothesizing such a relationship, but the hypothesis requires further testing (see Section 16.2.3).

### 16.2.3 Biological Bases Relating Nitrogen Dioxide Exposure to Respiratory Morbidity: Effects of Nitrogen Dioxide on the Respiratory Host Defense System

The lung is one of the common sites of attack of microorganisms. Although many types of microorganisms are implicated in respiratory infection, viruses represent a major cause, particularly for infants and children. In a viral respiratory infection, viral replication and altered immune responses to viral infections produce signs and symptoms of respiratory illness (Douglas, 1986). The respiratory system has several defense mechanisms against
inhaled infectious and chemical agents  Host defense mechanisms comprise a complex, cooperative response system of several cell types, cell products, tissues, and organs  Two major approaches (discussed below) have been used to demonstrate the effects of NO₂ on host defenses: (1) evaluation of effects on selected mechanisms of host defenses, and (2) use of infectivity models, which reflect the overall functioning of all host defense mechanisms against the infectious agent used

Animal studies provide important evidence indicating that several defense system components are targets for inhaled NO₂, including key elements of host defenses such as alveolar macrophages (AMs) and the humoral and cell-mediated immune system  Animal toxicological studies (Chapter 13) further show that NO₂ exposure can impair the respiratory host defense system sufficiently so as to result in the host being more susceptible to respiratory infection  Human clinical studies (Chapter 15) of host defenses are rare and their results are equivocal, but suggestive of the potential for NO₂ effects

Although the ciliated epithelial cells involved in mucociliary transport in the conducting airways exhibit morphological changes at NO₂ concentrations as low as 0.5 ppm for 7 mo of exposure (Yamamoto and Takahashi, 1984), mucociliary clearance is not affected by NO₂ exposures at <5.0 ppm (9,400 μg/m³) (Schlesinger et al, 1987)  As a foreign agent deposits below the mucociliary region in the gaseous exchange region of the lung, host defenses are provided primarily by the AM, which acts to remove or kill viable particles, to remove nonviable particles, and to process and present antigens to lymphocytes for antibody production  Exposure to NO₂ has produced a variety of effects on AMs in several animal species. For example, Schlesinger et al (1987) and Schlesinger (1987a,b) observed a decrease in the phagocytic ability of rabbit AMs after a 13-day (2 h/day) exposure to 0.3 ppm (560 μg/m³) and an increase in phagocytosis after 2 days of exposure to 1.0 ppm (1,880 μg/m³)  Additional effects observed at higher concentrations (e.g., between 0.5 and 5 ppm) include decreased pulmonary bactericidal activity, altered metabolism, increases in numbers of macrophages, and morphological changes (Rombout et al, 1986, Aranyi et al, 1976; Goldstein et al, 1974, Suzuki et al, 1986, Chang et al, 1986, Mochitate et al, 1986, Robison et al, 1990)  Decreases in the ability of AMs to engulf foreign particles (phagocytosis) and bactericidal activity are likely highly related to increased susceptibility to pulmonary infections  Controlled human exposure studies (0.6 ppm for 3 h) have also
examined AM function and show that these cells, when exposed to NO₂, tended \( p = 0.07 \) to inactivate influenza virus in vitro less effectively than cells collected after air exposure (Frampton et al., 1989a). Also, Devlin et al. (1992) reported that macrophages recovered from the predominantly alveolar fraction of bronchoalveolar lavage fluid showed a 42% decrease in ability to phagocytose *Candida albicans*.

Together, the humoral and cell-mediated immune systems are essential for antibody production and the secretion of cellular products that (1) regulate normal defense responses and/or (2) are lethal to certain invading organisms. Although the pulmonary immune system would better reflect defenses against respiratory infection, it has not been adequately studied after NO₂ exposure. However, there is some indication that exposure to NO₂ suppresses some of the systemic immune responses and that the effects are both concentration- and time-dependent. For example, a significant suppression of antibody production by spleen cells has been reported in experimental animals exposed for 1 mo to NO₂ concentrations as low as 0.4 ppm (Fujimaki et al., 1982). Subchronic exposure (7 weeks) to NO₂ also resulted in decreased numbers of circulating T lymphocytes, T-helper/inducer lymphocytes, and T-cytotoxic/suppressor lymphocytes in mice at NO₂ levels as low as 0.25 ppm (470 µg/m³) (Richters and Damji, 1988). The cause of this suppression is not clear.

Animal infectivity studies present key data relating NO₂ exposure to effects on the overall functioning of host defense mechanisms. In these studies, animals were exposed to varying concentrations and durations of NO₂, followed by exposure to an aerosol containing an infectious agent. Microbiologically induced mortality was used as the health end point. Exposure to NO₂ increased both bacteria- and influenza-induced mortality after subchronic exposures to levels as low as 0.5 to 1.0 ppm NO₂ (Ehrlich and Henry, 1968, Ito, 1971, Ehrlich et al., 1977). After acute (2-h) exposure, 2.0 ppm NO₂ has been the lowest effective concentration measured using the bacterial infectivity model (Ehrlich et al., 1977). Nitrogen dioxide increases microbiologically induced mortality by impairing the host’s ability to defend the respiratory tract from infectious agents, thereby increasing susceptibility to viral, mycoplasma, and bacterial infections (Ehrlich and Henry, 1968, Ito, 1971, Ehrlich et al., 1977, Parker et al., 1989, Gardner et al., 1977a,b, 1979, 1980, 1982, Graham et al., 1987, Jakab, 1987a,b, Motomiya et al., 1973, Miller et al., 1987). Using an animal model designed to evaluate the effects of NO₂ on nonfatal respiratory infection, NO₂ decreased the
intrapulmonary bactericidal activity in mice in a concentration-related manner, without a change in mucociliary clearance (Goldstein et al., 1973) Exposure to NO₂ was found to increase the severity of mycoplasma-induced lesions within the lung, but did not increase the susceptibility of the mice to the infection (Parker et al., 1989) Animal studies have also shown that influenza infection is exacerbated with NO₂ exposure (Ito, 1971) In studies with cytomegalovirus and paramyxovirus (Jakab, 1987a,b, Rose et al., 1988), the pathogenesis of these infections was enhanced.

The animal toxicology literature also provides evidence that the host's response to inhaled NO₂ can be significantly influenced by the exposure duration, concentration, and temporal pattern of exposure. The relationship of concentration (C) times duration (T, time) to susceptibility to respiratory infections indicates that, when the product of C × T is held constant and the individual C's and T's are varied, a difference in response occurs. The incidence of mortality was significantly more influenced by the concentration of NO₂ than by the duration of the exposure (Gardner et al., 1977a,b) The exposure pattern of NO₂ is also important when comparing and determining the effects of continuous versus intermittent exposure. When such data were adjusted for differences in C × T, the incidence of respiratory infection was essentially the same for both groups (Gardner et al., 1979) When animal studies were designed to mimic a typical urban outdoor exposure environment having periodic spikes of NO₂ superimposed on a lower continuous background level of NO₂, the evidence indicates that the animals exposed to the baseline plus short-term spikes were significantly more susceptible to a laboratory-induced infection than either the control or the background-NO₂-exposed mice (Miller et al., 1987, Gardner et al., 1982, Graham et al., 1987). It should be noted that the exposure patterns tested in animals are likely to be different from indoor NO₂ exposure patterns. This body of work for host defenses in mice shows that an average exposure value (C × T) is not an exact index or predictor of effects, rather, actual patterns of exposure more accurately represent the causative exposure.

It is also of interest that morphological studies, too, indicate that NO₂ concentrations play a more important role in inducing lung lesions than do exposure durations when the product of C × T is constant (Rombout et al., 1986). The influence of concentration was greater with intermittent NO₂ exposure than with continuous exposure.
Recent controlled human exposure studies examining the effects of NO₂ on pulmonary host defense systems have reported a trend (not statistically significant) toward an elevated rate of infection by a laboratory-induced, live attenuated influenza (A/Korea/reassortment) virus (Goings et al., 1989) Frampton et al. (1989a) also reported a trend (p < 0.07) for less effective inactivation of virus by AMs obtained from human subjects exposed continuously to 0.60 ppm NO₂, but no effects of virus inactivation were seen in subjects exposed continuously to 0.05 ppm with 2.0-ppm spikes. Exposure to NO₂ may transiently increase levels of antiprotease alpha-2-macroglobulin (α₂M) in lung lavage fluid. Although serving as an indicator of changes in the protease-antiprotease balance, alterations in α₂M in alveoli may have significance for local immunoregulation and may alter AM defenses against infection (Frampton et al., 1989b). These findings suggest, but do not prove, that NO₂ may play a role in increasing the susceptibility of adults to respiratory virus infections.

There is a hypothesis that the epidemiological associations between NO₂ exposure and respiratory symptoms/disease represent increased risk for respiratory infection. The weight of the evidence from several animal toxicological and human clinical studies, as summarized above, shows that NO₂ decreases host defense mechanisms against bacterial and viral infections. Some of these host defense studies are on mechanisms active in humans (e.g., AM functions). Other studies are on net defense functioning using outcome measures not valid for humans (i.e., mortality in the infectivity model), but nonetheless involving mechanisms shared with humans. Thus, these animal and human clinical studies provide a biologically plausible basis for the hypothesis. However, to test the hypothesis, it would be necessary to perform additional animal toxicological studies, to conduct more diagnostic tests for infection in future epidemiological studies, and to apply additional approaches in controlled human exposure studies.

16.2.4 Emphysema and Exposure to Nitrogen Dioxide

Studies on several animal species have shown that chronic exposure to high NO₂ levels (relative to ambient) can cause emphysema. Because emphysema is an irreversible disease, representing an important public health concern, whether NO₂ creates a risk for this disease in humans is a major question. Although this question cannot be definitively answered yet, the potential for risk warrants discussion here.

The definition of emphysema as used in the
United States is an anatomic one best characterized by National Institutes of Health (NIH) (1985) criteria. "An animal model of emphysema is defined as an abnormal state of the lungs in which there is enlargement of the airspace distal to the terminal bronchiole. Airspace enlargement should be determined qualitatively in appropriate specimens and quantitatively by stereologic methods." An additional essential criterion for human emphysema is the destruction of alveolar walls.

Several studies (Haydon et al., 1967, Freeman et al., 1972, Port et al., 1977) relate long-term (1- to more than 30-mo) exposure of rats and rabbits to high concentrations of NO₂ (> 8 ppm, much greater than ambient levels) with morphologic lung lesions that meet the 1985 NIH workshop criteria for a human model of emphysema (i.e., alveolar wall destruction occurred in addition to other characteristic changes). One study (Hyde et al., 1978) reported on dogs exposed to a mixture of 0.64 ppm NO₂ and 0.25 ppm nitric oxide (NO) for 68 mo. Upon examination 32 to 36 mo after exposure ceased, the dogs had morphologic lesions that meet the 1985 NIH workshop criteria for human emphysema. In the same dogs, pulmonary function was also measured. Pulmonary function decrements observed at the end of exposure progressed postexposure. This suggests that the morphological effects may also have been progressive. Another group of dogs in the same study was exposed to a mixture of "low" NO₂ (0.14 ppm) and "high" NO (1.1 ppm), but emphysema was not observed. Because the study did not include an NO₂-only group, it is not possible to discern the effects of NO₂ in the mixture. However, the presence of emphysema in the "high" NO₂-"low" NO group and its absence in the "low" NO₂-"high" NO group implies that NO₂ was a significant etiologic factor.

Emphysema was reportedly observed in numerous other NO₂ studies with several species of animals, but either the reports lacked sufficient detail for independent conclusions to be drawn or only the criteria for animal (not human) emphysema were met. Several other studies discussed in Chapter 13 were negative for emphysema. Various factors such as the exposure protocol and morphologic methods may also play a role in the outcome of studies. Potential differences may relate to the animal species used, age of the animals during exposure, concentration and duration of exposure, and the duration after exposure ceases before the animals are evaluated for emphysematous pathology.
In spite of the fact that there is a fairly extensive toxicologic data base concerning morphologic effects of NO$_2$, it is still not possible to establish a reasonably accurate "no-observed-effect" level for emphysema. This is likely due to a combination of factors: the complexity of changes occurring with NO$_2$ exposure, the lack of published papers utilizing highly sensitive morphometric techniques, interspecies differences in response, and inadequate description of methods and findings in some published reports. Qualitatively, then, it is clear that NO$_2$ can cause emphysema in animals. However, although the lowest effective NO$_2$ concentrations/exposure durations that induce emphysematous lung lesions can not yet be determined from available studies, the NO$_2$ exposures that have been found to cause emphysema (according to the NIH criteria) are far higher than those currently reported in ambient air.

16.3 CONCENTRATION-RESPONSE RELATIONSHIPS: HEALTH EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE

16.3.1 Clinical Studies

Table 16-1 summarizes key health effects observed in controlled human exposure (clinical) studies with NO$_2$ exposure durations of 0.5 to 3 h. The physiological end point that, to date, appears to be the most sensitive indicator of response is a change in airway responsiveness to bronchoconstrictors in asthmatics. This increase in airway responsiveness has been observed in some, but not all studies, and only at relatively low NO$_2$ concentrations within the range 0.2 to 0.3 ppm. Additionally, small decreases in functional expiratory volume in 1 s (FEV$_1$) or forced vital capacity (FVC) in adult or adolescent asthmatics have been observed in response to the same levels of NO$_2$. However, NO$_2$ concentration-response relationships are not evident for either airway responsiveness or pulmonary function changes. A second category of sensitive subjects are patients with chronic obstructive pulmonary disease (COPD). Although small decreases have been observed in FVC and FEV$_1$ in COPD patients exposed to 0.3 ppm in one study, no effects were seen in other studies at higher exposure levels. At higher exposure levels (more than 1.5 ppm), NO$_2$ exposure results in increased airway responsiveness and increased airway resistance in healthy adults. However,
### TABLE 16-1. KEY HUMAN HEALTH EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE—CLINICAL STUDIES

<table>
<thead>
<tr>
<th>NO₂ (ppm)</th>
<th>Observed Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 2-0 3 (0 5-2 0 h)</td>
<td>Trend toward increased airway responsiveness to challenges in asthmatics (Folinsbee, 1992) However, no significant effects observed by some or other investigators at NO₂ levels up to 4 ppm Small (4-6%) decreases in FEV₁ or FVC in adult or adolescent asthmatics, in response to NO₂ alone</td>
<td>Klemman et al (1983) Bauer et al (1986a,b) Koeng et al (1988) Bylin et al (1985, 1988) Mohsenn (1987a) Jorres and Magnussen (1990)</td>
</tr>
<tr>
<td>0 3 (3 75 h)</td>
<td>Small decreases (5-9%) in FVC and FEV₁ in COPD patients with mild exercise No effects seen by other investigators for COPD patients at 0 5-2 0 ppm NO₂</td>
<td>Morrow and Utell (1989)</td>
</tr>
<tr>
<td>1 5-2 0 (2-3 h)</td>
<td>Increased airway responsiveness to bronchoconstrictors in healthy adults However, effects not detected by other investigators at 2-4 ppm</td>
<td>Mohsenn (1987b) Frampton et al (1991)</td>
</tr>
<tr>
<td>≥2 00 (1-3 h)</td>
<td>Lung function changes (e.g., increased airway resistance) in healthy subjects Effects not found by others at 2-4 ppm</td>
<td>Beil and Ulmer (1976) Von Nieding et al (1979) Von Nieding and Wagner (1977) Von Nieding et al (1980)</td>
</tr>
</tbody>
</table>

*NO₂ = Nitrogen dioxide  
FEV₁ = Functional expiratory volume in 1 s  
FVC = Forced vital capacity  
COPD = Chronic obstructive pulmonary disease*

Some researchers have not observed any NO₂-induced changes in airway resistance at NO₂ levels between 2 and 4 ppm.

#### 16.3.2 Epidemiological Studies

The collective, combined evidence from epidemiology studies examining relationships between estimates of exposure to NO₂ and lower respiratory symptoms and disease in children aged 5 to 12 years (as evaluated by an EPA meta-analysis yielding quantitative...
estimates of effects) tends to demonstrate that increased risk for respiratory illness among children is associated with exposure to \( \text{NO}_2 \), as summarized in Table 16-2. In individual indoor studies of infants 2 years of age and younger, no consistent relationship was found between estimates of \( \text{NO}_2 \) exposure and the prevalence of respiratory symptoms and disease. Based on a meta-analyses of these infant studies, the combined odds ratio for the increase in respiratory disease per increase of 0.015 ppm \( \text{NO}_2 \) was 1.09 with a 95% confidence interval of 0.95 to 1.26. Thus, although the overall combined estimate is positive, it clearly contains the no-effect value of 1.0, (i.e., is not statistically significant), and so we cannot conclude that the evidence suggests an effect in infants comparable to that seen in older children (see Table 16-2). Higher levels (>0.3 ppm during a shift at work) in an occupational setting were related to an elevated prevalence of acute respiratory symptoms in adults. Also, episodic exposures occurring over a period of 1-h or longer at levels possibly as high as 1.5 ppm or higher have resulted in the occurrence of acute respiratory symptoms. Lastly, exceptionally high acute occupational exposures of 25 to 100 ppm \( \text{NO}_2 \) result in bronchial pneumonia, bronchitis, or bronchiolitis, and very extreme occupational \( \text{NO}_2 \) exposures (>200 ppm) have been associated with effects that range from hypoxemia and transient obstruction of the airways to death.

### 16.3.3 Animal Toxicological Studies

Numerous concentration-response studies have been conducted with animals using a wide range of exposure durations and end points, all of which influence the outcome. The major classes of effects observed at concentrations less than 1.0 ppm include decrements in host defenses, alterations in lung metabolism (e.g., increased lipid peroxidation and antioxidant metabolism), epithelial remodeling of the lower respiratory tract, thickening of the centriacinar interstitium, and a variety of extrapulmonary changes. Such findings can be qualitatively extrapolated to humans, but major uncertainties in respiratory tract dosimetry and species sensitivity currently preclude a quantitative extrapolation. Substantially higher \( \text{NO}_2 \) concentrations (≥12 ppm) have caused emphysema as defined by NIH criteria.

In infectivity studies examining \( C \times T \) and pattern of exposure, concentration had more influence than time of exposure in increasing susceptibility to respiratory bacterial infection in mice. Furthermore, the exact pattern of exposure played a major role in experimental
### TABLE 16-2. KEY HUMAN HEALTH EFFECTS OF EXPOSURE TO NITROGEN DIOXIDE—EPIDEMIOLOGICAL STUDIES

<table>
<thead>
<tr>
<th>NO₂ (ppm)</th>
<th>Observed Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015-ppm increase, where mean weekly concentrations in bedrooms in studies reporting levels were predominately between 0.008 and 0.065 ppm NO₂ (in 1- and 2-week integrated average NO₂ concentration estimating an unspecified long-term average)</td>
<td>A meta-analysis shows increased risk of lower respiratory symptoms/disease in children 5 to 12 years old associated with exposure estimates of NO₂ levels. The 95% confidence interval of the odds ratio estimated by Hasselblad et al. (1992) was 1.1 to 1.3. Predominant source of exposure contrast is homes with gas stoves vs homes with electric stoves.</td>
<td>Melia et al. (1977, 1979, 1980, 1982) Ware et al. (1984) Neas et al. (1991) Ekwo et al. (1983) Dijkstra et al. (1990) Keller et al. (1979)</td>
</tr>
<tr>
<td>0.015-ppm increase in annual average of 2-week NO₂ levels, where mean weekly concentrations in bedrooms were predominately between 0.005 and 0.050 ppm NO₂</td>
<td>In individual indoor studies of infants 2 years of age and younger, no consistent relationship was found between estimates of NO₂ exposure and the prevalence of respiratory symptoms and disease. Based on a meta-analyses of these infant studies, the combined odds ratio for the increase in respiratory disease per increase of 0.015 ppm NO₂ was 1.09 with a 95% confidence interval of 0.95 to 1.26. Thus, although the overall combined estimate is positive, it clearly contains the no-effect value of 1.0, (i.e., is not statistically significant), and so we cannot conclude that the evidence suggests an effect in infants comparable to that seen in older children (see Chapter 14).</td>
<td>Samet et al. (1993) Margolis et al. (1992) Dockery et al. (1989) Ogston et al. (1985) Ware et al. (1984) Ekwo et al. (1983) Melia et al. (1983)</td>
</tr>
<tr>
<td>&gt;0.3 ppm (average exposure during work shift)</td>
<td>Elevated prevalence of acute respiratory symptoms</td>
<td>Gamble et al. (1987)</td>
</tr>
<tr>
<td>Episodic exposure during hockey game to NO₂ levels of 1.5 ppm or higher</td>
<td>Occurrence of acute respiratory symptoms (cough, chest pain, dyspnea)</td>
<td>Smith et al. (1992) Hedberg et al. (1989)</td>
</tr>
<tr>
<td>25 to 100 ppm (episodic occupational exposure)</td>
<td>Bronchial pneumonia, bronchitis, and bronchiolitis induced by exceptionally high NO₂ exposure</td>
<td>Grayson (1956)</td>
</tr>
<tr>
<td>&gt;200 ppm (extreme episodic exposures)</td>
<td>Extreme exposure health outcomes range from hypoxemia/transient airway obstruction to death</td>
<td>Douglas et al. (1989)</td>
</tr>
</tbody>
</table>
outcomes Even so, duration is still important For example, as exposure proceeds from weeks to months at a given concentration, structural changes in the lung become more severe Also, at longer exposure durations, lower NO₂ concentrations cause effects Due to the large number of animal toxicological studies and the variety of exposure regimes, it is not possible to succinctly display the full range of concentration-responses Therefore Table 16-3 lists a few key studies showing the lowest concentrations that caused several types of effects.

16.4 SUBPOPULATIONS POTENTIALLY AT RISK FOR NITROGEN DIOXIDE HEALTH EFFECTS

Certain groups within the population may be more susceptible to the effects of NO₂ exposure, including persons with preexisting respiratory disease, children, and the elderly The reasons for paying special attention to these groups is that (1) they may be affected by lower levels of NO₂ than other subpopulations and (2) the impact of an effect of given magnitude may be greater Some causes of heightened susceptibility are better understood than others Subpopulations that already have reduced ventilatory reserves (e.g., the elderly and persons with asthma, emphysema, and chronic bronchitis) will be more impacted than other groups by decrements in pulmonary function For example, a healthy young person may not even notice a small percentage change in pulmonary function, but a person whose activities are already limited by reduced lung function may not have the reserve to compensate for the same percentage change

The National Institutes of Health (1991) estimates that approximately 10 million persons in the United States have asthma In the general population, asthma prevalence rates increased by 29% from 1980 to 1987 For those under 20 years old, asthma rates increased from approximately 35 to 50 per 1,000 persons, a 45% increase The airways of asthmatics may be hyperresponsive to a variety of inhaled materials, including pollens, cold-dry air, allergens, and air pollutants Asthmatics have the potential to be among the most susceptible members of the population with regard to respiratory responses to NO₂ (Section 15.3.1) On the average, asthmatics are much more sensitive to inhaled bronchoconstrictors such as histamine, methacholine, or carbachol The potential addition of an NO₂-induced increase in
<table>
<thead>
<tr>
<th>NO₂ (ppm)</th>
<th>Species</th>
<th>Observed Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Exposure Duration)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.04 ppm (continuous, 9 mo)</td>
<td>Rat</td>
<td>Increased lipid peroxidation (ethane in exhaled breath)</td>
<td>Sagar et al (1984)</td>
</tr>
<tr>
<td>0.2 ppm (continuous, base for 1 year) plus</td>
<td>Mouse</td>
<td>Increased susceptibility to respiratory infection and decreased vital capacity and respiratory system compliance, compared to control or baseline only</td>
<td>Miller et al (1987)</td>
</tr>
<tr>
<td>0.8 ppm (1-h peak, 2×/day, 5 days/week)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 ppm (7 h/day, 5 days/week, 7 weeks)</td>
<td>Mouse</td>
<td>Systemic effect on cell-mediated immunity</td>
<td>Richters and Damji (1988, 1990)</td>
</tr>
<tr>
<td>0.3 ppm (2 h/day, 2 days)</td>
<td>Rabbit</td>
<td>Decreased phagocytosis of alveolar macrophages</td>
<td>Schlesinger (1987a,b)</td>
</tr>
<tr>
<td>0.4 ppm (continuous, Mouse 4 weeks)</td>
<td></td>
<td>Decreased systemic humoral immunity</td>
<td>Fujimaki et al. (1982)</td>
</tr>
<tr>
<td>0.4 ppm (continuous, Rat 9 mo)</td>
<td></td>
<td>Increased antioxidants and antioxidant metabolism</td>
<td>Sagar et al (1984)</td>
</tr>
<tr>
<td>0.4 ppm (continuous, Rat up to 27 mo)</td>
<td></td>
<td>Slight increase in thickness of air-blood barrier at 18 mo, becoming significant by 27 mo, also alterations in bronchiolar and alveolar epithelium by 27 mo</td>
<td>Kubota et al (1987)</td>
</tr>
<tr>
<td>0.5 ppm (continuous, Mouse 3 mo)</td>
<td></td>
<td>Increased susceptibility to respiratory infection</td>
<td>Ehrlich and Henry (1968)</td>
</tr>
<tr>
<td>0.5-28 ppm (6 min to 1 year)</td>
<td>Mouse</td>
<td>Linear increase in susceptibility to respiratory infection with time, increased slope of curve with increased concentration, C more important than T</td>
<td>Gardner et al (1977a,b)</td>
</tr>
<tr>
<td>0.5 ppm (continuous Rat base, 6 weeks) plus 1.5 ppm (1-h peak, 2×/day, 5 days/week)</td>
<td></td>
<td>Alterations in Type 2 cells and increased interstitial matrix of proximal alveolar region, no changes in terminal bronchiolar region of adults</td>
<td>Crapo et al (1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chang et al (1986, 1988)</td>
</tr>
</tbody>
</table>

*NO₂ = Nitrogen dioxide
| C  | Concentration of exposure
| T  | Duration (time) of exposure

Airway response to the already heightened responsiveness to other substances raises the possibility of exacerbation of this pulmonary disease by NO₂, as discussed in Section 15 4. Other potentially susceptible groups include patients with COPD, such as emphysema and chronic bronchitis. Some of these patients have airway hyperresponsiveness to physical and chemical stimuli. A major concern with COPD patients is the absence of an adequate...
ventilatory reserve, a susceptibility factor described above. In addition, the poor distribution of respiratory tract ventilation in COPD may lead to a greater delivery of NO$_2$ to the segment of the lung that is well ventilated, thus resulting in a greater regional tissue dose. Also, NO$_2$ exposure may alter already impaired defense mechanisms, making this population potentially susceptible to respiratory infection. It is estimated (U.S. Department of Health and Human Services, 1990, Collins, 1988) that 14 million persons ($\approx 6\%$) suffer from COPD in the United States.

Because more than 2 million Americans have emphysema, it would be important to know whether NO$_2$ has the potential to exacerbate the disease. Lafuma et al. (1987) exposed both normal hamsters and hamsters with laboratory-induced (with elastase) emphysema to 2.0 ppm NO$_2$ for 8 h/day, 5 days/week for 8 weeks. Nitrogen dioxide exposure appeared to aggravate the elastase-induced emphysematous lesion. The investigators suggested that the results may imply a role for NO$_2$ in enhancing preexisting emphysema. In contrast, Mauderly et al. (1989, 1990) found that when elastase-induced emphysematous rats were chronically (7 h/day, 5 days/week, 2 years) exposed to 9.5 ppm NO$_2$, they were not more susceptible to NO$_2$ in terms of exacerbation of the elastase-induced lesions. Therefore, it is not clear what the potential would be for exacerbation of emphysema in humans at ambient concentrations.

Based upon epidemiology studies, children aged 5 to 12 years constitute a subpopulation potentially susceptible to an increase in respiratory morbidity associated with NO$_2$ exposure (Chapter 14). Data on the resident population of the United States provide information on the number of children in various age ranges (Table 16-4). Approximately 18 million children are in the age group 5 to 9 years, whereas around 17 million children are in the age group 10 to 14 years. However, the fraction of the numbers of potentially-at-risk children in various age groups that are actually exposed to NO$_2$ concentrations/patterns sufficient to induce respiratory morbidity has not been determined.

Another potential susceptible subpopulation group is immunocompromised individuals, who would have an increased susceptibility for infectious pulmonary disease as well as other health effects. Such people would hypothetically be more susceptible to agents, such as NO$_2$, that further compromise host defenses. Immunocompromised groups could include those people with abnormalities in polymorphonuclear leukocyte (PMN) number or function.
### TABLE 16-4. ESTIMATES OF THE RESIDENT POPULATION OF CHILDREN AND YOUNG ADULTS OF THE UNITED STATES, BY AGE AND SEX, JULY 1, 1989

<table>
<thead>
<tr>
<th>Age</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Ages</td>
<td>248,239,000</td>
<td>120,982,000</td>
<td>127,258,000</td>
</tr>
<tr>
<td>&lt;1</td>
<td>3,945,000</td>
<td>2,020,000</td>
<td>1,925,000</td>
</tr>
<tr>
<td>1 to 4</td>
<td>14,808,000</td>
<td>7,578,000</td>
<td>7,229,000</td>
</tr>
<tr>
<td>5 to 9</td>
<td>18,212,000</td>
<td>9,321,000</td>
<td>8,891,000</td>
</tr>
<tr>
<td>10 to 14</td>
<td>16,950,000</td>
<td>8,689,000</td>
<td>8,260,000</td>
</tr>
<tr>
<td>15 to 19</td>
<td>17,812,000</td>
<td>9,091,000</td>
<td>8,721,000</td>
</tr>
</tbody>
</table>

Source: Centers for Disease Control (1990)

and those with humoral and/or cell-mediated immunity dysfunctions. Hopewell (1989) discusses potentially immunocompromised groups in general, without regard to pollutant exposures. Reduction in the number of circulating PMNs is particularly common in patients undergoing chemotherapy for malignancies, in patients with acute leukemia, and in patients who have had bone marrow transplantation. Also, the use of corticosteroids, antineoplastic drugs, irradiation, and alcohol can decrease the effectiveness of PMNs by decreasing chemotaxis or adherence. In addition, alterations in PMN chemotaxis have been described in cirrhosis, renal failure, and Hodgkin’s disease.

Antibody production is primarily a function of B lymphocytes, thus decreased antibody production occurs when numbers of B cells are critically reduced or when the ability to respond to specific antigens is impaired. Conditions associated with defective antibody production include patients (1) infected with the human immunodeficiency virus (HIV), (2) with multiple myeloma, (3) with chronic lymphocytic leukemia, and (4) in the splenectomized state.

Cell-mediated immunity is mainly responsible for fending off intracellular pathogens and neoplastic disease. Killing of microbes within the lung is accomplished primarily by AMs and secondarily by various T lymphocytes. Abnormalities of cell-mediated immunity are produced by various diseases and include acquired immune deficiency syndrome (AIDS) and untreated Hodgkin’s disease. Also, therapeutic interventions that cause defects of cell-
mediated immunity are radiation treatment, corticosteroids, azathioprine, and cytotoxic/immunosuppressive drugs

The number of people with reduced immune function related to kidney transplants, AIDS, and chemotherapy can be estimated. The U.S. Bureau of the Census (1991) indicates that, based on reports of procurement programs and transplant centers in the United States, approximately 8,890 kidney transplant procedures were done in 1989. Also, Karon et al. (1990) reported that the Centers for Disease Control (CDC) estimated in 1990 that (1) approximately 1 million persons in the United States were then currently infected with HIV and (2) an estimated 52,000 to 57,000 cases of AIDS were expected to be diagnosed during 1990. As of October 1991, state and local health departments had reported to the CDC 196,034 AIDS cases among persons of all ages in the United States (Centers for Disease Control, 1992). As for potentially at-risk chemotherapy patients, in 1990, approximately 1 million new cases of cancer occurred in the United States (U.S. Bureau of the Census, 1991). Steele et al. (1991) estimate that about 25% of the total number of patients diagnosed with cancer in 1 year are prescribed chemotherapy as a first course of treatment. In addition, many cancer patients are treated by radiation.

Although the above immunocompromised groups represent potentially at-risk susceptible populations for NO\(_2\) effects, no human research has examined NO\(_2\) exposure in these groups. Thus, there only now exists a hypothesized association with increased susceptibility to NO\(_2\). Although it is clear that NO\(_2\) can affect AMs, humoral immunity, and cell-mediated immunity in otherwise normal animals (Chapter 13), the animal-to-human extrapolation cannot yet be made quantitatively. Nevertheless, it may be prudent to consider including such reduced immune function groups as susceptible subpopulations at potentially increased risk for NO\(_2\)-induced health effects.

16.5 NITROGEN DIOXIDE LEVELS, EXPOSURES, AND ESTIMATES

16.5.1 Ambient and Indoor Nitrogen Dioxide Levels

In urban areas, hourly NO\(_2\) patterns at fixed-site, ambient air monitors often show a bimodal pattern of morning and evening peaks, related to motor vehicular traffic patterns, superimposed on a lower baseline level. Sites affected by large stationary sources of NO\(_2\)
(or NO that rapidly converts to NO₂) are often characterized by short episodes at relatively high concentrations. Electric generating stations provide a source of NO₂ in such rural and urban areas. Occurrences of hourly average NO₂ concentrations ≥ 0.10 ppm are very infrequent. In fact, only about 5% of hourly average NO₂ concentrations exceed ≥ 0.05 ppm (Aerometric Information Retrieval System, 1992, U.S. Environmental Protection Agency, 1991a,b).

The highest hourly and annual ambient NO₂ levels reported are from monitoring stations in California. The seasonal patterns at California stations are usually quite marked and reach their highest levels through the fall and winter months, whereas stations elsewhere in the United States usually have less prominent seasonal patterns and may peak in the winter, in the summer, or contain little discernable variation. One-hour NO₂ values can exceed 0.2 ppm, but, in 1988, only 16 stations (12 in California) reported an apparently credible second high 1-h value greater than 0.2 ppm. Because at least 98% of 1-h values at most stations are below 0.1 ppm, such values above 0.2 ppm are quite rare excursions. Thus, ambient 1-h values of 0.1 ppm are more typical 1-h maximums (Aerometric Information Retrieval System, 1992, U.S. Environmental Protection Agency, 1991a,b).

Since 1980, the U.S. nationwide mean annual-average level among reporting NO₂ stations has been consistently below 0.03 ppm. For the period 1980 to 1990, there were indications of a downward trend for the composite annual-average NO₂ concentration (U.S. Environmental Protection Agency, 1991b). The 1990 composite NO₂ average was 8% less than the 1981 level, a statistically significant difference. For 103 Metropolitan Statistical Areas reporting a valid year's data for at least one station in 1988, 1989, and 1990, annual averages ranged from 0.007 to 0.061 ppm. The collective mode for the peak annual average in the 1988 and 1989 period was approximately 0.02 ppm. The only recently measured exceedances of the current 0.053 ppm NO₂ annual National Ambient Air Quality Standard occurred at stations in Southern California (Aerometric Information Retrieval System, 1992, U.S. Environmental Protection Agency, 1991a,b).

Most people, however, spend a significant portion of their time indoors. This can result in increased NO₂ exposure, depending on the presence and use of indoor sources (e.g., gas stoves, kerosene heaters, and unvented gas space heaters) or reduced exposure, depending on the absence of such sources and on the tightness of home construction and the
use of air conditioning and other building features that affect the degree of penetration of outdoor NO$_2$ into buildings. Harlos et al. (1987a) report maximum kitchen NO$_2$ values for 1-h of 0.419 ppm during gas stove use, and a mean maximum of 0.182 ppm averaged over the 4-h sampling period.

Several studies have examined the issue of human exposure to NO$_2$ and the relationship of indoor/outdoor air quality for occupants of homes with and without significant indoor sources of NO$_2$ (Quackenboss et al., 1986, Sexton et al., 1983, Colome et al., 1987, Leaderer et al., 1987). Quackenboss et al. (1986) found that in the winter in Portage, WI, indoor weekly average NO$_2$ concentrations were 3.2 times higher than outdoor NO$_2$ levels in gas stove homes, while indoor levels were 0.6 times outdoor NO$_2$ levels in electric stove homes during the same period. The fact that indoor NO$_2$ levels in electric stove homes were below measured outdoor levels may be due to chemical reactions of NO$_2$ with indoor surfaces.

Given the large amount of time spent at home by most subjects, Quackenboss et al. (1986) found relatively high correlations between weekly measurements of indoor NO$_2$ concentrations and total personal exposure in gas stove homes ($r = 0.85$ for summer and $0.87$ for winter) and to a lesser extent in electric stove homes ($r = 0.68$ for summer and $0.61$ for winter). Correlation between outdoor NO$_2$ levels and total personal exposure is less in the summer ($r = 0.55$ for gas stove homes and $r = 0.68$ for electric stove homes) and much lower in the winter ($r = 0.20$ and $0.28$, respectively). Another factor that would affect total exposure is respiratory ventilation rate, which may differ indoors and outdoors depending on levels and patterns of human activities.

Colome et al. (1987), in a study of over 600 randomly sampled residences in Southern California, report that outdoor concentrations of NO$_2$ are found to be the single most important determinant of average indoor levels of NO$_2$ in Southern California. Outdoor NO$_2$ levels accounted for between 15 to 40% of the variation in indoor concentrations in this study. Based on the regression analysis of data from multiple homes, indoor/outdoor ratios varied from 0.46 to 1.00, depending on the season of the year.

It is remarkable that the contribution of gas cooking to indoor NO$_2$ levels is as highly consistent as it is among studies for locations (kitchens, bedrooms, activity rooms) within the residences and by season, given the great variability of the factors that govern the emissions (source type, source condition, source use, and source venting) and dilution and removal of...
NO₂ indoors (house volume, infiltration, etc) This consistency is not observed until the impact of outdoor concentrations is corrected for because background levels can vary considerably over time and geographic area. The impact of gas cooking and possibly other unvented or improperly vented combustion sources on indoor NO₂ levels is superimposed upon the indoor background level resulting from outdoor levels. In areas where outdoor levels are low, concentrations indoors from gas appliances will be higher than, and in many cases, much higher than outdoor levels (e.g., Marbury et al., 1988, Quackenboss et al., 1987, 1988, Spengler et al., 1983, Leaderer et al., 1986, Ryan et al., 1988a,b) If outdoor concentrations are high, then indoor levels in homes with gas appliances can be closer to or even lower than the outdoor levels (Wilson et al., 1986).

A by-product of NO₂, HONO, may be a factor contributing to observed health effects, however, no health data and limited exposure data are available to enable further evaluation of this possibility (see Chapter 7). Brauer et al. (1991) suggested that HONO is potentially less likely to be neutralized by ammonia than other acids. Additionally, HONO may be absorbed at a higher rate in the pulmonary tract than is NO₂ due to its greater solubility. The contrast in both mean and hourly peak values between NO₂ and HONO (e.g., respective peak and mean levels of 0.280 ppm and 0.069 ppm for NO₂ versus 0.029 ppm and 0.019 ppm for HONO), as indicated by data from Brauer et al. (1990), suggests that for HONO to be a significant risk factor, it would have to be more toxicologically potent than NO₂ (Neas et al., 1991).

### 16.5.2 Patterns of Potential Exposure to Nitrogen Dioxide and Related Health Effects

Relationships between estimates of NO₂ exposure and increased respiratory symptoms and diseases have been observed in epidemiological studies of children aged 5 to 12 years (see Chapter 14). Actual exposure would optimally be measured with personal exposure monitors with rather brief averaging times, but this is not yet technically feasible. Even so, delivered dose is responsible for effects. Although dose has an obvious broad relationship to exposure, it is very significantly influenced by ventilation rates/patterns and respiratory tract anatomy. For example, even in the same microenvironment, a sedentary child would receive a different dose than a sedentary adult, and exercise would result in still different doses.
Lacking such precise estimates of dose, it is necessary to use available exposure estimates. Here exposure is defined as the combination of an individual’s average NO₂ exposure concentration over time for all settings or environments. Average indoor residential concentrations (e.g., whole-house average or bedroom level) tend to be the best predictors of personal NO₂ exposure (see Chapter 8). Exposure was estimated in the NO₂ epidemiological studies by either direct measure of pollutant levels or by use of the surrogate measure provided by differences in NO₂ levels observed in homes with gas cookers and homes with electric stoves. The concentration measures for both are typically derived from 1- to 2-week averages as determined by Palms tubes.

Measuring NO₂ levels averaged over 1 to 2 weeks presents two concepts that require discussion. First, how does such a measure provide data on discerning the hypothesized difference between two patterns of exposure and their potentially different relationships to observed health effects, and, second, how adequately does such a measure estimate long-term exposures?

16.5.2.1 Patterns of Exposure

Exposure to NO₂ can be characterized by different patterns. For example, one exposure pattern, best typified by a home without an NO₂ emission source, is a cumulative average level that estimates exposure resulting from a mildly fluctuating background level without transient higher levels being experienced. A second exposure pattern is usually associated with the use of a gas cooking stove in the home. It consists both of a cumulative average fluctuating background level and short-term (1 to 2-h) peak concentrations. Although hour-by-hour characterization of exposure would be most helpful in examining exposure-response data, in both cases, cumulative averages are the available human exposure data, typically 2-week averages.

It is important to consider evidence useful in evaluating the relationship between NO₂ exposure patterns and health effects to discern the potential effects of ambient air patterns. The epidemiology studies reviewed in Chapter 14 predominantly contrast exposures between electric stove homes and gas stove homes, except for those studies examining ambient exposures. Spengler et al. (1979) and several key studies reviewed in Chapters 7, 8, and 14 show that a striking difference in NO₂ levels exists between homes using gas versus electric...
cooking. Short-term NO\textsubscript{2} levels in homes with emission sources have been characterized for 1-h time periods (see Chapter 7). Harlos et al. (1987a) report maximum 1-h kitchen NO\textsubscript{2} levels of 0.419 ppm during gas stove use, and mean maximums of 0.182 ppm averaged over the 4-h sampling period. As discussed in Chapter 7, both short-term peak concentrations and longer term mean concentrations are lower in activity rooms and bedrooms than in kitchens. Being out of the kitchen or house during gas stove use would thusly limit exposure to high short-term levels. House and bedroom averages correlate better than kitchen averages with personal exposure data (see Chapter 7). This mainly reflects the small amount of time spent in the kitchen (with high peaks) as opposed to other indoor locations and the smaller contribution that peaks make to the total exposure.

Again, as discussed above, ambient levels are typically characterized by 1-h and annual averages. Annual averages in 1988 and 1989 in United States Metropolitan Statistical Areas ranged from 0.007 to 0.061 ppm, with a collective mode representing a U.S. annual average of approximately 0.02 ppm. Typical 1-h values range from zero to 0.1 ppm (188 \text{\mu g/m}^3), with a mode of 0.05 ppm (see Figure 7-8). Thus, outdoor 1-h values more typically peak near 0.1 ppm.

Available data do not adequately examine the relative contributions of peak and average exposures and their relationship with observed health effects. Advances in monitoring and in the determination of health outcome measures are needed to better examine the potential relationship between peak exposures and health outcomes as contrasted to longer term pollutant measures. These needs represent important areas for future research.

16.5.2.2 Long-Term Exposure Estimates

Several of the epidemiology studies in the quantitative analysis in Chapter 14 used a single 2-week NO\textsubscript{2} average or used two 2-week NO\textsubscript{2} averages to characterize long-term exposure. The representativeness of such estimates of long-term exposure (e.g., 1 year) is a consideration in generalizing the results of these studies to the long-term ambient situation. To roughly estimate the possible divergence from an actual annual ambient mean resulting from selecting one or two 2-week averages, data from the U.S. Environmental Protection Agency’s Aerometric Information and Retrieval System data bank (AIRS, 1991) for
10 stations, selected from among those having fairly complete records for 1988 or 1989 (see Chapter 7), have been analyzed for the variability in their 2-week averages (Table 16-5). These stations represent various regions of the United States but emphasize California, where high levels most frequently occur. For each station, 2-week averages were calculated week by week, these were then ranked and the 10th and 90th percentiles were determined. These two percentiles were then divided by the station’s annual mean to provide a common frame of reference. For example, the 10th and 90th percentile fractions for a single 2-week average for Los Angeles in 1988 were 0.77 and 1.27 times the annual mean, respectively. This would indicate that 80% of the 2-week averages were between 77 and 127% of the annual average of 0.061 ppm (0.047 to 0.078 ppm, respectively). In a similar manner, percentiles were calculated from two 2-week averages, using the means of 2-week averages that were 26 weeks apart, thus providing data for two opposite seasons such as winter and summer. The 26 possible averages were ranked as before. Because these averages represent more weeks of data taken at two different times, it is expected that they would come closer to the true annual average, this is generally the case.

The results suggest that most (80%) of the single 2-week averages are within 80 and 125% of the mean, except possibly for cities (such as Dallas) with very low means. The two 2-week averages produce a better estimate, with most lying between 85 and 120% of the mean. The reader is cautioned that these numbers and analyses may not be representative of actual exposure situations. Also, the use of a single outdoor monitor may not produce a representative exposure estimate. These selected data and analyses are offered only to describe potential relationships between ambient NO₂ annual averages and 2-week data periods.

Further, forty sites were chosen (for their availability of data) to try to roughly estimate the uncertainty of these estimates. The sites were split into three categories depending on the value of the annual average, that is, (1) 0.001 to 0.020 ppm, (2) 0.020 to 0.040 ppm, or (3) >0.040 ppm NO₂. For each site, up to 52 2-week averages (starting at the beginning of each week) were calculated, along with the overall annual average. Each 2-week average can be thought of as an estimate of the annual average, and the standard error of this estimate was calculated across all sites within a particular exposure range. The standard error was estimated from the absolute deviations instead of the deviations squared to avoid
TABLE 16-5. U.S. ENVIRONMENTAL PROTECTION AGENCY
ANALYSIS OF VARIABILITY IN TWO-WEEK AMBIENT AVERAGES OF
ONE-HOUR NITROGEN DIOXIDE DATA AT 10 SELECTED LOCATIONS

<table>
<thead>
<tr>
<th>City</th>
<th>Year</th>
<th>Annual Average (ppm)</th>
<th>2-Week Average 10th Percentile</th>
<th>2-Week Average 90th Percentile</th>
<th>Two 2-Week Average 10th Percentile</th>
<th>Two 2-Week Average 90th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Los Angeles, CA</td>
<td>1988</td>
<td>0.061</td>
<td>0.77 1.27</td>
<td></td>
<td>0.87 1.20</td>
<td></td>
</tr>
<tr>
<td>Azusa, CA</td>
<td>1989</td>
<td>0.051</td>
<td>0.81 1.27</td>
<td></td>
<td>0.83 1.17</td>
<td></td>
</tr>
<tr>
<td>Upland, CA</td>
<td>1988</td>
<td>0.047</td>
<td>0.79 1.38</td>
<td></td>
<td>0.87 1.22</td>
<td></td>
</tr>
<tr>
<td>Anaheim, CA</td>
<td>1988</td>
<td>0.046</td>
<td>0.71 1.23</td>
<td></td>
<td>0.86 1.10</td>
<td></td>
</tr>
<tr>
<td>New York, NY</td>
<td>1988</td>
<td>0.034</td>
<td>0.86 1.18</td>
<td></td>
<td>0.91 1.16</td>
<td></td>
</tr>
<tr>
<td>Chicago, IL</td>
<td>1989</td>
<td>0.030</td>
<td>0.84 1.23</td>
<td></td>
<td>0.88 1.11</td>
<td></td>
</tr>
<tr>
<td>Cincinnati, OH</td>
<td>1989</td>
<td>0.029</td>
<td>0.81 1.19</td>
<td></td>
<td>0.83 1.23</td>
<td></td>
</tr>
<tr>
<td>Worcester, MA</td>
<td>1988</td>
<td>0.018</td>
<td>0.77 1.26</td>
<td></td>
<td>0.90 1.18</td>
<td></td>
</tr>
<tr>
<td>Miami, FL</td>
<td>1989</td>
<td>0.011</td>
<td>0.45 1.46</td>
<td></td>
<td>0.75 1.31</td>
<td></td>
</tr>
</tbody>
</table>

Source: Aerometric Information Retrieval System (1991)

possible problems from nonnormally distributed data. In a similar manner, standard errors were estimated from two 2-week averages, using the average of 2-week averages that were 26 weeks apart. The number of sites, ranges of annual means, and average standard errors of the one 2-week and two 2-week estimates are given in Table 16-6.

Table 16-6 shows that the standard error of the estimate goes up with the annual average itself. In general, one 2-week estimate has a standard error of about 25% of the mean, whereas the two 2-week estimate has a standard error of about 15% of the mean.

A similar analysis was made for indoor sites using data from homes in the Albuquerque area supplied by Lambert (1991). One hundred sites were selected (by Lambert), including 25 electric stove homes and 75 gas stove homes. The extensive data set consisted of 26 2-week averages for each home. Standard errors were calculated for three different estimates. (1) one 2-week average, (2) two 2-week averages, and (3) a fixed value estimate depending on whether the home used a gas stove versus an electric stove. When all the electric stove homes were combined, the overall average NO₂ for the electric stove homes was 6.51 μg/m³ (0.003 ppm). The gas stove homes averaged 32.67 μg/m³ (0.017 ppm).
TABLE 16-6. AVERAGE ANNUAL NITROGEN DIOXIDE MEANS (ppm) AND STANDARD ERRORS OF ESTIMATES FOR 40 SITES IN THE UNITED STATES BY ANNUAL AVERAGE AS DERIVED BY THE U.S. ENVIRONMENTAL PROTECTION AGENCY FROM THE AEROMETRIC INFORMATION RETRIEVAL SYSTEM (1991)

<table>
<thead>
<tr>
<th>Ranges of Annual Averages</th>
<th>Number of Sites</th>
<th>Average Mean</th>
<th>One 2-Week Average</th>
<th>Two 2-Week Average</th>
<th>Average Standard Error of Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 001-0 020</td>
<td>7</td>
<td>0 0159</td>
<td>0 0046</td>
<td>0 0028</td>
<td></td>
</tr>
<tr>
<td>0 020-0 040</td>
<td>16</td>
<td>0 0313</td>
<td>0 0080</td>
<td>0 0041</td>
<td></td>
</tr>
<tr>
<td>&gt;0 040</td>
<td>17</td>
<td>0 0495</td>
<td>0 0111</td>
<td>0 0056</td>
<td></td>
</tr>
</tbody>
</table>

These two overall averages were used as the fixed estimate of each home’s annual $\text{NO}_2$ level. The estimated standard errors were calculated in the same manner as was done for the outdoor data. The results are presented in Table 16-7.

TABLE 16-7. AVERAGE ANNUAL NITROGEN DIOXIDE MEANS (ppm) AND STANDARD ERRORS OF ESTIMATES FOR 100 HOMES BASED ON DATA OF LAMBERT (1991) AS DERIVED BY THE U.S. ENVIRONMENTAL PROTECTION AGENCY

<table>
<thead>
<tr>
<th>Source of Exposure</th>
<th>Number of Homes</th>
<th>Overall Average</th>
<th>One 2-Week</th>
<th>Two 2-Week</th>
<th>Fixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electric Stove</td>
<td>25</td>
<td>0 003</td>
<td>0 001</td>
<td>0 008</td>
<td>0 014</td>
</tr>
<tr>
<td>Gas Stove</td>
<td>75</td>
<td>0 017</td>
<td>0 010</td>
<td>0 005</td>
<td>0 012</td>
</tr>
</tbody>
</table>

The standard errors from the estimates of indoor exposure are somewhat higher than the standard errors of the outdoor estimates. The standard error of the one 2-week average is about 50% of the mean, and the standard error of the two 2-week average is about 25% of the mean. The fixed estimate (using an overall average for electric homes and a separate overall average for gas stove homes) had a slightly larger standard error than did the one 2-week average. This suggests that measured 2-week averages are better for characterizing exposure than a fixed average based only on the presence or absence of a gas stove.
However, a single 2-week average is only slightly better. Although the standard errors are quite large (compared with the mean), the estimates are adequate to distinguish a high exposure (usually with a gas stove present) from a low exposure (usually with an electric stove present) household.

16.5.3 Nitrogen Dioxide Exposure Estimates

The following exposure analysis estimates the potential impact of outdoor NO₂ concentrations on exposure in children and adults. This analysis is based on a simple approach that does not adequately take into account the interaction of activity or location and the NO₂ level in that location during the time period in that microenvironment. Instead, averages are used. Further efforts are beyond the scope of this document, but would be necessary to produce a more appropriate analysis. This limited exposure estimate provides a perspective for this document. Two factors are needed for this analysis. The first factor is the fraction of time the children spent outdoors. This, of course, will vary by age, season, and locality. The second factor is the increase in indoor concentrations of NO₂ (e.g., in the bedroom) as a function of the outdoor concentration, which also varies by season and locality.

The fraction of time spent outdoors is estimated by several studies (see Table 16-8), and the estimates are highly dependent on age. In general, the fractions ranged from about 0.3 to 20% of the time being spent outdoors. Rather than assume that a single value applies, several different percents (0.3, 1, 3, 5, 10, 15, and 20) are evaluated in the analysis.

The contribution of outdoor NO₂ concentrations to indoor levels must be estimated. To start, average outdoor values for the whole season are used. The analysis assumes no indoor sources. The model used is based on a physical mass balance equation (Butler et al., 1990; Drye et al., 1989). The model (see Chapter 8) states that the indoor concentration (Cₚᵢₙ) will be a linear function of the outdoor concentration (Cₜₒᵤₜ), with a constant term (A).

For purposes of estimating the increase in indoor NO₂ from outdoor NO₂, only the regression coefficient for the indoor/outdoor (I/O) ratio itself is needed. An indoor/outdoor ratio of 0.59 may be an appropriate value (see Chapter 7). Combining the estimates derived by Drye et al. (1989) and Butler et al. (1990) for NO₂ concentration in a bedroom (Cₜₑₐ₉) for summer and winter using a simple average into a single equation yields.
TABLE 16-8. NITROGEN DIOXIDE EXPOSURE ESTIMATES IN PARTS PER MILLION (ppm) DERIVED BY THE U.S. ENVIRONMENTAL PROTECTION AGENCY AS FUNCTION OF OUTDOOR NITROGEN DIOXIDE CONCENTRATION AND PERCENT TIME OUTDOORS, WHERE INDOOR/OUTDOOR RATIO EQUALS 0.59 AND ASSUMING A BASELINE CONCENTRATION OF 0.005 ppm

<table>
<thead>
<tr>
<th>Outdoor NO₂ ppm</th>
<th>0 3\textsuperscript{a}</th>
<th>1\textsuperscript{b}</th>
<th>3\textsuperscript{c}</th>
<th>5\textsuperscript{d}</th>
<th>10\textsuperscript{e}</th>
<th>15\textsuperscript{f}</th>
<th>20\textsuperscript{g,h}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>0.010</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>0.015</td>
<td>0.011</td>
<td>0.011</td>
<td>0.011</td>
<td>0.011</td>
<td>0.011</td>
<td>0.012</td>
<td>0.012</td>
</tr>
<tr>
<td>0.020</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.015</td>
<td>0.015</td>
</tr>
<tr>
<td>0.025</td>
<td>0.017</td>
<td>0.017</td>
<td>0.017</td>
<td>0.017</td>
<td>0.018</td>
<td>0.018</td>
<td>0.018</td>
</tr>
<tr>
<td>0.030</td>
<td>0.020</td>
<td>0.020</td>
<td>0.020</td>
<td>0.020</td>
<td>0.021</td>
<td>0.021</td>
<td>0.022</td>
</tr>
<tr>
<td>0.035</td>
<td>0.023</td>
<td>0.023</td>
<td>0.023</td>
<td>0.023</td>
<td>0.024</td>
<td>0.025</td>
<td>0.025</td>
</tr>
<tr>
<td>0.040</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.026</td>
<td>0.027</td>
<td>0.028</td>
<td>0.029</td>
</tr>
<tr>
<td>0.045</td>
<td>0.029</td>
<td>0.029</td>
<td>0.029</td>
<td>0.029</td>
<td>0.030</td>
<td>0.031</td>
<td>0.032</td>
</tr>
<tr>
<td>0.050</td>
<td>0.032</td>
<td>0.032</td>
<td>0.032</td>
<td>0.032</td>
<td>0.033</td>
<td>0.034</td>
<td>0.035</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Harlos et al (1987b) Children <1 year old  
\textsuperscript{b}Dorre et al (1990) Children 2 to 3 years old, minimum time  
\textsuperscript{c}Dorre et al (1990) Children 2 to 3 years old, mean time  
\textsuperscript{d}Noy et al (1986) Housewives  
\textsuperscript{e}Clausing et al (1986) High school students  
\textsuperscript{f}Quackenboss et al (1982) Family members  
\textsuperscript{g}Schwab et al (1986) Fourth through sixth graders  
\textsuperscript{h}Adar and Spengler (1989) Children can spend as much as 50% of their time outdoors in the summer

\[ C_{bed} = 0.59 \times C_{out} + A_1 \]  \hspace{1cm} (16-1)

From Equation 16-1, the total NO₂ exposure (\( C_{total} \)) can then be estimated for a child who spends \( P_{out} \) fraction of his time outdoors, assuming a baseline indoor concentration of \( C_{in} \)

\[ C_{total} = \{C_{in} + 0.59 \times (C_{out} - C_{in})\} \times (1 - P_{out}) \]  \hspace{1cm} (16-2)

\[ + C_{out} \times P_{out} \]
The increase in NO$_2$ exposure ($C_{incr}$) is estimated by

\[ C_{incr} = 0.59 \times (C_{out} - C_{in}) \times (1 - P_{out}) \]
\[ + (C_{out} - C_{in}) \times P_{out} \]  

(16-3)

Table 16-8 presents values for this exposure estimate as a function of outdoor NO$_2$ concentration and percent of time outdoors for the I/O ratio 0.59. As long as the regression coefficient is high (0.59 in this case), the fraction of time outdoors will have little impact on the exposure. For smaller values of the coefficient (e.g., near 0.3), the activity pattern would have a much larger impact.

Table 16-9 shows changes in the exposure estimate in a nest of subtables where the percent time outdoors and the outdoor NO$_2$ level vary for specific I/O ratios of 0.1 to 0.8. Indoor/outdoor ratios depend on several factors, such as season, use of air conditioning, tight versus loose homes, and location in the United States (see Chapter 7).
<table>
<thead>
<tr>
<th>Outdoor NO$_2$ (ppm)</th>
<th>Percentage of Time Outdoors</th>
<th>Outdoor NO$_2$ (ppm)</th>
<th>Percentage of Time Outdoors</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.020</td>
<td>0.007 0.007 0.007 0.007</td>
<td>0.020</td>
<td>0.013 0.013 0.013 0.013</td>
</tr>
<tr>
<td>0.025</td>
<td>0.009 0.009 0.009 0.009</td>
<td>0.025</td>
<td>0.015 0.015 0.015 0.015</td>
</tr>
<tr>
<td>0.030</td>
<td>0.010 0.010 0.010 0.010</td>
<td>0.030</td>
<td>0.018 0.018 0.018 0.018</td>
</tr>
<tr>
<td>0.035</td>
<td>0.011 0.011 0.011 0.011</td>
<td>0.035</td>
<td>0.020 0.020 0.020 0.020</td>
</tr>
<tr>
<td>0.040</td>
<td>0.012 0.012 0.012 0.012</td>
<td>0.040</td>
<td>0.023 0.023 0.023 0.023</td>
</tr>
<tr>
<td>0.045</td>
<td>0.013 0.013 0.013 0.013</td>
<td>0.045</td>
<td>0.025 0.025 0.025 0.025</td>
</tr>
<tr>
<td>0.050</td>
<td>0.014 0.014 0.014 0.014</td>
<td>0.050</td>
<td>0.028 0.028 0.028 0.028</td>
</tr>
<tr>
<td>0.055</td>
<td>0.015 0.015 0.015 0.015</td>
<td>0.055</td>
<td>0.030 0.030 0.030 0.030</td>
</tr>
<tr>
<td>0.060</td>
<td>0.016 0.016 0.016 0.016</td>
<td>0.060</td>
<td>0.033 0.033 0.033 0.033</td>
</tr>
</tbody>
</table>

$^{a}$NO$_2$ = Nitrogen dioxide
I/O R = Indoor/outdoor ratio
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APPENDIX A.
GLOSSARY OF TERMS AND SYMBOLS

ABBREVIATIONS, ACRONYMS, AND SYMBOLS

Å Ångstrom ($10^{-10}$ meter)
"A" strain A particular type of influenza virus
9-AA 9-Amino-acridine
AaDO$_2$ Difference between alveolar and arterialized partial pressure of oxygen
AAS Atomic absorption spectroscopy
AATCC American Association of Textile Chemists and Colorists
ACH Air changes per hour
Ad A particular strain of laboratory mouse
AD Annular denuder
AICHE American Institute of Chemical Engineers
AIDS Acquired immune deficiency syndrome
AIRS Aerometric Information Retrieval System
Al Aluminum
Al$^{3+}$ Aluminum ion
Al$_2$(SO$_4$)$_3$ Aluminum sulfate
AM Alveolar macrophage
$\alpha_2$-M Alpha-2-macroglobulin
AMP Adenosine monophosphate, adenosine 5' phosphate
AMT Arithmetic mean thickness
ANC Acid-neutralizing capacity
ANOVA Analysis of variance
ANSA 8-amino-1-naphthalene-sulfonic acid
ANSI American National Standards Institute
APCD Air Pollution Control District
APHA  American Public Health Association
\(\alpha_1\text{PI}\)  Alpha-1-protease inhibitor
A/PR/8  A particular strain of influenza virus
A/PR/8/34  A particular strain of influenza virus
AQCR  Air Quality Control Region
AQIRP  Air Quality Improvement Research Program
AQSM  Air Quality Simulation Model
ASTM  American Society for Testing and Materials
atm  One atmosphere, a unit of pressure
ATP  Adenosine triphosphate
avg  Average
BAKI  Potassium iodide solution acidified with boric acid
BAL  Bronchoalveolar lavage
BaSO\(_4\)  Barium sulfate
BHA  Butylated hydroxyanisole
BHPN  N-bis(2-hydroxypropyl)nitrosamine
BHR  Bronchial or airways hyperresponsiveness
BHT  Butylated hydroxytoluene
BMRC  British Medical Research Council
BP  Blood pressure
Br  Bromine
BrNO\(_3\)  Bromine nitrate
BrO  Bromine monoxide
\(b_{\text{scat}}\)  Extinction coefficient due to scatter by aerosols
\(^\circ\text{C}\)  Degrees Celsius (Centigrade)
\(^{13}\text{C}\)  Carbon-13
\(^{14}\text{C}\)  Carbon-14, a radioactive form of carbon
Ca  Calcium
Ca\(^{2+}\)  Calcium ion
CAA  Clean Air Act
cAMP  Cyclic adenosine monophosphate, adenosine 5'-phosphate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>CAMP</td>
<td>Community Air Monitoring Program</td>
</tr>
<tr>
<td>CASAC</td>
<td>Clean Air Scientific Advisory Committee</td>
</tr>
<tr>
<td>C57BL</td>
<td>A particular strain of laboratory mouse</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>A particular strain of laboratory mouse</td>
</tr>
<tr>
<td>CD-1</td>
<td>A particular strain of laboratory mouse</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
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<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate, guanosine 5'‐phosphate</td>
</tr>
<tr>
<td>CH₄</td>
<td>Methane</td>
</tr>
<tr>
<td>C3H</td>
<td>A particular strain of laboratory mouse</td>
</tr>
<tr>
<td>C₃H₆</td>
<td>Propylene</td>
</tr>
<tr>
<td>ChE</td>
<td>Cholinesterase</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>Cl</td>
<td>Chlorine</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>Chloride ion</td>
</tr>
<tr>
<td>Cl₂</td>
<td>Chlorine molecule</td>
</tr>
<tr>
<td>Cₖ</td>
<td>Lung compliance</td>
</tr>
<tr>
<td>Cₖdyn</td>
<td>Dynamic lung compliance</td>
</tr>
<tr>
<td>CLM</td>
<td>Chemiluminescence</td>
</tr>
<tr>
<td>CLM-PC</td>
<td>Chemiluminescence with photolytic converter</td>
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<tr>
<td>ClNO₃</td>
<td>Chlorine nitrate</td>
</tr>
<tr>
<td>ClO</td>
<td>Chlorine monoxide</td>
</tr>
<tr>
<td>Cₖstat</td>
<td>Static lung compliance</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
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<tr>
<td>CNG</td>
<td>Compressed natural gas</td>
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<tr>
<td>CNS</td>
<td>Central nervous system, the brain and spinal cord</td>
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<tr>
<td>CO</td>
<td>Carbon monoxide</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>¹³CO₂</td>
<td>Carbon-13 labeled carbon dioxide</td>
</tr>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
</tr>
<tr>
<td>COH</td>
<td>Coefficient of haze</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
</tbody>
</table>
CPK Creatine phosphokinase
CR-1 A particular strain of laboratory mouse
CRD Chronic respiratory disease
CRD Completely randomized design
CSTR Continuously stirred tank reactor
C × T Exposure concentration in ppm multiplied by time of exposure in hours or other time measurement
Cu-Cd Copper-cadmium
CV Closing volume
CV Coefficient of variance
CVM Contingent valuation method
D10 Dose that would cause a 10% decrease in functional expiratory volume in 1 second
D = CT Dose equals concentration multiplied by time
DD Denuder difference
DEN Diethylnitrosamine (also DENA)
DIAL Differential absorption lidar
DIFKIN Diffusion Kinetics Model
DIN Total inorganic nitrogen
DLco Diffusion capacity of the lung for carbon monoxide
DMA Dimethylamine
DMN Dimethylnitrosamine
DNA Deoxyribonucleic acid
DOAS Differential optical absorption spectroscopy
DON Dissolved organic nitrogen
DPPD N,N-diphenylphenylenediamine
EC Prefix of International Commission on Enzymes’ identification numbers
EDF Environmental Defense Fund
EGR Exhaust-gas recirculation
EKG Electrocardiogram
EPA U S Environmental Protection Agency
EPRI Electric Power Research Institute
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>EV</td>
<td>Electric vehicle</td>
</tr>
<tr>
<td>°F</td>
<td>Degrees Fahrenheit</td>
</tr>
<tr>
<td>FAV</td>
<td>Final acute value</td>
</tr>
<tr>
<td>FCV</td>
<td>Final chronic value</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>FEF</td>
<td>Forced expiratory flow</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>Iron sulfate</td>
</tr>
<tr>
<td>FET</td>
<td>First-edge time</td>
</tr>
<tr>
<td>FEV</td>
<td>Forced expiratory volume</td>
</tr>
<tr>
<td>FEV₀.₇₅</td>
<td>0.75-Second forced expiratory volume</td>
</tr>
<tr>
<td>FEV₁.₀</td>
<td>One-second forced expiratory volume</td>
</tr>
<tr>
<td>FEV₂₅-₇₅%</td>
<td>Forced expiratory volume at 25 to 75% of vital capacity</td>
</tr>
<tr>
<td>FP</td>
<td>Filter pack</td>
</tr>
<tr>
<td>FRM</td>
<td>Federal Reference Method for air quality measurement</td>
</tr>
<tr>
<td>ft</td>
<td>Foot</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform spectroscopy (also FS)</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>FW</td>
<td>Fresh weight of plant material</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>Gₐw</td>
<td>Airway conductance</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GC-ECD</td>
<td>Gas chromatography with electron capture detection</td>
</tr>
<tr>
<td>GC-FID</td>
<td>Gas chromatography with flame ionization detection</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatograph in combination with mass spectrometry</td>
</tr>
<tr>
<td>GDH</td>
<td>Glutamase dehydrogenase</td>
</tr>
<tr>
<td>GM</td>
<td>General Motors Corporation</td>
</tr>
<tr>
<td>GMP</td>
<td>Guanosine 5′-phosphate, guanosine monophosphate</td>
</tr>
<tr>
<td>GOGAT</td>
<td>Glutamine oxoglutarate aminotransferase (or glutamate synthase)</td>
</tr>
<tr>
<td>G-6-P</td>
<td>Glucose-6-phosphate</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>GP-CLM</td>
<td>Gas-phase chemiluminescence</td>
</tr>
<tr>
<td>GPT</td>
<td>Gas-phase titration</td>
</tr>
<tr>
<td>GS</td>
<td>Glutamine synthetase</td>
</tr>
<tr>
<td>GSH</td>
<td>A tripeptide, glutathione (reduced form)</td>
</tr>
<tr>
<td>GSSG</td>
<td>The disulfide (oxidized) form of GSH</td>
</tr>
<tr>
<td>GTE/CITE</td>
<td>Global Tropospheric Experiment/Chemical Instrumentation Test and Evaluation</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>H·</td>
<td>Hydrogen (free radical)</td>
</tr>
<tr>
<td>H⁺</td>
<td>Hydrogen ion</td>
</tr>
<tr>
<td>³H</td>
<td>Tritium, a radioactive form of hydrogen</td>
</tr>
<tr>
<td>ha</td>
<td>Hectare</td>
</tr>
<tr>
<td>HBEF</td>
<td>Hubbard Brook Experimental Forest</td>
</tr>
<tr>
<td>HbO₂</td>
<td>Oxyhemoglobin</td>
</tr>
<tr>
<td>HC</td>
<td>Hydrocarbon</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HCN</td>
<td>Hydrogen cyanide</td>
</tr>
<tr>
<td>HDV</td>
<td>Heavy-duty vehicle</td>
</tr>
<tr>
<td>HF</td>
<td>Hydrogen fluoride</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>5-Hydroxyindoleacetic acid</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>hν</td>
<td>Planck’s constant (h) times the frequency of radiated energy (ν) = Quanta of energy (E)</td>
</tr>
<tr>
<td>HNO₂</td>
<td>Nitrous acid (liquid form)</td>
</tr>
<tr>
<td>HNO₃</td>
<td>Nitric acid (also HONO₂)</td>
</tr>
<tr>
<td>HO·</td>
<td>Hydroxyl free radical (also OH)</td>
</tr>
<tr>
<td>HO₂·</td>
<td>Hydroperoxyl free radical</td>
</tr>
<tr>
<td>H₂O</td>
<td>Water</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>HONO</td>
<td>Nitrous acid (gaseous form)</td>
</tr>
<tr>
<td>HONO₂NO₂</td>
<td>Peroxynitric acid</td>
</tr>
</tbody>
</table>
HPIC  High performance ion chromatograph
HPLC  High performance liquid chromatograph
HR  Heart rate
H₂S  Hydrogen sulfide
H₂SO₄  Sulfuric acid
5-HT  Serotonin
³H-thymidine  Tritiated thymidine
IARC  International Agency for Research on Cancer
IC  Ion chromatography
IF  Irrigation and fertilization
IFS  Integrated Forest Study
Ig  Immunoglobulins
IgA  Immunoglobulin A fraction
IgG  Immunoglobulin G fraction
IgG₁  Immunoglobulin G₁ fraction
IgG₂  Immunoglobulin G₂ fraction
IgM  Immunoglobulin M fraction
IL-1  Interleukin-1
in  Inch
IR  Infrared
IRGA  Infrared gas analysis
k  Rate constant or dissociation constants
K  Potassium
K⁺  Potassium ion
kg  Kilogram
km  Kilometer
L  Liter (also ℓ)
LAR  Leaf area ratio
LC₅₀  Lethal concentration 50%, that concentration which is lethal to 50% of test subjects
LD₅₀  Lethal dose 50%, dose which is lethal to 50% of the subjects
LDH  Lactic acid (lactate) dehydrogenase
LDV  Light-duty vehicle
LIF  Laser-induced fluorescence
LM  Light microscope
LNG  Liquefied natural gas
log EF  Base 20 logarithm of the emission factor
LPG  Liquefied petroleum gas
LPS  Bacterial lipopolysaccharide
LT50  The time required for 50% of the test animals to die when given a lethal dose
LTB4  Leukotriene B4
m  Meter
M  Molar
M  Third body (in a reaction)
M85  Fuel blended from 85% methanol and 15% gasoline
M100  Methanol
MAK  Maximum permissible concentration (in Germany)
max  Maximum
MDL  Minimum detection limit
MERL  Marine Ecosystem Research Laboratory
MFR  Maximal flow rate
Mg  Magnesium
Mg2+  Magnesium ion
μg  Microgram
μg/m³  Micrograms per cubic meter
mg/m³  Milligrams per cubic meter
MgO  Magnesium oxide
MgSO4  Magnesium sulfate
min  Minute
MIT  Massachusetts Institute of Technology
mL  Milliliter
μL  Microliter
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>µm</td>
<td>Micrometer</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
</tr>
<tr>
<td>MMD</td>
<td>Mass median diameter</td>
</tr>
<tr>
<td>MMEF</td>
<td>Maximal midexpiratory flow</td>
</tr>
<tr>
<td>MMFR</td>
<td>Mid-maximal flow rate</td>
</tr>
<tr>
<td>mo</td>
<td>Month</td>
</tr>
<tr>
<td>MPC</td>
<td>Maximum permissible concentration (in the USSR)</td>
</tr>
<tr>
<td>MSA</td>
<td>Metropolitan Statistical Area</td>
</tr>
<tr>
<td>MSCET</td>
<td>Month and State current emission trends</td>
</tr>
<tr>
<td>MT</td>
<td>Metric ton</td>
</tr>
<tr>
<td>N</td>
<td>Atomic nitrogen</td>
</tr>
<tr>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td>N₂</td>
<td>Molecular nitrogen</td>
</tr>
<tr>
<td>^13N</td>
<td>Nitrogen-13, a radioactive form of nitrogen</td>
</tr>
<tr>
<td>^15N</td>
<td>Nitrogen-15</td>
</tr>
<tr>
<td>Na⁺</td>
<td>Sodium ion</td>
</tr>
<tr>
<td>NA</td>
<td>Not applicable</td>
</tr>
<tr>
<td>NAAQS</td>
<td>National Ambient Air Quality Standard</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride, common table salt</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>Sodium carbonate</td>
</tr>
<tr>
<td>NAD⁺</td>
<td>Nicotinamide-adenine dinucleotide (+ indicates oxidized form)</td>
</tr>
<tr>
<td>NADB</td>
<td>National Air Data Bank</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide-adenine dinucleotide (reduced form)</td>
</tr>
<tr>
<td>NADP</td>
<td>National Acid Deposition Program</td>
</tr>
<tr>
<td>NADP⁺</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide-adenine dinucleotide phosphate (reduced form)</td>
</tr>
<tr>
<td>NaF</td>
<td>Sodium fluoride</td>
</tr>
<tr>
<td>NAMS</td>
<td>National Air Monitoring Station</td>
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<tr>
<td>NaNO₂</td>
<td>Sodium nitrite</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>Sodium nitrate</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
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</tr>
<tr>
<td>NAPAP</td>
<td>National Acid Precipitation Assessment Program</td>
</tr>
<tr>
<td>NaR</td>
<td>Nitrate reductase</td>
</tr>
<tr>
<td>NAR</td>
<td>Net assimilation ratio</td>
</tr>
<tr>
<td>NAS</td>
<td>National Academy of Sciences</td>
</tr>
<tr>
<td>NASA</td>
<td>National Aeronautics and Space Administration</td>
</tr>
<tr>
<td>NASN</td>
<td>National Air Surveillance Network</td>
</tr>
<tr>
<td>NCF</td>
<td>Neutrophil chemotactic factor</td>
</tr>
<tr>
<td>NDIR</td>
<td>Nondispersive infrared</td>
</tr>
<tr>
<td>NDIR</td>
<td>Nondispersive infrared</td>
</tr>
<tr>
<td>NDMA</td>
<td>Nitrosodimethylamine</td>
</tr>
<tr>
<td>NEDA</td>
<td>$N$-(1-Naphthyl)-ethylenediamine dihydrochloride</td>
</tr>
<tr>
<td>NEDS</td>
<td>National Emissions Data System</td>
</tr>
<tr>
<td>NEIC</td>
<td>National Enforcement Investigations Center</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>NGV</td>
<td>Natural gas vehicle</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>Ammonia</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>Ammonium ion</td>
</tr>
<tr>
<td>$^{15}$NH$_4^+$</td>
<td>Nitrogen-15 labeled ammonium ion</td>
</tr>
<tr>
<td>NHLBI</td>
<td>National Heart, Lung, and Blood Institute</td>
</tr>
<tr>
<td>NH$_4$NO$_3$</td>
<td>Ammonium nitrate</td>
</tr>
<tr>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>Ammonium sulfate</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>NiR</td>
<td>Nitrite reductase</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer cell</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>NMHC</td>
<td>Nonmethane hydrocarbon</td>
</tr>
<tr>
<td>N-6-MI</td>
<td>$N$-nitrosoheptamethyleneimine</td>
</tr>
<tr>
<td>NMOR</td>
<td>$N$-nitrosomorpholine</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>Nitrogen dioxide</td>
</tr>
<tr>
<td>$^{15}$NO$_2$</td>
<td>Nitrogen-15 labeled nitrogen dioxide</td>
</tr>
</tbody>
</table>
\begin{align*}
\text{NO}_2^- & \quad \text{Nitrite ion} \\
\text{NO}_3^- & \quad \text{Nitrate ion} \\
\text{NO}_3^- & \quad \text{Nitrate ion} \\
\text{NO}_3^- & \quad \text{Nitrogen-15 labeled nitrate ion} \\
\text{N}_2\text{O} & \quad \text{Nitrous oxide} \\
\text{N}_2\text{O}_3 & \quad \text{Dinitrogen trioxide} \\
\text{N}_2\text{O}_4 & \quad \text{Dinitrogen tetroxide} \\
\text{N}_2\text{O}_5 & \quad \text{Dinitrogen pentoxide} \\
\text{NOHb} & \quad \text{Nitrosylhemoglobin} \\
\text{NO}_x & \quad \text{Nitrogen oxides} \\
\text{NO}_y & \quad \text{Sum of oxides of nitrogen and other oxidized nitrogen compounds, excluding nitrous oxide} \\
\text{NPN} & \quad n\text{-Propyl nitrate} \\
\text{4-NQO} & \quad 4\text{-Nitroquinoline-1-oxide} \\
\text{NRA} & \quad \text{Nitrate reductase activity} \\
\text{NSA} & \quad \text{Nitrosating agent} \\
\text{NSF} & \quad \text{National Science Foundation} \\
\text{NSS} & \quad \text{National Stream Survey} \\
\text{NSWS} & \quad \text{National Surface Water Survey} \\
\text{O} & \quad \text{Atomic oxygen} \\
\text{O}_2 & \quad \text{Molecular oxygen} \\
\text{O}_3 & \quad \text{Ozone} \\
\text{OAQPS} & \quad \text{Office of Air Quality Planning and Standards} \\
\text{O}(^{1}\text{D}) & \quad \text{Excited atomic oxygen} \\
\text{ODS} & \quad \text{Oxygen depletion sensor} \\
\text{OH} & \quad \text{Hydroxyl group} \\
\text{OH}^- & \quad \text{Hydroxide ion} \\
\text{ON}^+ & \quad \text{Nitrosonium ion} \\
\text{[}^{15}\text{O}]\text{-NO}_2 & \quad \text{Oxygen-15 labeled nitrogen dioxide} \\
\text{O}(^{3}\text{P}) & \quad \text{Ground state atomic oxygen} \\
\text{OR} & \quad \text{Odds ratio} \\
\text{P} & \quad \text{Phosphorus}
\end{align*}
$^{32}$P  Phosphorus-32, a radioactive form of phosphorus
PaCO₂  Arterial partial pressure of carbon dioxide
PACO₂  Alveolar partial pressure of carbon dioxide
PAH  p-Aminohippuric acid
PAN  Peroxyacetyl nitrate
PaO₂  Arterial partial pressure of oxygen
PAO₂  Alveolar partial pressure of oxygen
PARS  Precise Accuracy Reporting System
PBzN  Peroxybenzoyl nitrate
PD40  Dose required to reduce specific airway conductance by 40%
PD₁₀₀  Dose of methacholine required to double specific airway resistance
PD₁₀₀RHE  Provocative dose in respiratory heat exchange units needed to decrease functional expiratory volume in 1 second by 10%
PD₈₈SO₂  Concentration of sulfur dioxide required to increase specific airway resistance by 8 units
PEF  Peak expiratory flow
PEFR  Peak expiratory flow rate
PEFV  Partial expiratory flow volume
PEF₄₀ₐ₉C  Partial expiratory flow at 40% of vital capacity
PF  Photofragmentation
PFC  Plaque-forming cell
6-P-G  6-Phosphogluconate
pH  Log of the reciprocal of the hydrogen ion concentration
PHA  Phytohemagglutinin
P₁  Inorganic phosphate
PM  Particulate Matter
PM  Photomultiplier
PMN  Polymorphonuclear leukocyte
PN  Particulate nitrate
PO₂  Partial oxygen pressure
ppb  Parts per billion
pphm  Parts per hundred million
ppm  Parts per million
PPN  Peroxypropionyl nitrate
ppt  Parts per trillion
PSC  Polar stratospheric cloud
PSD  Passive sampling device
P value  Probability
PV_{100SR_{aw}(SO_2)}  Ventilation of sulfur dioxide required to produce a 100% increase in specific airway resistance
Q  Cardiac output
QRS  A complex of three distinct electrocardiogram waves which represent the beginning of ventricular contraction
RAMS  Regional Air Monitoring System
RAPS  Regional Air Pollution Study
R_{aw}  Airway resistance
RBC  Red blood cell, erythrocyte
RCBD  Randomized complete block diagram
RC(O)O_2NO_2  Peroxyacylnitrate
RD  Relative duration
RGR  Relative growth rate
RH  Relative humidity
RHE  Respiratory heat exchange
RIBD  Randomized incomplete block diagram
RM  Reference method for air quality measurement
RNA  Ribonucleic acid
RO_2^{•}  Organic peroxy radical (where R is an organic moiety)
RR  Relative risk
RSD  Relative standard deviation
RSV  Respiratory syncytial virus
R_T  Total respiratory resistance
RUBISCO  Ribulose-1,5-biphosphate carboxylase-oxygenase
RV  Residual volume
S  Sulfur
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAI</td>
<td>Science Applications, Inc</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic status</td>
</tr>
<tr>
<td>SF₆</td>
<td>Sulfur hexafluoride</td>
</tr>
<tr>
<td>SGₐw</td>
<td>Specific airway conductance</td>
</tr>
<tr>
<td>SGOT</td>
<td>Serum glutamic-oxaloacetic transaminase</td>
</tr>
<tr>
<td>SGPT</td>
<td>Serum glutamic-pyruvic transaminase</td>
</tr>
<tr>
<td>SH⁻</td>
<td>Sulfhydryl group</td>
</tr>
<tr>
<td>SMSA</td>
<td>Standard Metropolitan Statistical Area</td>
</tr>
<tr>
<td>SN</td>
<td>Suspended nitrates</td>
</tr>
<tr>
<td>SO₂</td>
<td>Sulfur dioxide</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>Sulfate ion (also SO₄⁻)</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SOₓ</td>
<td>Sulfur oxides</td>
</tr>
<tr>
<td>SP</td>
<td>Single photon</td>
</tr>
<tr>
<td>SPF</td>
<td>Specific pathogen free</td>
</tr>
<tr>
<td>SRₐw</td>
<td>Specific airway resistance</td>
</tr>
<tr>
<td>SRBC</td>
<td>Sheep red blood cell</td>
</tr>
<tr>
<td>SRM</td>
<td>Standard reference material</td>
</tr>
<tr>
<td>SS</td>
<td>Suspended sulfates</td>
</tr>
<tr>
<td>STP</td>
<td>Standard temperature and pressure</td>
</tr>
<tr>
<td>TA</td>
<td>Tungstic acid</td>
</tr>
<tr>
<td>TBA</td>
<td>Thio-barbituric acid</td>
</tr>
<tr>
<td>TDLAS</td>
<td>Tunable-diole laser spectroscopy</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethanolamine</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscope</td>
</tr>
<tr>
<td>TFR</td>
<td>Transition flow reactor</td>
</tr>
<tr>
<td>Tg</td>
<td>Terragram, 10⁶ metric tons or 10¹² grams</td>
</tr>
<tr>
<td>TGS-ANSA</td>
<td>A 24-hour method for the detection of analysis of NO₂ in ambient air</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>TGV</td>
<td>Thoracic gas volume</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>T-NH₃</td>
<td>Total ammonia</td>
</tr>
<tr>
<td>TNT</td>
<td>Trinitrotoluene</td>
</tr>
<tr>
<td>TP</td>
<td>Total phosphate</td>
</tr>
<tr>
<td>TP</td>
<td>Two photon</td>
</tr>
<tr>
<td>TPTT</td>
<td>20% transport time</td>
</tr>
<tr>
<td>TSP</td>
<td>Total suspended particulate</td>
</tr>
<tr>
<td>TTFMS</td>
<td>Two-tone frequency modulated spectroscopy</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UVGSH</td>
<td>Unvented gas space heater</td>
</tr>
<tr>
<td>VC</td>
<td>Vital capacity</td>
</tr>
<tr>
<td>VE</td>
<td>Ventilatory volume</td>
</tr>
<tr>
<td>Vₑ</td>
<td>Minute ventilation</td>
</tr>
<tr>
<td>VEEE</td>
<td>Venezuelan equine encephalomyelitis (virus)</td>
</tr>
<tr>
<td>V/FFV</td>
<td>Variable- or flexible-fuel vehicle</td>
</tr>
<tr>
<td>Vmax</td>
<td>Maximum expiratory flow rate</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile organic compound</td>
</tr>
<tr>
<td>Vₜ</td>
<td>Total volume</td>
</tr>
<tr>
<td>V/V</td>
<td>Volume per volume</td>
</tr>
<tr>
<td>V₅₀%VC</td>
<td>Ventilation at 50% vital capacity</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell</td>
</tr>
<tr>
<td>WTP</td>
<td>Willingness to pay</td>
</tr>
<tr>
<td>Xe</td>
<td>Xenon</td>
</tr>
<tr>
<td>ZEV</td>
<td>Zero Emission Vehicle</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc</td>
</tr>
<tr>
<td>&gt;</td>
<td>Greater than</td>
</tr>
<tr>
<td>&lt;</td>
<td>Less than</td>
</tr>
<tr>
<td>≈</td>
<td>Approximately</td>
</tr>
</tbody>
</table>
GLOSSARY

AaDO₂  Alveolar-arterial difference or gradient of the partial pressure of oxygen  An overall measure of the efficiency of the lung as a gas exchanger  In healthy subjects, the gradient is 5 to 15 millimeters of mercury (torr)

A/PR/8 virus  A type of virus capable of causing influenza in laboratory animals, also, A/PR/8/34

Abscission  The process whereby leaves, leaflets, fruits, or other plant parts become detached from the plant

Absorption coefficient  A quantity which characterizes the attenuation with distance of a beam of electromagnetic radiation (like light) in a substance

Absorption spectrum  The spectrum that results after any radiation has passed through an absorbing substance

Abstraction  Removal of some constituent of a substance or molecule

Acetaldehyde  CH₃CHO, an intermediate in yeast fermentation of carbohydrate and in alcohol metabolism, also called acetic aldehyde, ethaldehyde, ethanal

Acetate rayon  A staple or filament fiber made by extrusion of cellulose acetate  It is saponified by dilute alkali, whereas viscose rayon remains unchanged

Acetylcholine  A naturally occurring substance in the body that can cause constriction of the bronchi in the lungs

Acid:  A substance that can donate hydrogen ions

Acid dyes  A large group of synthetic coal-tar-derived dyes that produce bright shades in a wide color range  Low cost and ease of application are features that make them the most widely used dyes for wool  Also used on nylon  The term acid dye is derived from their precipitation in an acid bath

Acid mucopolysaccharide  A class of compounds composed of protein and polysaccharide  Mucopolysaccharides comprise much of the substance of connective tissue

Acid phosphatase  An enzyme (EC 3 1 3 2) that catalyzes the disassociation of phosphate (PO₄) from a wide range of monoesters of orthophosphoric acid  Acid phosphatase is active in an acidic pH range

Acid rain  Rain having a pH less than 5.6, the minimum expected from atmospheric carbon dioxide
Acrolein $\text{CH}_2=\text{CHCHO}$, a volatile, flammable, oily liquid giving off irritant vapor. Strong irritant of skin and mucous membranes. Also called acrylic aldehyde or 2-propenal.

Acrylics (plastics) Plastics that are made from acrylic acid and are light in weight, have great breakage resistance, and lack odor and taste. Not resistant to scratching, burns, hot water, alcohol, or cleaning fluids. Examples include Lucite and Plexiglas. Acrylics are thermoplastics and are softened by heat and hardened into definite shapes by cooling.

Acrylic fiber The generic name of manufactured fibers derived from acrylic resins (minimum of 85% acrylonitrile units).

Actinic A term applied to wavelengths of light too small to affect one's sense of sight, such as ultraviolet.

Actinomycetes Members of the genus Actinomyces, nonmotile, nonsporeforming, anaerobic bacteria, including both soil-dwelling saprophytes and disease-producing parasites.

Activation energy The energy required to bring about a chemical reaction.

Acute respiratory disease Respiratory infection, usually with rapid onset and of short duration.

Acute toxicity Any poisonous effect produced by a single short-term exposure that results in severe biological harm or death.

Acyl Any organic radical or group that remains intact when an organic acid forms an ester.

Adenoma An ordinarily benign neoplasm (tumor) of epithelial tissue, usually well circumscribed, tending to compress adjacent tissue rather than infiltrating or invading.

Adenosine monophosphate (AMP) A nucleotide found among the hydrolysis products of all nucleic acids, also called adenylic acid.

Adenosine triphosphatase (ATPase) An enzyme (EC 3.6.1.3) in muscle and elsewhere that catalyzes the release of the high-energy, terminal phosphate group of adenosine triphosphate.

Adrenalectomy Removal of an adrenal gland. This gland is located near or upon the kidney and is the site of origin of a number of hormones.

Adsorption Adhesion of a thin layer of molecules to a liquid or solid surface.

Advecton Horizontal flow of air at the surface or aloft, one of the means by which heat is transferred from one region of the earth to another.
Aerodynamic diameter: The diameter of a unit density sphere having the same settling speed (under gravity) as the particle in question of whatever shape and density.

Aerosol: Solid particles or liquid droplets that are dispersed or suspended in a gas.

Agglutination: The process by which suspended bacteria, cells, or similar particles adhere and form into clumps.

Airborne pathogen: A disease-causing microorganism that travels in the air or on particles in the air.

Air pollutant: A substance present in the ambient atmosphere, resulting from human activity or from natural processes, which may cause damage to human health or welfare, the natural environment, or materials or objects.

Air spaces: All alveolar ducts, alveolar sacs, and alveoli. To be contrasted with airways.

Airway conductance ($G_{aw}$): Reciprocal of airway resistance $G_{aw} = (1/R_{aw})$.

Airway resistance ($R_{aw}$): The (frictional) resistance to airflow afforded by the airways between the airway opening at the mouth and the alveoli.

Airways: All passageways of the respiratory tract from mouth or nares down to and including respiratory bronchioles. To be contrasted with air spaces.

Alanine aminotransferase: An enzyme (EC 2.6.1.2) transferring amino groups from L-alanine to 2-ketoglutamate. Also known as alanine transaminase.

Albumin: A type of simple, water-soluble protein widely distributed throughout animal tissues and fluids, particularly serum.

Aldehyde: An organic compound characterized by the group -CHO.

Aldolase: An enzyme (EC 4.1.2.7) involved in metabolism of fructose that catalyzes the formation of two three-carbon intermediates in the major pathway of carbohydrate metabolism.

Algal bloom: Sudden spurt in growth of algae that can adversely affect water quality.

Alkali: A salt of sodium or potassium capable of neutralizing acids.

Alkaline phosphatase: A phosphatase (EC 3.1.3.1) with an optimum pH of 8.6, present ubiquitously.

Allergen: A material that, as a result of coming into contact with appropriate tissues of an animal body, induces a state of sensitivity resulting in various reactions, generally associated with idiosyncratic hypersensitivities.
Alpha-hydroxybutyrate dehydrogenase  An enzyme (EC 1.1.1.30), present mainly in mitochondria, that catalyzes the conversion of hydroxybutyrate to acetoacetate intermediate biochemical pathways

Alpha rhythm  A rhythmic pulsation obtained in brain waves exhibited in the sleeping state of an individual

Alveolar capillary membrane  Finest portion of alveolar capillaries, where gas transfer to and from blood takes place

Alveolar macrophage (AM)  A large, mononuclear, phagocytic cell found on the alveolar surface, responsible for particle clearance from the deep lung and for viral and bacterial killing

Alveolar oxygen partial pressure (PAO₂)  Partial pressure of oxygen in the air contained in the air sacs of the lungs

Alveolar septa  The tissue between two adjacent pulmonary alveoli, consisting of a close-meshed capillary network covered on both surfaces by thin alveolar epithelial cells

Alveolus  An air cell, a terminal, sac-like dilation in the lung  Gas exchange (oxygen/carbon dioxide) occurs here

Ambient  The atmosphere to which the general population may be exposed  Construed here not to include atmospheric conditions indoors, or in the workplace

Amine  A substance that may be derived from ammonia (NH₃) by the replacement of one, two or three of the hydrogen (H) atoms by hydrocarbons or other radicals (primary, secondary, or tertiary amines, respectively)

Amino acids  Molecules consisting of a carboxyl group, a basic amino group, and a residue group attached to a central carbon atom  Serve as the building blocks of proteins

p-Aminohippuric acid (PAH)  A compound used to determine renal plasma flow

Aminotriazole  A systemic herbicide (C₂H₄N₄) used in areas other than croplands, that also possesses some antithyroid activity, also called amitrole

Ammonification  Decomposition with production of ammonia or ammonium compounds, especially by the action of bacteria on nitrogenous organic matter

Ammonium  Anion (NH₄⁺) or radical (NH₄) derived from ammonia by combination with hydrogen Present in rainwater, soils and many commercial fertilizers

Amnestic  Pertains to immunologic memory upon receiving a second dose of antigen, the host "remembers" the first dose and responds faster to the challenge

A-19
Anaerobic: Living, active or occurring in the absence of free oxygen

Anaerobic bacteria: A type of microscopic organism that can live in an environment not containing free oxygen

Anaphylactic dyspneic attack: Difficulty in breathing associated with a systemic allergic response

Anaphylaxis: A term commonly used to denote the immediate, transient kind of immunological (allergic) reaction characterized by contraction of smooth muscle and dilation of capillaries due to release of pharmacologically active substances

Angiosperm: A plant having seeds enclosed in an ovary, a flowering plant

Angina pectoris: Severe constricting pain in the chest that may be caused by depletion of oxygen delivery to the heart muscle, usually caused by coronary disease

Angstrom (Å): A unit (10^-8 centimeter) used in the measurement of the wavelength of light

Anhydride: A compound resulting from removal of water from two molecules of a carboxylic (-COOH) acid. Also, may refer to those substances (anhydrous) that do not contain water in chemical combination

Anion: A negatively charged atom, radical, or ion

Anorexia: Diminished appetite, aversion to food

Anoxic: Without or deprived of oxygen

Antagonism: When the effects of a mixture are less than the sum of the effects of each individual chemical

Anthraquinone: A yellow crystalline ketone (C_{14}H_{8}O_2) derived from anthracene and used in the manufacture of dyes

Anthropogenic: Of, relating to, or influenced by humans. An anthropogenic source of pollution is one caused by human actions

Antibody: Any body or substance evoked by the stimulus of an antigen and that reacts specifically with an antigen in some demonstrable way

Antigen: A material such as a foreign protein that, as a result of coming in contact with appropriate tissues of an animal, after a latent period, induces a state of sensitivity and/or the production of an antibody

Antistatic agent: A chemical compound applied to fabrics to reduce or eliminate accumulation of static electricity
Arachidonic acid  Long-chain fatty-acid that serves as a precursor of prostaglandins

Area source  In air pollution, any small individual fuel combustion or other pollutant source, also, all such sources grouped over a specific area

Aromatic  Belonging to that series of carbon-hydrogen compounds in which the carbon atoms form closed rings containing unsaturated bonds (as in benzene)

Arterial partial pressure of oxygen (PaO₂)  Portion of total pressure of dissolved gases in arterial blood as measured directly from arterial blood

Arterialized partial pressure of oxygen  The portion of total pressure of dissolved gases in arterial blood attributed to oxygen, as measured from nonarterial (e.g., ear-prick) blood

Arteriosclerosis  Commonly called hardening of the arteries  A condition that exists when the walls of the blood vessels thicken and become infiltrated with excessive amounts of minerals and fatty materials

Artifact  A spurious measurement produced by the sampling or analysis process

Ascorbic acid  Vitamin C, a strong reducing agent with antioxidant properties

Aspartate transaminase  Also known as aspartate aminotransferase (EC 2.6.1.1)  An enzyme catalyzing the transfer of an amine group from glutamic acid to oxaloacetic acid, forming aspartic acid in the process  Serum level of the enzyme is increased in myocardial infarction and in diseases involving destruction of liver cells

Asphyxia  Impaired exchange of oxygen and carbon dioxide, excess of carbon dioxide, and/or lack of oxygen, usually caused by ventilatory problems

Asthma  A disease characterized by an increased responsiveness of the airways to various stimuli and manifested by slowing of forced expiration that changes in severity either spontaneously or as a result of therapy  The term asthma may be modified by words or phrases indicating its etiology, factors provoking attacks, or duration

Asymptomatic  Presenting no subjective evidence of disease

Atmosphere  The body of air surrounding the earth  Also, a measure of pressure (atm) equal to the pressure of air at sea level, 14.7 pounds per square inch

Atmospheric deposition  Removal of pollutants from the atmosphere onto land, vegetation, water bodies, or other objects by absorption, sedimentation, Brownian diffusion, impaction, or precipitation in rain
Atomic absorption spectrometry  A measurement method based on the absorption of radiant energy by gaseous ground-state atoms. The amount of absorption depends on the population of the ground state, which is related to the concentration of the sample being analyzed.

Atopic  Clinical hyperreactivity of the airways associated with asthma and allergies.

Atropine: A poisonous, white crystalline alkaloid (C₁₇H₂₃NO₃) derived from belladonna and related plants, used to relieve spasms of smooth muscles. It is an anticholinergic agent that blocks the parasympathetic actions of acetylcholine and other cholinergic agents.

Autocorrelation  Statistical interdependence of variables being analyzed, produces problems, for example, when observations may be related to previous measurements or other conditions.

Autoimmune disease  A condition in which antibodies are produced against the subject’s own tissues.

Autologous  A term referring to cellular elements, such as red blood cells and alveolar macrophages, from the same organism, also, something naturally and normally occurring in some part of the body.

Autotrophic  A term applied to those microorganisms that are able to maintain life without an exogenous organic supply of energy, or which only need carbon dioxide or carbonates and simple inorganic nitrogen.

Autotrophic bacteria  A class of microorganisms that require only carbon dioxide or carbonates and a simple inorganic nitrogen compound for carrying on life processes.

Auxin: An organic substance that causes lengthening of the stem when applied in low concentrations to shoots of growing plants.

Awn: One of the slender bristles that terminate the glumes of the spikelet in some cereals and other grasses.

Azo dye: Dyes in which the azo group is the chromophore and joins benzene or naphthalene rings.

Background measurement  A measurement of pollutants in ambient air due to natural sources, usually taken in remote areas.

Bactericidal activity  The process of killing bacteria.

Barre. Bars or stripes in a fabric, caused by uneven weaving, irregular yarn, or uneven dye distribution.

Basal cell: One of the innermost cells of the deeper epidermis of the skin.
Base cation $\text{Ca}^{2+}, \text{Mg}^{2+}, \text{K}^+, \text{or Na}^+$

Base saturation The degree to which soil cation exchange capacity is occupied by base cations. This is expressed as a percent, using charge-equivalents.

Benzenethiol A compound of benzene and a hydrosulfide group.

Beta $(\beta)$-lipoprotein A biochemical complex or compound containing both lipid and protein and characterized by having a large molecular weight, rich in cholesterol. Found in certain fractions of human plasma.

Bilateral renal sclerosis A hardening of both kidneys of chronic inflammatory origin.

Biomass That part of a given habitat consisting of living matter.

Biosphere The part of the earth's crust, waters, and atmosphere where living organisms can subsist.

Biphasic Having two distinct successive stages.

Bleb A collection of fluid beneath the skin, usually smaller than bullae or blisters.

Blood urea The chief end product of nitrogen metabolism in mammals, excreted in human urine in the amount of about 32 grams (1 ounce) a day.

Bloom A greenish-gray appearance imparted to silk and pile fabrics either by nature of the weave or by the finish, also, the creamy white color observed on some good cottons.

Blue-green algae A group of simple plants that are the only nitrogen-fixing organisms that photosynthesize as do higher plants.

Brightener A compound, such as a dye, that adheres to fabrics in order to provide better brightness or whiteness by converting ultraviolet radiation to visible light. Sometimes called optical bleach or whitening agent. The dyes used are of the fluorescent type.

Broad bean The large flat edible seed of an Old World upright vetch ($Vicia faba$), or the plant itself, widely grown for its seeds and for fodder.

Bronchi The first subdivisions of the trachea, which conduct air to and from the bronchioles of the lungs.

Bronchiole One of the finer subdivisions of the bronchial (trachea) tubes, less than 1 millimeter in diameter, and having no cartilage in its wall.

Bronchiolitis Inflammation of the bronchioles, which may be acute or chronic. If the etiology is known, it should be stated. If permanent occlusion of the lumens is present, the term bronchiolitis obliterans may be used.
Bronchiolitis fibrosa obliterans syndrome  Obstruction of the bronchioles by fibrous granulation arising from an ulcerated mucosa, the condition may follow inhalation of irritant gases

Bronchitis  A nonneoplastic disorder of structure or function of the bronchi resulting from infectious or noninfectious irritation  The term bronchitis should be modified by appropriate words or phrases to indicate its etiology, its chronicity, the presence of associated airways dysfunction, or the type of anatomic change  The term chronic bronchitis, when unqualified, refers to a condition associated with prolonged exposure to nonspecific bronchial irritants and accompanied by mucus hypersecretion and certain structural alterations in the bronchi  Anatomic changes may include hypertrophy of the mucous-secreting apparatus and epithelial metaplasia, as well as more classic evidences of inflammation  In epidemiologic studies, the presence of cough or sputum production on most days for at least three months of the year for at least two consecutive years has sometimes been accepted as a criterion for the diagnosis

Bronchoconstrictor  An agent that causes a reduction in the caliber (diameter) of a bronchial tube

Bronchodilator  An agent that causes an increase in the caliber (diameter) of a bronchus or bronchial tube

Bronchopneumonia  Acute inflammation of the walls of the smaller bronchial tubes, with irregular area of consolidation due to spread of the inflammation into peribronchiolar alveoli and the alveolar ducts

Bronchospasm  Temporary narrowing of the bronchi due to a violent, involuntary contraction of the smooth muscle of the bronchi

Bronchus  One of the subdivisions of the trachea serving to convey air to and from the lungs  The trachea divides into right and left main bronchi, which in turn form lobar, segmental, and subsegmental bronchi

Brownian diffusion  Diffusion by random movement of particles suspended in liquid or gas, resulting from the impact of molecules of the fluid surrounding the particles

BTPS conditions (BTPS)  Body temperature, barometric pressure, and saturated with water vapor  These are the conditions existing in the gas phase of the lungs  For humans, the normal temperature is 37 °C, the pressure is based on the barometric pressure, and the partial pressure of water vapor is 47 torr

Buffer  A substance in solution capable of neutralizing both acids and bases and thereby maintaining the original pH of the solution

Buffering  In reference to soil acidification, this is resistance to change resulting from reserves of acid or base cations on the soil cation-exchange sites
Buffering capacity  Ability of a body of water and its watershed to neutralize introduced acid

Butanol  A four-carbon, straight-chain alcohol, C₄H₉OH, also known as butyl alcohol

Butylated hydroxytoluene (BHT)  A crystalline phenolic antioxidant

Butylated hydroxyanisole (BHA)  An antioxidant

1⁴C labeling  Use of a radioactive form of carbon as a tracer, often in metabolic studies

1⁴C-proline  An amino acid that has been labeled with radioactive carbon

Calciteous  Resembling or consisting of calcium carbonate (lime), or growing on limestone or lime-containing soils

Calorie  Amount of heat required to raise temperature of 1 gram of water at 15 °C by 1 °C

Cannula  A tube that is inserted into a body cavity, or other tube or vessel, usually to remove fluid

Capillary  The smallest type of vessel, resembles a hair. Usually in reference to a blood or lymphatic capillary vessel

Carbachol  A cholinergic parasympathetic stimulant, carbamoylcholine chloride (C₆H₁₅CΙN₂O₂), that produces constriction of the bronchial smooth muscles similar to acetylcholine

Carbon monoxide  An odorless, colorless, toxic gas with a strong affinity for hemoglobin and cytochrome, it reduces oxygen absorption capacity, transport, and utilization

Carboxyhemoglobin  A fairly stable union of carbon monoxide with hemoglobin that interferes with the normal transfer of carbon dioxide and oxygen during circulation of blood. Increasing levels of carboxyhemoglobin result in various degrees of asphyxiation, including death

Carcinogen  Any agent producing or playing a stimulatory role in the formation of a malignancy

Carcinoma  Malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and giving rise to metastases

Cardiac output  The volume of blood passing through the heart per unit time

Cardiovascular  Relating to the heart and the blood vessels or the circulation

Carotene  Lipid-soluble yellow-to-orange-red pigments universally present the photosynthetic tissues of higher plants, algae, and the photosynthetic bacteria
Cascade impactor  A device for measuring the size distribution of particulates and/or aerosols, consisting of a series of plates with orifices of graduated size that separate the sample into a number of fractions of decreasing aerodynamic diameter

Catabolism  Destructive metabolism involving the release of energy and resulting in breakdown of complex materials in the organism

Catalase  An enzyme (EC 1 11 1 6) catalyzing the decomposition of hydrogen peroxide to water and oxygen

Catalysis  A modification of the rate of a chemical reaction by some material, which is unchanged at the end of the reaction

Catalytic converter  An air pollution abatement device that removes organic contaminants by oxidizing them into carbon dioxide and water

Catecholamine  A pyrocatechol with an alkalamine side chain, functioning as a hormone or neurotransmitter, such as epinephrine, norepinephrine, or dopamine

Cathepsins  Enzymes that have the ability to hydrolyze certain proteins and peptides, occur in cellular structures known as lysosomes

Cation  A positively charged ion

Cation exchange capacity  The ability of a soil to absorb positively charged ions by electrostatic forces  This absorption occurs on negatively charged sites on clays and organic matter in soils

Cellular permeability  Ability of gases to enter and leave cells, a sensitive indicator of injury to deep-lung cells

Cellulose  The basic substance that is contained in all vegetable fibers  It is a carbohydrate and constitutes the major substance in plant life  Used to make cellulose acetate and rayon

Cellulose acetate  Commonly refers to fibers or fabrics in which the cellulose is only partially acetylated with acetate groups  An ester made by reacting cellulose with acetic anhydride with sulfate as a catalyst

Cellulose rayon  A regenerated cellulose that is chemically the same as cellulose except for physical differences in molecular weight and crystallinity

Cellulose triacetate  A cellulose fiber that is completely acetylated  Fabrics of triacetate have higher heat resistance than acetate and may be safely ironed at higher temperature  Such fabrics have improved ease-of-care characteristics because after heat treatment during manufacture, a change in the crystalline structure of the fiber occurs
Cellulosics  Cotton, viscose rayon, and other fibers made of natural-fiber raw materials

Celsius scale  The thermometric scale in which the freezing point of water is 0 and the boiling point is 100

Central hepatic necrosis  The pathologic death of one or more cells, or of a portion of the liver, involving the cells adjacent to the central veins

Central nervous system (CNS)  The brain and the spinal cord

Centroacinar area  The center portion of a gland shaped as a bunch of grapes

Cerebellum  The large posterior brain-mass lying above the pons and medulla and beneath the posterior portion of the cerebrum

Cerebral cortex  The layer of gray matter covering the entire surface of the cerebral hemisphere of mammals

Chain reaction  A reaction that stimulates its own repetition

Challenge  Exposure of a test organism to a virus, bacteria, or other stress-causing agent, used in conjunction with exposure to a pollutant of interest, to explore possible susceptibility brought on by the pollutant

Chamber study  Research conducted using a closed vessel in which pollutants are reacted or substances are exposed to pollutants

Chemiluminescence  A measurement technique in which radiation is produced as a result of chemical reaction

Chemotactic  Relating to attraction or repulsion of living protoplasm by chemical stimuli

Chlorophyll  A group of closely related green photosynthetic pigments occurring in leaves, bacteria, and organisms

Chloroplast  A plant cell inclusion body containing chlorophyll

Chlorosis  Discoloration of normally green plant parts that can be caused by disease, lack of nutrients, or various air pollutants, resulting in the failure of chlorophyll to develop

Cholesterol  A steroid alcohol (C_{27}H_{45}OH), the most abundant steroid in animal cells and body fluids

Cholinesterase (CHE)  One (EC 3.1.1.8) of a family of enzymes capable of catalyzing the hydrolysis of acylcholines
Chondrosarcoma. A malignant neoplasm derived from cartilage cells, occurring most frequently near the ends of long bones.

Chromatid. Each of the two strands formed by longitudinal duplication of a chromosome that becomes visible during an early stage of cell division.

Chromophore. A chemical group that produces color in a molecule by absorbing near ultraviolet or visible radiation when bonded to a nonabsorbing, saturated residue that possesses no unshared, nonbonding valence electrons.

Chromosome. One of the bodies (46 in humans) in the cell nucleus that is the bearer and carrier of genetic information.

Chronic obstructive pulmonary disease (COPD). This term refers to diseases of uncertain etiology characterized by persistent slowing of airflow during forced expiration. It is recommended that a more specific term, such as chronic obstructive bronchitis or chronic obstructive emphysema, be used whenever possible. Synonymous with chronic obstructive lung disease (COLD).

Cilia. Motile, often hairlike extensions of a cell surface.

Ciliary action. Movements of cilia in the upper respiratory tract, which move mucus and foreign material upward.

Ciliogenesis. The formation of cilia.

Citric acid (Krebs) cycle. A major biochemical pathway in cells, involving terminal oxidation of fatty acids and carbohydrates. It yields a major portion of energy needed for essential body functions and is the major source of carbon dioxide. It couples the glycolytic breakdown of sugar in the cytoplasm with those reactions producing adenosine triphosphate in the mitochondria. It also serves to regulate the synthesis of a number of compounds required by a cell.

Clara cell. A nonciliated cell in the epithelium of the respiratory tract.

Closing capacity (CC). Closing volume plus residual volume, often expressed as a ratio of total lung capacity (TLC) (i.e., CC/TLC%).

Closing volume (CV). The volume exhaled after the expired gas concentration is reflected from an alveolar plateau during a controlled breathing maneuver. (Most commonly obtained during a single-breath nitrogen washout test.) Because the value obtained is dependent on the specific test technique, the method used must be designated in the text, and when necessary, specified by a qualifying symbol. Closing volume is often expressed as a ratio of the vital capacity (VC) (i.e., CV/VC%).

Codon. A sequence of three nucleotides that encodes information required to direct the synthesis of one or more amino acids.
Coefficient of haze (COH)  A measurement of visibility interference in the atmosphere

Cohort  A group of individuals or vital statistics about them having a statistical factor in common in a demographic study (e.g., year of birth, sex, level of exposure to a pollutant, etc.)

Collagen  The major protein of the white fibers of connective tissue, cartilage, and bond
Comprises over half the protein of the mammal.

Collisional deactivation  Reduction in energy of excited molecules caused by collision with other molecules or other objects such as the walls of a container

Colorimetric  A chemical analysis method relying on measurement of the degree of color produced in a solution by reaction with the pollutant of interest

Community exposure  A situation in which people in a sizeable area are subjected to ambient pollutant concentrations

Compliance (C_L,C_R)  A measure of distensibility
Pulmonary compliance is given by the slope of a static volume-pressure curve at a point, or the linear approximation of a nearly straight portion of such a curve, expressed as the change in volume per unit change in distending pressure (liters per centimeter of water or milliliters per centimeter of water) Because the static volume-pressure characteristics of lungs are nonlinear (static compliance decreases as lung volume increases) and vary according to the previous volume history (static compliance at a given volume increases immediately after full inflation and decreases following deflation), careful specification of the conditions of measurement are necessary Absolute values also depend on organ size See also dynamic compliance

Complement  Thermolabile substance present in serum that is destructive to certain bacteria and other cells that have been sensitized by specific complement-fixing antibody

Compound  A substance with its own distinct properties, formed by the chemical combination of two or more elements in fixed proportion

Concanavalin-A  One of two crystalline globulins occurring in the jack bean, a potent hemagglutinin

Conductance (G)  The reciprocal of resistance  See airway conductance

Conifer  A plant, generally evergreen, needle-leafed, bearing naked seeds singly or in cones

Converter  See catalytic converter

Coordination number  The number of bonds formed by the central atom in a complex
Copolymer  The product of the process of polymerization in which two or more monomeric substances are mixed prior to polymerization. Nylon is a copolymer.

Copro por phyrin  One of two porphyrin compounds found normally in feces as a decomposition product of bilirubin (a bile pigment). Porphyrin is a widely distributed pigment consisting of four pyrrole nuclei joined in a ring.

Cordage. A general term which includes banding, cable, cord, rope, string, and twine made from fibers. Synthetic fibers used in making cordage include nylon and dacron.

Corrosion. Destruction or deterioration of a material because of reaction with its environment.

Corticosterone: A steroid obtained from the adrenal cortex. It induces some deposition of glycogen in the liver, sodium conservation, and potassium excretion.

Cosmopolitan  In the biological sciences, a term denoting worldwide distribution.

Coulometric: Chemical analysis performed by determining the amount of a substance released in electrolysis by measuring the number of coulombs used.

Coumarin  A toxic white crystalline lactone (C₉H₆O₂) found in plants.

Coupler. A chemical used to combine two others in a reaction (e.g., to produce the azo dye in the Griess-Saltzman method for nitrogen dioxide).

Crevice corrosion  Localized corrosion occurring within crevices on metal surfaces exposed to corrosives.

Critical Load  A quantitative estimates of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the ecosystem do not occur according to present knowledge.

Crosslink  To connect, by an atom or molecule, parallel chains in a complex chemical molecule, such as a polymer.

Cryogenic trap. A pollutant sampling method in which a gaseous pollutant is condensed out of sampled air by cooling (e.g., traps in one method for nitrosamines are maintained below −79 °C, using solvents maintained at their freezing points).

Cuboidal  Resembling a cube in shape.

Cultivar  An organism produced by parents belonging to different species or to different strains of the same species, originating and persisting under cultivation.

Cuticle. A thin outer layer, such as the thin continuous fatty film on the surface of many higher plants.
Cyanosis  A dark bluish or purplish coloration of the skin and mucous membrane due to deficient oxygenation of the blood

Cyclic GMP  Guanosine 5’-phosphoric acid

Cytochrome  A class of hemoprotein whose principal biological function is electron and/or hydrogen transport

Cytology  The anatomy, physiology, pathology, and chemistry of the cell

Cytoplasm  The substance of a cell exclusive of the nucleus

Dacron  The trade name for polyester fibers made by E I du Pont de Nemours and Co., Inc., made from dimethyl terephthalate and ethylene glycol

Dark adaptation  The process by which the eye adjusts under reduced illumination and the sensitivity of the eye to light is greatly increased

Dark respiration  Metabolic activity of plants at night, consuming oxygen to use stored sugars and releasing carbon dioxide

Deciduous plants  Plants that drop their leaves at the end of the growing season

Degradation (textiles)  The decomposition of fabric or its components or characteristics (color, strength, elasticity) by means of light, heat, or air pollution

Denitrification  A bacterial process occurring in soils, or water, in which nitrate is used as the terminal electron acceptor and is reduced primarily to molecular nitrogen. It is essentially an anaerobic process, it can occur in the presence of low levels of oxygen only if the microorganisms are metabolizing in an anoxic microzone

De novo  Over again.

Deoxyribonucleic acid (DNA)  A nucleic acid considered to be the carrier of genetic information coded in the sequence of purine and pyrimidine bases (organic bases). It has the form of a double-stranded helix of a linear polymer

Depauperate  Falling short of natural development or size

Deposition

Acidic  Removal of acidic pollutants from the atmosphere by dry and wet deposition

Dry  Removal of pollutants from the atmosphere through interactions with various surfaces of plants, land, and water
Respiratory tract  The depositing of inhaled pollutants within the respiratory tract, which depends on breathing patterns, airway geometry, and the physical and chemical properties of the inhaled pollutants

Wet  Removal of pollutants from the atmosphere by precipitation (e.g., rain or snow)

Derivative spectrophotometer  An instrument with an increased capability for detecting overlapping spectral lines and bands and also for suppressing instrumentally scattered light

Desorb: To release a substance that has been taken into another substance or held on its surface, the opposite of absorption or adsorption

Desquamation  The shedding of the outer layer of any surface

Detection limit  A level below which an element or chemical compound cannot be reliably detected by the method or measurement being used for analysis

Detritus  Loose material that results directly from disintegration

DeVarda alloy  An alloy of 50% copper, 45% aluminum, and 5% zinc

Diastolic blood pressure  The blood pressure as measured during the period of filling the cavities of the heart with blood

Diazonium salt  A chemical compound (usually colored) of the general structure $\text{ArN}_2^+\text{Cl}^-$, where Ar refers to an aromatic group

Diazotizer  A chemical that, when reacted with amines ($\text{RNH}_2$, for example), produces a diazonium salt (usually a colored compound)

Dichotomous sampler  A device used to collect separately fine and coarse particles from an aerosol and to measure gravimetrically the concentration of such different-sized particles in the ambient air

Differentiation  The process by which a cell, such as a fertilized egg, divides into specialized cells, such as the embryonic types that eventually develop into an entire organism

Diffusion: The process by which molecules or other particles intermingle as a result of their random thermal motion

Diffusing capacity of the lung ($D_L$, $D_L\text{O}_2$, $D_L\text{CO}_2$, $D_L\text{CO}$)  Amount of gas (oxygen, carbon monoxide, carbon dioxide) commonly expressed as milliliters of gas (standard temperature and pressure, dry) diffusing between alveolar gas and pulmonary capillary blood per torr mean gas pressure difference per minute (such as mL O$_2$/min-torr) Synonymous with transfer factor and diffusion factor

A-32
Dimer  A compound formed by the union of two like radicals or molecules

Dimerize  Formation of dimers

1,6-diphosphofructose aldolase  An enzyme (EC 4.1.1.13) cleaving fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate

D-2,3-diphosphoglycerate  A salt or ester of 2,3-diphosphoglyceric acid, a major component of certain mammalian erythrocytes involved in the release of oxygen from oxyhemoglobin  Also a postulated intermediate in the biochemical pathway involving the conversion of 3- to 2-phosphoglyceric acid

Diplococcus pneumoniae  A species of spherical-shaped bacteria belonging to the genus Streptococcus  May be a causal agent in pneumonia

Direct dye  A dye with an affinity for most fibers; used mainly when color resistance to washing is not important

Disperse dyes  Also known as acetate dyes, these dyes were developed for use on acetate fabrics, and are now also used on synthetic fibers

Distal  Far from some reference point such as median line of the body, point of attachment, or origin

Diurnal  Having a repeating pattern or cycle 24 hours long

DLCO  The diffusing capacity of the lungs for carbon monoxide  The ability of the lungs to transfer carbon monoxide from the alveolar air into the pulmonary capillary blood

Dorsal kyphosis  Abnormal curvature of the spine, hunchback

Dose  The quantity of a substance to be taken all at one time or in fractional amounts within a given period, also the total amount of a pollutant delivered or concentration per unit time times time

Dose-response curve  A curve on a graph based on responses occurring in a system as a result of a series of stimuli intensities or doses

Dry deposition  The processes by which matter is transferred to ground from the atmosphere, other than precipitation, includes surface absorption of gases and sedimentation, Brownian diffusion, and impaction of particles

Dyeing  A process of coloring fibers, yarns, or fabrics with either natural or synthetic dyes

Dynamic calibration  Testing of a monitoring system using a continuous sample stream of known concentration
Dynamic compliance (C_{dyn}) is the ratio of the tidal volume to the change in intrapleural pressure between the points of zero flow at the extremes of tidal volume (L/cm H₂O or mL/cm H₂O). Because at the points of zero airflow at the extremes of tidal volume, volume acceleration is usually other than zero, and because, particularly in abnormal states, flow may still be taking place within lungs between regions that are exchanging volume, dynamic compliance may differ from static compliance, the latter pertaining to condition of zero volume acceleration and zero gas flow throughout the lungs. In normal lungs at ordinary volumes and respiratory frequencies, static and dynamic compliance are the same.

Dynel. A trademark for a modacrylic staple fiber spun from a copolymer of acrylonitrile and vinyl chloride. It has high strength, quick-drying properties, and resistance to alkalies and acids.

Dyspepsia. Indigestion, upset stomach.

Dyspnea. Shortness of breath, difficulty or distress in breathing, rapid breathing.

Ecosystem. The interacting system of a biological community and its environment.

Eddy. A current of water or air running contrary to the main current.

Edema. Pressure of excess fluid in cells, intercellular tissue, or cavities of the body.

Elastance (E). The reciprocal of compliance (expressed in centimeters of water per liter or centimeters of water per milliliter).

Elastomer. A synthetic rubber product that has the physical properties of natural rubber.

Electrocardiogram. The graphic record of the electrical currents that initiate the heart's contraction.

Electrode. One of the two extremities of an electric circuit.

Electrolyte. A nonmetallic electric conductor in which current is carried by the movement of ions; also a substance that displays these qualities when dissolved in water or another solvent.

Electronegativity. Measure of affinity for negative charges or electrons.

Electron microscopy. A technique that utilizes a focused beam of electrons to produce a high-resolution image of minute objects such as particulate matter, bacteria, viruses, and DNA.

Electronic excitation energy. Energy associated in the transition of electrons from their normal low-energy orbitals to orbitals of higher energy.
Electrophilic  Having an affinity for electrons

Electrophoresis  A technique by which compounds can be separated from a complex mixture by their attraction to the positive or negative pole of an applied electric potential

Eluant  A liquid used in the process of elution

Elute  To perform an elution

Elution  Separation of one material from another by washing or by dissolving one in a solvent in which the other is not soluble

Elutriate  To separate a coarse, insoluble powder from a finer one by suspending them in water and pouring off the finer powder from the upper part of the fluid

Emission spectrometry  A rapid analytical technique based on measurement of the characteristic radiation emitted by thermally or electrically excited atoms or ions

Emphysema  A condition of the lung characterized by abnormal, permanent enlargement of airspaces distal to the terminal bronchiole, accompanied by the destruction of their walls, and without obvious fibrosis

Emphysematous lesions  A wound or injury to the lung as a result of emphysema

Empirical modeling  Characterization and description of a phenomena based on experience or observation

Encephalitis  Inflammation of the brain

Endoplasmic reticulum  An elaborate membrane structure extending from the nuclear membrane or eucaryotic cells to the cytoplasmic membrane

Endothelium  A layer of flat cells lining especially blood and lymphatic vessels

Entropy  A measure of disorder or randomness in a system  Low entropy is associated with highly ordered systems

Enzyme  Any of numerous proteins produced by living cells that catalyze biological reactions

Enzyme Commission (EC)  The International Commission on Enzymes, established in 1956, developed a scheme of classification and nomenclature under which each enzyme is assigned an EC number that identifies it by function

Eosinophils  Leukocytes (white blood cells) that stain readily with the dye eosin
Epidemiology. A study of the distribution and determinants of disease in human population groups.

Epidermis. The outermost living layer of cells of any organism

Epididymal fat pads The fatty tissue located near the epididymis The epididymis is the first convoluted portion of the excretory duct of the testis

Epiphyte. A plant growing on another plant but obtaining food from the atmosphere

Epithelial: Relating to epithelium, the membranous cellular layer that covers free surfaces or lines tubes or cavities of an animal body, which encloses, protects, secretes, excretes and/or assimilates

Erosion corrosion Acceleration or increase in rate of deterioration or attack on a metal because of relative movement between a corrosive fluid and the metal surface Characterized by grooves, gullies, or waves in the metal surface

Erythrocyte: A mature red blood cell

Escherichia coli A short, gram-negative, rod-shaped bacteria common to the human intestinal tract A frequent cause of infections in the urogenital tract

Esophageal Relating to the portion of the digestive tract between the pharynx and the stomach

Estrus. That portion or phase of the sexual cycle of female animals characterized by willingness to permit coitus

Estrus cycle The series of physiologic uterine, ovarian, and other changes that occur in higher animals

Etiolation Paleness and/or altered development resulting from the absence of light

Etiology The causes of a disease or condition, also, the study of causes

Eucaryotic: Pertaining to those cells having a well-defined nucleus surrounded by a double-layered membrane

Eutrophication Elevation of the level of nutrients in a body of water, which can contribute to accelerated plant growth and filling

Excited state: A state of higher electronic energy than the ground state, usually a less stable one

Expiratory (maximum) flow rate The maximum rate at which air can be expelled from the lungs
Exposure level  Concentration of a contaminant to which an individual or a population is exposed

Extinction coefficient  A measure of the space rate of diminution, or extinction, of any transmitted light, thus, it is the attenuation coefficient applied to visible radiation

Extramedullary hematopoiesis  The process of formation and development of the various types of blood cells and other formed elements not including that occurring in bone marrow

Extravasate  To exclude from or pass out of a vessel into the tissues, applies to urine, lymph, blood, and similar fluids

Far ultraviolet  Radiation in the range of wavelengths from 100 to 190 nanometers

Federal Reference Method (FRM)  For nitrogen dioxide, the EPA-approved analyzers based on the gas-phase chemiluminescent measurement principle and associated calibration procedures, regulatory specifications prescribed in Title 40, Code of Federal Regulations, Part 50, Appendix F

Fenestrae  Anatomical apertures often closed by a membrane

FEV₁/FVC  A ratio of timed (t = 0, 5, 1, 2, 3 seconds) forced expiratory volume (FEV₁) to forced vital capacity (FVC)  The ratio is often expressed in percent (100 × FEV₁/FVC)  It is an index of airway obstruction

Fiber  A fine, threadlike piece, as of cotton, jute, or asbestos

Fiber-reactive dye  A water-soluble dyestuff that reacts chemically with the cellulose in fibers under alkaline conditions; the dye contains two chlorine atoms that combine with the hydroxyl groups of the cellulose

Fibrin  A white insoluble elastic filamentous protein derived from fibrinogen by the action of thrombin, especially in the clotting of blood

Fibroadenoma  A benign neoplasm derived from glandular epithelium, involving proliferating fibroblasts, cells found in connective tissue

Fibroblast  An elongated cell with cytoplasmic processes present in connective tissue, capable of forming collagen fibers

Fibrosis  The formation of fibrous tissue, usually as a reparative or reactive process and not as a normal constituent of an organ or tissue

Fine particles  Airborne particles smaller than 2 to 3 micrometers in aerodynamic diameter
Flocculation  Separation of material from a solution or suspension by reaction with a flocculant to create fluffy masses containing the material to be removed.

Flow volume curve  Graph of instantaneous forced expiratory flow recorded at the mouth, against corresponding lung volume. When recorded over the full vital capacity, the curve includes maximum expiratory flow rates at all lung volumes in the vital capacity range and is called a maximum expiratory flow-volume curve (MEFV). A partial expiratory flow-volume curve (PEFV) is one which describes maximum expiratory flow rate over a portion of the vital capacity only.

Fly ash: Fine, solid particles of noncombustible ash carried out of a bed of solid fuel by a draft.

Fogs: Suspension of liquid droplets formed by condensation of vapor or atomization, the concentration of particles is sufficiently high to obscure visibility.

Folded-path optical system  A long (e.g., 8 to 22 meters) chamber with multiple mirrors at the ends which can be used to reflect an infrared beam through an ambient air sample many times, a spectrometer can be used with such a system to detect trace pollutants at very low levels.

Forced expiratory flow (FEF)  Related to some portion of the forced vital capacity (FVC) curve. Modifiers refer to the amount of the FVC already exhaled when the measurement is made. For example:

\[ \text{FEF}_{75\%} = \text{Instantaneous forced exhaled flow after 75\% of the forced vital capacity has been exhaled} \]

\[ \text{FEF}_{200-1,200} = \text{Mean forced expiratory flow between 200 milliliters and 1,200 milliliters of the forced vital capacity (formerly called the maximum expiratory flow rate [MEFR])} \]

\[ \text{FEF}_{25-75\%} = \text{Mean forced expiratory flow during the middle half of the forced vital capacity (formerly called the maximum midexpiratory flow rate [MMFRI])} \]

\[ \text{FEF}_{\text{max}} = \text{The maximal forced expiratory flow achieved during an forced vital capacity} \]

Forced expiratory volume (FEV)  Denotes the volume of gas that is exhaled in a given time interval from the beginning of the execution of a forced vital capacity. Conventionally, the times used are 0.5, 0.75, or 1 second, symbolized FEV\(_{0.5}\), FEV\(_{0.75}\), and FEV\(_{1.0}\), respectively. These values are often expressed as a percent of the forced vital capacity, for example, \((\text{FEV}_{1.0}/\text{FVC}) \times 100\).

Forced inspiratory vital capacity (FIVC)  The maximal volume of air inspired with a maximally forced effort from a position of maximal expiration.
Forced vital capacity (FVC)  The maximum volume of air that can be forcibly expelled from the lungs after the deepest inspiration

Fractional threshold concentration  The portion of the concentration at which an event or a response begins to occur, expressed as a fraction

Free radical  Any of a variety of highly reactive atoms or molecules characterized by having an unpaired electron

Fritted bubbler  A porous glass device used in air pollutant sampling systems to introduce small bubbles into solution

Functional residual capacity (FRC)  The volume of gas remaining in the lungs at the end of a normal expiration  It is the sum of expiratory reserve volume and residual volume (see pulmonary measurements)

Gas chromatography (GC)  A method of separating and analyzing mixtures of chemical substances  A flow of gas causes the components of a mixture to migrate differentially from a narrow starting zone in a special porous, insoluble sorptive medium  The pattern formed by zones of separated pigments and of colorless substances in this process is called a chromatogram, and can be analyzed to obtain the concentration of identified pollutants

Gas exchange  Movement of oxygen from the alveoli into the pulmonary capillary blood as carbon dioxide enters the alveoli from the blood  In broader terms, the exchange of gases between alveoli and lung capillaries

Gas-liquid chromatography  A method of separating and analyzing volatile organic compounds in which a sample is vaporized and swept through a column filled with solid support material covered with a nonvolatile liquid  Components of the sample can be identified and their concentrations can be determined by analysis of the characteristics of their retention in the column because compounds have varying degrees of solubility in the liquid medium

Gas trapping  Trapping of gas behind small airways that were opened during inspiration but closed during forceful expiration  It is a volume difference between forced vital capacity and vital capacity

Gastric juice  A thin watery digestive fluid secreted by glands in the mucous membrane of the stomach

Gastroenteritis  Inflammation of the mucous membrane of stomach and intestine

Genotype  The type of genes possessed by an organism

Geometric mean  An estimate of the average of a distribution  Specifically, the nth root of the product of n observations
Geometric standard deviation. A measure of variability of a distribution. It is the antilogarithm of the standard deviation of the logarithms of the observations.

Globulins (a, b, q). A family of proteins precipitated from plasma (or serum) by half-saturation with ammonium sulfate, or separable by electrophoresis. The main groups are the a, b, and q fractions, differing with respect to associated lipids and carbohydrates and in their content of antibodies (immunoglobulins).

Glomerular nephrotic syndrome. Dysfunction of the kidneys characterized by excessive protein loss in the urine, accumulation of body fluids, and alteration in albumin/globulin ratio.

Glucose. A sugar that is a principal source of energy for humans and other organisms.

Glucose-6-phosphate dehydrogenase. An enzyme (EC 1.1.1.49) catalyzing the dehydrogenation of glucose-6-phosphate to 6-phosphogluconolactone.

Glutamic-oxaloacetic transaminase (SGOT). An enzyme (EC 2.6.1.1) whose serum level increases in myocardial infarction and in diseases involving destruction of liver cells. Also known as aspartate aminotransferase.

Glutamic-pyruvic transaminase (SGPT). Now known as alanine aminotransferase (EC 2.6.1.2), the serum levels of this enzyme are used in liver function tests.

Glutathione (GSH). A tripeptide composed of glycine, cystine, and glutamic acid.

Glutathione peroxidase. An enzyme (EC 1.11.1.9) that catalyzes the destruction of hydroperoxides formed from fatty acids and other substances. Protects tissues from oxidative damage. It is a selenium-containing protein.

Glutathione reductase. The enzyme (EC 1.6.4.2) that reduces the oxidized form of glutathione.

Glycolytic pathway. The biochemical pathway by which glucose is converted to lactic acid in various tissues, yielding energy as a result.

Glycoside. A type of chemical compound formed from the condensation of a sugar with another chemical radical via a hemiacetal linkage.

Goblet cells. Epithelial cells that have been distended with mucus and when this is discharged as mucus, a goblet-shaped shell remains.

Golgí apparatus. A membrane system involved with secretory functions and transport in a cell. Also known as a dictyosome.

Grana. The lamellar stacks of chlorophyll-containing material in plant chloroplasts.
Griege carpet  A carpet in its unfinished state (i.e., before it has been scoured and dyed)
The term also is used for woven fabrics in the unbleached and unfinished state

Ground state  The state of minimum electronic energy of a molecule or atom

Guanylate cyclase (GC)  An enzyme (EC 4.6.2.1) catalyzing the transformation of guanosine triphosphate to guanosine 3'-5'-cyclic phosphate

\(^3\text{H}-\text{Thymidine}  \text{ Thymine deoxynucleoside}  \text{ One of the four major nucleosides in DNA}  
\(^3\text{H}-\text{thymidine} \text{ has been uniformly labeled with tritium, a radioactive form of hydrogen}

Haze  Fine dust, smoke, or fine vapor reducing transparency of air

Hemagglutination  The agglutination of red blood cells  Can be used as a measurement of antibody concentration

Hematocrit  The percentage of the volume of a blood sample occupied by cells

Hematology  The medical specialty that pertains to the blood and blood-forming tissues

Hemochromatosis  A disease characterized by pigmentation of the skin possibly due to inherited excessive absorption of iron

Hemoglobin (Hb)  The red, respiratory protein of the red blood cells, hemoglobin transports oxygen from the lungs to the tissues as oxyhemoglobin (HbO\(_2\)) and returns carbon dioxide to the lungs as hemoglobin carbamate, completing the respiratory cycle

Hemolysis  Alteration or destruction of red blood cells, causing hemoglobin to be released into the medium in which the cells are suspended

Hepatectomy  Complete removal of the liver in an experimental animal

Hepatic  Relating to the liver

Hepatocyte  A liver cell

Heterogeneous process  A chemical reaction involving reactants of more than one phase or state, such as one in which gases are absorbed into aerosol droplets, where the reaction takes place

Heterologous  A term referring to donor and recipient cellular elements from different organisms, such as red blood cells from sheep and alveolar macrophage from rabbits

Heterotrophs  Fungi and bacteria that rely on organic matter for their energy source
Hexose monophosphate shunt  Also called the phosphogluconate oxidative pathway of glucose metabolism, which affords a total combustion of glucose independent of the citric acid cycle. It is the important generator of nicotinamide-adenine dinucleotide phosphate (reduced form) necessary for synthesis of fatty acids and the operation of various enzymes. It serves as a source of ribose and 4- and 7-carbon sugars.

High volume (hi-vol) sampler  A high flow-rate device used to collect particles from the atmosphere and to gravimetrically measure the concentration of particles across a broad range of sizes in ambient air.

Histamine  A depressor amine derived from the amino acid histidine and found in all body tissues, with the highest concentration in the lung, a powerful stimulus of gastric secretion, a constrictor of bronchial smooth muscle, and a vasodilator that causes a fall in blood pressure.

Homogenate  Commonly refers to tissue ground into a creamy consistency in which the cell structure is disintegrated.

Host defense mechanism  Inherent means by which a biologic organism protects itself against infection, such as antibody formation, macrophage action, ciliary action, etc.

Host resistance  The resistance exhibited by an organism, such as a human, to an infecting agent, such as a virus or bacteria.

Humoral:  Relating to the extracellular fluids of the body, blood and lymph.

Hybrid:  An organism descended from parents belonging to different varieties or species.

Hydrocarbons  A vast family of compounds containing carbon and hydrogen in various combinations, found especially in fossil fuels. Some contribute to photochemical smog.

Hydrolysis  Decomposition involving splitting of a bond and addition of the H and OH parts of water to the two sides of the split bond.

Hydrometeor  A product of the condensation of atmospheric water vapor (e.g., fog, rain, hail, snow).

Hydroxyproline  An amino acid found among the hydrolysis products of collagen.

Hygroscopic  Pertaining to a marked ability to accelerate the condensation of water vapor.

Hygroscopic growth  Growth induced by moisture, often applied in reference to the growth in size of inhaled particles within the respiratory tract in combination with resident moisture.

Hyperplasia  Increase in the number of cells in a tissue or organ excluding tumor formation.
Hyperplastic  Relating to hyperplasia, an increase in the number of cells

Hypertrophy  Increase in the size of a tissue element, excluding tumor formation

Hypertension  Abnormally elevated blood pressure

Hypolimnion  Portions of a lake below the thermocline in which water is stagnant and uniform in temperature.

Hypoxia  A lower than normal amount of oxygen in the air, blood, or tissues

Immunoglobulin (Ig)  A class of structurally related proteins consisting of two pairs of polypeptide chains. Antibodies are immunoglobulins and all immunoglobulins probably function as antibodies.

Immunoglobulin A (IgA)  A type of antibody that comprises approximately 10 to 15% of the total amount of antibodies present in normal serum

Immunoglobulin G (IgG)  A type of antibody that comprises approximately 80% of the total amount of antibodies present in normal serum. Subfractions of IgG are fractions G₁ and G₂.

Immunoglobulin M (IgM)  A type of antibody that comprises approximately 5 to 10% of the total amount of antibodies present in normal serum

Impaction  An impinging or striking of one object against another, also, the force transmitted by this act

Impactor  An instrument which collects samples of suspended particulates by directing a stream of the suspension against a surface, or into a liquid or a void

Index of proliferation  Ratio of promonocytes to polymorphic monocytes in the blood

Infarction  Sudden insufficiency of arterial or venous blood supply due to emboli, thrombi, or pressure

Infectivity model  A testing system in which the susceptibility of animals to airborne infectious agents with and without exposure to air pollutants is investigated to produce information related to the possible effects of the pollutant on humans

Inflorescence  The arrangement and development of flowers on an axis, also, a flower cluster or a single flower

Influenza A₂/Taiwan Virus  An infectious-viral disease, believed to have originated in Taiwan, characterized by sudden onset, chills, fevers, headache, and cough
Infrared Light invisible to the human eye, between the wavelengths of $7 \times 10^{-7}$ and $10^{-3}$ meter ($7,000$ and $10,000,000$ Angstroms)

Infrared laser A device that utilizes the natural oscillations of atoms or molecules to generate coherent electromagnetic radiation in the infrared region of the spectrum

Infrared spectrometer An instrument for measuring the relative amounts of radiant energy in the infrared region of the spectrum as a function of wavelength

Ingestion To take in for digestion

In situ In the natural or original position

Instrumental averaging time The time over which a single example or measurement is taken, resulting in a measurement that is an average of the actual concentrations over that period

Insult An injury or trauma

Intercostal Between the ribs, especially of a leaf

Interferant A substance that a measurement method cannot distinguish completely from the one being measured, which therefore can cause some degree of false response or error

Interferon A macromolecular substance produced in response to infection with active or inactivated virus, capable of inducing a state of resistance

Intergranular corrosion A type of corrosion that takes place at and adjacent to grain boundaries, with relatively little corrosion of the grains

Interstitial edema An accumulation of an excessive amount of fluids in a space within tissues

Interstitial pneumonia A chronic inflammation of the interstitial tissue of the lung, resulting in compression of air cells

Intraluminal mucus Mucus that collects within any tubule

Intraperitoneal injection An injection of material into the serous sac that lines the abdominal cavity

In utero Within the womb, not yet born

In vitro Refers to experiments conducted outside the living organism

In vivo Refers to experiments conducted within the living organism
Irradiation Exposure to any form of radiation

Ischemia Local anemia due to mechanical obstruction (mainly arterial narrowing) of the blood supply

Isoenzymes Also called isozymes One of a group of enzymes that are very similar in catalytic properties, but may be differentiated by variations in physical properties, such as isoelectric point or electrophoretic mobility Lactic acid dehydrogenase is an example of an enzyme having many isomeric forms

Isopleth A line on a map or chart connecting points of equal value

Jacobs-Hochheiser method The original Federal Reference Method for nitrogen dioxide, currently unacceptable for air pollution work

*Klebsiella pneumoniae* A species of rod-shaped bacteria found in soil, water, and in the intestinal tract of humans and other animals Certain types may be causative agents in pneumonia

Kyphosis An abnormal curvature of the spine, with convexity backward

Lactate A salt or ester of lactic acid

Lactic acid (lactate) dehydrogenase (LDH) An enzyme (EC 1 1 1 27) with many isomeric forms that catalyzes the oxidation of lactate to pyruvate via transfer of hydrogen to nicotinamide-adenine dinucleotide Isomeric forms of lactic acid dehydrogenase in the blood are indicators of heart damage

Lamellar bodies Arranged in plates or scales One of the characteristics of Type II alveolar cells

Lavage fluid Any fluid used to wash out hollow organs, such as the lung

Leaching The removal of elements from soil, litter, or plant foliage by water

Lecithin Any of several waxy hygroscopic phosphatides that are widely distributed in animals and plants, they form colloidal solutions in water and have emulsifying, wetting, and hygroscopic properties

Legume A plant with root nodules containing nitrogen-fixing bacteria

Lesion A wound, injury, or other more or less circumscribed pathologic change in the tissues

Leukocyte Any of the white blood cells
Lewis base  A base, defined in the Lewis acid-base concept, is a substance that can donate an electron pair

Lichens  Perennial plants which are a combination of two plants, an alga and a fungus, growing together in an association so intimate that they appear as one

Ligand: Those molecules or anions attached to the central atom in a complex

Light-fastness  The ability of a dye to maintain its original color under natural or indoor light

Linolenic acid  An unsaturated fatty acid essential in nutrition

Lipase  An enzyme that accelerates the hydrolysis or synthesis of fats or the breakdown of lipoproteins

Lipids: A heterogeneous group of substances that occur widely in biological materials. They are characterized as a group by their extractability in nonpolar organic solvents

Lipofuscin  Brown pigment granules representing lipid-containing residues of lysosomal digestion. Proposed to be an end product of lipid oxidation that accumulates in tissue

Lipoprotein  Complex or protein containing lipid and protein

Loading rate  The amount of a nutrient available to a unit area of body of water over a given period of time

Locomotor activity  Movement of an organism from one place to another of its own volition

Long path length infrared absorption  A measurement technique in which a system of mirrors in a chamber is used to direct an infrared beam through a sample of air for a long distance (up to 2 kilometers), the amount of infrared light absorbed is measured to obtain the concentrations of pollutants present

Lung compliance (CI)  The volume change produced by an increase in a unit change in pressure across the lung (i.e., between the pleural surface and the mouth)

Lycra  A spandex textile fiber created by E I du Pont de Nemours & Co., Inc., with excellent tensile strength, a long flex life and high resistance to abrasion and heat degradation. Used in brassieres, foundation garments, surgical hosiery, swim suits, and military and industrial applications

Lymphocytes  White blood cells formed in lymphoid tissue throughout the body, they comprise about 22 to 28% of the total number of leukocytes in the circulating blood and function in immunity
Lymphocytogram  The ratio, in the blood, of lymphocytes with narrow cytoplasm to those with broad cytoplasm

Lysosomes  Organelles found in cells of higher organisms that contain high concentrations of degradative enzymes and are known to destroy foreign substances that cells engulf by pinocytosis and phagocytosis  Believed to be a major site where proteins are broken down

Lysozymes  Lytic enzymes destructive to cell walls of certain bacteria  Present in some body fluids, including tears and serum

*Macaca speciosa*  A species of monkeys used in research

Macrophage  Any large, ameboid, phagocytic cell having a nucleus without many lobes, regardless of origin

Malaise  A feeling of general discomfort or uneasiness, often the first indication of an infection or disease

Malate dehydrogenase  An enzyme (EC 1 1 1 37) with at least six isomeric forms that catalyze the dehydrogenation of malate to oxaloacetate or its decarboxylation (removal of a carbon dioxide group) to pyruvate  Malate, oxaloacetate, and pyruvate are intermediate components of biochemical pathways

Mannitol  An alcohol derived from reduction of the sugar fructose  Used in renal function testing to measure glomerular (capillary) filtration

Manometer  An instrument for the measurement of pressure of gases or vapors

Mass median diameter (MMD)  Geometric median size of a distribution of particles based on weight

Mass spectrometry (MS)  A procedure for identifying the various kinds of particles present in a given substance by ionizing the particles and subjecting a beam of the ionized particles to an electric or magnetic field such that the field deflects the particles in angles directly proportional to the masses of the particles

Maximal expiratory flow ($V_{max}$)  Forced expiratory flow, related to the total lung capacity or the actual volume of the lung at which the measurement is made  Modifiers refer to the amount of lung volume remaining when the measurement is made  For example

$V_{max75\%}$ = Instantaneous forced expiratory flow when the lung is at 75% of its total lung capacity

$V_{max3\;0}$ = Instantaneous forced expiratory flow when the lung volume is 3.0 liters
Maximal expiratory flow rate (MEFR)  Obsolete terminology  See FEF<sub>200-1,200</sub> under forced expiratory flow

Maximal midexpiratory flow rate (MMFR or MMEF)  See FEF<sub>25-75%</sub> under forced expiratory flow

Maximal ventilation (max V<sub>E</sub>)  The volume of air breathed in 1 minute during repetitive maximal respiratory effort  Synonymous with maximum ventilatory minute volume

Maximal voluntary ventilation (MVV)  The volume (liters per minute at body temperature and pressure, saturated) of air breathed by a subject during voluntary maximum hyperventilation (rapid deep breathing) lasting a specific period of time  Replaces maximal breathing capacity

Mean (arithmetic)  The sum of observations divided by sample size

Mechanical clearance  See mucociliary action

Median  A value in a collection of data values that is exceeded in magnitude by one-half the entries in the collection

MEFR  See FEF<sub>200-1,200</sub> under forced expiratory flow

Mesoscale  Of or relating to meteorological phenomena from 1 to 100 kilometers in horizontal extent

Messenger RNA  A type of RNA that conveys genetic information encoded in the DNA to direct protein synthesis

Metaplasia  The abnormal transformation of an adult, fully differentiated tissue of one kind into a differentiated tissue of another kind

Metaproterenol  A bronchodilator used for the treatment of bronchial asthma

Metastases  The shifting of a disease from one part of the body to another, the appearance of neoplasms in parts of the body remote from the seat of the primary tumor

Meteorology  The science that deals with the atmosphere and its phenomena

Methacholine  A parasympathomimetic bronchoconstrictor drug with similarities to carbachol and acetylcholine

Methemoglobin: A form of hemoglobin in which the normal reduced state of iron (Fe<sup>2+</sup>) has been oxidized to ferric iron (Fe<sup>3+</sup>)  It contains oxygen in firm union with Fe<sup>3+</sup> and is not capable of exchanging oxygen in normal respiratory processes

Methimazole  An antithyroid drug similar in action to propylthiouracil
Methyltransferase  Any enzyme transferring methyl groups from one compound to another

Microcoulometric  Capable of measuring millionths of coulombs used in electrolysis of a substance, to determine the amount of a substance in a sample

Microflora  A small or strictly localized plant

Micron  One-millionth of a meter

Microphage  A small phagocyte, a polymorphonuclear leukocyte that is phagocytic

Millimolar  One-thousandth of a molar solution  A solution of one-thousandth of a mole (in grams) per liter

Mineral acid amon  An amon associated with strong, or mineral acids such as sulfuric, nitric, or hydrochloric acid  These anions include nitrate \( \text{NO}_3^- \), sulfate \( \text{SO}_4^{2-} \), and chloride \( \text{Cl}^- \)

Minute ventilation (\( V_E \))  See pulmonary measurements

Minute volume  The minute volume of breathing, a product of tidal volume times the respiratory frequency in one minute, synonymous with minute ventilation

Mitochondria  Organelles of the cell cytoplasm that contain enzymes active in the conservation of energy obtained in the aerobic part of the breakdown of carbohydrates and fats, in a process called respiration

MMFR  Maximal midexpiratory flow  See FEF\(_{25-75\%}\) under forced expiratory flow

Mobile sources.  Automobiles, trucks, and other pollution sources that are not fixed in one location

Modacrylic fiber  A manufactured fiber in which the fiber-forming substance is any long chain synthetic polymer composed of less than 85% but at least 35% by weight of acrylonitrile units

Moieties  One of two or more parts into which something is divided

Mole  The mass, in grams, numerically equal to the molecular weight of a substance

Molecular correlation spectrometry  A spectrophotometric technique that is used to identify unknown absorbing materials and measure their concentrations by using preset wavelengths

Molecular weight  The weight of one molecule of a substance obtained by adding the gram-atomic weights of each of the individual atoms in the substance
Monocyte  A relatively large mononuclear leukocyte, normally constituting 3 to 7% of the leukocytes of the circulating blood

Morbidity.  The quantity or state of being diseased, also, used in reference to the ratio of the number of sick individuals to the total population of a community (i.e., morbidity rate)

Mordant  A substance that acts to bind dyes to a textile fiber of fabric

Morphological.  Relating to the form and structure of an organism or any of its parts

Morphology  Structure and form of an organism at any stage of its life history

Morphometry  The quantitative measurement of structure (morphology)

Mortality rate  For a given period of time, the ratio of the number of deaths occurring per 1,000 population  Also known as death rate

Moving average:  A procedure involving taking averages over a specific period prior to and including a year in question, so that successive averaging periods overlap (e.g., a three-year moving average would include data from 1967 through 1969 for the 1969 average and from 1968 through 1970 for 1970)

Mucociliary action  Ciliary action of the mucous membranes lining respiratory tract airways that aids in removing particles from the lungs

Mucociliary clearance  Removal of materials from the upper respiratory tract via ciliary action

Mucociliary transport  The process by which mucus is transported, by ciliary action, from the lungs

Mucosa  The mucous membrane  It consists of epithelium, lamina propria, and, in the digestive tract, a layer of smooth muscle

Mucous membrane  A mucus-secreting membrane that lines passages and cavities communicating with the exterior of the body

Mucus  The clear, viscid secretion of mucous membranes, consisting of mucin, epithelial cells, leukocytes, and various inorganic salts suspended in water

Murine  Relating to mice

Mutagen  A substance capable of causing, within an organism, biological changes that affect potential offspring through genetic mutation
Mutagenic  Having the power to cause mutations  A mutation is a change in the character of a gene (a sequence of base pairs in DNA) that is perpetuated in subsequent divisions of the cell in which it occurs.

Myocardial infarction  Infarction of any area of the heart muscle usually as a result of occlusion of a coronary artery.

Mycorrhizae  Fungi that live in association with plant roots and assist in the uptake of water and nutrients in exchange for carbohydrates.

Nares  The nostrils.

Nasopharyngeal  Relating to the nasal cavity and the pharynx (throat).

National Air Surveillance Network (NASN)  Network of monitoring stations for sampling air to determine extent of air pollution, established jointly by federal and state governments.

Near ultraviolet  Radiation of the wavelengths 2,000 to 4,000 Ångstroms.

Necrosis  Death of cells that can discolor areas of a plant or kill the entire plant.

Necrotic  Pertaining to the pathologic death of one or more cells, or of a portion of tissue or organ, resulting from irreversible damage.

Neonate  A newborn.

Neoplasm  An abnormal tissue that grows more rapidly than normal, synonymous with tumor.

Neoplasia  The pathologic process that results in the formation and growth of a tumor.

Neutrophil  A mature white blood cell formed in bone marrow and released into the circulating blood. Neutrophils normally account for 54 to 65% of the total number of leukocytes.

Ninhydrin  An organic reagent used to identify amino acids.

Nitramine  A compound consisting of a nitrogen attached to the nitrogen of amine.

Nitrate  A salt or ester of nitric acid (NO_3^- is used to symbolize the ionic form, NO_3 is used for the radical).

Nitrification  The principal natural source of nitrate in which ammonium ions (NH_4^+) are oxidized to nitrates by specialized microorganisms. Other organisms oxidize nitrates to nitrates.
Nitrifiers. Soil microorganisms that convert ammonium ions (NH₄⁺) or organic nitrogen to nitrate ions (NO₃⁻), a process referred to as nitrification. Organisms that convert NH₄⁺ to NO₃⁻ are referred to as autotrophic nitrifiers, and organisms that convert organic nitrogen to NO₃⁻ are referred to as heterotrophic nitrifiers.

Nitrite. A salt or ester of nitrous acid (NO₂⁻).

Nitrocellulose. Any of several esters of nitric acid formed by its action on cellulose, used in explosives, plastics, varnishes, and rayon, also called cellulose nitrate.

Nitrogen cycle: Refers to the complex pathways by which nitrogen-containing compounds are moved from the atmosphere into organic life, into the soil, and back to the atmosphere.

Nitrogen fixation. The metabolic assimilation of atmospheric nitrogen by soil microorganisms, which becomes available for plant use when the microorganisms die, also, industrial conversion of free nitrogen into combined forms used in production of fertilizers and other products.


Nitrogen saturation. A condition in which ecosystems are unable to accumulate any more nitrogen.

Nitrogen washout. The multiple breath curve obtained by plotting the fractional concentration of nitrogen in expired alveolar gas versus time for a subject switched from breathing ambient air to an inspired mixture of pure oxygen. A progressive decrease of nitrogen concentration ensues, which may be analyzed into two or more exponential components.

Nitrosamine: A compound consisting of a nitrosyl group connected to the nitrogen of an amine.

Nitrosation. Addition of a nitrosyl group.

N-Nitroso compounds. Compounds carrying the functional nitrosyl group.

Nitrosyl: A group composed of one oxygen and one nitrogen atom (-N=O).

Nitrosylhemoglobin (NOHb). The red, respiratory protein of erythrocytes to which a nitrosyl group is attached.

N/P Ratio. Ratio of nitrogen to phosphorous dissolved in lake water, important due to its effect on plant growth.

Nucleolus. A small spherical mass of material within the substance of the nucleus of a cell.
Nucleophilic  Having an affinity for atomic nuclei, electron-donating

Nucleoside  A compound that consists of a purine or pyrimidine base combined with deoxyribose or ribose and found in RNA and DNA

5'-Nucleotidase  An enzyme (EC 3.1.3.5) that hydrolyzes nucleoside 5'-phosphates into phosphoric acid (H₃PO₄) and nucleosides

Nucleotide  A compound consisting of a sugar (ribose or deoxyribose), a base (a purine or a pyrimidine), and a phosphate, a basic structural unit of RNA and DNA

Nylon  A generic name chosen by E I du Pont de Nemours & Co, Inc for a group of protein-like chemical products classed as synthetic linear polymers, two main types are Nylon 6 and Nylon 66

Oclusion  A point at which an opening is closed or obstructed

Olefin  An open-chain hydrocarbon having at least one double bond

Olfactory  Relating to the sense of smell

Olfactory epithelium  The inner lining of the nose and mouth that contains neural tissue sensitive to smell

Oligotrophic  A body of water deficient in plant nutrients, also generally having abundant dissolved oxygen and no marked stratification

Orbitals  Areas of high electron density in an atom or molecule

Orlon  An acrylic fiber produced by E I du Pont de Nemours and Co, Inc, based on a polymer of acrylonitrile, used extensively for outdoor uses, it is resistant to chemicals and withstands high temperatures.

Oronasal breathing  Breathing through the nose and mouth simultaneously, typical human breathing pattern at moderate to high levels of exercise versus normally predominant nasal breathing while at rest

Osteogenic osteosarcoma  The most common and malignant of bone sarcomas (tumors) It arises from bone-forming cells and affects chiefly the ends of long bones

Ovarian primordial follicle  A spheroidal cell aggregation in the ovary in which the primordial oocyte (immature female sex cell) is surrounded by a single layer of flattened follicular cells
Oxidant: A chemical compound that has the ability to remove electrons from another chemical species, thereby oxidizing it, also, a substance containing oxygen that reacts in air to produce a new substance, or one formed by the action of sunlight on oxides of nitrogen and hydrocarbons.

Oxidation: An ion or molecule undergoes oxidation by donating electrons.

Oxidative deamination: Removal of the amine (NH₂) group from an amino compound by reaction with oxygen.

Oxidative phosphorylation: The mitochondrial process by which "high-energy" phosphate bonds form from the energy released as a result of the oxidation of various substrates. Principally occurs in the tricarboxylic acid pathway.

Oxyhemoglobin: Hemoglobin in combination with oxygen. It is the form of hemoglobin present in arterial blood.

Ozone layer: A layer of the stratosphere from 20 to 50 kilometers above the earth's surface characterized by high ozone content produced by ultraviolet radiation.

Ozone scavenging: Removal of ozone from ambient air or plumes by reaction with nitric oxide, producing nitrogen dioxide and molecular oxygen.

Paired electrons: Electrons having opposite intrinsic spins about their own axes.

Parenchyma: The essential and distinctive tissue of an organ or an abnormal growth, as distinguished from its supportive framework.

Parenchymal: Referring to the distinguishing or specific cells of a gland or organ.

Partial pressure: The pressure exerted by a single component in a mixture of gases.

Particle: Any object, solid or liquid, having definite physical boundaries in all directions, includes, for example, fine solid particles, such as dust, smoke, fumes, or smog, found in the air or in emissions.

Particulate matter (PMₓ): Matter in the form of small airborne liquid or solid particles. In the abbreviation, the subscript "x" indicates the particulate mean aerodynamic diameter.

Particulates: Fine liquid or solid particles, such as dust, smoke, mist, fumes, or smog, found in the air or in emissions.

Pascal: A unit of pressure in the International System of Units. One pascal is equal to 7.4 × 10⁻³ torr. The pascal is equivalent to 1 N per square meter.

Pathogen: Any virus, microorganism, or other substance causing disease.
Pathophysiologic Derangement of function seen in disease, alteration in function as distinguished from structural defects

Peak expiratory flow (PEF) The highest forced expiratory flow measured with a peak flow meter

Peptide bond The bond formed when two amino acids react with each other

Percentiles The percentage of all observations exceeding or preceding some point, thus, 90th percentile is a level below which 90% of the observations will fall

Perennial Trees and other plants that live more than one year are called perennials

Perfusate A liquid, solution or colloidal suspension that has been passed over a special surface or through an appropriate structure

Perfusion Artificial passage of fluid through blood vessels

Permanent-press fabrics Fabrics in which applied resins contribute to the easy care and appearance of the fabric and to the crease and seam flatness by reacting with the cellulose on pressing after garment manufacture

Permeation tube A tube that is selectively porous to specific gases

Peroxidation Refers to the process by which certain organic compounds are converted to peroxides

Peroxyacetyl nitrate (PAN) Pollutant created by action of sunlight on hydrocarbons and nitrogen oxides in the air, an ingredient of photochemical smog

pH A measure of the effective acidity or alkalinity of a solution It is expressed as the negative logarithm of the hydrogen ion concentration Pure water has a hydrogen ion concentration equal to $10^{-7}$ M per liter at standard conditions (25 °C) The negative logarithm of this quantity is 7 Thus, pure water has a pH value of 7 (neutral) The pH scale is usually considered as extending from 0 to 14 A pH less than 7 denotes acidity, and a pH more than 7 denotes alkalinity

Phagocytosis A mechanism by which alveolar macrophages and polymorphonuclear leukocytes engulf particles, one of several lung defense mechanisms by which foreign agents (biological and nonbiological) are removed from the respiratory tract

Phenotype The observable characteristics of an organism, resulting from the interaction between an individual genetic structure and the environment in which development takes place

Phenylthiourea A crystalline compound (C$_7$H$_5$N$_2$S) that is bitter or tasteless depending on a single dominant gene in the taster

A-55
Phlegm: Viscid mucus secreted in abnormal quantity in the respiratory passages

Phosphatase Any of a group of enzymes that liberate inorganic phosphate from phosphoric esters (EC sub-subclass 3 1 3)

Phosphocreatine kinase An enzyme (EC 2 7 3 2) catalyzing the formation of creatine and adenosine triphosphate, its breakdown is a source of energy in the contraction of muscle; also called creatine phosphate

Phospholipid A molecule consisting of lipid and phosphoric acid group(s) An example is lecithin Serves as an important structural factor in biological membranes

Photochemical oxidants Primary ozone, nitrogen dioxide, and peroxyacetyl nitrate, with lesser amounts of other compounds, formed as products of atmospheric reactions involving organic pollutants, nitrogen oxides, oxygen, and sunlight

Photochemical smog Air pollution caused by chemical reaction of various airborne chemicals in sunlight

Photodissociation The process by which a chemical compound breaks down into simpler components under the influence of sunlight or other radiant energy

Photolysis Decomposition upon irradiation by sunlight

Photomultiplier tube An electron multiplier in which electrons released by photoelectric emission are multiplied in successive stages by dynodes that produce secondary emissions

Photon A quantum of electromagnetic energy

Photostationary A substance or reaction that reaches and maintains a steady state in the presence of light

Photosynthesis The process in which green parts of plants, when exposed to light under suitable conditions of temperature and water supply, produce carbohydrates using atmospheric carbon dioxide and release oxygen

Phyllosphere Usually refers to the leaf surface of plants

Phytotoxic Poisonous to plants

Phytoplankton Minute aquatic plant life

Pi (Π) bonds Bonds in which electron density is not symmetrical about a line joining the bonded atoms
Pinocytotic  Refers to the cellular process (pinocytosis) in which the cytoplasmic membrane forms invaginations in the form of narrow channels leading into the cell. Liquids can flow into these channels and the membrane pinches off pockets that are incorporated into the cytoplasm and digested.

Pitting  A form of extremely localized corrosion that results in holes in the metal. One of the most destructive forms of corrosion.

Pituitary  A stalk-like gland near the base of the brain that is attached to the hypothalamus. The anterior portion is a major repository for hormones that control growth, stimulate other glands, and regulate the reproductive cycle.

Placenta  The organ in the uterus that provides metabolic interchange between the fetus and mother.

Plasmid  Replicating unit, other than a nucleus gene, that contains nucleoprotein and is involved in various aspects of metabolism in organisms, also called paragenes.

Plasmolysis  The dissolution of cellular components, or the shrinking of plant cells by osmotic loss of cytoplasmic water.

Plastic  A plastic is one of a large group of organic compounds synthesized from cellulose, hydrocarbons, proteins, or resins and capable of being cast, extruded, or molded into various shapes.

Plasticizer  A chemical added to plastics to soften, increase malleability, or make them more readily deformable.

Platelet (blood)  An irregularly-shaped disk with no definite nucleus, about one-third to one-half the size of an erythrocyte and containing no hemoglobin. Platelets are more numerous than leukocytes, numbering from 200,000 to 300,000 per cubic millimeter of blood.

Plethysmograph  A device for measuring and recording changes in volume of a part, organ, or the whole body. A body plethysmograph is a chamber apparatus surrounding the entire body.

Pleura  The serous membrane enveloping the lungs and lining the walls of the chest cavity.

Plume  Emission from a flue or chimney, usually in a stream-like distribution downwind of the source, which can be distinguished from the surrounding air by appearance or chemical characteristics.

Pneumonia (interstitial)  A chronic inflammation of the interstitial tissue of the lung, resulting in compression of the air cells. An acute, infectious disease.
Pneumonocytes  A nonspecific term sometimes used in referring to types of cells characteristic of the respiratory part of the lung

Podzol: Any of a group of zonal soils that develop in a moist climate, especially under coniferous or mixed forests

Point source  A single stationary location of pollutant discharge

Polarography  A method of quantitative or qualitative analysis based on current-voltage curves obtained by electrolysis of a solution with steadily increasing voltage

Pollution gradient  A series of exposure situations in which pollutant concentrations range from high to low

Polyacrylonitrile  A polymer made by reacting ethylene oxide and hydrocyanic acid  Dynel and Orlon are examples

Polyamides  Polymerization products of chemical compounds which contain amino \((-\text{NH}_2)\) and carboxyl \((-\text{COOH})\) groups  Condensation reactions between the groups form amides \((-\text{CONH}_2)\)  Nylon is an example of a polyamide

Polycarbonate  Any of various tough transparent thermoplastics characterized by high impact strength and high softening temperature

Polycythemia  An increase above the normal in the number of red cells in the blood

Polyester fiber  A manufactured fiber in which the fiber-forming substance is any long-chain synthetic polymer composed of at least 85% by weight of an ester of a dihydric alcohol and terephthalic acid  Dacron is an example

Polymer  A large molecule produced by linking together many like molecules

Polymerization  In fiber manufacture, converting a chemical monomer (simple molecule) into a fiber-forming material by joining many like molecules into a stable, long-chain structure

Polymorphic monocyte  Type of leukocyte with a multilobed nucleus

Polymorphonuclear leukocytes  Cells that represent a secondary nonspecific cellular defense mechanism  They are transported to the lungs from the bloodstream when the burden handled by the alveolar macrophages is too large

Polysaccharides  Polymers made up of sugars  An example is glycogen, which consists of repeating units of glucose

Polystyrene  A thermoplastic plastic that may be transparent, opaque, or translucent  It is light in weight, tasteless, and odorless, it also is resistant to ordinary chemicals
Polyurethane  Any of various polymers that contain NHCOO linkages and are used especially in flexible and rigid foams, elastomers, and resins

Pores of Kohn  Also known as interalveolar pores, pores between air cells  Assumed to be pathways for collateral ventilation

Precipitation  Any of the various forms of water particles that fall from the atmosphere to the ground, rain, snow, etc

Precursor  A substance from which another substance is formed, specifically, one of the anthropogenic or natural emissions or atmospheric constituents that reacts under sunlight to form secondary pollutants comprising photochemical smog

Probe  In air pollution sampling, the tube or other conduit extending into the atmosphere to be sampled, through which the sample passes to treatment, storage, and/or analytical equipment

Proline  An amino acid (C₅H₉NO₂) that can be synthesized from glutamate by animals

Promonocyte  An immature monocyte not normally seen in the circulating blood

Proteinuria  The presence of more than 0.3 gram of urinary protein in a 24-hour urine collection

Pulmonary  Relating to the lungs

Pulmonary edema  An accumulation of excessive amounts of fluid in the lungs

Pulmonary lumen  The spaces in the interior of the tubular elements of the lung (bronchioles and alveolar ducts)

Pulmonary measurements  Measurements of the volume of air moved during a normal or forced inspiration or expiration  Specific lung volume measurements are defined independently

\[
\text{Lung volume measurements} = \text{Tidal volume, inspiratory reserve volume, expiratory reserve volume, residual volume (four basic independent volumes)}
\]

\[
\text{Capacities} = \text{Combinations of basic volumes}
\]

\[
\text{Total lung capacity (TLC)} = \text{Tidal volume} + \text{inspiratory reserve volume} + \text{expiratory reserve volume} + \text{residual volume, the volume of gas in the lungs at the time of maximal inspiration or the sum of all volume compartments} \quad \text{The method of measurement should be indicated, as with residual volume}
\]
Vital capacity (VC) = Tidal volume + inspiratory reserve volume + expiratory reserve volume, the greatest volume of gas that can be expelled by voluntary effort after maximal inspiration. Also forced vital capacity and forced inspiratory vital capacity.

Functional residual capacity (FRC) = Residual volume + expiratory reserve volume, the volume of gas remaining in the lungs at the resting, end-tidal expiratory position. Equivalent to the sum of residual volume and expiratory reserve volume. The method of measurement should be indicated as with residual volume.

Inspiratory capacity (IC) = Tidal volume + inspiratory reserve volume.

Inspiratory vital capacity (IVC) = The maximal volume that can be inspired from the resting end-expiratory position, also forced inspiratory vital capacity.

Expiratory reserve volume (ERV) = The maximal volume that can be exhaled from the resting end-tidal expiratory position. See also Functional residual capacity.

Residual volume (RV) = That volume of air remaining in the lungs after maximal exhalation. The method of measurement should be indicated in the text or, when necessary, by appropriate qualifying symbols.

Residual volume to total lung capacity ratio (RV/TLC) = A ratio that expresses the percentage of the total lung capacity occupied by residual volume, varies somewhat with age, but ordinarily should be no more than 20 to 30%.

Tidal volume = That volume of air inhaled or exhaled with each breath during quiet breathing, used only to indicate a subdivision of lung volume. When tidal volume is used in gas exchange formulations, the symbol $V_T$ should be used.

Minute ventilation (MV) = The volume of gas exchanged per minute at rest or during any stated activity, it is the tidal volume times the number of respirations per minute. See ventilation.

Pulmonary resistance = Sum of airway resistance and viscous tissue resistance.

Purine bases = Organic bases that are constituents of DNA and RNA, including adenine and guanine.

Purulent = Containing or forming pus.

Pyrimidine bases = Organic bases found in DNA and RNA. Cytosine and thymine occur in DNA and cytosine and uracil are found in RNA.

QRS: Graphical representation on the electrocardiogram of a complex of three distinct waves that represent the beginning of ventricular contraction.
Quasistatic compliance  Time dependent component of elasticity, compliance is the reciprocal of elasticity

Rain out  Removal of particles and/or gases from the atmosphere by their involvement in cloud formation (particles act as condensation nuclei, gases are absorbed by cloud droplets), with subsequent precipitation

Rayleigh scattering  Coherent scattering in which the intensity of the light of wavelength $\lambda$ scattered in any direction, making an angle with the incident direction, is directly proportional to $1 + \cos^2 \theta$ and inversely proportional to $\lambda^4$

Reactive dyes  Dyes that react chemically with cellulose in fibers under alkaline conditions. Also called fiber reactive or chemically reactive dyes

Reduction  Acceptance of electrons by an ion or molecule

Reference method (RM)  For nitrogen dioxide, an EPA-approved gas-phase chemiluminescent analyzer and associated calibration techniques, regulatory specifications are described in Title 40, Code of Federal Regulations, Part 50, Appendix F. Formerly, Federal Reference Method

Residual capacity  The volume of air remaining in the lungs after a maximum expiratory effort, same as residual volume

Residual volume (RV):  The volume of air remaining in the lungs after a maximal expiration. The residual volume is equal to the total lung capacity minus the vital capacity

Resin  Any of various solid or semisolid amorphous natural organic substances, usually derived from plant secretions, which are soluble in organic solvents but not in water, also any of many synthetic substances with similar properties used in finishing fabrics, for permanent press shrinkage control or water repellency

Resistance flow (R)  The ratio of the flow-resistive components of pressure to simultaneous flow (in centimeters of water per liter per second). Flow-resistive components of pressure are obtained by subtracting any elastic or inertial components, proportional respectively to volume and volume acceleration. Most flow resistances in the respiratory system are nonlinear, varying with the magnitude and direction of flow, with lung volume and lung volume history, and possibly with volume acceleration. Accordingly, careful specification of the conditions of measurement is necessary, see airway resistance and total pulmonary resistance

Ribosomal RNA  The most abundant RNA in a cell and an integral constituent of ribosomes

Ribosomes  Discrete units of RNA and protein that are instrumental in the synthesis of proteins in a cell. Aggregates are called polysomes

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Runoff: Water from precipitation, irrigation, or other sources that flows over the ground surface to streams

Sclerosis Pathological hardening of tissue, especially from overgrowth of fibrous tissue or increase in interstitial tissue

Secondary particles (or secondary aerosols) Dispersion aerosols that form in the atmosphere as a result of chemical reactions, often involving gases

Selective leaching The removal of one element from a solid alloy by corrosion processes

Septa. A thin wall dividing two cavities or masses of softer tissue

Seromucoid Pertaining to a mixture of watery and mucinous material such as that of certain glands

Serum antiprotease A substance, present in serum, that inhibits the activity of proteinases (enzymes that destroy proteins)

Sigma (s) bonds. Bonds in which electron density is symmetrical about a line joining the bonded atoms

Silo-filler’s disease Pulmonary lesion produced by oxides of nitrogen produced by fresh silage

Single breath nitrogen elimination rate Percentage rise in nitrogen fraction per unit of volume expired

Single breath nitrogen technique A procedure in which a vital capacity inspiration of 100% oxygen is followed by examination of nitrogen in the vital capacity expired

Singlet state The highly reactive energy state of an atom in which certain electrons have unpaired spins

Sink: A reactant with or absorber of a substance

Sodium arsinite (Na$_3$AsO$_3$) A compound used with sodium hydroxide in the absorbing solution of a 24-hour integrated manual method for nitrogen dioxide

Sodium dithionite A strong reducing agent (a supplier of electrons)

Sodium metabisulfite (Na$_2$S$_2$O$_5$) A compound used in absorbing solutions of nitrogen dioxide analysis methods

Sorb. To take up and hold by absorption or adsorption

Sorbent A substance that takes up and holds another by absorption or adsorption
Sorbitol dehydrogenase  An enzyme that interconverts the sugars sorbitol and fructose

Sorption  The process of being sorbed

Spandex  A manufactured fiber in which the fiber-forming substance is a long chain synthetic elastomer composed of at least 85% of a segmented polyurethane

Specific airway conductance ($SG_{aw}$)  Airway conductance divided by the lung volume at which it was measured, that is, normalized airway conductance  Airway conductance ($G_{aw}$)/thoracic gas volume (TGV)

Specific airway resistance ($SR_{aw}$)  Airway resistance multiplied by the volume at which it was measured  $SR_{aw} = \text{airway resistance} (R_{aw}) \times \text{thoracic gas volume} (TGV)$, liter (L) $\times$ centimeter of water per liter per second (cm H$_2$O/L/s)

Spectrometer  An instrument used to measure radiation spectra or to determine wavelengths of the various radiations

Spectrophotometry  A technique in which visible, ultraviolet, or infrared radiation is passed through a substance or solution and the intensity of light transmitted at various wavelengths is measured to determine the spectrum of light absorbed

Spectroscopy  Use of the spectrometer to determine concentrations of an air pollutant

Spermatocytes  A cell destined to give rise to spermatozoa (sperm)

Sphingomyelins  A group of phospholipids found in brain, spinal cord, kidney, and egg yolk

Sphygmomanometer  An apparatus, consisting of a cuff and a pressure gauge, that is used to measure blood pressure

Spirometer  A mechanical device, including bellows or other sealed, moving parts, that collects and stores gases and provides a graphical record of lung volume changes over time  See breathing pattern and respiratory cycle

Spirometry  The measurement, by a form of gas meter (spirometer), of volumes of air that can be moved in and out of the lungs

Spleen  A large vascular organ located on the upper left side of the abdominal cavity It is a blood-forming organ in early life It is a storage organ for red corpuscles and because of the large number of macrophages, acts as a blood filter

Sputum  Expectorated matter, especially mucus or mucopurulent matter expectorated as a result of diseases of the air passages

Squamous  Scale-like, scaly
Standard deviation

Measure of the dispersion of values about a mean value. It is calculated as the positive square root of the average of the squares of the individual deviations from the mean.

Standard temperature and pressure

0 °C, 760 millimeters of mercury.

Standard temperature and pressure, dry (STPD) conditions

These are the conditions of a volume of gas at 0 °C and 760 torr, without water vapor. An STPD volume of a given gas contains a known number of moles of that gas.

Staphylococcus aureus

A spherically shaped, infectious species of bacteria found especially on nasal mucous membrane and skin.

Static lung compliance ($C_{\text{Lstat}}$)

Measure of lung’s elastic recoil (volume change resulting from change in pressure) with no or insignificant airflow.

Steady state exposure

Exposure to air pollutants whose concentration remains constant for a period of time.

Steroids

A large family of chemical substances comprising many hormones and vitamins and having large ring structures.

Stilbene

An aromatic hydrocarbon ($C_{14}H_{12}$) used as a phosphor and in making dyes.

Stoichiometric factor

Used to express the conversion efficiency of a nonquantitative reaction, such as the reaction of nitrogen dioxide with azo dyes in air monitoring methods.

Stoma

A minute opening or pore (plural is stomata).

Stratosphere

That region of the atmosphere extending from 11 kilometers above the surface of the earth to 50 kilometers. At 50 kilometers above the earth, temperature rises to a maximum of 0 °C.

Streptococcus pyogenes

A species of bacteria found in the human mouth, throat, and respiratory tract and in inflammatory exudates, blood stream, and lesions in human diseases. It causes formation of pus or even fatal septicemias.

Stress corrosion cracking

Cracking caused by simultaneous presence of tensile stress and a specific corrosive medium. The metal or alloy is virtually unattached over most of its surface, while fine cracks progress through it.

Strong interactions

Forces or bond energies holding molecules together. Thermal energy will not disrupt the formed bonds.

Sublobular hepatic necrosis

The pathologic death of one or more cells, or of a portion of the liver, beneath one or more lobes.
Succession  The progressive natural development of vegetation towards a climax, during which one community is gradually replaced by others

Succinate  A salt of succinic acid involved in energy production in the citric acid cycle

Sulfadiazine  One of a group of sulfa drugs Highly effective against pneumococcal, staphylococcal, and streptococcal infections

Sulfadiazine  An antibacterial agent of the sulfonamide group, active against homolytic streptococci, staphylococci, pneumococci, and meningococci

Sulfanilamide  A crystalline sulfonamide (C₆H₈N₂O₂S), the amide of sulfanilic acid and parent compound of most sulfa drugs

Sulfhydryl group  A chemical radical consisting of sulfur and hydrogen (-SH) that confers reducing potential to the chemical compound to which it is attached

Sulfur dioxide (SO₂)  Colorless gas with pungent odor released primarily from burning of fossil fuels, such as coal, containing sulfur

Sulfur dyes  Used only on vegetable fibers, such as cottons They are insoluble in water and must be converted chemically in order to be soluble They are resistant (fast) to alkalies and washing and fairly fast to sunlight

Supernatant  The clear or partially clear liquid layer that separates from the homogenate upon centrifugation or standing

Surfactant  A substance capable of altering the physiochemical nature of surfaces, such as one used to reduce surface tension of a liquid

Symbiotic  A close association between two organisms of different species in which at least one of the two benefits

Synergistic  A relationship in which the combined action or effect of two or more components is greater than that of the components acting separately.

Systolic  Relating to the rhythmical contraction of the heart

Tachypnea  Very rapid breathing

Teragram (Tg)  One million metric tons, 10¹² grams

Teratogenesis  The disturbed growth processes resulting in a deformed fetus

Teratogenic  Causing or relating to abnormal development of the fetus

Threshold  The level at which a physiological or psychological effect begins to be produced
Thylakoid: A membranous lamella of protein and lipid in plant chloroplasts where the photochemical reactions of photosynthesis take place.

Thymidine: A nucleoside (C\textsubscript{10}H\textsubscript{14}N\textsubscript{2}O\textsubscript{5}) that is composed of thymine and deoxyribose, occurs as a structural part of DNA.

Tidal volume (V\textsubscript{T}). The volume of air that is inspired or expired in a single breath during regular breathing.

Titer: The standard of strength of a volumetric test solution. For example, the titration of a volume of antibody-containing serum with another volume containing virus.

Tocopherol: \(\alpha\)-\(d\)-Tocopherol is one form of Vitamin E prepared synthetically. The \(\alpha\) form exhibits the most biological activity. It is an antioxidant and retards rancidity of fats.

Torr: A unit of pressure sufficient to support a 1-millimeter column of mercury, 760 torr = 1 atmosphere.

Total lung capacity (TLC): The sum of all the compartments of the lung, or the volume of air in the lungs at maximum inspiration.

Total pulmonary resistance (R\textsubscript{L}): Resistance measured by relating flow-dependent transpulmonary pressure to airflow at the mouth. Represents the total (frictional) resistance of the lung tissue (R\textsubscript{t}) and the airways (R\textsubscript{aw}), \(R\textsubscript{L} = R\textsubscript{aw} + R\textsubscript{t}\).

Total suspended particulates (TSP): Solid and liquid particles present in the atmosphere.

Trachea: Commonly known as the windpipe, a cartilaginous air tube extending from the larynx (voice box) into the thorax (chest), where it divides, serving as the entrance to each of the lungs.

Tracheobronchial region: The area encompassed by the trachea to the gas exchange region of the lung; the conducting airways.

Transaminase: Aminotransferase, an enzyme transferring an amino group from an \(\alpha\)-amino acid to the carbonyl carbon atom of an \(\alpha\)-keto acid.

Transmissivity (UV): The percent of ultraviolet radiation passing through a medium.

Transmittance: The fraction of the radiant energy entering an absorbing layer that reaches the layer's further boundary.

Transpiration: The process of the loss of water vapor from plants.

Triethanolamine: An amine ([HOCH\textsubscript{2}CH\textsubscript{2}]\textsubscript{3}N) used in the absorbing solution of one analytical method for nitrogen dioxide.
Troposphere  That portion of the atmosphere in which temperature decreases rapidly with altitude, clouds form, and mixing of air masses by convection takes place. Generally extends to about 7 to 10 miles above the earth's surface.

Type 1 cells  Thin, alveolar surface, epithelial cells across which gas exchange occurs.

Type 2 cells  Thicker, alveolar surface, epithelial cells that produce surfactant and serve as progenitor cells for Type 1 cell replacement.

Ultraviolet light  Invisible to the human eye of wavelengths between $4 \times 10^{-7}$ and $5 \times 10^{-7}$ meter (4,000 to 50 Ångstroms).

Urea-formaldehyde resin  A compound composed of urea and formaldehyde in an arrangement that conveys thermosetting properties.

Urobilinogen  One of the products of destruction of blood cells, found in the liver, intestines, and urine.

Uterus  The womb, the hollow muscular organ in which the impregnated ovum (egg) develops into the fetus.

Vacuole  A minute space in any tissue.

Vagal  Refers to the vagus nerve. This mixed nerve arises near the medulla oblongata and passes down from the cranial cavity to supply the larynx, lungs, heart, esophagus, stomach, and most of the abdominal viscera.

Valence  The number of electrons capable of being bonded or donated by an atom during bonding.

Van Slyke reactions  Reaction of primary amines, including amino acids, with nitrous acid, yielding molecular nitrogen.

Variance  A measure of dispersion or variation of a sample from its expected value, it is usually calculated as the square root of a sum of squared deviations about a mean divided by the sample size.

Vat dyes  Dyes that have a high degree of resistance to fading by light, nitrogen oxides, and washing. Widely used on cotton and viscose rayon. Colors are brilliant and of almost any shade. The name was originally derived from their application in a vat.

Venezuelan equine encephalomyelitis  A form of equine encephalomyelitis found in parts of South America, Panama, Trinidad, and the United States, and caused by a virus. Fever, diarrhea, and depression are common. In humans, there is fever and severe headache after an incubation period of 2 to 5 days.
Ventilation  Physiological process by which gas is exchanged between the outside air and the lungs  The word ventilation sometimes designates ventilatory flow rate (or ventilatory minute volume), which is the product of the tidal volume multiplied by the ventilatory frequency  Conditions are usually indicated as modifiers, for example

\[ \dot{V}_E = \text{Expired volume per minute (liters per minute, body temperature and pressure, saturated [BTPS])} \]

\[ \dot{V}_I = \text{Inspired volume per minute (liters per minute, BTPS)} \]

Ventilation is often referred to as "total ventilation" to distinguish it from "alveolar ventilation" (see ventilation, alveolar)

Ventilation, alveolar \((V_A)\)  The portion of the total ventilation that is involved in gas exchange with the blood, alveolar ventilation is less than total ventilation because when a tidal volume of gas leaves the alveolar spaces, the last part does not get expelled from the body but occupies the dead space, to be respired with the next inspiration  Thus, the volume of alveolar gas actually expelled completely is equal to the tidal volume minus the volume of the dead space  Thus truly complete expiration volume times the ventilatory frequency constitutes the alveolar ventilation

Ventilation, dead-space \((V_D)\)  Ventilation per minute of the physiologic dead space (volume of gas not involved in gas exchange with the blood), at body temperature and pressure, saturated conditions, defined by the following equation

\[ \dot{V}_D = V_E(PaCO_2 - P_ECO_2)/(PaCO_2 - P_lCO_2) \]

Ventilation/perfusion ratio \((V_A/Q)\)  Ratio of the alveolar ventilation to the blood perfusion volume flow through the pulmonary parenchyma, such as pulmonary blood flow or right heart cardia output, this ratio is a fundamental determinant of the oxygen and carbon dioxide pressure of the alveolar gas and of the end-capillary blood  Throughout the lungs, the local ventilation/perfusion ratios vary, and, consequently, the local alveolar gas and end-capillary blood compositions also vary

Villus  A projection from the surface, especially of a mucous membrane

Vinyl chloride  A gaseous chemical suspected of causing at least one type of cancer  It is used primarily in the manufacture of polyvinyl chloride, a plastic

Viscose rayon  Filaments of regenerated cellulose coagulated from a solution of cellulose xanthate  Raw materials can be cotton linters or chips of spruce, pine, or hemlock

Visible region  Light between the wavelengths of 4,000 and 8,000 Ångstroms

Visual range  The distance at which an object can be distinguished from background
Vital capacity (VC)  The greatest volume of air that can be exhaled from the lungs after a maximum inspiration (see Pulmonary measurements)

Vitamin E  Any of several fat-soluble vitamins (tocopherols) essential in nutrition of various vertebrates

Washout  The capture of gases and particles by falling raindrops

Weak interactions  Forces, electrostatic in nature, that bind atoms and/or molecules to each other  Thermal energy will disrupt the interaction  Also called Van der Waal’s forces

Weathering  In this context, weathering refers to the releases of base cations from soil minerals to cationic forms, which can be taken up by plants, leached, or absorbed to cation-exchange sites

Wet deposition  The process by which atmospheric substances are returned to earth in the form of rain or other precipitation

Wheat germ lipase  An enzyme, obtained from wheat germ, that is capable of cleaving a fatty acid from a neutral fat, a lipolytic enzyme

X-ray fluorescence spectrometry  A nondestructive technique that utilizes the principle that every element emits characteristic x-ray emissions when excited by high-energy radiation

Zeolites  Hydrous silicates analogous to feldspars, occurring in lavas and various soils

Zooplankton  Minute animal life floating or swimming weakly in a body of water