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External Review Draft

EPA

Air Quality Criteria for Oxides of Nitrogen

Review Draft

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Volume III of III

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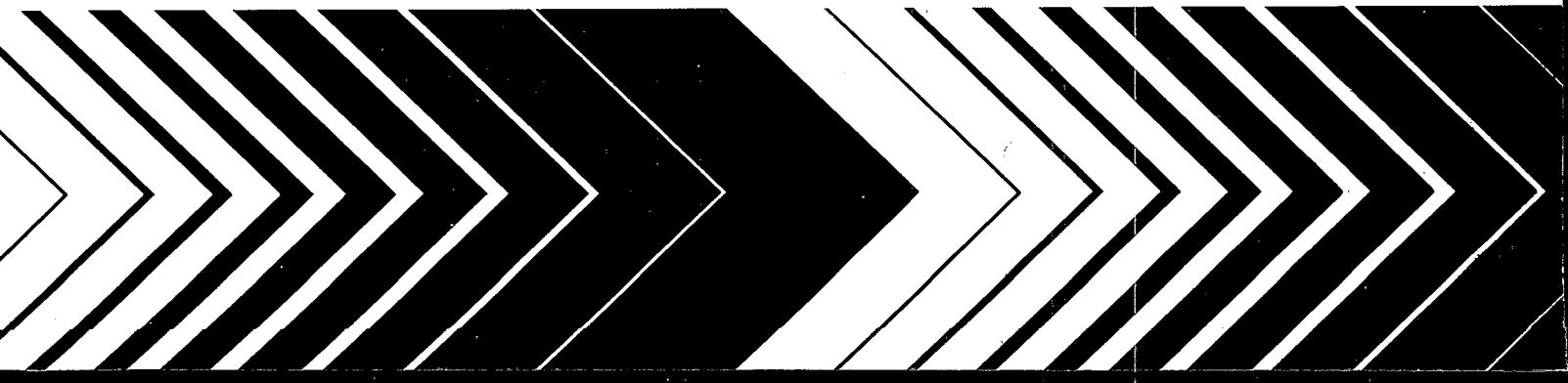

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Air Quality Criteria for Oxides of Nitrogen

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13. STUDIES OF THE EFFECTS OF NITROGEN COMPOUNDS ON ANIMALS

13.1 INTRODUCTION

This chapter discusses the effects of the oxides of nitrogen (NO_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, 1987).

Most of the data presented in this chapter relate to the effects of nitrogen dioxide (NO_2) on experimental animals because the vast majority of the NO_x literature is on NO_2. The results of the few comparative NO_x studies show that NO_2 appears to be the most toxic. The majority of the literature describes the effects of NO_2 on the respiratory tract; however, extrapulmonary effects also have been observed and are included here. A broad range of NO_2 concentrations have been evaluated, but emphasis has primarily been placed on those studies at exposure concentrations of 9,400 $\mu g/m^3$ (5 ppm) or less, with the exception of studies on dosimetry, mortality, and emphysema. Related studies demonstrating the potential of NO_2 to produce structural, functional, and biochemical changes in mammalian species at higher concentrations are incorporated in a series of tables.

Discussions of the available literature on the effects of other NO_x compounds and mixtures containing NO_2 also are 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 measuring or estimating 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 to be transformed to dose-response relationships. However, to 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 make up, metabolic rates, detoxification systems, and/or other factors. At present, our knowledge of dosimetry is more advanced than that of species sensitivity, inhibiting quantitative animal-to-human extrapolation of effective NO_2 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 NO_2, 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 inter- or intraspecies dosimetric extrapolation, a further confounding aspect 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 carried out to obtain direct measurements of absorbed NO_2 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 NO_2 occur (see Section 13.2.3.4), is extremely difficult, and may not be possible in the near future due to 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 a gas acting at a given site in the lung is related to the airway luminal or airspace concentration at that site. As a result, NO_2 does not immediately interact with cellular components of the respiratory tract. Instead, NO_2 first comes into contact with the liquid or fluid that lines the cells or tissues of the respiratory tract (mucus plus periciliary fluid in the upper respiratory tract and tracheobronchial regions; surfactant film plus serous fluid layer 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 is 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.

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 capilliary 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 physiological processes and structural variations occur between species, laboratory animals and humans are very similar. So much so that dosimetry models can be and are 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, since 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 is very little 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 a gas similar to NO_2, namely 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 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 the transport of gases 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 process. 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).

For the NO_2 dosimetry investigation of Miller et al. (1982), reactions were modeled as instantaneous and only in the liquid lining. (The NO_2 concentration at the air-liquid lining interface was set to zero, and the tissue dose was defined in terms of the flux of NO_2 to the tissue at the interface.) Mucociliary processes have been considered too slow to be effective and are usually ignored. Because NO_2 is highly reactive, only a small fraction of the NO_2 would be assumed to enter the capilliary blood, where it would not survive to be recirculated to the lung. Thus, the blood compartment would be a sink for NO_2. However, if the products of chemical reactions and their distribution in the rest of the body are important, as is possible with NO_2, then relevant systemic processes would have to be taken into account.

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 location. 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

Morphological studies on animals suggest 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 77,080 $\mu g/m^3$ (4 to 41 ppm). The upper respiratory tract of the two species was observed to remove 42.1% (standard deviation = 14.9%) of the NO_2 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 $\mu g/m^3$; 40.4 ppm NO_2) 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 since a MANOVA, and not an ANOVA, test should have been used to analyze the data. Kleinman and Mautz (1987) exposed dogs to 1,880 or 9,400 $\mu g/m^3$ (1 or 5 ppm) NO_2 in air at 85% relative humidity. Uptake was measured only during inhalation. They found that more NO_2 was absorbed in the upper respiratory tract of dogs with nasal breathing than with oral breathing. They also found that the percent uptake of NO_2 by the upper respiratory tract of dogs decreased with increasing ventilation rates (during exercise for both nasal and oral breathing). Specifically, nasal uptakes ranged from approximately 85% to less than 80% and oral breathing ranged from about 60% to approximately 45% as the ventilation increased from resting values to four times this value. The negative correlation between percent uptake and ventilation is what would be expected based on the work of Aharonson et al. (1974).

Lower Respiratory Tract Absorption

Corn et al. (1976) estimated the average mass transfer coefficient (K) of NO_2 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 NO_2 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 Corn et al. (1976) value of K to theoretical values inferred from Overton et al. (1987) for O_3 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 $\mu g/m^3$ (5 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 $\mu g/m^3$ (4 to 20 ppm) and the minute volume 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 (1987) also measured lower respiratory tract uptake. They found that at ventilatory parameters corresponding to rest about 85% of the inhaled NO_2 entering the lungs was absorbed by this region; this increased to 100% with high ventilation rates.

Results of simulating the uptake of NO_2 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 chemistry of reaction 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 NO_2 is absorbed throughout the lower respiratory tract, but the major dose to tissue would be delivered in the centriacinar 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 13.2.3.4). Beyond this region there is a rapid fall in the NO_2 dose delivered to tissue. Depending on the tracheal concentration and tidal volume, the model predicted that 75 to 95% of the NO_2 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 NO_2 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 NO/NO_2 mixture containing 550 to 13,500 $\mu g/m^3$ (0.29 to 7.2 ppm) NO_2 for brief (but unspecified) periods. Of the inhaled NO_2, 81 to 90% was absorbed during normal respiration; this increased to 91 to 92% with maximal ventilation. For 30 min Bauer et al. (1984) exposed adult asthmatics to 560 $\mu g/m^3$ (0.3 ppm) NO_2. The exposed subjects inhaled NO_2 by mouthpiece for 20 min at rest, then exercised for 10 min on a bicycle ergometer. The inspired and expired NO_2 concentrations were measured showing that at rest the average uptake was 73%; whereas, during exercise the average uptake was 88%, a statistically significant increase. The effects of NO_2 exposure on pulmonary function in humans following this exposure regime are reported in Chapter 15.

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 NO_2 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 NO_2 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 NO_2 to the lower respiratory tract because the percent of NO_2 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 the assumption 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 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 O_3, a reactive gas whose uptake processes are similar to those of NO_2. 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 O_3 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 bronchiole 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 other hand, the maximal increase in mass absorbed by the tracheobronchial region which was by a factor of 1.4 as compared to the increase of absorbed O_3 in the pulmonary region which was by a factor greater than 13 (1.5 times the ratio of exercise to rest minute volumes). The simulation results for rats (Overton et al., 1987) are in agreement with these predictions. Since the upper respiratory tract delivers a greater proportion of inhaled NO_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_2 are reasonable.

Systemic Dosimetry

Once deposited, NO_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_2 entered the bloodstream based upon estimates of radiolabelled nitrate (NO_3^-) and nitrite (NO_2^-) levels in the blood and urine of rabbits following exposure to 45,120 $\mu g/m^3$ (24 ppm) NO_2 for 4 h. The initial transformation products following NO_2 absorption have been the subject of some speculation, however.

The distribution of $^{13}NO_2$ (560 to 1,710 $\mu g/m^3$ [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_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 (HNO_2) and nitric acid (HNO_3); the authors suggested that the acids were responsible for subsequent toxic effects. Based upon the absorption of NO_2 by isolated ventilated perfused rat lungs, Postlethwait and Mustafa (1981) proposed that the main reaction of NO_2 was not with lung water but with readily oxidizable tissue components (e.g., proteins and lipids) to produce NO_2^-. These investigators found that 70% of absorbed NO_2 appeared as NO_2^- in perfusate and lung tissue, and that the concentration of NO_2^- produced increased with time during exposure. They also hypothesized that NO_2^- in the blood may then be oxidized to NO_3^- by interaction with hemoglobin in red blood cells. Saul and Archer (1983) provided support for this pathway using an in vivo system in which rats were exposed for 24 h to NO_2 at 2,260 to 16,540 $\mu g/m^3$ (1.2 to 8.8 ppm). They also concluded that the main reaction pathway of absorption in the lungs was the reaction of NO_2 with oxidizable tissue components to produce NO_2^-. This product may then serve as a precursor for other chemical reactions at extrarespiratory sites.

The current data base indicates that once NO_2 is absorbed in lung fluids, the subsequent reaction products are rapidly taken up and then translocated via the bloodstream. For example, intratracheally instilled NO_2^- 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 20,870 to 112,610 $\mu g/m^3$ (11.1 to 59.9 ppm) $^{15}NO_2$ for 0.5 to 53.9 h, found increased levels of labelled nitrogen in the lungs, kidneys, plasma, and urine, as well as an increased level of NO_2^- in plasma. In a later study, Oda et al. (1981) noted a dose-dependent increase in both

NO_2^- and NO_3^- levels in the blood of mice during 1-h exposures to 9,400 to 75,200 $\mu g/m^3$ (5 to 40 ppm) NO_2. The blood levels of NO_2^- and NO_3^- declined rapidly after exposures ended, with the decay half-times of a few minutes for NO_2^- and about 1 h for NO_3^-. The shorter time for the former was ascribed to its rapid reaction (oxidation) with hemoglobin, producing NO_3^- and methemoglobin (Oda et al., 1981; Kosaka et al., 1979; Case et al., 1979), although such measurements were not made. Free NO_3^- in blood is generally excreted in urine (Green and Hiatt, 1954; Hawksworth and Hill, 1971).

Summary

The important physical, chemical, and biological factors involved in the uptake of NO_2 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 NO_2 uptake for extrapolation purposes. With respect to dosimetry, the following has been observed. Total respiratory tract uptake in humans ranged from 73 to 92% depending on the investigation and the breathing state. The percent total uptake was found to increase with increasing exercise level. Upper respiratory tract uptakes have been measured in dogs, rabbits, and rats. Uptake values ranged from as low as 25% to as high as 85% depending on the study, species, air flow 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, uptake values of 36 and 65% have been reported for isolated, ventilated, perfused rat lungs. Experimental evidence indicated that NO_2 chemically reacted with lung tissue, but did not penetrate directly to blood. However, the reaction products, NO_2^- and NO_3^-, were found throughout the body of experimental animals.

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. 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. 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 Mortality

Studies with various species of experimental animals (Table 13-1) have shown that single exposures to NO_2 for ≤24 h are lethal only at exposure concentrations far exceeding those routinely found in ambient air (i.e., >37.6 mg/m^3 [20 ppm]). The acute lethal effect of NO_2 is due primarily to pulmonary edema; however, laryngeal spasm has been reported in guinea pigs. Delayed deaths are often the result of bronchiolitis and pneumonia. Continuous or repeated exposures to NO_2 result in little or no mortality in most species, with the possible exception of the guinea pig, until concentrations exceed ~18.8 mg/m^3 (10 ppm) (Table 13-2). Furthermore, the data base suggests that brief exposures to high concentrations are more lethal than equivalent exposures to lower levels for longer periods of time.

There is evidence for differences in sensitivity between animal species. This is seen in the series of acute exposures reported by Steadman et al. (1966), and summarized in Table 13-1. Species sensitivity was also mathematically assessed by Book (1982), who used the data of Hine et al. (1970) and Steadman et al. (1966) to calculate estimated concentrations (LC_{50}) of NO_2 which would result in 50% lethality after a 1-h exposure; the values obtained were 186.12 mg/m^3 (99 ppm) for mice, 206.8 mg/m^3 (110 ppm) for rats, 171.08 mg/m^3 (91 ppm) for guinea pigs, 263.2 mg/m^3 (140 ppm) for rabbits, and 244.4 mg/m^3 (130 ppm) for dogs.

There are, however, reported variations in interspecies and strain sensitivity to NO_2 exposure (e.g., different LC_{50} values for similar exposures). These may be due to a number of factors, which include differences in age, sex, or strain of animals, genetic differences within different strains of animals, differences in analytical procedures for measuring NO_2 and even failure to distinguish between NO_2 and mixed oxides of nitrogen in some studies, and differences in environmental conditions (e.g., temperature, humidity, and diet). The influence of diet (e.g., Vitamin E) on NO_2 toxicity is discussed more fully in the section on lung biochemistry (Section 13.2.3.2).

TABLE 13-1. MORTALITY FROM ACUTE (≤ 24 h) EXPOSURE TO NO_2

NO_2 Concentration		Exposure		Mortality Due		
mg/m^3	ppm	(min)	Species	to Exposure	Remarks	Reference
≤940	≤500	5	Rat	LC_{50} = 416 ppm	Death occurred from 0.5 h to 3 days after exposure.	Carson et al. (1962)
		15		LC_{50} = 201 ppm		
		30		LC_{50} = 162 ppm		
		60		LC_{50} = 115 ppm		
		15	Rabbit	LC_{50} = 315 ppm	--	
56.4-1,880	30-1,000	Various	Cat Rabbit Guinea pig Mouse Rat	30 ppm for 3 h: no mortality; 100 ppm: death after 318 min exposure.	--	LaTowsky et al. (1941)
141	75	360	Rat	100%		Crapo et al. (1978)
multiple exposures		2-240	Rat	2 min LC_{50} = 1,445 ppm*	Most deaths occurred within 24 h after exposure.	Gray et al. (1954)
9.4-470	5-250	5-1,440	Rat Mouse Guinea pig Rabbit Dog	0% at ≤20 ppm for up to 1,440 min; 40 ppm for 480 min: mortality only in guinea pigs (33%).	--	Hine et al. (1970)
123	65.3	480	Monkey Rat Guinea pig Dog Rabbit	100% 40% 87% 100% 33%	All deaths in monkeys occurred by 6.5 h after exposure; all others by 72 h after exposure.	Steadman et al. (1966)

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TABLE 13-1 (cont'd). MORTALITY FROM ACUTE (≤ 24 h) EXPOSURE TO NO_2

NO_2 Concentration		Exposure (min)	Species	Mortality Due to Exposure	Remarks	Reference
mg/m^3	ppm					
94	50	480	Rat (adult)	50%	Deaths by end of exposure.	Stephens et al. (1982)
			Rat (juvenile)	0%	--	
188-1,800	100-1,000	30	Mouse	LC_{50} = 250-500 ppm	--	Boren (1964)

*Calculated LC_{50} value.

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TABLE 13-2. MORTALITY FROM REPEATED OR CHRONIC EXPOSURE TO NO_2

NO_2 Concentration						
mg/m^3	ppm	Exposure	Species	Mortality Due to Exposure	Remarks	Reference
23.5	12.5	Continuous	Rat	11% at 213 days	--	Freeman and Haydon (1964)
47	25	Continuous		89% between 121-180 days of exposure		
94	50	Continuous		78% between 48-68 days of exposure		
188	100	Continuous		100% within 24 h		
1.88 9.4	1 5	6 h/day, 5 days/wk, 18 mo	Dog	0%	--	Wagner et al. (1965)
1.88 9.4 47	1 5 25	6 h/day, 5 days/wk, up to 18 mo	Rat Guinea pig Rabbit Hamster	0%	--	Wagner et al. (1965)
9.4	5	6 h/day, 5 days/wk, 15 mo	Mouse	0%	--	Wagner et al. (1965)
67	35.6	8 h/day, 5 days/wk, 6 wk	Rat Guinea pig Rabbit Dog Monkey	27% 53% 67% 0% 100%	All deaths occurred between Days 2-44 except monkey; all deaths on Day 1 for monkey.	Steadman et al. (1966)
0.9 9.2 21.4	0.48 4.9 11.4	24 h/day, 90 days	Rat Guinea pig Dog Monkey	0-53% 0-93% 0% 0-33%	--	Steadman et al. (1966)

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TABLE 13-2 (cont'd). MORTALITY FROM REPEATED OR CHRONIC EXPOSURE TO NO_2

NO_2 Concentration						
mg/m^3	ppm	Exposure	Species	Mortality Due to Exposure	Remarks	Reference
7.5	4	Continuous, 112 days	Rat	0%	--	Haydon et al. (1965)
22.7	12	Continuous, 815 days	Rat	0%	--	Haydon et al. (1965)
3.8	2	Continuous, >700 days (lifetime)	Rat	0%	--	Freeman et al. (1968c)
1.5	0.8	Continuous, >900 days (lifetime)	Rat	0%	--	Freeman et al. (1966)
9.4	5	4 h/day-7.5 h/day, 5 days/wk, 6 mo	Guinea pig	0%	--	Balchum et al. (1965)
47	25	3 days	Rat	0%	--	Stephens et al. (1982)
3.8 18.8	2 10	3 days	Rat Guinea pig	0% 67%	--	Azoulay-Dupuis et al. (1983)

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13.2.3 Respiratory Effects

13.2.3.1 Host Defense Mechanisms

The respiratory tract defenses encompass many interrelated responses; however, for simplicity, they can be divided into two major parts: the physical defense mechanisms and the cellular defense mechanisms. The first part, the physical defense mechanisms, includes structural and physicochemical adaptations in the proximal upper and lower airways and their lining layers. The pulmonary defense mechanisms (mucociliary system) in this part begins 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 μm). 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 or coughing or both (Breeze and Wheeldon, 1977; Green, 1970; Newhouse et al., 1976; Proctor, 1977). The majority of airways have an epithelial lining of ciliated columnar cells and mucus-secreting goblet cells and mucous glands. The number of mucus-secreting cells increases in disease states or after inhalation of irritants, while the number of ciliated cells decreases. Ciliary movement directs overlying mucus, particles, and absorbed gases toward the pharynx where they are swallowed or expectorated. The trapping of potential pathogens on the mucous layer followed by the ciliary escalator action and expulsion from the airways is the lung's first line of defense.

The second part 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

digestion of particles and 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 (MPO) stored in the primary granules and, therefore, are an important source of $OH^{\bullet}$ 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 which 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 (IgG, IgA, IgM) into the blood and body fluids. Secretory immunoglobulin A (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 (IgG) is the predominant class of immunoglobulins of the lower respiratory tract. These immunoglobulins act to enhance macrophage functioning. Immunoglobulin M (IgM) 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. There is little replacement of T cells

in the adult human. 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 (PAF). The interferons 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. Interferons are protein substances produced by virus-invaded cells that prevent the replication of the virus (Lefkowitz et al., 1984; Lefkowitz et al., 1983). It was later determined to be a cytokine (IL6) (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 NO_2 exposure, their proper functioning is essential in humoral and cell-mediated immunity against viral disease. These functions include: (a) interferon production in bronchoalveolar lavage fluid; (b) AM function; (c) natural killer activity; (d) cytotoxic T lymphocyte activity; and (e) antibody activity (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 natural killer activity. Interferon, AMs, and natural killer cells all contribute to the nonspecific antiviral immunity of the host. The first specific immunological activity important for defense against viral disease is cytotoxic T lymphocytes. 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 chemicophysical properties of the mucus, and the rate of mucus transport. Viral and bacterial infections and various chemicals can lead to over or under secretion of mucus and to 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 are known to result in an unwanted accumulation of cellular secretions, increased acute bacterial and viral infections, chronic bronchitis, and prolonged pulmonary complications possibly associated with the pathogenesis of chronic obstructive lung disease or bronchial cancer through the unwanted accumulation of inhaled carcinogens (Schlesinger et al., 1987). Continual loss of these ciliated cells will eventually result in replacement of the normal ciliated epithelium with squamous metaplasia of other nonciliated cell types.

Most evident are the reports of significant loss of cilia in the bronchiolar epithelium. These effects, as reported in earlier studies, were at NO_2 concentrations greatly exceeding those encountered in the environment. When lower levels of NO_2 (3,760 $\mu g/m^3$ [2 ppm]) were studied, the loss of cilia was not detected until after 6 weeks of exposure when small areas devoid of cilia became evident (Azoulay et al., 1978). The loss of cilia may be accompanied by a prolonged aberration of ciliogenesis. A description of histopathological changes of the epithelial cells of the conducting airways can be found in Section 13.2.3.4, addressing the morphological effects of NO_2 exposure. This section and Table 13-3 below review those studies that have shown that the mucociliary clearance defenses are vulnerable to the effects of NO_2.

Rombout et al. (1986), in a series of experiments covering both continuous (24 h/day) and intermittent (6 h/day) exposure regimens, reported that a loss of cilia was evident in rats after 2 days of exposure to 5,000 $\mu g/m^3$ (2.7 ppm) NO_2. No effects were observed in rats exposed to 1,000 and 2,500 $\mu g/m^3$ (0.5 and 1.3 ppm) NO_2. Reduction in number, evidence of swelling, hypertrophy, and focal hyperplasia of ciliated epithelial cells in rats exposed to

TABLE 13-3. MUCOCILIARY ACTIVITY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
560-1,880	0.3-1.0	14 days	Rabbit	No effect on tracheobronchial clearance.	Schlesinger et al. (1987)
940 1,880 7,520	0.5 1.0 4.0	7 mo	Rat	Loss of cilia and structural effects seen at 0.5 ppm; effects increased in a concentration-related manner.	Yamamoto and Takahashi (1984)
940 2,500 5,000 20,000	0.5 1.3 2.7 10.5	6 h/day or 24 h/day, 4 wk	Rat	Loss of cilia at 2.7 and 10 ppm. Short-term, intermittent exposure may be more effective than continuous exposure.	Rombout et al. (1986)
3,760	2.0	72 h	Rat	Reduction in cilia number, evidence of cellular swelling, hypertrophy, and hyperplasia.	Stephens et al. (1972)
3,760	2	6 wk	Rat	Loss of cilia.	Azoulay et al. (1978)
3,760	2	In vitro; 1.5 h/day, 5 days/wk, 14 wk	Hamster	After 2-wk exposure, ciliary beating activity was decreased and morphological changes were observed.	Schiff (1977)
9,400	5	In vitro	N/A	Decrease in ciliary beating activity.	Kita and Omichi (1974)
11,280	6	7 days/wk, 6 wk	Rat	Decrease in mucociliary velocity; functional impairment reversed by 1 wk postexposure.	Giordano and Morrow (1972)
14,100 28,200	7.5 15.0	2 h	Sheep	Slowing of mucus velocity at highest concentration.	Abraham et al. (1980)
18,000	10.0	2 h	Rabbit	No effect on bronchial clearance rate.	Schlesinger et al. (1986)

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TABLE 13-3 (cont'd). MUCOCILIARY ACTIVITY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
37,600	20	23 h/day, 28 days	Mouse	Number of cilia reduced; focal swelling of cilia shaft; effects reversible in clean air.	Ranga and Kleinerman (1981)
37,600	20	24 h	Rat Hamster	Deciliation of epithelial cells.	Foster et al. (1985)

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3,760 $\mu g/m^3$ (2.0 ppm) NO_2 for 72 h was reported by Stephens et al. (1972). The effects became more pronounced as the length of exposure increased up to 14 days, at which time the recovery process began. In another study, there was a reduction in the number of cilia and the cilia appeared swollen in rats continuously exposed to 940 $\mu g/m^3$ (0.5 ppm) NO_2 for 7 months (Yamamoto and Takahashi, 1984). When the exposure concentration was increased to 1,800 and 7,520 $\mu g/m^3$ (1.0 and 4.0 ppm), the effects became more pronounced and additional morphological changes were evident, indicating a concentration-response relationship.

Clearance of marker substances deposited in the airways has been used to assess the effects of NO_2 on mucociliary clearance. This method has merit as an index of overall efficiency of mucociliary clearance. The mucocilary clearance of inhaled tracer particles deposited into the tracheobronchial tree of rabbits exposed for 2 h/day for 14 days to 0, 560, and 1,880 $\mu g/m^3$ (0, 0.3 and 1.0 ppm) NO_2 did not alter the normal clearance rate (Schlesinger et al., 1987).

Giordano and Morrow (1972) demonstrated significant impairment of tracheobronchial clearance rates in rats following exposure to 11,280 $\mu g/m^3$ (6.0 ppm) NO_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 $\mu g/m^3$ (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).

Thus, exposures to NO_2 found to compromise this protective function are not only dependent upon the concentration of the NO_2, but the duration of the exposure. It appears that it would take prolonged exposure to higher 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_2 on isolated ciliated epithelium cells and tracheal rings. Kita and Omichi (1974) reported that exposure to NO_2 at concentrations greater than 9,400 $\mu g/m^3$ (5.0 ppm) resulted in a decreased rate of ciliary beating. Schiff (1977) isolated and exposed hamster tracheal ring cultures to 3,760 $\mu g/m^3$ (2.0 ppm) NO_2 for 1.5 h/day, 5 days/week for 1 to 4 weeks. After 14 days of exposure, the NO_2 produced decreased ciliary beating and various morphological changes.

Alveolar Macrophages

Besides altering the protective function of the epithelial cells of the conducting airways, NO_2 may also produce structural, biochemical, and functional changes in AMs. The effectiveness of AMs depends on the type, number, and viability of the cells. The cells must maintain an intact membrane, mobility, phagocytic activity, and have functioning enzyme systems. Evidence from both in vivo and in vitro studies indicate that NO_2 exposure alters many of these functions, thereby increasing the risk of the host to disease.

In Vivo Exposure. Alveolar macrophages isolated from mice continuously exposed to 3,760 $\mu g/m^3$ (2.0 ppm) NO_2 for 33 weeks or exposed to 940 $\mu g/m^3$ (0.5 ppm) NO_2 continuously with a 1-h peak to 3,760 $\mu g/m^3$ (2.0 ppm) for 5 days/week showed distinctive morphological changes as compared to controls after 21 weeks of exposure (Aranyi et al., 1976). Examples of structural changes observed included the loss of surface processes, appearance of fenestrae, bleb formation, and denuded surface areas. Continuous exposure to a lower level (i.e., 940 $\mu g/m^3$ [0.5 ppm] or 188 $\mu g/m^3$ [0.1 ppm] with a 1-h peak to 1,880 $\mu g/m^3$ [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 functional changes such as chemotaxis, phagocytosis, and bactericidal ability.

Numerous studies have shown that NO_2 exposure at concentrations greater than 9,400 $\mu g/m^3$ (5.0 ppm) increases, in a concentration-related manner, the number of AMs (Foster et al., 1985; Kleinerman et al., 1982; DeNicola et al., 1981; Sherwin et al., 1968; Busey et al., 1974; Wright et al., 1982). Alveolar macrophage accumulation has also been reported after 15 weeks exposure at lower concentrations such as to either 9,400 $\mu g/m^3$ (5.0 ppm) NO_2 or to 1,880 $\mu g/m^3$ (1.0 ppm) with two 1.5-h spikes to 9,400 $\mu g/m^3$ (5.0 ppm)/day (Gregory et al., 1983). After a 1-day exposure to 5,000 $\mu g/m^3$ (2.7 ppm) NO_2, Rombout et al. (1986) reported an increase in the number of AMs in the terminal bronchioles and proximal alveoli of rats. The accumulation of AMs was accompanied by an increase in mitosis. This accumulation of AMs reached a peak after 8 days of exposure. Two days after the cessation of this exposure, the accumulation was no longer evident. No effect was seen at 1,000 or 2,500 $\mu g/m^3$ (0.5 or 1.3 ppm).

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 ppm) NO_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. The AMs from exposed animals also exhibited increased metabolic activity as measured by the activity of glucose-6-phosphate dehydrogenase, glutathione peroxidase, and pyruvate kinase, all of 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_2 exposure significantly increased the number of AMs that could be lavaged from rats exposed to either 7,520 $\mu g/m^3$ (4 ppm) or 15,040 $\mu g/m^3$ (8 ppm). In this study the exposure continued for 10 days, but the effect became most significant between the third and fifth days 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. Schlesinger et al. (1987) 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 ppm) NO_2.

The morphometric methods used to examine for altered lung structure failed to find any changes in the number of AMs per meter squared or the average cell volume (μm^3) in rats following exposure for 23 h/day to 3,700 $\mu g/m^3$ (2.0 ppm) NO_2 for 6 weeks with two daily spikes of 0.5 h each to 11,280 $\mu g/m^3$ (6.0 ppm) NO_2 (Crapo et al., 1984). Chang et al. (1986) studied the response of 1-day-old and 6-week-old rats following exposure to 0.5 ppm (940 $\mu g/m^3$) NO_2, 23 h/day for 6 weeks. Another group of adult rats was exposed to 2.0 ppm (3,760 $\mu g/m^3$) for the same duration. Two daily 1-h spikes that were three times the baseline level (i.e., 2,820 $\mu g/m^3$ [1.5 ppm] and 11,280 $\mu g/m^3$ [6.0 ppm]) were applied Monday-Friday. In these studies, the adult rats were more sensitive to NO_2 than young 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 10.0 ppm) NO_2 than adults based on changes in lung structure and superoxide dismutase activity in isolated AMs.

There have been numerous reports demonstrating a decrease in phagocytic activity of AMs from experimental animals following exposure to NO_2 concentrations greater than 18,800 $\mu g/m^3$ (10 ppm) (Gardner et al., 1969; Acton and Myrvik, 1972; and Foster et al., 1985); however, few studies evaluated the effect of NO_2 on phagocytosis at lower exposure levels. Suzuki et al. (1986) proposed that the suppression of phagocytosis might be due to the ability of NO_2 to mediate membrane lipid peroxidation. The study by Dowell et al. (1971) adds support to this hypothesis. They reported seeing evidence of swelling of AMs exposed to 5,640 $\mu g/m^3$ (3 ppm), which became most evident when exposed to 13,160 $\mu g/m^3$ (7 ppm) NO_2. The authors described such swelling 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 phagocytic activity of rat AMs was significantly depressed after 5 days of exposure to 15,040 $\mu g/m^3$ (8 ppm) (Suzuki et al., 1986). A similar suppression was noted following exposure to 7,520 $\mu g/m^3$ (4 ppm) but only after 7 days of exposure. In all cases, the phagocytic activity of these affected cells recovered to the control value at 10 days of exposure. Lefkowitz et al. (1986) failed to find any depression in phagocytosis after mice were exposed for 7 days to 9,400 $\mu g/m^3$ (5 ppm) NO_2.

Macrophages isolated from humans exposed for 3 h to 1,120 $\mu g/m^3$ (0.6 ppm) NO_2 were less able to inactivate influenza virus than controls cells. However, when the concentration was reduced to 94 $\mu g/m^3$ (0.05 ppm) NO_2 with three 15 min spikes to 3,760 $\mu g/m^3$ (2.0 ppm), the rate of viral inactivation was similar to controls (Frampton et al., 1987; Smeglin et al., 1986).

Two independent studies have reported that NO_2 exposure significantly decreases the ability of rat AMs to produce superoxide anion radical. Oxygen radicals are believed to be essential for phagocytosis. Failure of the AM to produce this free radical anion limits the antibacterial activity of these cells, making them less able to defend the lung from infectious agents. Amoruso et al. (1981) presented evidence of such an effect at NO_2 concentrations ranging from 2,444 to 31,960 $\mu g/m^3$ (1.3 to 17.0 ppm). The duration of the NO_2 exposure was not given. In these studies, the authors' objective was to compare the measured NO_2

response with O_3 effects, and thus all data were expressed in terms of parts per million-hours (ppm-h) making it impossible to determine the specific concentration and duration of exposure that elicited this effect. A decrease of superoxide anion radical production began after exposure to 18.3 ppm-h NO_2. Production was reduced by 50% after 29.1 ppm-h and at 51 ppm-h, the highest exposure tested, the production was decreased by 85%. Similar responses were reported by Suzuki et al. (1986), who 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 $\mu g/m^3$ (4.0 or 8.0 ppm) NO_2. At the highest concentration, the effect was significant each day, but at the lower concentration the depression was significant only on exposure Days 3, 5, and 10.

Alveolar macrophages obtained by lavage from baboons exposed to 3,760 $\mu g/m^3$ (2.0 ppm) NO_2 for 8 h/day, 5 days/week for 6 months 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 was significantly depressed in rabbits following a 2 h/day exposure for 13 days to 560 $\mu g/m^3$ (0.3 ppm) but not at 1,880 $\mu g/m^3$ (1.0 ppm) (Schlesinger et al., 1987). 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 ^{85}Sr-tagged polystyrene latex spheres from the lungs of rabbits following a single 2-h exposure to 560, 1,880, 5,600, or 18,800 $\mu g/m^3$ (0.3, 1.0, 3.0, or 10.0 ppm) NO_2. An acceleration in clearance (decreased daily retention) was evident immediately after exposure to the two lowest NO_2 concentrations; at the higher levels of NO_2, an acceleration in clearance was not evident until midway through the 14-day postexposure period. Repeated exposure for 14 days for 2 h/day to 1,880 or 18,800 $\mu g/m^3$ NO_2 produced a response similar to a single exposure at the same concentration, indicating that some attenuation (tolerance) may be produced after the initial exposure providing some degree of protection during subsequent exposures.

A number of animal studies have been performed to induce various structural, functional, and chemical changes in AMs by exposing the test animals to exceedingly high

concentrations (i.e, greater than 9,400 $\mu g/m^3$ [5 ppm] NO_2). Some of these studies, along with the studies conducted at lower exposure concentrations, are listed in Table 13-4 to familiarize the reader with the broad spectrum of biological responses that AMs can exhibit following NO_2 exposure.

In Vitro Exposure. A number of investigators have utilized in vitro models to determine the direct effects of NO_2 on specific target cells. Voisin et al. (1977) have shown concentration-related effects after exposure for 30 min to NO_2 concentrations as low as 188 $\mu g/m^3$ (0.1 ppm). In this system, the AMs, attached to cellulose acetate fibers, were floated on top of a nutrient medium which diffused through the filter, maintaining cell viability without submerging the cells. These floating cells were then exposed to NO_2. In these studies, guinea pig AMs seemed to be sensitive to NO_2, exhibiting a reduction in phagocytic and normal bactericidal activity, reduced ATP content, and major morphological changes. The severity of these changes was related to the NO_2 concentration (Voisin et al., 1977). Increasing either the exposure concentration to 9,400 $\mu g/m^3$ (5.0 ppm) or the exposure duration to 24 h resulted in complete destruction of the cell (Voisin et al., 1984). Normal human AMs exposed in vitro to high levels of NO_2 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).

Humoral and Cell-Mediated Immunuity

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 NO_2 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 NO_2. 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

TABLE 13-4. ALVEOLAR MACROPHAGES

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
94 (with peak) and 1,120	0.05 with 3×15 min peaks to 2.0 ppm and 0.6	3 h	Human	AMs lost ability to inactivate influenza virus at highest concentration.	Frampton et al. (1989) Smeglin et al. (1986)
940, 188 with 3-h peak to 1,880 for 5 days/wk	0.5, 0.1 with 3-h peak to 1 for 5 days/wk	Continuous, 24 wk	Mouse	No observable effects on morphology of AMs.	Aranyi et al. (1976)
3,760 or 940 (3,760 with 1-h peak)	2.0 or 0.5 (with 1-h peak to 2.0 5 days/wk)	Continuous, 21, 28, or 37 wk	Mouse	After 21 wks of exposure, morphological changes were identified, such as loss of surface processes, appearance of fenestrae, bleb formation, and denuded surface areas.	
560 1,880	0.3 1.0	2 h/day, 13 days	Rabbit	Depressed phagocytic ability of AMs; no effect on cell number or viability; random mobility reduced at lowest concentration.	Schlesinger et al. (1987)
560 1,880 5,600 18,800	0.3 1.0 3.0 10.0	2 h/day, 1 or 14 days	Rabbit	Acceleration in clearance of particles from lung at two lowest concentrations; effects from repeated exposures to two highest concentrations equivalent to effects seen at lower concentrations.	Vollmuth et al. (1986)

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TABLE 13-4 (cont'd). ALVEOLAR MACROPHAGES

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
940 (with two peaks of 2,820); 3,760 (with two peaks of 11,280)	0.5 (with two 1-h peaks to 1.5); 2.0 (with two 1-h peaks to 6.0)	6 wk (23 h/day)	Rat	Increase in number of AMs and cell volume; adults were more sensitive than young.	Crapo et al. (1984) Crapo et al. (1986)
1,880 4,320 12,400	1.0 2.3 6.6	17 h	Mouse	Bactericidal activity significantly decreased by 6 and 35% at the two highest exposures, respectively; no effect at 1.0 ppm.	Goldstein et al. (1974)
1,880 28,200 45,120	1.0 15.0 24.0	22 h/day	Rat	Stimulated clearance of particles from lung at lowest concentration, but decreased clearance rate at two highest concentrations.	Ferin and Leach (1977)
1,880 with two 1.5 h peaks to 9,400	1.0 with two 1.5-h peaks to 5.0	7 h/day, 5 days/wk for 15 wk	Rat	Accumulation of AMs. Superimposed spikes produced changes that may persist with continued exposures.	Gregory et al. (1983)
2,444-31,960	1.3-17.0	-	Rat	Decreased production of superoxide anion radical.	Amoruso et al. (1981)
3,700 19,000	2.0 10.0	3 days	Guinea pig Rat	Newborns were less affected than adults when AM were tested for SOD levels.	Azoulay-Dupuis et al. (1983)
3,700	2.0	8 h/day, 5 days/wk, 6 mo	Baboon	Impaired AM responsiveness to migration inhibitory factor.	Greene and Schenider (1978)
5,000	2.7	24 h	Rat	Increase in number of AMs.	Rombout et al. (1986)

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TABLE 13-4 (cont'd). ALVEOLAR MACROPHAGES

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
6,768 22,748	3.6 12.1	2 h	Rat	Enhanced macrophage agglutination with concanavavalin A at both concentrations tested.	Goldstein et al. (1977a)
7,520 19,000 47,000	4.0 10 25	6 h/day, 7, 14, or 21 days	Rat	Changes in morphology at all concentrations; increase in number of AMs at two highest concentrations; phagocytic capacity reduced after 14 and 21 days of exposure to highest concentration.	Hooftman et al. (1988)
7,520	4.0	10 days	Rat	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.	Mochitate et al. (1986)
7,520 15,040	4.0 8.0	Up to 10 days	Rat	Increase in number of AMs at most concentrations, reaching a peak on Day 3 and 5; no increase in number of PMNs; decrease in AM viability throughout exposure period. Suppression of phagocytic activity after 7 days of exposure to the lowest level and after 5 days of exposure to the higher level; returned to normal value at 10 days. Both concentrations caused decrease in superoxide radical production, but at the lowest concentration the effect became significant on Days 3, 5, and 10; at the higher concentration, the effect was significant at all time periods tested.	Suzuki et al. (1986)
9,400	5.0	7 days	Mouse	No effect on phagocytic activity.	Lefkowitz et al. (1986)

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TABLE 13-4 (cont'd). ALVEOLAR MACROPHAGES

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
9,400 28,200	5 15	3 h exposure after infections with parainfluenza3 virus. Challenge with rabbit pox virus.	Rabbit	AMs lost resistance to pox virus after exposure to 15 ppm.	Acton and Myrvik (1972)
9,400 19,000 28,200	5 10 15	3 h	Humans (In vitro exposure)	No change in cell viability, release of neutrophil chemotactic factor, or interleukin-1.	Pinkston et al. (1988)
9,400- 75,000	5-40	2 days to 15 wk	Mouse Rat Monkey Rat	Concentration-related increase in total number of AMs.	Kleinerman et al. (1982) DeNicola et al. (1981) Busey et al. (1974) Wright et al. (1982)
13,200	7.0	24 h	Rabbit	Increased rosette formation in AMs treated with lipase.	Hadley et al. (1977)
19,000	10	35 days	Guinea pig	63% increase in epithelial cells positive for macrophage congregation.	Sherwin et al. (1968)
19,000	10	4 h	Mouse	Increase in total pulmonary cells.	Jakab (1988)
19,000 47,000	10 25	24 h	Rat	Appearance of intracellular dense bodies in exposed AMs.	Katz and Laskin (1976)

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TABLE 13-4 (cont'd). ALVEOLAR MACROPHAGES

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
19,000 28,200 94,000	10 15 50	3 h	Rabbit	Inhibition of phagocytic activity.	Gardner et al. (1969) Acton and Myrvik (1972)
37,600	20	18 h	Mouse	Impairment of pulmonary antibacterial activity of AMs.	Niki (1981)
15,000- 112,800	≥20 ppm	3 h to 30 days	Rabbit Mouse Hamster	Concentration-related influx of PMNs following NO_2 exposure.	Gardner et al. (1969) DeNicola et al. (1981) Kleinerman et al. (1982) Niki (1981)
37,600	20	24 h	Hamster Rat	Membrane changes indicated by increase in cell size of AMs and ability to spread; influx of PMNs and AMs. Inhibitory effect on phagocytosis and rosette formation in the rat.	Foster et al. (1985)

*AMs = alveolar macrophages.
SOD = superoxide dismutase.
PMNs = polymorphonuclear leukocytes.
DNA = deoxyribonucleic acid.

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exposed host. Such changes can result in a decrease in immune function (suppression) following exposure to toxic chemicals and may lead to a partial or complete deficiency. Enhanced immune activity may result in an exaggerated response to an antigenic stimulus causing an unwanted 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 far more serious than the original lesions.

Because the immune system is a multicomponent, carefully regulated system, it offers numerous target sites for NO_2 action and there are some studies that indicate that high concentrations of NO_2 (>9,400 $\mu g/m^3$; [5.0 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 ppm) 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. In this study, the authors assessed immunological response by monitoring the daily output of PFC in the efferent lymph of caudal mediastinal lymph nodes. Although the number of animals used was small and the data were not analyzed statistically, it would appear that in the animals that were immunized (with sheep red blood cells, SRBC) (a T cell dependent antigen) 2 days after NO_2 exposure the output of PFC was consistently below controls.

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) NO_2 on cellular immunity in rats after intratracheal immunization of the lung with SRBC. 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 an impact on NO_2 on the humoral immune response to intratracheally delivered influenza vaccine. In monkeys exposed for 493 days to 1,880 $\mu g/m^3$ (1 ppm) 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 controls (Fenters et al., 1973; Ehrlich and Fenters, 1973). In monkeys exposed to 9,400 $\mu g/m^3$ (5 ppm NO_2) for a total of 169 days and immunized with mouse-adapted influenza virus (A/PR/8), serum neutralization titers were lower than controls initially; 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 viral strain used for immunization played a role, along with exposure differences. The authors also hypothesized that exposure to 1,880 $\mu g/m^3$ (1 ppm) 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) also suggest that both the nature of and rate of change in immunological function from NO_2 exposure can be both concentration- and time-dependent. At 7-week intervals, tests were conducted 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 vs. 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 SRBC was reported by Fujimaki and Shimizu (1981). Initially 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 primary antibody response to SRBC at the two highest levels of exposure. Expanding on these studies, they later demonstrated that NO_2, in addition to reducing the primary antibody response, also significantly altered the host's secondary response to this antigen. The data led the authors to speculate that the suppression of the primary response was due to a reduction in the functioning of the B cells, whereas the depression of the secondary response was related to a T cell defect (Fujimaki et al., 1981).

Using this same model, Fujimaki et al. (1982) reported a similar effect in mice (i.e., suppression of primary antibody response in the spleen) after a 4-week exposure to 752 and 3,000 $\mu g/m^3$ (0.4 and 1.6 ppm) NO_2. At the higher concentration, there was no significant difference observed in the activities of the T and B lymphocytes. Secondary antibody response was not affected at 752 $\mu g/m^3$ (0.4 ppm) but was slightly enhanced at 3,000 $\mu g/m^3$ (1.6 ppm) NO_2. However, Maigetter et al. (1978) found that the normal transformation response of mouse splenic T and B cells to phytohemagglutinin and bacterial lipopolysaccharide, respectively, was suppressed following a 3-month exposure to 940 $\mu g/m^3$ (0.5 ppm) NO_2.

Lefkowitz et al. (1986) employed several methods to measure immunoactivity of mice exposed to 9,400 $\mu g/m^3$ (5.0 ppm) NO_2 for 24 h/day for 6 days and injected with SRBC 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 PFC to SRBC. 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 ppm) NO_2 for 6 months 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 NO_2 had decreased serum levels of IgA and exhibited nonspecific increases in serum IgM, IgG, and IgG_2 (Ehrlich et al., 1975).

Extrapulmonary effects on the functional activity of the immune system was tested in mice by Richters and Damji (1988). The percentage of the total T lymphocyte population was lower in the spleens of AKR/cum 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 and T cytotoxic/suppressor lymphocytes were also lower in the spleen of exposed animals. There were no statistically significant changes in the percentages of natural killer cells or mature T cells. C57BL/6J 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.

Richters and Damji (1990) reported similar findings in AKR/cum mice exposed for up to 181 days, 7 h/day, 5 days/week to 470 $\mu g/m^3$ (0.25 ppm) NO_2. The T-helper/inducer ($CD4^+$) lymphocytes (spleen) were reduced; no effects were observed on T cytotoxic/ suppressor cells. Spontaneously developing lymphomas in NO_2-exposed animals had progressed more slowly than those in control animals. This was attributed to the NO_2-induced reduction in the T-helper/inducer lymphocytes.

Mice that were vaccinated with influenza virus (A-2/Taiwan/1/64) after 3 months of continuous exposure to 3,760 $\mu g/m^3$ (2.0 ppm) or to 940 $\mu g/m^3$ (0.5 ppm) NO_2 with a 1-h daily 5 days/week spike exposure to 3,760 $\mu g/m^3$ (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. (1973) findings in monkeys exposed to 1,880 $\mu g/m^3$ (1.0 ppm) for over 1 year.

Few studies have been undertaken to assess the effects of NO_2 on interferon production. Mice exposed to either 9,400 or 47,000 $\mu g/m^3$ (5 or 25 ppm) NO_2 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 $\mu g/m^3$ (5 ppm) and 28,200 $\mu g/m^3$ (15 ppm) NO_2. 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 concentration and duration of exposure to NO_2.

The effects of NO_2 exposure on the immune system appear to be concentration- and time-dependent. Some studies suggest little effect, while others suggest suppression or activation, depending not only on concentration, but also on length of exposure, species tested, and specific end points measured. Although we lack a thorough understanding of the potential effects of NO_2 on the immune system, it is evident that the immune system is sensitive to NO_2. Table 13-5 summarizes the reported immunological effects following exposure to NO_2.

TABLE 13-5. IMMUNOLOGICAL EFFECTS

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
470	0.25	7 h/day, 5 days/wk, 7 wk	Mouse (AKR/cum)	Reduced percentage of total T cell population and trend towards reduced percentage of certain T cell subpopulations; no reduction of mature T cells or natural killer cells.	Richters and Damji (1988)
470	0.25	7 h/day, 5 days/wk, 181 days	Mouse (AKR/cum)	Reduced percentage of total T cell population and percentages of T helper/inducer cells on Days 37 and 181 of exposure.	Richters and Damji (1990)
670	0.35	7 h/day, 5 days/wk, 12 wk	Mouse (C57BL/6J)	Trend towards suppression in total percentage of T cells. No effects on percentages of other T cell subpopulations.	Richters and Damji (1988)
752 3,000	0.4 1.6	4 wk	Mouse	Primary antibody response was suppressed. At both concentrations, no difference in T and B cell activity.	Fujimaki et al. (1982)
940	0.5	3 mo	Mouse	Suppression of T and B cell responsiveness.	Maigaetter et al. (1978)
940 with 1-h daily peak to 3,760, or 3,760	0.5 with 1-h daily (5 days/wk) peak to 2.0 ppm, or 2.0	24 h/day, 5 days/wk, 3 mo followed by vaccination with influenza A2/Taiwan virus	Mouse	Decrease in serum neutralizing antibody; hemagglutination inhibition titers unchanged; before virus challenge NO_2 exposure decreased serum IgA and increased IgG_1, IgM, and IgG_2; after virus, serum IgA unchanged and IgM increased.	Ehrlich et al. (1975)
1,880	1.0	493 days; challenged five times with monkey-adapted influenza virus during NO_2 exposure	Monkey	Hemagglutination inhibition antibody titers not altered. Compared to controls, NO_2 caused an earlier and greater increase in serum neutralization antibody titers to the virus.	Fenters et al. (1973)

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TABLE 13-5 (cont'd). IMMUNOLOGICAL EFFECTS

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
1,880	1.0	6 mo followed by intranasal challenge with *K. pneumoniae*	Guinea pig	Decreased hemolytic activity of complement; decrease in all immunoelectrophoretic fractions.	Kosmider et al. (1973b)
2,820 9,400	1.5 5.0	24 h/day, 6, 14 or 21 days	Mouse	Reduction in number of splenic plaque-forming cells; lowering the concentration to 1.5 ppm and extending the length to 14 or 21 day decreased cell number by 33 and 50%, respectively; no effect on cell-mediated immune system or hemagglutinating titers.	Lefkowitz et al. (1986)
9,400	5.0	1.5 h/day, 10-11 days	Sheep	Reduction in splenic plaque-forming cells in immunized animals.	Joel et al. (1982)
9,400 28,200	5.0 15.0	4 h/day, 5 days/wk, 5.52 mo	Guinea pig	Serum antibodies against lung tissue increased with concentration and duration of exposure.	Balchum et al. (1965)
9,400	5.0	Continuous, 169 days, challenged 4× with mouse-adapted influenza virus	Monkey	Initial depression in serum neutralization titers with return to normal by Day 133; no effect on secondary response on hemmagglutin inhibition titer.	Fenters et al. (1971)
10,000	5.3	Continuous, 33 days	Guinea pig	No effect on antibody production.	Antweiller et al. (1975)
9,400 47,000	5.0 25.0	3-7 days	Mouse	No effect on serum interferon levels.	Lefkowitz et al. (1983, 1984)

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TABLE 13-5 (cont'd). IMMUNOLOGICAL EFFECTS

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
9,400 18,800 48,900	5.0 10.0 26.0	24 h	Rat	Concentration-related elevation of cellular immunity after immunizing the lung with sheep RBC.	Hillam et al. (1983)
9,400	5.0	Continuous, 6 mo	Monkey	Depressed postvaccination serum neutralizing antibody formation.	Ehrlich and Fenters (1973)
9,400 37,600 75,200	5.0 20 40	12 h	Mouse	No effect of 5 ppm; at higher levels, suppression in primary and secondary antibody response; and depression of number of plaque-forming cells.	Fujimaki and Shimizu (1981) Fujimaki et al. (1981)
18,800	10.0	2 h/day, 30 wk	Mouse	Marked depression in ability of T cells to respond to nonspecific stimuli; reduced ability to reject tumors.	Holt et al. (1979)
37,600	20.0	4 days	Mouse	NO_2 did not alter natural immunity, but impaired acquired resistance to Klebsiellia infection.	Bouley et al. (1986)
37,600	20.0	96 h	Mouse	Both hemagglutin inhibition titers and numbers of splenic plaque-forming cells were depressed; returned to normal 2 days after cessation of exposure.	Azouley-Dupuis et al. (1985)
45,000	25.0	3 h	Rabbit	Reduced ability to produce interferon.	Valand et al. (1970)

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Interaction With Infectious Agents

Different experimental approaches using intact animals have been employed in an effort to determine the functional efficiency of the host's pulmonary defenses following NO_2 exposure. Animals are randomly selected for exposure to either a pollutant, in this case NO_2, 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 NO_2-exposed and the control 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, 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 infectious disease 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 are capable of epidemiological confirmation. One can believe that similar alterations in these basic defense mechanisms that occur in animals could also occur in humans, since 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 (epidemiological studies). Such a comparison is proper, since both mortality (animals) and morbidity (humans) result from a loss of pulmonary defenses. However, one should understand that different exposure levels may be required to produce a similar

response in humans. Table 13-6 summarizes effects of exposure to NO_2 and an infectious agent.

An enhancement in mortality following exposure to NO_2 and a pathogenic microorganism could be due to several factors. Studies by Goldstein et al. (1973) showed decreases in pulmonary bactericidal activity following NO_2 exposure. In their first experiments, mice breathed aerosols of *Staphylococcus aureus* (*S. aureus*) labelled with radioactive phosphorus and then were exposed to NO_2 for 4 h. Physical removal of the bacteria was not affected by any of the NO_2 concentrations used up to 27,800 $\mu g/m^3$ (14.8 ppm). Concentrations of 13,200, 17,200, and 27,800 $\mu g/m^3$ (7.0, 9.2, and 14.8 ppm) NO_2 did lower the bactericidal activity by 7, 14, and 50%, respectively, when compared to controls. Lower concentrations (3,570 and 7,140 $\mu g/m^3$ [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., 1974), mice breathed 1,800, 4,320, and 12,400 (1.0, 2.3, and 6.6 ppm) NO_2 for 17 h and then were exposed to an aerosol of *S. aureus*. Four hours later the animals were examined for the number of organisms present in the lungs. No difference in the number of bacteria inhaled was found in the NO_2-exposed animals. Concentrations of 4,320 and 12,400 $\mu g/m^3$ NO_2 decreased pulmonary bactericidal activity by 6 and 35%, respectively, compared to controls. Exposure to 1,800 $\mu g/m^3$ NO_2 had no significant effect. Goldstein et al. (1974) hypothesized that the decreased bactericidal activity was due to defects in AM function. Research conducted by Jakab (1987) indicate that the concentration of NO_2 required to suppress normal pulmonary bactericidal activity in mice probably depends on the specific invading organism. For example, exposure to >7,520 $\mu g/m^3$ (4 ppm) NO_2 for 4 h significantly depressed normal bactericidal defenses of the lungs against deposited *S. aureus*, but it required a concentration of 19,000 $\mu g/m^3$ (10 ppm) before the lung's ability to kill deposited *Pasteurella* and *Proteus* was impaired.

A 4-h exposure to 9,400 $\mu g/m^3$ (5.0 ppm) NO_2 significantly impaired the ability of mice to kill staphylococcus organisms. This effect became more significant with increasing concentrations. A similar exposure to 4,700 and 7,500 $\mu g/m^3$ (2.5 and 4.0 ppm) produced no significant response (Jakab, 1988). The combination of corticosteroid (subcutaneous injection) and NO_2 exposure (4 h) significantly impaired the intrapulmonary killing of

TABLE 13-6. INTERACTION OF NO_2 WITH INFECTIOUS AGENTS

NO_2 Concentration						
$\mu g/m^3$	ppm	Exposure	Species	Infective Agent*	Effects	Reference
100 plus 100 O_3 with spikes twice/day (188 plus 200 O_3)	0.05 plus 0.05 O_3 with spikes (0.1 plus 0.1 O_3)	Continuous, 15 days 5 (spikes-1 h, twice/day, 5 days/wk)	Mouse	*Streptococcus* sp.	No effect.	Gardner (1980) Gardner et al. (1982) Graham et al. (1987)
940 plus 100 O_3 with spikes (1,880 plus 200 O_3)	0.5 plus 0.05 O_3 with spikes (1.0 plus 0.1 O_3)				Increased mortality with NO_2 alone; no effect with O_3 alone; synergistic effect with O_3 plus NO_2.	
2,300 plus 200 with spikes (4,700 plus 600 O_3)	1.2 plus 0.1 O_3 with spikes (2.5 plus 0.3 O_3)				Increased mortality with NO_2 alone and O_3 alone; additive effect of O_3 plus NO_2.	
370 plus spike of 1,500	0.2 plus 0.8 spike twice/day for 1 h	5 days/wk, 1 yr	Mouse	*Streptococcus* sp.	Spike plus baseline caused significantly greater mortality than baseline; spike exposure group may have induced lung lesion.	Miller et al. (1987)

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TABLE 13-6 (cont'd). INTERACTION OF NO_2 WITH INFECTIOUS AGENTS

NO_2 Concentration						
$\mu g/m^3$	ppm	Exposure	Species	Infective Agent*	Effects	Reference
560-940	0.3-0.5	Continuous, 3 mo	Mouse	A/PR/8 virus	High incidence of adenomatous proliferation of peripheral and bronchial epithelial cells; NO_2 alone and virus alone caused less severe alterations.	Motomiya et al. (1973)
		Continuous, 6 mo			No enhancement of effect of NO_2 and virus.	
940	0.5	Intermittent, 6 or 18 h/day, to 12 mo	Mouse	*K. pneumonia*	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.	Ehrlich and Henry (1968)
		Continuous, 90 days				
940-1,880	0.5-1.0	Continuous, 39 days	Mouse (female)	A/PR/8 virus	Increased susceptibility to infection.	Ito (1971)
18,800	10.0	2 h/day, 1, 3, and 5 days				
940-52,670	0.5-28.0	Varied	Mouse	*Streptococcus* sp.	Increased mortality with increased time and concentration; concentration is more important than time.	Gardner et al. (1977a,b) Coffin et al. (1977)
940	0.5	3 h/day, 3 mo	Mouse	*Streptococcus* sp.	Increase in mortality with reduction in mean survival time.	Ehrlich et al. (1979)

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TABLE 13-6 (cont'd). INTERACTION OF NO_2 WITH INFECTIOUS AGENTS

NO_2 Concentration						
$\mu g/m^3$	ppm	Exposure	Species	Infective Agent*	Effects	Reference
940 1,880 2,820	0.5 1.0 1.5	24 h/day, 7 days/wk, 3 mo	Mouse	*K. pneumoniae*	Significant increase in mortality after 3-day exposure to 5.0 ppm; no effect at other concentrations.	McGrath and Oyervides (1985)
9,400	5.0	3 days				
1,880 4,320 12,400	1.0 2.3 6.6	17 h	Mouse	*S. aureus* after exposure	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 1.0 ppm.	Goldstein et al. (1974)
1,880-4,700	1.0-2.5	4 h	Mouse	*S. aureus*	Impaired bactericidal activity between 1.0 and 2.5 ppm in animals injected with corticosteroids.	Jakab (1988)
4,320	2.3				6% decrease in bactericidal activity.	
12,400	6.6				35% decrease in bactericidal activity.	
1,880	1.0	48 h	Mouse	*Streptococcus* sp. *S. aureus*	Increased proliferation of organism in lung of exposed mice but no effect with Staphylococcus.	Sherwood et al. (1981)
1,880 5,600	1.0 3.0	3 h	Mouse	*Streptococcus* sp.	Exercise on continuously moving wheels during exposure; increased mortality at 3 ppm.	Illing et al. (1980)

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TABLE 13-6 (cont'd). INTERACTION OF NO_2 WITH INFECTIOUS AGENTS

NO_2 Concentration						
$\mu g/m^3$	ppm	Exposure	Species	Infective Agent*	Effects	Reference
2,820	1.5	Continuous or intermittent, 7 h/day, 7 days/wk	Mouse	*Streptococcus* sp.	After 1 wk, mortality with continuous exposure was greater than that for intermittent; after 2 wk, no significant difference between continuous and intermittent exposure.	Gardner et al. (1979)
6,600	3.5				Increased mortality with increased duration of exposure; no significant difference between continuous and intermittent exposure; with data adjusted for total difference in C × T, mortality essentially the same.	
2,800 plus 8,100 spike	1.5 plus 4.5 spike	Continuous 60 h then spike for 1, 3.5, or 7 h, then continuous 18 h	Mouse	*Streptococcus* sp.	Mortality increased with 3.5- and 7-h single spike when bacterial challenge was immediate, but not 18-h postexposure.	Gardner (1980) Gardner et al. (1982) Graham et al. (1987)
8,100	4.5	1, 3.5, or 7 h			Mortality proportional to duration when bacterial challenge was immediate, but not 18 h postexposure.	
2,800	1.5	7 h/day, 4, 5, and 7 days	Mouse	*Streptococcus* sp.	Elevated temperature (32 °C) increased mortality after 7 days.	Gardner et al. (1981)

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TABLE 13-6 (cont'd). INTERACTION OF NO_2 WITH INFECTIOUS AGENTS

NO_2 Concentration		Exposure	Species	Infective Agent*	Effects	Reference
$\mu g/m^3$	ppm					
3,570 7,140	1.9 3.8	4 h	Mouse	*S. aureus* prior to NO_2 exposure	Physical removal of bacteria unchanged at 1.9-14.8 ppm.	Goldstein et al. (1973)
13,160	7.0				7% lower bactericidal activity.	
17,200	9.2				14% lower bactericidal activity.	
27,800	14.8				50% lower bactericidal activity.	
3,760	2.0	1.5 h/day for 1, 2 and 3 wk	Hamster (in vitro tracheal explants)	Influenza virus	Peak virus production occurred earlier.	Schiff (1977)
3,760	2.0	3 h	Mouse	*Streptococcus* sp.	Increased mortality.	Ehrlich et al. (1977) Ehrlich (1980)
2,820 4,700 6,600 9,400 18,800 28,200	1.5 2.5 3.5 5.0 10.0 15.0	2 h	Mouse	*K. pneumoniae*	No effect of 1.5 or 2.5 ppm; increased mortality at 3.5 ppm and above. Increase in mortality when *K. pneumoniae* challenge 1 and 6 h after 5 or 10 ppm NO_2 exposure; when *K. pneumoniae* challenge 27 h following NO_2 exposure, effect only at 15 ppm.	Purvis and Ehrlich (1963) Ehrlich (1980)
4,700 7,500 9,400 18,800 28,200	2.5 4.0 5.0 10.0 15.0	4 h	Mouse	*S. aureus*	Impaired bactericidal activity at 4.0 ppm and above.	Jakab (1987)

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TABLE 13-6 (cont'd). INTERACTION OF NO_2 WITH INFECTIOUS AGENTS

NO_2 Concentration						
$\mu g/m^3$	ppm	Exposure	Species	Infective Agent*	Effects	Reference
6,580	3.5	2 h	Mouse	*K. pneumoniae*	Increased mortality of all species.	Ehrlich (1975)
65,830	35.0	2 h	Hamster			
94,050	50.0	2 h	Squirrel monkey			
7,520	4.0	4 h	Mouse	Staphylococcus, Pasteurella, and Proteus	Effect on bactericidal activity of the lung depends on microorganism.	Jakab (1987)
9,400	5.0	6 h/day, 6 days	Mouse	Cytomegalo-virus	Increase in virus susceptibility.	Rose et al. (1988)
9,400	5.0	Continuous, 2 mo	Squirrel monkey	*K. pneumoniae* or A/PR/8 virus	Increased viral-induced mortality (1/3). Increase in Klebsiella-induced mortality (2/7); no control deaths.	Henry et al. (1970)
19,000	10.0	Continuous, 1 mo			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.	
9,400	5.0	2 mo	Squirrel monkey	*K. pneumoniae*	Mortality 2/7; bacteria present in lung of survivors at autopsy.	Henry et al. (1969)
65,800	35.0	1 mo			Mortality 1/4; bacteria present in lungs of survivors at autopsy.	
94,000	50.0	2 h			Mortality 3/3.	
37,600	20.0	18 h	Mouse	*K. pneumoniae*	Bacterial proliferation due to impaired pulmonary bactericidal activity but no effect on physical removal. Increase in PMNs.	Niki (1981)

S. aureus = *Staphylococcus aureus*.
K. pneumoniae = *Klebsiella pneumoniae*.

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staphylococci at concentrations between 1,880 and 4,700 $\mu g/m^3$ (1.0 and 2.5 ppm) (Jakab, 1988). 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).

Differences in species sensitivity 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. pneumonia* (Ehrlich, 1975). However, differences in sensitivity were noted between the species. Concentrations of 9,400 to 65,830 $\mu g/m^3$ (5.0 to 35.0 ppm) NO_2 had no effect on monkeys. Squirrel monkeys exposed continuously to NO_2 levels of 9,400 and 18,800 $\mu g/m^3$ (5.0 and 10.0 ppm) for 2 and 1 months, respectively, showed increased susceptibility to a challenge with *K. pneumoniae* and reduced lung clearance of viable bacteria (Henry et al., 1970). Two of seven monkeys exposed to 9,400 $\mu g/m^3$ (5.0 ppm) for 2 months died and the rest had bacteria in the lungs on autopsy. Hamsters exhibited enhanced mortality at concentrations of $\geq$65,830 $\mu g/m^3$ (35 ppm) but not at 9,400 to 47,000 $\mu g/m^3$ (5.0 to 25.0 ppm) NO_2. The mouse model was the most sensitive to NO_2 exposure, as evidenced by enhanced mortality following exposure to 6,580 $\mu g/m^3$ (3.5 ppm) but not to 2,820 to 4,700 $\mu g/m^3$ (1.5 to 2.5 ppm) NO_2 for 2 h (Ehrlich, 1975). Purvis and Ehrlich (1963) also reported no effect on mortality in mice exposed for 2 h to 2,820 or 4,700 $\mu g/m^3$ (1.5 or 2.5 ppm); effects occurred at 6,600 $\mu g/m^3$ (3.5 ppm) and higher. However, when *Streptococcus* sp. was the infectious agent, a 3-h exposure to 3,760 $\mu g/m^3$ (2.0 ppm) NO_2 caused an increased in mortality in mice (Ehrlich et al., 1977). This suggests that the normal pulmonary defenses had not recovered sufficiently to successfully clear the lung of these viable microbes even after several weeks in clean air.

Squirrel monkeys exposed to 9,400 or 18,800 $\mu g/m^3$ (5 or 10 ppm) NO_2 for 2 or 1 month, 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 NO_2 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 NO_2 on respiration. Because no spontaneous changes in respiratory patterns were produced following a 3-day exposure to 9,400 $\mu g/m^3$ (5 ppm) NO_2, the authors concluded that any such relationship was unlikely.

The importance of the test organism used with the infectivity models was demonstrated by Sherwood et al. (1981). These researchers illustrated that exposure to 1,880 $\mu g/m^3$ (1 ppm) NO_2 for 48 h increased the propensity of virulent group C streptococci, but not *S. aureus*, to proliferate within the murine lungs and cause earlier mortality.

In a series of investigations, 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), Gardner et al. (1982), and Coffin et al. (1977). The concentrations of NO_2 varied from 1,880 to 26,320 $\mu g/m^3$ (1 to 14 ppm), and the duration of exposure ranged from 0.5 to 7 h so that the product of concentration and time (C $\times$ T) equalled a value of 7. In these studies exposure to high concentrations of NO_2 for brief periods of time resulted in greater mortality than did prolonged exposures to lower concentrations of NO_2. This indicated that susceptibility to infection was influenced more by the concentration of NO_2 than by the duration of the exposure.

Using the same model, Gardner et al. (1977b) examined the effect of varying durations of continuous exposure on the mortality of mice exposed to 6 concentrations of NO_2 (940 to 52,670 $\mu g/m^3$ [0.5 to 28.0 ppm]). *Streptococcus* sp. was used for all concentrations, except 940 $\mu g/m^3$ (0.5 ppm), in which case *K. pneumoniae* was used. A linear concentration-response indicated that mortality increases with increasing length of exposure to a given concentration of NO_2 (Figure 13-1). Mortality also increased with increasing concentration of NO_2 as indicated by the steeper slopes with higher concentrations. When C $\times$ T was held constant, the relationship between concentration and time produced significantly different mortality responses. At a constant C $\times$ T of approximately 21 (ppm $\times$ h), a 14-h exposure to 2,800 $\mu g/m^3$ (1.5 ppm) NO_2 increased mortality by 12.5% whereas a 1.5-h exposure to 27,300 $\mu g/m^3$ (14.0 ppm) NO_2 enhanced mortality by 58.5%. These studies confirmed the previous conclusion that concentration is more important than time in determining the degree of injury induced by NO_2 in this model. According to Larsen et al. (1979), NO_2 modeling

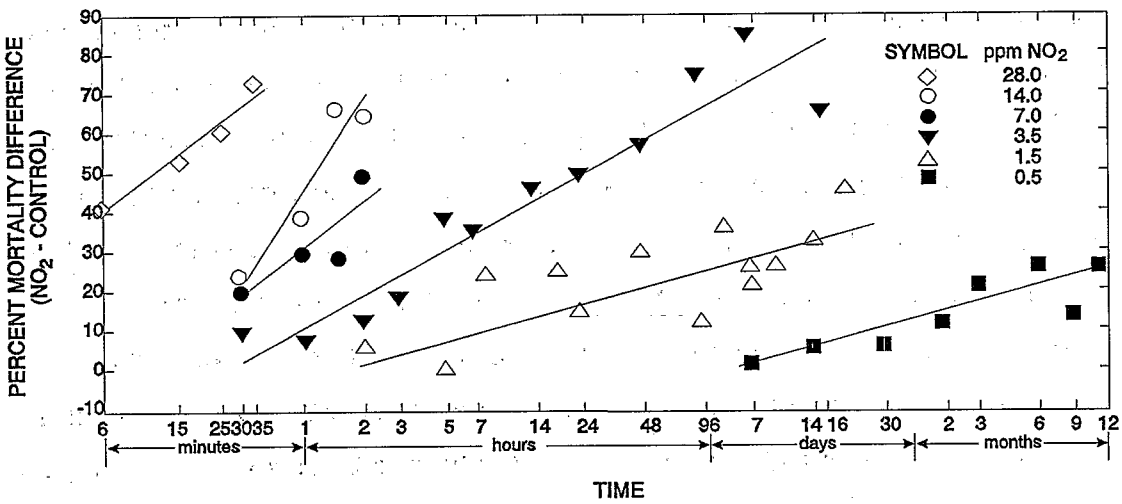

Figure 13-1. Mortality enhancement for mice exposed to NO_2 at various concentrations and for various durations prior to challenge with streptococci. At all concentrations, prolonged exposure results in enhanced mortality, but the severity of resistance reduction is more directly related to concentration.

Source: Gardner et al. (1977b).

studies have shown that the concentration (c) of NO_2 expected to cause a certain mortality level (z) as a function of the hours of exposure (t) can be expressed as $c = 9.55\ (2.42)^z t^{-0.33}$.

Gardner et al. (1979) also compared the effect of continuous versus intermittent exposure to NO_2 followed by bacterial challenge with *Streptococcus* sp. Mice were exposed either continuously or intermittently (7 h/day, 7 days/week) to 2,800 or 6,600 $\mu g/m^3$ (1.5 or 3.5 ppm) NO_2. The results of continuous and intermittent exposure to 6,600 $\mu g/m^3$ (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 2,800 $\mu g/m^3$ (1.5 ppm) NO_2 increased mortality after 24 h of exposure. During the first week of exposure, the mortality was significantly higher in mice exposed continuously to NO_2 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 NO_2 may ultimately be as toxic as sustained high levels (Gardner et al., 1979).

Mice were exposed continuously or intermittently (6 or 18 h/day) to 940 $\mu g/m^3$ (0.5 ppm) NO_2 for up to 12 months (Ehrlich and Henry, 1968). Neither exposure regimen affected murine resistance to *K. pneumoniae* infection during the first month. Those exposed continuously exhibited decreased resistance to the infectious agent as demonstrated by a significant enhancement in mortality at 3, 6, 9, and 12 months. In another experiment, a significant enhancement did not occur at 3 months but was observed after 6 months of exposure. After 6 months, mice exposed intermittently (6 or 18 h/day) to NO_2 showed significant increases in mortality over controls (18%). Only the continuously exposed animals showed increased mortality (23%) over controls following 12 months of exposure. After 12 months of exposure, mice in the three experimental groups showed a reduced capacity to clear viable bacteria from their lung. This was first observed after 6 months in the continuously exposed groups and after 9 months in the intermittently exposed groups. These changes, however, were not statistically tested for significance. Therefore, while it is not possible to directly compare the results of the studies using *Streptococcus* sp. to those using *K. pneumoniae*, the data suggest that as the concentration of NO_2 is decreased, a longer exposure time is necessary for the intermittent exposure regimen to produce a level of effect equivalent to that of a continuous exposure.

Mice exposed continuously to 9,400 $\mu g/m^3$ (5.0 ppm) for 3 days had both a significant increase in percent mortality and a decrease in relative mean survival time (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 months did not demonstrate a difference in either of these parameters. These subchronic exposure results do not agree with Ehrlich and Henry (1968) who found excess mortality after continuous exposure to 2,280 $\mu g/m^3$ (1.5 ppm) for 3 months, 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 study had 95% mortality in the control groups, making it virtually impossible to detect a NO_2-induced enhancement in mortality.

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 spike exposures superimposed on a lower continuous background level of NO_2. Such a regimen approximates the pattern of exposure which humans receive in the urban environment. Mice were exposed to spikes of 8,100 $\mu g/m^3$ (4.5 ppm) for 1, 3.5, or 7 h and then challenged with *Streptococcus* sp. either immediately or 18 h postexposure. Mortality was proportional to the duration of the spike when the mice were exposed to bacteria immediately postexposure, but mice had recovered from the exposure by 18 h. Similar findings were reported by Purvis and Ehrlich (1963) who found a significant increase in excess mortality in mice exposed for 2 h to 9,400 $\mu g/m^3$ (5.0 ppm) NO_2, followed by a challenge with *K. pneumoniae*. He reported that the increased mortality due to the infectious challenge was present at 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. When a spike of 8,100 $\mu g/m^3$ (4.5 ppm) was superimposed on a continuous background of 2,800 $\mu g/m^3$ (1.5 ppm) for 62 h preceding and 18 h following the spike, mortality was significantly enhanced by a spike lasting 3.5 or 7 h when the infectious agent was administered 18 h after the spike (Gardner, 1980; Gardner et al., 1982; Graham et al., 1987). 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 NO_2. The effect of multiple spikes was examined by exposing mice for 2 weeks to two daily 1-h spikes (morning and afternoon) of 8,100 $\mu g/m^3$ (4.5 ppm) superimposed on a continuous background of 2,800 $\mu g/m^3$ (1.5 ppm) NO_2. Spikes were not superimposed on the continuous background during the weekends. Mice were exposed to the infectious agent either immediately before or after the morning spike. When the infectious agent was given before the morning spike, the increase in mortality did not closely approach that of a continuous exposure to 2,800 $\mu g/m^3$ (1.5 ppm) NO_2. However, in mice exposed after the morning spike, by 2 weeks of exposure, the increased mortality over controls approached that equivalent to continuous exposure to 2,800 $\mu g/m^3$ (1.5 ppm) NO_2. These findings demonstrate that the pattern of exposure determines the response and that the response is not predictable based on a simple C $\times$ T relationship.

Further investigations into the effects of NO_2 spikes on murine antibacterial lung defenses have been conducted using a spike 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 370 $\mu g/m^3$ (0.2 ppm) or to this baseline level on which was superimposed two times a day a 1-h spike of 1,500 $\mu g/m^3$ (0.8 ppm) NO_2, 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 spike regimen was significantly greater than that of either the NO_2 background exposed mice or the control mice. This chronic study indicates that short-term spikes of NO_2 can cause detectable effects on antibacterial lung defenses. It is of interest that such spikes also may have induced a subtle lung lesion as indicated by pulmonary function analyses of these exposed animals (see Section 13.2.3.3) (Miller et al., 1987).

The persistence of the effects of NO_2 was investigated by Purvis and Ehrlich (1963) who exposed mice for 2 h to NO_2 before or after an aerosol challenge with *K. pneumoniae*. At 9,400, 18,800, and 28,200 $\mu g/m^3$ (5.0, 10.0, and 15.0 ppm) NO_2 there was a significant enhancement of mortality in mice challenged with bacteria 1 h and 6 h after the NO_2 exposure. When bacteria challenge was delayed for 27 h, there was an effect only in the group exposed to the highest concentration.

Mice exposed continuously for 3 months to 560 to 940 $\mu g/m^3$ (0.3 to 0.5 ppm) NO_2 followed by a challenge with A/PR/8 influenza virus caused 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 NO_2 than with either the viral or NO_2 exposures alone. Continuous NO_2 exposure for an additional 3 months did not enhance the effect of NO_2 or the subsequent virus challenge.

Ito (1971) challenged mice with influenza A/PR/8 virus after continuous exposure to 940 to 1,880 $\mu g/m^3$ (0.5 to 1.0 ppm) NO_2 for 39 days and to 18,800 $\mu g/m^3$ (10.0 ppm) NO_2, 2 h daily for 1, 3, and 5 days. Acute and intermittent exposure to 18,880 $\mu g/m^3$ (10.0 ppm) NO_2 as well as continuous exposure to 940 to 1,880 $\mu g/m^3$ (0.5 to 1.0 ppm) NO_2 increased the susceptibility of mice to influenza virus as demonstrated by increased mortality. Further, when isolated hamster tracheal organ explants were exposed 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) NO_2 (Rose et al., 1988). The data would indicate no effects at exposures to levels of 4,700 $\mu g/m^3$ (2.5 ppm) and below.

Exposure to a NO_2 concentration of 9,400 $\mu g/m^3$ (5.0 ppm) did not significantly alter the course of a parainfluenza (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).

Environmental stress, in addition to influencing the lethality of a particular exposure concentration, as discussed in the section on mortality, has been shown to enhance the toxic effect of NO_2. Mice placed on continuously moving exercise wheels during exposure to 5,600 $\mu g/m^3$ (3.0 ppm) NO_2, but not 1,880 $\mu g/m^3$ (1.0 ppm), for 3 h showed enhanced mortality over nonexercised NO_2-exposed mice using the streptococcal infectivity model (Illing et al., 1980). The presence of other environmental factors, such as ozone (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 NO_2 on host resistance to infection. When mice were stressed by elevated temperature (32 °C) for 7 days, but not for 4 days, and exposed to 2,800 $\mu g/m^3$ (1.5 ppm) NO_2, there was a significant enhancement in mortality rate (Gardner et al., 1982).

Summary

A wide variety of mammalian species, including humans, depend on a similar array of host defense mechanisms necessary to protect or reduce the risk to infectious disease. Since animals and humans share similar host defense systems, generally it is assumed that if a particular effect has been identified in a number of animal species then it is likely that a similar effect also may occur in humans.

The host defense system is one of the many potential targets whose function has been shown to be altered significantly by exposure to NO_2. 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. The host defense parameters which have been most widely used to examine the association between NO_2 exposure and pulmonary host defenses include the functional activity of AMs, mucociliary clearance, immunological competence, and susceptibility to experimentally induced respiratory infectious disease.

The ciliated cells in the conducting airways seem to be the most vulnerable cells of the tracheobronchial region to the effects of NO_2 exposure. In animals, NO_2 causes structural alterations in the ciliated cells of the airways. The lowest concentration and exposure duration that showed a reduction in total number of cilia was a 7-month exposure of rats to 940 $\mu g/m^3$ (0.5 ppm) NO_2. This study and others have shown that when the concentrations of NO_2 were increased, the effects became significantly more pronounced. Clearance of marker substances have been used to determine if the number and structural effects on cilia also resulted in a significant reduction in the rate of mucociliary clearance. Significant impairment of tracheobronchial clearance rates were not seen at levels below 9,400 $\mu g/m^3$ (5.0 ppm) NO_2. 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 animals to NO_2 would have to be greater than 9,400 $\mu g/m^3$ to induce any significant alterations that would have detrimental health effects. These effects may be due to the direct cytotoxic action of NO_2 on the ciliated cells, the alteration in amount and viscosity of the mucus layer, or to the chemical paralysis of the cilia.

Within the pulmonary region of the lung, the primary cellular defense affected by both acute and long-term exposure to NO_2 is the AM. A common functional assay evaluates the capacity of these cells to conduct phagocytic and bactericidal activities. Nitrogen dioxide causes a marked depression of phagocytic activity, reduces cell viability, disrupts macrophage membrane integrity, reduces the total number of available cells, produces morphological changes, and decreases bactericidal activity. While a few of these effects were seen following exposure to concentrations $<1,880$ $\mu g/m^3$ (1.0 ppm), most of the studies showed effects at concentrations between 1,880 and 9,400 $\mu g/m^3$ (1.0 and 5.0 ppm) NO_2. Evidence clearly indicates that these cells are no longer capable of isolating, transporting, detoxifying, or clearing inhaled substances, and, because of the effects on host defenses, animals exposed

to NO_2 could be expected to succumb to a bacterial or viral infection when compared with clean air control animals.

The systemic cell-mediated and humoral immune system is also a target for NO_2, as evidenced by animal studies. The immunological effects reported seem to be variable. Some studies show effects while others do not. It has been suggested that long-term exposure may result in a suppression of the various humoral and cell-mediated functions, while shorter exposures may cause an enhancement of immunological reactivity. 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. Nevertheless, the data suggest that NO_2 or one of its reactive products may penetrate the lung epithelial and endothelial layers to enter the blood and produce some response in the systemic immune system. Research on the systemic immune system in mice, guinea pigs, and monkeys indicate that subchronic and chronic exposure at or below 1,880 $\mu g/m^3$ (1.0 ppm) can suppress T and B cell responsiveness to mitogens and decrease the number of T cells. Nitrogen dioxide influences the production of serum-neutralizing antibodies to viruses and humoral primary antibody response to red blood cells. Other immunological effects attributed to NO_2 include an increase in IgM and IgG, and a decrease in IgA serum levels.

The significance of many of these changes is uncertain, and studies conducted at lower levels of exposure and for longer periods are needed to improve our understanding of these immunological responses. Future studies need to take into account the kinetics of the immune response vis-a-vis toxicokinetics and dose-rate influences on toxicity. Unfortunately, being able to apply state-of-the-art methods for measurement of the pulmonary immune system have not been reported for NO_2. In the absence of adequate data, one can only speculate that if NO_2 affects the systemic immune system, it is likely that it also would affect the pulmonary immune system.

A great amount of evidence exists in the literature that illustrates the relationship between an increased susceptibility to viral, fungal, or bacterial infections and the suppression of various host defenses. Research with NO_2 clearly indicates that exposure to NO_2 can impair a number of the body's defense mechanisms. The consequence of such a suppression would lead ultimately to increased microbial proliferation within the lung, resulting in an increase in the incidence and severity of pulmonary infections.

Animal studies have demonstrated that both acute and chronic exposures to NO_2 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 NO_2-exposed mice are challenged with a viable microbial aerosol and mortality measured is the most sensitive. For example, a 39-day exposure to 940 $\mu g/m^3$ (0.5 ppm) NO_2 increased influenza-induced mortality, and a 6-month exposure to 940 $\mu g/m^3$ (0.5 ppm) NO_2 increased bacterial-induced mortality. After an acute exposure, 3,760 $\mu g/m^3$ (2.0 ppm) is the lowest effective level tested for the bacterial model.

The mouse streptococcal infectivity model has been applied extensively to elucidate C × T relationships. Concentration has a predominant influence over time of exposure. In the urban air, the typical pattern of NO_2 is a low-level baseline exposure on which are superimposed peaks corresponding to peaks of NO_x 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. 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 $\mu g/m^3$ (0.2 ppm) NO_2 baseline exposure (21 h/day, 7 days/week) was compared to baseline plus two daily 1-h spikes of 1,504 $\mu g/m^3$ (0.8 ppm) NO_2 for (5 days/week). Only the baseline plus spike group exhibited significant increased susceptibility to bacterial infection.

The effects associated with NO_2 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 animal data, it is understood that in humans, different exposure levels of NO_2 may be required to produce effects similar to those seen in animals.

13.2.3.2 Lung Biochemistry

Studies of lung biochemistry in animals exposed to NO_2 have focussed on either the putative mechanisms of toxic action of NO_2 or on detection of indicators of tissue and cell damage. The currently popular theory to explain NO_2 toxicity is that NO_2 initiates lipid peroxidation in unsaturated fatty acids in membranes of target cells. These changes are thought to cause cell injury or death and the symptoms associated with NO_2 inhalation. An alternative theory is that NO_2 oxidizes water-soluble, low molecular weight reducing substances and proteins, resulting in a metabolic dysfunction that evidences itself as the toxic symptom. Nitrogen dioxide may, in fact, act by both means and, as a consequence, may affect the intermediary metabolism of animals and their growth and maturation. Nitrogen dioxide dissolves in water to produce nitrous (HNO_2) and nitric (HNO_3) acids; thus, the possibility of an acid or a pH effect as a primary or secondary mechanism of injury should also be considered. In addition, several potential biochemical mechanisms related to detoxification of NO_2 or to responses to NO_2 intoxication have been proposed and are outlined in Tables 13-7, 13-8, and 13-9.

Lipid Metabolism

Various investigators have performed in vitro experiments to evaluate the effects of NO_2 on isolated lung cells or subcellular components to examine the possible relevance of these effects as they relate to the NO_2 oxidation of unsaturated fatty acids. In a recent series of studies, Patel and Block (1986a, 1986b, 1987, 1988) examined the effects of NO_2 exposure on cultured endothelial cells from either pulmonary arteries or aortae of pigs. After exposing the lung cells to 9,400 $\mu g/m^3$ (5 ppm) for 24 h, they observed changes in membrane fluidity, lipid peroxide formation, 5-hydroxytryptamine uptake, and release of lactate dehydrogenase (a marker for cell membrane abnormalities). The authors concluded that injury of endothelial cells by NO_2 can be ascribed to decreased membrane fluidity secondary to lipid peroxidation,

TABLE 13-7. EFFECTS OF NO_2 ON LIPID METABOLISM

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
75 750 7,500	0.04 0.4 4.0	Continuous, 9, 18, or 27 mo	Rat	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. No changes in total lipid, phospholipid, total cholesterol, or triglyceride contents.	Sagai et al. (1984)
75 225 750	0.04 0.12 0.4	Continuous, 6, 9, and 18 mo		Increased ethane exhalation after 9 and 18 mo.	
940 plus 61 NO	0.5 plus 0.05 NO	8 h/day, 122 days	Guinea pig	No effect on total lung content of phospholipid; alterations in individual phospholipid classes. Ammonia had no impact.	Trzeciak et al. (1977)
NO_x + equal amount ammonia	NO_x + equal amount ammonia				
750 1,880 5,640 9,400	0.4 1.0 3.0 5.0	72 h	Guinea pig	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.	Selgrade et al. (1981)
9,400	5.0	3 h		Increased lung lipid content in vitamin C-depleted guinea pigs 18 h postexposure.	
750	0.4	72 h or 1 wk		No mortality or effect on lung lavage fluid composition in normal or vitamin C-depleted animals..	

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TABLE 13-7 (cont'd). EFFECTS OF NO_2 ON LIPID METABOLISM

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
5,640	3.0	Continuous, 17 days	Rat	Decrease in linoleic and linolenic acid content of lung lavage fluid.	Menzel et al. (1972)
18,800	10.0	Continuous, 4 wk		Decrease in unsaturated fatty acids in lavage and lung tissue.	
5,600	3.0	7 days	Rat	Increased lipid peroxidation with Vitamin E deficiency.	Sevanian et al. (1982)
9,400	5.0	24 h	Pig (in vitro)	Changes in membrane fluidity, lipid peroxide formation, 5-hydroxytryptamine uptake, and release of lactate dehydrogenase.	Patel and Block (1986a,b, 1987, 1988)
9,400	5.0	48 h	Pig (in vitro)	Increased degradation of phosphatidylethanolamine to lyso-phosphatidylethanolamine in epithelial cells from pulmonary artery; also, increased concentration of phosphatidylserine in plasma membrane.	Sekharam et al. (1991)
18,800	10.0	5 h/wk, 4-8 wk	Hamster (Vitamin A deficient)	Lipid droplets in alveolar walls; reduced DNA uptake.	Kim (1977, 1978)
18,800	10.0	12 h	Rat	Changes in fatty acids of lung lavage phospholipids.	Kobayashi et al. (1984)
18,800	10.0	1, 3, 5, 7, 14 days	Rat	Changes in prostaglandins and thromboxane B_2 in lung lavage fluid.	Kobayashi (1986)
56,400	30.0	1 or 5 h	Rat	Increased incorporation of [^{3}H]thymidine in lung DNA and increased phosphatidylcholine content.	Blank et al. (1982)

TBA = Thiobarbituric acid.

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TABLE 13-8. EFFECTS OF NO_2 ON LUNG AMINO ACIDS, PROTEINS, AND ENZYMES

NO_2 Concentration		Exposure	Species	Effects	Reference
$\mu g/m^3$	ppm				
750 1,880 5,640 9,400	0.4 1.0 3.0 5.0	72 h	Guinea pig	No effect at 0.4 ppm; increase in lung lavage protein in vitamin C-depleted but not normal animals at 1.0 ppm and above.	Selgrade et al. (1981)
9,400	5.0	3 h		Increased lung lavage protein in vitamin C-depleted guinea pigs 15-h postexposure.	-
750	0.4	Continuous, 1 wk		No effect on lung lavage protein in vitamin C-depleted guinea pigs.	
750 2,250 7,500 18,800	0.4 1.2 4.0 10.0	2 wk 1-16 wk	Rat	Changes in TBA-reactive substances, exhaled ethane, and enzyme activities in lung homogenates.	Ichinose et al. (1983)
75, 750 7,500	0.04 0.4 4.0	9, 18, 27 mo			
750	0.4	Continuous, 1 wk	Guinea pig	Increase in lung protein content of guinea pigs with an unquantified vitamin C deficiency.	Sherwin and Carlson (1973)
750 2,250 7,500	0.4 1.2 4.0	1 to 14 wk	Rat	Changes in succinate-cytochrome c reductase and cytochrome P-450 activities.	Takahashi et al. (1986)
750 2,250 7,500	0.4 1.2 4.0	4 mo	Rat	Changes in ethane exhalation, TBA-reactive substances and enzyme activities in lung homogenates.	Ichinose and Sagai (1982)
845	0.45	7 h/day, 4 wk	Mouse	No changes in lung serotonin levels; increase in brain serotonin and 5-hydroxyindoleacetic acid content.	Sherwin et al. (1986)
935	0.47	Continuous, 10, 12, 14 days	Mouse	Increased content of serum proteins in homogenized whole lung tissue.	Sherwin and Layfield (1976)

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TABLE 13-8 (cont'd). EFFECTS OF NO_2 ON LUNG AMINO ACIDS, PROTEINS, AND ENZYMES

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
1,880	1.0	Continuous, 2 wk	Rabbit	Decrease in lecithin synthesis after 1 wk; less marked depression after 2 wk.	Seto et al. (1975)
1,880 9,400	1.0 5.0	15 wk	Rat	Changes in lung lavage fluid and tissue levels of enzymes early in exposure; resolved by 15 wk.	Gregory et al. (1983)
1,880- 18,800	1.0-10	2 h	Rabbit	1 ppm: elevated thromboxane B_2. 3 ppm: depressed thromboxane B_2. 10 ppm: depressed 6-keto-$PGF_{1\alpha}$ and thromboxane B_2.	Schlesinger et al. (1990)
3,760	2.0	Continuous, 1-3 wk	Guinea pig	Increase in number of lactic acid dehydrogenase—positive cells with time of exposure. Suggest Type 1 (LDH negative) cells decrease as Type 2 (LDH positive) cells increase.	Sherwin et al. (1973)
3,760 18,800	2.0 10.0	3 days	Rat Guinea pig	Decreased lung superoxide dismutase activity.	Azoulay-Dupuis et al. (1983)
3,760 7,500 18,800	2.0 4.0 10	7-14 days	Rat	Increased activity of lung glycolytic enzymes.	Mochitate et al. (1985)
5,450	2.9	Continuous, 5 days/wk, 9 mo	Rat	Increase in lung wet weight (12.7%) and decrease in total lipid (8.7%); decrease in saturated fatty acid content of lung lavage fluid and tissue; increase in surface tension of lung lavage fluid.	Arner and Rhoades (1973)
5,640	3.0	7 days	Rat	Various changes in lung homogenate protein and DNA content and enzyme activities; changes more severe in vitamin E-deficient rats.	Elsayed and Mustafa (1982)
5,640	3.0	4 h/day, 4 days	Squirrel monkey	Thickening of collagen fibrils.	Bils (1976)

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TABLE 13-8 (cont'd). EFFECTS OF NO_2 ON LUNG AMINO ACIDS, PROTEINS, AND ENZYMES

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
7,520 18,800 47,000	4.0 10.0 25.0	7, 14, and 21 days	Rat	Increased gamma-glutamyl transferase on Days 14 and 21; no consistent effect on akaline phosphatase, lactate dehydrogenase, or total protein.	Hooftman et al. (1988)
9,024	4.8	3 h	Guinea pig	Increased lung lavage fluid protein content.	Hatch et al. (1986)
8,100	4.5	16 h		Increased lung wet weight, alterations in lung antioxidant levels.	
9,024	4.8	7 days	Mouse	No significant changes in lung homogenate parameters; possible synergistic interaction with 0.45 ppm O_3.	Mustafa et al. (1984)
9,400	5.0	14-72 h	Mouse	Increase in lung protein (14 to 58 h) by radioactive label incorporation.	Csallany (1975)
9,400	5.0	2 wk	Rat	Increased amounts of the trytophan metabolites, xanthurenic and kynurenic acid, were excreted in urine during Week 2 of exposure.	Suzuki et al. (1988)
9,400-47,000	5.0-25.0	Continuous, 7 days	Rat	Concentration-related increase in rate of collagen synthesis.	Last et al. (1983)
9,400 37,600 94,000	5.0 20.0 50.0	3 h	Rabbit	Benzo(a)pyrene hydroxylase activity of tracheal mucosa not affected.	Palmer et al. (1972)
9,400	5.0	3 days	Rat	Increased protein content of lung lavage fluid.	Last and Warren (1987)
15,000	8.0	Continuous, 14 days	Mouse	Increase in lung protein.	Csallany (1975)
18,800	10.0	Continuous, 14 days	Rat	Changes in several enzymes in whole lung homogenates.	Sagai et al. (1982)
18,800 7,500	10 4.0	1-7 days	Rat	Increased lung protein content; changes in microsomal enzyme activities.	Mochitate et al. (1984)

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TABLE 13-8 (cont'd). EFFECTS OF NO_2 ON LUNG AMINO ACIDS, PROTEINS, AND ENZYMES

NO_2 Concentration		Exposure	Species	Effects	Reference
$\mu g/m^3$	ppm				
18,800 37,600 56,400 75,000	10.0 20.0 30.0 40.0	4 h	Rat	Increased activities of various enzymes, sialic acid, and protein content in lung lavage fluid; attenuation of these responses by high dietary levels of vitamin E.	Guth and Mavis (1985, 1986)
37,600	20.0	16 h	Mouse	Increased activity of various enzymes in lung and increased TBA-reactive material.	Ichinose et al. (1982)
56,400	30.0	Continuous, to 30 days	Hamster	Increase in lung proteolytic activity and in serum antiprotease at 2 days; declined to normal values at 50 days.	Kleinerman and Rynbrandt (1976)
56,400	30.0	Continuous, to 30 days	Hamster	Increase in lung proteolytic activity at 2 and 5 days, but the optimum pH was acidic (3.0); not active at physiological pH of 7.2; attributed to cathepsins, A, B, C, D, and E.	Rynbrandt and Kleinerman (1977)
56,400	30.0	22 h/day, 3 wk	Hamster	Loss of body weight; increased dry lung weight; decrease in total lung collagen within 4 days and total lung elastin within 10 days; collagen levels return to normal by Day 14 of exposure; three wk after exposure ceased lung elastin levels had returned to normal.	Kleinerman and Ip (1979)
75,200	40.0	5 h	Rat	Increased incorporation of ^{14}C-palmitic acid in lung lecithin; accumulation of disaturated lecithin by 6 h after NO_2 with maximum accumulation by 48 h.	Blank et al. (1978)
75,200 132,000	40.0 70.0	2 h	Rat	Benzo(a)pyrene hydroxylase, phenol-0-methyl transferase, and catechol-0-methyl transferase not affected.	Law et al. (1975)
56,400	30.0	3 wk + 3 wk recovery in air	Hamster	Changes in apparent levels of lung collagen and elastin.	Kleinerman and Ip (1979)

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TABLE 13-9. EFFECTS OF NO_2 ON ANTIOXIDANT METABOLISM AND INFLUENCE OF ANTIOXIDANTS

NO_2 Concentration		Exposure	Species	Effects*	Reference
$\mu g/m^3$	ppm				
75 750 7,500	0.04 0.4 4.0	Continuous, 9 and 18 mo	Rat	NPSH increased at the 2 higher NO_2 levels 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; G-6-PD was increased after a 9- and 18-mo exposure to 4.0 ppm; no effects on 6-phosphogluconate dehydrogenase, superoxide dismutase, or disulfide reductase; some GSH S-transferase had decreased activities after 18-mo exposure to 0.4 and 4.0 ppm.	Sagai et al. (1984)
750-940	0.4-0.5	Continuous, 1.5 yr	Mouse	Growth reduced; vitamin E (30 or 300 mg/kg diet) improved growth.	Csallany (1975)
940 1,880	0.5 1.0	Continuous, 17 mo	Mouse	Decreased body weight with vitamin E-deficient, vitamin E-supplemented (30 and 300 ppm) and DPPD-supplemented (30 ppm); no change in tissue weight with exception of increased kidney weight in 1 ppm exposed animals with vitamin E-deficient diet; slightly decreased survival rate.	Csallany and Ayaz (1978)
940	0.5	Continuous, 17 mo	Mouse	No change in blood and lung GSH-peroxidase activity.	Ayaz and Csallany (1978)
1,880	1.0	4 h/day, 6 days	Rat	Vitamin E supplement reduced the lipid peroxidation in exposed rats.	Thomas et al. (1967)
1,880 4,330 11,560	1.0 2.3 6.2	Continuous, 4 days	Rat	Activities of GSH reductase and G-6-PD increased at 6.2 ppm proportional to duration of exposure. Plasma lysozyme and GSH peroxidase not affected; no effects at 1.0 or 2.3 ppm.	Chow et al. (1974)

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TABLE 13-9 (cont'd). EFFECTS OF NO_2 ON ANTIOXIDANT METABOLISM AND INFLUENCE OF ANTIOXIDANTS

NO_2 Concentration		Exposure	Species	Effects*	Reference
$\mu g/m^3$	ppm				
5,600	3.0	7 days	Rat	No effects on parameters tested.	Mustafa et al. (1979a,b)
13,200	7.0	4 days		Increase in lung weight, G-6-PD, GSH reductase, and GSH peroxidase.	
18,800	10.0	4 days		Increase in lung weight, G-6-PD, 6-P-gluconate dehydrogenase, and GSH reductase.	
28,200	15.0	4 days		Increase in lung weight, DNA content, G-6-PD, 6-P-gluconate dehydrogenase, GSH reductase, disulfide reductase, GSH peroxidase, succinate oxidase, and cytochrome oxidase; no effect on lung protein.	
11,000	6.0	4 h/day, 30 days	Mouse	Increase in GSH reductase and G-6-PD activities.	Csallany (1975)
28,000 53,000	15.0 28.0	7 days		Increase in GSH levels, GSH reductase, G-6-PD, and GSH peroxidase.	
26,320	14.0	Continuous, 12, 24, 48, 72, or 96 h	Rat	Significant elevation in G-6-PD, GSH reductase, and GSH peroxidase activities in young adult and older exposed rats; less effect on enzyme activities in weanling rats and no change in enzyme activities in exposed neonates.	Tyson et al. (1982)
62,040	33.0	Continuous, to 23 days	Rat	High level of dietary antioxidants increased the time until 50% mortality occurred (LT_{50}). LT_{50} of vitamin E depleted rats was 11.1 days versus 170 days for vitamin E-supplemented rats.	Menzel et al. (1972)
37,600-47,000	20.0-25.0	8 h	Rat	Increased survival rate in rats fed vitamin E-supplemented diet (1,500, 150, 45, or 10.5 mg/kg diet.	Fletcher and Tappel (1973)

*GSH = Glutathione.
G-6-PD = Glucose-6-phosphate dehydrogenase..
NPSH = Non-protein sulfhydryls.

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and that the altered physical state of the cell membrane causes impaired functionality of the membrane, leading to cellular abnormalities of metabolism and biochemistry. In a more recent study, Sekharam et al. (1991) found that NO_2-induced oxidative damage in epithelial cells (from the pulmonary artery of pigs) exposed to 9,400 $\mu g/m^3$ (5 ppm) for 48 h activates phospholipase A_1 and increases degradation of phosphatidylethanolamine to lyso-phosphatidylethanolamine. The plasma membrane concentration of phosphatidylserine was also increased in these cells.

Rietjens and co-workers (1986, 1987) exposed cultured rat AMs to NO_2 or O_3 by gas diffusion through a Teflon® film. Ozone appeared to be about 10 times more toxic than NO_2 in the system. Based on various experiments using different radical scavengers, the authors concluded that O_3 and NO_2 acted by different mechanisms, where NO_2 exerted its toxicity via a free radical-mediated peroxidative pathway and O_3 via a pathway involving the formation of lipid ozonides. Both O_3 and NO_2 appear to act at the level of lipid oxidation in causing AM toxicity. However, is should be noted that the chemical reactions of O_3 or NO_2 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, NO_2 or O_3 may react with unsaturated fatty acids, 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 NO_2 on AMs appears in the sections on host defense mechanisms and morphological effects, respectively.

Severe malnutrition of animals can drastically affect their response to toxicants, including NO_2. Experimental interest in this area has mainly focussed on dietary lipids, vitamin E and other lipid-soluble antioxidants, and vitamin C and other water-soluble antioxidants. Roehm et al. (1971) studied the in vitro oxidation of unsaturated fatty acids by O_3 and NO_2. Both NO_2 and O_3 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 2,800 $\mu g/m^3$ (1.5 ppm) NO_2. The addition of free radical-scavenging agents such as vitamin E, butylated hydroxytoluene, or butylated hydroxyanisol delayed the onset of oxidation in vitro. The rate of oxidation of linolenic acid in thin films was proportional to concentrations of NO_2 from 940 to 10,200 $\mu g/m^3$ (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 NO_2 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 NO_2. Phenolic antioxidants can prevent the autoxidation of unsaturated fatty acids by NO_2 by reacting with both fatty acid hydroperoxyl free radicals and nitrohydroperoxyl free radicals generated by the addition of NO_2 to unsaturated fatty acids. It is not known whether this sequence of reactions is important in the lung in vivo.

Sagai et al. (1984) reported an increase in thiobarbituric acid (TBA) reactants in rats exposed to 7,520 $\mu g/m^3$ (4.0 ppm) NO_2 continuously for 9 months. When exposure was increased to 18 months, an exposure-related increase in TBA reactants was seen in rats exposed to 75, 752, and 7,520 $\mu g/m^3$ (0.04, 0.4, and 4.0 ppm) NO_2, but the increase was only significant in animals exposed to the two highest concentrations. Total lung protein content was not affected by the NO_2 exposure. The exhalation of ethane in the breath was measured in an assay of in vivo lipid peroxidation. 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 not a major difference between room and chamber control values. At 75, 750, and 7,500 $\mu g/m^3$, 9 and 18 months of exposure increased the exhalation of ethane. The two lower NO_2 concentrations also increased ethane exhalation after 27 months of exposure, but at 7,500 $\mu g/m^3$, ethane was within the control range. 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 750 $\mu g/m^3$ NO_2, supporting the interpretation of the ethane results. In a second series of experiments, chamber control rats were used and rats were exposed to 75, 225, and 750 $\mu g/m^3$ (0.04, 0.12, and 0.4 ppm) for 6, 9, and 18 months. After 6 months, ethane exhalation only increased at 750 $\mu g/m^3$. All NO_2 concentrations increased ethane exhalation after 9 and 18 months of exposure. These studies show 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). Based on their body of work and

other related studies, Sagai et al. (1984) hypothesize that the cause of the increased lipid peroxidation is also related to NO_2-induced thickening of alveolar walls and decreased O_2 tension in arterial blood.

Arner and Rhoades (1973) exposed rats for 9 months 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. Values for specific unsaturated fatty acids of biological importance were not reported. The surface tension of extracts of the lung increased, and the authors suggested that the increased surface tension corresponded to a decrease in the lung surfactant concentration.

Trzeciak et al. (1977) exposed guinea pigs to 940 $\mu g/m^3$ (0.8 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. No differences were found in the total weight of phospholipids of exposed vs. control lungs. It should be noted that the vast majority of lung phospholipids contain saturated fatty acids. Significant alterations were found in the individual phospholipid classes. Decreases were noted in phophatidylethanolamine, sphingomyelin, phosphatidyl serine, phosphatidyl glycerol-3-phosphate, and phosphatidic acid. Increases were noted in the lysophosphatidyl ethanolamine content, while the phosphatidyl choline (lecithin) content remained constant or was slightly depressed. Such changes could be indicative of changes in cell content of the lung. The presence of ammonia did not significantly influence the results.

Lecithin synthesis appeared to be depressed in the lungs of rabbits exposed to 1,880 $\mu g/m^3$ (1 ppm) of NO_2 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.

Csallany (1975) exposed mice continuously for 1.5 years to 750 to 940 $\mu g/m^3$ (0.4 to 0.5 ppm) or 1,790 to 1,880 $\mu g/m^3$ (0.95 to 1.0 ppm) NO_2. Animals were fed a basal diet that was either deficient in, or supplemented with, vitamin E at 30 or 300 mg/kg of diet. Nitrogen dioxide reduced the growth rate in all four diet groups, but the vitamin E-supplemented groups grew faster than the nonsupplemented groups. The higher level of vitamin E in the diet gave the same growth rate as the intermediate vitamin E content diet.

In other studies from this group, (Ayaz and Csallany, 1977, 1978; Csallany and Ayaz, 1978) female weanling mice were exposed to 940 or 1,880 $\mu g/m^3$ (0.5 or 1.0 ppm) NO_2 continuously for 17 months. Animals were divided into three groups receiving the basal diet with either a normal supplement of vitamin E (30 mg/kg), or 300 mg/kg, or 30 mg/kg of the synthetic antioxidant N,N-diphenylphenylenediamine. After 17 months 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.

Exposure of vitamin A-deficient hamsters to 18,800 $\mu g/m^3$ (10 ppm) NO_2 for 5 h once a week for 4 to 8 weeks reportedly caused lung damage as compared to NO_2-exposed hamsters with sufficient vitamin A intake (Kim 1977, 1978).

Normal or vitamin C-depleted guinea pigs were exposed to 752, 1,880, 5,460, or 9,400 $\mu g/m^3$ (0.4, 1.0, 3.0, or 5.0 ppm) NO_2 for 72 h and the lung lavage protein and lipid content were determined (Selgrade et al., 1981). No effect was observed in guinea pigs having normal blood contents of vitamin C, but vitamin C-depleted guinea pigs, having an average of 25% of the normal blood vitamin C content, had greater lavagable protein and lipid content, except for those guinea pigs exposed to 752 $\mu g/m^3$ (0.4 ppm) NO_2. In animals exposed to 9,400 $\mu g/m^3$ (5.0 ppm) NO_2, the changes in lavage fluid composition were correlated with mortality (50%) and alveolar edema as observed by conventional light microscopic histopathology.

Effects on Sulfhydryl Compounds and Pyridine Nucleotides

Oxidation of sulfhydryl compounds and pyridine nucleotides in the lung is well-established for O_3 exposures (Evans et al., 1974), but little evidence has been reported for NO_2 (Guth and Mavis, 1985).

Effects on Lung Amino Acids, Proteins, and Enzymes

Nitrogen dioxide can oxidize various reducible amino acids or side chains of proteins in aqueous solution (Freeman and Mudd, 1981). For example, Prutz et al. (1985) showed that NO_2 could oxidize tyrosine in peptides or proteins in pulse radiolysis experiments. Suzuki et al. (1988) have reported increased amounts of trytophan metabolites in the urine of rats exposed for 2 weeks to 9,400 $\mu g/m^3$ (5 ppm) NO_2. Concentrations of NO_2 above

9,400 $\mu g/m^3$ produce lung edema with concomitant infiltration of serum protein and enzymes. Influx of inflammatory cells (predominantly leukocytes) from blood may occur. Alterations in the epithelial cell types of the lung also can 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 NO_2 on lung cell enzymes.

Nitrogen dioxide also increased the protein content of lung lavage in Vitamin C-depleted guinea pigs (Selgrade et al., 1981; Sherwin and Carlson, 1973). Selgrade et al. (1981) found effects as low as 1,880 $\mu g/m^3$ (1.0 ppm) after a 72-h exposure, but a 1-week exposure to 750 $\mu g/m^3$ (0.4 ppm) did not increase protein in lung lavage. 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 deficiencies may explain the difference between the two studies.

Sherwin et al. (1972) exposed guinea pigs to 3,760 $\mu g/m^3$ (2 ppm) NO_2 for 1, 2, or 3 weeks. They examined lung sections histochemically for lactic acid dehydrogenase (LDH). With this technique, LDH is suggested 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 NO_2 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 morphological studies (see Section 13.2.3.4).

Benzo(a)pyrene hydroxylase activity of the tracheobronchial region of the lung was studied by Palmer et al. (1972) in rabbits that had been exposed to 9,400, 37,600, or 94,000 $\mu g/m^3$ (5, 20, or 50 ppm) NO_2 for 3 h. No effect was observed on the benzo(a)pyrene hydroxylase activities with NO_2 exposure. Law et al. (1975) studied the effect of NO_2 on benzo(a)pyrene hydroxylase, microsomal O-methyl transferase, catechol O-methyl transferase, and supernatant catechol O-methyl transferase activities of rat lungs. While benzo(a)pyrene hydroxylase activity of the lung could be induced by treatment with the

carcinogen 3-methylcholanthrene, exposure to 75,200 or 132,000 $\mu g/m^3$ (40 to 70 ppm) NO_2 for 2 h had no effect. Thus, the studies of Palmer et al. (1972) and Law et al. (1975) agree that NO_2 exposure does not effect 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, and does not appear to be affected by NO_2 treatment.

A major concern has been the effect of NO_2 on the structural proteins of the lungs, since elastic recoil is lost after exposure. Bils (1976) reported a thickening of the collagen fibrils in squirrel monkeys exposed to 5,640 $\mu g/m^3$ (3 ppm) NO_2, 4 h/day for 4 days. Kosmider et al. (1973a) reported that the urinary hydroxyproline and acid mucopolysaccharide content of guinea pigs exposed to 1,880 $\mu g/m^3$ (1 ppm) NO_2 for 6 months were significantly increased. Since the remodeling of bone is the major source (>90%) of urinary hydroxyproline in normal animals and dietary ascorbate status would affect hydroxyproline homeostasis in guinea pigs, the significance of these observations to lung structure and function remains to be shown.

Last and co-workers have examined the response of rats to exposure to O_3 or NO_2 by quantitative analysis of apparent collagen synthesis rates by 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 $\mu g/m^3$ (5 to 25 ppm) NO_2 for 7 days. The authors found a linear exposure concentration-response curve for plots of collagen synthesis rate vs. NO_2 concentration over this range of NO_2 exposures, with a correlation coefficient (least squares analysis) for fit of the data to a straight line of 0.92. Interestingly, the ratio of the slope of this line to a similar exposure concentration-response curve for O_3, a measure of the relative toxicity of O_3 to NO_2, was about 18:1—in good agreement with calculations of their relative toxicity based upon histological indices of damage (Freeman et al., 1974a). Extrapolation (linear) of the line to an estimated no-observable-effect level (NOEL) gave a value of about 1,880 to 5,640 $\mu g/m^3$ (1 to 3 ppm) NO_2, which is in good agreement with results presented in a subsequent paper by this group (Last and Warren, 1987). 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.

Effects on Antioxidant Metabolism and Influence of Antioxidants

Menzel (1970) proposed that antioxidants might protect the lung from NO_2 damage by inhibiting lipid peroxidation. Data related to this hypothesis have been reported Ayaz and Csallany (1978), Csallany (1975), Fletcher and Tappel (1973), Menzel et al. (1972), and Thomas et al. (1968). Many laboratories have observed changes in the activity of enzymes in the lungs of NO_2-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 NO_2. Buthionine sulfoxime, an inhibitor of GSH synthesis, has also been shown to cause increased lung damage in mice exposed to 1.0 ppm O_3, 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 O_3, involving coupled reactions of glucose-6-phosphate dehydrogenase (to produce NADPH), GSH reductase (to regenerate NADP), and GSH peroxidase (to regenerate GSH). Chow et al. (1974) exposed rats to 1,880, 4,330, or 11,560 $\mu g/m^3$ (1.0, 2.3, or 6.2 ppm) NO_2 continuously for 4 days to examine the effect on the GSH peroxidase system. They determined the activity of GSH reductase, glucose-6-phophate dehydrogenase, and GSH peroxidase in the soluble fraction of exposed rat lungs. Linear regression analysis of the correlation between the NO_2 concentration and enzymatic activity showed a significant positive correlation coefficient of 0.63 for GSH reductase and of 0.84 for glucose-6-phosphate dehydrogenase. No correlation was found between the GSH peroxidase activity and the NO_2 exposure concentration. The activities of GSH reductase and glucose-6-phosphate dehydrogenase were significantly increased during exposure to 11,560 $\mu g/m^3$ NO_2. The possible role of edema and cellular inflammation in these findings was not examined. These researchers concluded: "Since exposure of rats to NO_2 has insignificant effect on lung GSH peroxidase activity, but had significantly increased the activities of GSH reductase and G-6-P dehydrogenase, it appears that this oxidant attacks mainly glutathione and NADPH while O_3 not only initiates lipid peroxidation but also directly attacks these reducing substances."

Sagai et al. (1984) studied the effects of prolonged (9 and 18 months) exposures to 75, 750, and 7,500 $\mu g/m^3$ (0.04, 0.4, and 4.0 ppm) NO_2 on rats. Non-protein sulfhydryl levels were increased by 750 and 7,500 $\mu g/m^3$ after both exposure durations. Nine- and 18-month

exposures to 7,500 $\mu g/m^3$ caused a decrease in the activity of GSH peroxidase and an increase in glucose-6-phosphate dehydrogenase activity. Glutathione peroxidase activity was also decreased in rats exposed to 750 $\mu g/m^3$ NO_2 for 18 months. Three GSH S-transferases were also studied, two of which (aryl S-transferase and aralkyl S-transferase) exhibited decreased activities after 18 months of exposure to 750 and 7,500 $\mu g/m^3$ NO_2. No effects were observed on the activities of 6-phosphogluconate dehydrogenase, superoxide dismutase, or disulfide reductase. When effects were observed, they followed a concentration- and exposure duration-response. The decreases in antioxidant metabolism were inversely related to the formation of lipid peroxides (see lipid subsection).

Ayaz and Csallany (1978) exposed female mice continuously for 17 months to 940 or 1,880 $\mu g/m^3$ (0.5 or 1.0 ppm) NO_2 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 940 $\mu g/m^3$ NO_2 did not alter blood or lung GSH peroxidase; however, exposure to 1,880 $\mu g/m^3$ NO_2 suppressed enzyme activity. A combination of vitamin E deficiency and 1,880 $\mu g/m^3$ NO_2 exposure resulted in the lowest GSH peroxidase activity in blood and lung. Liver GSH peroxidase was unaffected by either vitamin E deficiency or NO_2 exposure.

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. Since 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.

Summary

The most popular theory describing NO_2 toxicity is that of lipid peroxidation of unsaturated fatty acids in target cell membranes. An alternate theory is that NO_2 oxidizes water-soluble low molecular weight reducing substances and proteins. Significant alterations in individual phospholipid classes have been reported in Vitamin C-deficient guinea pigs exposed to 750 $\mu g/m^3$ (0.4 ppm) for 72 h and Vitamin C-normal guinea pigs exposed for 8 h/day, for 122 days to 940 $\mu g/m^3$ (0.5 ppm) NO_2. Exposure to 75 (0.04 ppm) NO_2

increased lipid peroxidation (as indicated by increased ethane exhalation) in the lungs of rats exposed for 9 months or longer. No changes in blood and lung GSH peroxidase activity were reported in mice exposed to 940 $\mu g/m^3$ (0.5 ppm) NO_2 continuously for up to 17 months; however, when the exposure concentration was increased, a suppression of GSH peroxidase activity was noted. This enzyme activity was decreased in rats exposed to 750 $\mu g/m^3$ for 18 months; other antioxidant enzymes were also affected.

13.2.3.3 Pulmonary Function

The key issues addressed by investigators evaluating the effects of NO_2 on pulmonary function in experimental animals were (1) the effects of low-level, long-term exposures to an urban pattern of NO_2, the lowest concentrations which 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 NO_2 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. While hamster lungs at 40 days have reached adult volumes and elasticity, lung growth through alveolar enlargement continues in rats to 5 months of age (Mauderly, 1989).

Results of exposures to NO_2 are shown in Table 13-10. Although data in Table 13-10 are presented in order of increasing concentrations, results will be discussed in a slightly different order so that, where appropriate, papers using similar exposure patterns or by the same investigators can be discussed together.

Nitrogen dioxide concentrations in urban areas are not constant but consist of spikes superimposed on a relatively constant background level. Miller et al. (1987) evaluated this urban pattern of NO_2 exposure in mice using continuous 7 days/week, 23 h/day exposures to 380 $\mu g/m^3$ (0.2 ppm) NO_2 with twice daily (5 days/week), one-hour spike exposures to 1,500 $\mu g/m^3$ (0.8 ppm) NO_2 for 32 and 52 weeks. Mice exposed to clean air and to the constant background concentration of 380 $\mu g/m^3$ (0.2 ppm) served as controls. Data from

TABLE 13-10. EFFECTS OF NO_2 ON PULMONARY FUNCTION

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
380 380 (base) + 1,500 spike	0.2 0.2 (base) + 0.8 (spike)	23 h/day, 32 and 52 wk with 1-h spikes twice/day 4× base concentration	Mouse	Decreased end-expiratory volume and vital capacity following 0.2 ppm + 0.8 ppm spike NO_2 exposures compared with 0.2 ppm NO_2 exposures. Tendency toward increased residual volume following spike NO_2 exposures compared with control and 0.2 ppm NO_2 exposures.	Miller et al. (1987)
750 2,250 7,520	0.4 1.2 4.0	1, 2, and 3 mo	Rat	Decreased heart rate following 1-mo exposure to 1.2 and 4.0 ppm NO_2. Decreased body weight and PaO_2 following 3-mo exposure to 4.0 ppm NO_2.	Suzuki et al. (1981)
940 (base) + 2,820 (spike), 1,880 (base) + 5,640 (spike), 3,760 (base) + 11,280 (spike)	0.5 (base) + 1.5 (spike), 1.0 (base) + 3.0 (spike), 2.0 (base) + 6.0 (spike)	23 h/day, 7 days/wk base; 1-h spikes twice/day, 5 days/wk 3× base concentration; 1, 3, and 6 wk	Rat (1-day and 7-wk old)	Increased lung volume and compliance in neonates following 3, but not 6 wk exposure. Decreased body weight and lung compliance in adult rats following 6 wk exposure. Adults recovered by 3 wk postexposure.	Stevens et al. (1988)
940 (base) + 2,820 (spike)	0.5 (base) + 1.5 (spike)	23 h/day, 7 days/wk; 2-h spike, 5 days/wk, 1, 3, 12, 52 and 78 wk	Rat	Decreased ΔFEF_{25} following 78 wk exposure. Concentration-related increase in expiratory resistance and expiratory time and decrease in frequency of breathing during challenge with 4 and 8% CO_2 following 78 wk NO_2 exposure.	Winsett et al. (1990)

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TABLE 13-10 (cont'd). EFFECTS OF NO_2 ON PULMONARY FUNCTION

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
3,760	2.0	8 h/day, 5 days/wk, 8 wk	Hamster	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.	Lafuma et al. (1987)
9,400	5.0	30 min/day, twice/wk for 7 wk; last 5 wk of exposure followed by 10 min exposure to aerosolized albumin	Guinea pig	Increased dyspneic breathing during 4th through 7th wk of NO_2-albumin exposure. No substantial difference between NO- and NO_2-exposed animals.	Yoshida et al. (1980)
9,400 18,800 37,600 75,200	5.0 10 20 40	24 h	Mouse	Concentration-related decrease in forced swimming time immediately following exposure. Elevated blood lactic acid immediately and 24 h following 4-min forced swim in 5 ppm NO_2 exposed group.	Suzuki et al. (1982a)
9,400 18,800 37,600	5.0 10 20	24 h	Mouse	Concentration-related increase in respiration rate. Decrease in $PaCO_2$ following exposure to 5 ppm NO_2 and in PaO_2 following exposure to 10 and 20 ppm NO_2.	Suzuki et al. (1982b)
9,400 188,000	5.0 100	Continuous, 3 days 8 min	Mouse	The irritant response to 8-min, 100 ppm NO_2 exposure, typified by increased respiratory rate, decreased tidal volume and minute volume, was blunted by pre-exposure to 5 ppm NO_2 for 3 days.	McGrath and Smith (1984)

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TABLE 13-10 (cont'd). EFFECTS OF NO_2 ON PULMONARY FUNCTION

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
10,150	5.4	3 h/day for 7, 14, or 30 days Additional groups exposed to 1 ppm O_3 for 3 h	Rat	Tendency toward increased lung volume at low inflation pressures following 30-day NO_2 exposure. Increased pulmonary resistance following 30-day exposure to NO_2 plus O_3 or O_3 alone.	Yokoyama et al. (1980)
13,160-274,500	7.0-146	1 h	Guinea pig	Concentration-related increase in sensitivity to inhaled histamine aerosols 10 min following NO_2 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.	Silbaugh et al. (1981)
14,000 28,000	7.5 15.0	2 h 2 and 4 h	Sheep	Increased pulmonary resistance immediately following 4-h exposure to 15 ppm NO_2. Decreased tracheal mucus velocity immediately following 2-h exposure to 15 ppm NO_2. No consistent effects of exposure on airway reactivity to inhaled carbachol, arterial blood gases, and pulmonary and systemic hemodynamics.	Abraham et al. (1980)
17,860	9.5	7 h/day, 5 days/wk, 24 mo	Rats (normal and elastase-treated)	Increased lung volumes and lung compliance and decreased rate of forced exhalation in NO_2-exposed rats. No significant interactions between NO_2 and elastase treatment.	Mauderly et al. (1990)
17,860	9.5	7 h/day, 5 days/wk, 6 mo (beginning at birth, at conception, or at 6-mo of age)	Rats	No substantive effects of NO_2 exposure in animals exposed from conception until 6-mo after birth or in animals beginning exposure at 6-mo of age.	Mauderly et al. (1987)

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TABLE 13-10 (cont'd). EFFECTS OF NO_2 ON PULMONARY FUNCTION

NO_2 Concentration		Exposure	Species	Effects*	Reference
$\mu g/m^3$	ppm				
37,600	20	23 h/day, 20 days, 30 day recovery	Mouse	Mice with connective-tissue disease tended to have reduced lung distensibility immediately and 30 days following NO_2 exposure. Mice without genetic lung disease showed no consistent effects.	Suzuki et al. (1982c)
37,600	20	2.5 h	Rat	Bradycardia and arrhythmia produced in both tracheostomized and nontracheostomized rats, suggesting that upper airway irritation (trigeminal nerve stimulation) was not important.	Tsubone et al. (1984)
56,400	30	23 h/day, 7 days, 1 yr recovery	Hamster (neonate and 3 wk)	Following 1 yr recovery, hamsters exposed as newborns, but not those exposed at 3-wk of age, had increased lung volumes and lower recoil pressures at 40 and 60% of maximum lung volume.	Lam et al. (1983)
65,800	35	6 h/day, 25 days, 10 wk recovery	Rat	No change in arterial blood gases or lung volumes resulting from NO_2 exposures.	Stavert et al. (1986)

*$PaCO_2$ = Arterial carbon dioxide tension.
PaO_2 = Arterial oxygen tension.
ΔFEF_{25} = Change in forced expiratory flow at 25% of forced vital capacity.

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animals examined immediately and 30 days following both exposure durations were combined for analysis since 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 spikes and those exposed to constant background NO_2 levels, although the same pattern of effects was found when comparing spike 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 NO_2 with diurnal spikes than in mice exposed to the constant level of NO_2. Lung distensibility, measured as respiratory system compliance, also tended to be lower ($p = 0.072$) in mice exposed to diurnal spikes of NO_2 compared with constant NO_2 exposure. These changes suggest that up to 52 weeks of low-level NO_2 exposure with diurnal spikes produces some decrease in lung distensibility resulting in decreased respiratory-system compliance and vital capacity. Lung morphology in these mice was evaluated only by light microscopy and showed no exposure-related lesions. The absence of observable morphologic lesions using light microscopy does not preclude the presence of subtle morphologic changes detectable by more sensitive techniques of electron microscopy and qualitative morphometry (see Section 13.2.3.4). The decrease in lung distensibility measured in this study is consistent with the thickening of collagen fibrils in squirrel monkeys exposed to 5,640 $\mu g/m^3$ (3.0 ppm) NO_2, 4 h/day for 4 days (Bils, 1976) and the increase in in vitro lung collagen synthesis rates measured in lung minces from rats exposed in vivo for 7 days to 9,400 to 47,000 $\mu g/m^3$ (5.0 to 25.0 ppm) NO_2 (Last et al., 1983).

Winsett et al. (1990) exposed 60-day-old rats to 940 $\mu g/m^3$ (0.5 ppm) NO_2, 23 h/day, 7 days/week, with a 2-h spike of 2,820 $\mu g/m^3$ (1.5 ppm) NO_2, 5 days/week for up to 78 weeks. Evaluations of pulmonary function conducted at 1, 3, 12, and 52 weeks showed no significant changes. Following 78 weeks of exposure, delta flow at 25% forced vital capacity was 71% lower in NO_2-exposed animals compared with air-exposed animals. The lower delta flow in NO_2-exposed animals may indicate airway obstruction or premature airway closure in the lung periphery. This slowing of the terminal portion of forced expiration may or may not be related to progressive increases in expiratory resistance and expiratory time, and decreases in frequency of breathing between NO_2- and air-exposed rats during a 7-min challenge with 4 and 8% CO_2. Taken together, these results suggest that the

ability of the lung to empty rapidly, particularly at low lung volumes, has been compromised by exposure to NO_2. Morphometric data showing a loss of elastic tissue in the proximal alveolar region provides morphologic correlation to these functional changes.

Effects of exposure to diurnal spikes of NO_2 were also studied by Stevens et al. (1988). These investigators exposed 1-day and 7-week old rats to 940, 1,880, and 3,760 $\mu g/m^3$ (0.5, 1.0, and 2.0 ppm) NO_2 with twice daily 1-h spikes at three-times the baseline concentration for 1, 3, and 7 weeks. The effects on rats beginning exposures at 1 day and 7 weeks of age were substantially different. In rats beginning exposure at 1-day-old, vital capacity and respiratory system compliance were increased following 3 weeks but not 6 weeks of exposure to the 1,880 to 3,760 $\mu g/m^3$ NO_2 baselines with spikes. In rats beginning exposure at 7 weeks of age, respiratory system compliance was decreased following 6 weeks of exposure and body weight was decreased following 3- and 6-weeks of exposure to the 3,760 $\mu g/m^3$ baseline with spike. The decreased compliance in adult rats exposed to NO_2 was associated with a thickening of the alveolar interstitium, particularly in the proximal alveolar region (Chang et al., 1986). A more detailed description of the morphometric findings of this study is reported in Section 13.2.3.4. In the adult rats, pulmonary function changes returned to normal values by 3 weeks after exposure ceased.

Mauderly et al. (1987) measured no substantive effects on pulmonary function in rats exposed to 17,860 $\mu g/m^3$ (9.5 ppm) NO_2, 7 h/days, 5 days/week either beginning exposure at 6 months of age or beginning from the time of conception until 6 months after birth. Differences in the observed effects of NO_2 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 vs. 23 h/day with diurnal spikes), to differences in the stage of lung development at the time of sacrifice (alveolar expansion essentially complete at 6 months, but still continuing at 13 weeks), or to some other factor.

Lafuma et al. (1987) exposed 12-week-old hamsters to 3,760 $\mu g/m^3$ (2.0 ppm) NO_2, 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 H_2O with 2.5% glutaraldehyde) were significantly higher in NO_2-exposed animals than in air-exposed controls independent of elastase treatment. Vital capacity and pulmonary compliance were not affected by NO_2 exposure. Mauderly et al. (1990) exposed rats to

higher concentrations of NO_2 (17,860 $\mu g/m^3$ [9.5 ppm]) for longer times (7 h/day, 5 days/week for 24 months) and also found no increased susceptibility to NO_2 in elastase-treated animals.

Suzuki and Tsubone, along with their colleagues, have conducted extensive studies on the effects of NO_2 on respiratory and cardiac function in mice and rats. Since many cardiovascular effects observed following exposure to NO_2 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-month exposure to 2,250 and 7,520 $\mu g/m^3$ (1.2 and 4.0 ppm) NO_2, but not following 2 and 3 months exposure. Arterial O_2 tension (PaO_2) was decreased following 3 months exposure to 7,520 $\mu g/m^3$ NO_2. Respiratory rate was not affected by NO_2 exposures. Suzuki et al. (1981) also exposed rats for up to 3 months to between 752 and 7,520 $\mu g/m^3$ (0.4 and 4.0 ppm) NO_2. After 3 months of exposure to 7,520 $\mu g/m^3$ NO_2, anesthetized rats, artifically 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) NO_2 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 $\mu g/m^3$ NO_2 compared with controls. Exposures to higher concentrations of NO_2 ($\geq$ 18,800 $\mu g/m^3$) resulted in a concentration-related decrease in maximum forced swimming time. These results are similar to those of Campbell (1976) who measured decreased forced swimming times in rats exposed to 37,600 and 75,200 $\mu g/m^3$ NO_2 for 24 and 5 h, respectively, but not in rats exposed to 15,000 $\mu g/m^3$ (8 ppm) NO_2 for 19 days. Changes in blood lactic acid levels in the Suzuki et al. study indicate that although mice exposed to 9,400 $\mu g/m^3$ NO_2 were able to swim as long as control mice, the cardiorespiratory system was not able to supply sufficient O_2 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, and 37,600 $\mu g/m^3$ (5, 10, and 20 ppm) NO_2 for 24 h. The irritant effect of exposure to 9,400 $\mu g/m^3$ NO_2 resulted in increased respiratory rate and an

associated decrease in arterial carbon dioxide tension ($PaCO_2$), but no effect on PaO_2. Respiratory rates were increased at the two highest NO_2 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 months exposures of mice and rats to NO_2 concentrations of 9,400 $\mu g/m^3$ NO_2 and greater stimulated respiratory reflexes which slow the heart rate and produce concentration-related pulmonary edema which decreases blood oxygenation and impairs maximum exercise performance.

McGrath and Smith (1984) found that the irritant response in mice to an 8 min exposure to 188,000 $\mu g/m^3$ (100 ppm) NO_2 was lessened by a 3-day continuous preexposure to 9,400 $\mu g/m^3$ (5 ppm) NO_2. Considering both 188,000 $\mu g/m^3$ NO_2 exposures and phenyl diguanide injections as irritant challenges, 3 to 7 days exposure to 7,520 to 9,400 $\mu g/m^3$ (4 to 5 ppm) NO_2 lessened the response to 188,000 $\mu g/m^3$ NO_2 exposure, suggesting the development of a tolerance or attenuated response to NO_2 (McGrath and Smith, 1984), but heightened the response to phenyl diguanide injections (Tsubone and Suzuki, 1984). Several other studies have reported the development of a tolerance or attenuated response to subsequent exposures to NO_2 in experimental animals and humans and are discussed in the section on mortality and controlled human clinical studies. Certainly different processes are involved to account for these different responses.

Yoshida et al. (1980b) exposed guinea pigs to 5,898 $\mu g/m^3$ (5.02 ppm) NO or 9,400 $\mu g/m^3$ NO_2 (5.0 ppm). After four preliminary 30-min NO or NO_2 exposures, spread over 2 weeks, animals were exposed twice a week for 10 weeks to either 30 min of NO or NO_2, followed 20 min later by a 10-min exposure to aerosolized albumin. After 5 weeks of NO or NO_2 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 significantly more severe in animals exposed to NO or NO_2 followed by exposure to aerosolized albumin than in animals exposed to albumin alone, with the greatest differences occurring between the fourth and seventh week of exposure to albumin. Animals exposed to NO compared with NO_2 appeared to be slightly

more affected by subsequent albumin exposures, but differences between NO- and NO_2-exposed animals were not significant. Animals previously exposed to NO or NO_2 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

Pulmonary function following NO_2 exposures in experimental animals has shown consistent patterns among different treatment conditions and animal species. Exposures to diurnal spikes of NO_2 superimposed on a constant background level, simulating NO_2 patterns in the urban environment, produced a decrease in lung distensibility in both mice and rats. These changes were very subtle and in mice occurred at concentrations of 380 $\mu g/m^3$ (0.2 ppm) NO_2 with spikes of 1,500 $\mu g/m^3$ (0.8 ppm) after 1 year of exposure. Impaired gas exchange was a predominate feature following several months of exposure to 7,520 $\mu g/m^3$ (4.0 ppm) and was reflected in decreased PaO_2, impaired physical performance, and increased anaerobic metabolism. Newborn and mature animals are affected differently by NO_2 exposures, particularly rats exposed subchronically to continuous background NO_2 concentrations with diurnal spikes. Lung distensibility was increased transiently in rats exposed as newborns, but was decreased in rats exposed as young adults. Hamsters exposed to high NO_2 concentrations as newborns had functional changes 1 year later indicative of mild pulmonary emphysema, which were not found in hamsters exposed when older. All these studies taken together demonstrate that NO_2 produces subtle to dramatic changes in pulmonary function, depending on the concentration and duration of exposure. Lung distensibility and gas exchange are the parameters most consistently affected by exposure.

13.2.3.4 Morphologic Studies

Inhalation of NO_2 produces morphological alterations in the respiratory tract. Tables 13-11, 13-12, and 13-13 provide an overview of results of studies using NO_2 levels >9,400 $\mu g/m^3$ (5 ppm). This discussion is generally limited to those studies using NO_2

TABLE 13-11. EFFECTS OF ACUTE EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
940	0.5	4 h	Rat	Loss of cytoplasic granules in and rupture of mast cells.	Thomas et al. (1967)
940	0.5	Continuous, up to 6 days	Rat	Increased number of mast cells in trachea as exposure duration increased.	Hayashi et al. (1987)
1,880	1.0	1 h	Rat	Degranulation and decreased number of mast cells.	Thomas et al. (1967)
3,760	2.0	3 days	Rat	No pathology.	Azoulay-Dupuis et al. (1983)
3,760	2.0	3 days	Guinea pig	Thickening of alveolar walls; edema; increase in macrophage number; loss of bronchiolar cilia; inflammation.	Azoulay-Dupuis et al. (1983)
5,640-30,100	3-16	1 h	Dog	Edema at ≥7 ppm; some damage to alveolar cell mitochondria and cell membrane at ≥3 ppm.	Dowell et al. (1971)
9,400	5.0	24 h/day, 3 days	Rat	No pathology.	Messiha et al. (1983)
18,800	10.0	3 days	Rat	Fibrin deposition in alveoli; some bronchiolar cilia loss.	Azoulay-Dupuis et al. (1983)
18,800	10.0	3 days	Guinea pig	Severe loss of cilia in trachea and bronchioles; edema; hemorrhage; inflammation; focal emphysema; increased number of Type 2 cells.	Azoulay-Dupuis et al. (1983)
18,800-188,00	10-100	5-30 min	Rat	Type 2 cell hyperplasia at 25 ppm for 30 min and higher exposure levels.	Stavert and Lehnert (1990)
20,000	10.6	6 h	Rat	Cilia loss, shortening of cilia; focal hypertrophy of bronchiolar epithelium.	Rombout et al. (1986)

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TABLE 13-11 (cont'd). EFFECTS OF ACUTE EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
20,700	11	1 h	Rat	No pathology.	Carson et al. (1962)
22,600	12	48 h	Hamster	Mild, multifocal terminal bronchiolitis.	DeNicola et al. (1981)
22,600-33,800	12-18	Continuous, 1 day	Rat	Type 2 cell proliferation.	Evans et al. (1978)
26,300	14	Continuous, 24-72 h	Rat	Loss of ciliated cells, proliferation of nonciliated cells in terminal bronchioles; proliferation of Type 2 cells in adjacent alveoli.	Stephens et al. (1978)
28,200	15	24 h	Hamster	Increase in labelling of Type 2 cells, macrophages, and terminal bronchiolar epithelium.	Hackett (1979)
28,200	15	1-7 days	Rat	Loss of cilia and flattening of epithelium in terminal bronchioles; hyperplasia of Type 2 cells; increase in macrophage number.	Parkinsons and Stephens (1973)
28,200-32,000	15-17	24 h	Rat	Focal loss of cilia in terminal bronchioles; increased division of nonciliated cells in terminal bronchioles.	Evans et al. (1976)
28,200-32,000	15-17	48 h	Rat	Increase in number of labelled Type 2 cells.	Evans et al. (1973b)
32,000	17	1-24 h	Rat	Loss of cilia and hypertrophy of ciliated and nonciliated cells in terminal bronchioles; hypertrophy of Type 1 cells; interstitial edema; repair with Type 2 cells.	Stephens et al. (1972)
32,000	17	Continuous, 7 days	Rat	Increase labelling in terminal bronchioles and alveoli; normal by Day 7.	Evans et al. (1972)

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TABLE 13-11 (cont'd). EFFECTS OF ACUTE EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
32,000	17	48 h	Hamster	Moderate to severe terminal bronchiolitis.	DeNicola et al. (1981)
33,800-37,600	18-20	24 h	Rat	Increased labelling of nonciliated cells (goblet, serous, transitional) in bronchial epithelium.	Evans et al. (1986)
37,600	20	48 h	Mouse	Hyperplasia of terminal bronchiolar epithelium and Type 2 cells; inflammation of terminal bronchioles and adjacent alveoli; increased macrophage number; fibrin deposition.	Hastings et al. (1987)
37,600	20	20 h	Rat	Hypertrophy and hyperplasia of Type 2 cells; thickening of alveolar walls; edema.	Hayashi et al. (1987)
37,600	20	24 h	Rat	Cilia loss in bronchioli, reduction in secretory granules in Clara cells; thickening of Type 1 cells; increase in division of Type 2 cells; decreased rosette formation by macrophages; no change in macrophage number.	Foster et al. (1985)
37,600	20	24 h	Hamster	Desquamation of terminal bronchiolar epithelium; inflammation; loss of cilia; swelling of Type 1 cells; flattening of Clara cells; decrease in macrophage number; increase in rosette formation by macrophages; increase in lymphocytes.	Foster et al. (1985)
41,400	22	48 h	Hamster	Severe terminal bronchiolitis; edema; hemorrhage.	DeNicola et al. (1981)
47,000	25	72 h	Rat	Fibrin accumulation in proximal alveoli; Type 1 cell destruction; edema; increased macrophage number.	Stephens et al. (1982)

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TABLE 13-12. EFFECTS OF SUBCHRONIC EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
207 865 5,264 16,544	0.11 0.46 2.8 8.8	Continuous, 1 mo	Rat (1-, 3-, 12-, 21-mo old)	Various morphometric changes, depending on age and exposure level. Multiphasic pattern (e.g., decrease in air-blood barrier thickness from 1-12 mo of age, and increase in 21-mo old.	Kyono and Kawai (1982)
640	0.34	6 h/day, 5 days/wk, 6 wk	Mouse	Type 2 cell hypertrophy and hyperplasia; increase in mean linear intercept and amount of alveolar wall area.	Sherwin and Richters (1982)
940 + 1,820 3,760 + 11,280	0.5 (base) + 1.5 peak, 2.0 base + 6.0 peak	23 h/day, 7 days/wk base; 1-h peaks twice/day, 5 days/wk 3× base concentration; 6 wk	Rat (1-day and 6-wk old)	In proximal alveolar region: Base (0.5 ppm) + 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. Base (2.0 ppm) + peak (only adults studied) caused similar changes plus an increase in numbers of Type 1 cells, which were smaller than normal Type 1 cells. In terminal bronchiolar region: Base (0.5 ppm) + peak caused no effects in neonates or adults. Base (2.0 ppm) + peak (only adults studied) had fewer ciliated cells with a reduced surface area and alterations in the shape of Clara cells.	Crapo et al. (1984) Chang et al. (1986, 1988)
1,000 2,500 5,000 20,000	0.53 1.33 2.7 10.6	Continuous (24 h/day), 28 days	Rat	At 5.3 and 1.33 ppm: no pathology. At 2.7 ppm: focal thickening of centriacinar septa by 2 days; progressive loss of cilia and abnormal cilia in trachea and main bronchi at ≥4 days; hypertrophy of bronchiolar epithelium at ≥8 days. At 10.6 ppm: hypertrophy of ciliated and Clara cells in terminal bronchioles by 2 days; hyperplasia of bronchioli by 4 days; extensive shortening and loss of cilia in trachea through bronchioles by 4 days; necrosis of Type 1 cells; increase in number of Type 2 cells; thickening of proximal alveolar septa; increased number of macrophages and accumulation in bronchioles; alveolar dilatation.	Rombout et al. (1986)

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TABLE 13-12 (cont'd). EFFECTS OF SUBCHRONIC EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
1,000	0.53	24 h/day, 90 days	Guinea pig Rabbit Dog Monkey Rat	No pathology.	Steadman et al. (1966)
1,320-1,500	0.7-0.8	Continuous, 1 mo	Mouse	Mucous hypersecretion; focal degeneration and desquamation of mucous membrane; terminal bronchiolar epithelial hyperplasia; some alveolar enlargement; shortening of cilia.	Nakajima et al. (1980)
1,880-2,880	1-1.5	Continuous, 1 mo	Mouse	Terminal bronchiolar epithelial hyperplasia; some alveolar enlargement.	Nakajima et al. (1980)
3,760	2.0	8 h/day, 5 days/wk, 8 wk	Hamster	Moderate alveolar enlargement, primarily at bronchiolar-alveolar duct junction; increase in mean linear intercept; decrease internal surface area of lung; no lesions in bronchial, bronchiolar, alveolar duct, or alveolar epithelium; no change in macrophage number.	Lafuma et al. (1987)
3,760	2.0	Continuous, 7-21 days	Guinea pig	Type 2 cell hypertrophy at 7 or 21 days.	Sherwin et al. (1973)
3,760	2.0	Continuous, 6 wk	Rat	Minimal effect: some cilia loss in terminal bronchioles; some distended or disrupted alveolar walls.	Azoulay et al. (1978)

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TABLE 13-12 (cont'd). EFFECTS OF SUBCHRONIC EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
7,520 18,800 47,000	4.0 10.0 25.0	6 h/day, 5 days/wk, up to 21 days	Rat	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 bronchiolar epithelium by 7 days. At 25 ppm: no lesions in nasal cavity; hypertrophy or hyperplasia of small bronchi or bronchiolar 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.	Hooftman et al. (1988)
9,400 18,800	5.0 10.0	Continuous, 90 days	Cyno- molgus monkey	Bronchiolar epithelia hyperplasia; some focal pulmonary edema.	Busey et al. (1974)
18,880	10.0	Continuous, 14 days	Rat	3 days: Hypertrophy of Type 2 cells; desquamation of alveolar cells; thickening of alveolar walls; interstitial edema; goblet cell hyperplasia in trachea and bronchi. 7-14 days: Hypertrophy of bronchiolar cells; loss of cilia; partial desquamation of bronchiolar epithelium; increased edema; Type 2 cell hyperplasia; increase in lamellar bodies.	Hayashi et al. (1987)
18,800	10.0	Continuous, 1 mo	Rat	Increased arithmetic mean thickness of air-blood barrier.	Kyono and Kawai (1982)
18,800	10.0	Continuous, 6 wk	Guinea pig	Type 2 cell hyperplasia; increase in lipid bodies and lamellae in cells	Yuen et al. (1971)
20,000	10.6	6 h/day, 28 days	Rat	Loss of cilia; focal hypertrophy of bronchiolar epithelium.	Rombout et al. (1986)
22,600- 33,800	12-18	Continuous, up to 15 days	Rat	Cilia loss, sloughing of ciliated cells, and hyperplasia in terminal bronchioles; Type 1 cell loss in adjacent alveoli; increase in macrophage number.	Cabral-Anderson et al. (1977)

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TABLE 13-12 (cont'd). EFFECTS OF SUBCHRONIC EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
28,200	15	Continuous, 1-4 wk or 2-17 mo	Rat	Response related to exposure duration: stenosis of terminal and respiratory bronchioles; hypersecretion in terminal bronchioles; cellular debris and free cells in lumen; decrease in ciliated cells; and smoothing of surface in terminal bronchioles.	Juhos et al. (1980)
32,000	17	Continuous, up to 43 days	Rat	Loss of cilia in terminal bronchioles; abnormal ciliogenesis; crystalloid inclusions in bronchiolar epithelial cells.	Stephens et al. (1971a)
37,600	20	28 days	Mouse	Loss of cilia in bronchiolar epithelium; swelling of ciliary shaft; some compound cilia; changes reversible after 30 day recovery.	Ranga and Kleinerman (1981)
37,600	20	Continuous, 4 wk	Rat	Hypertrophy of bronchiolar epithelium; increase in alveolar size; thickening of alveolar septa; inflammatory cells in alveolar duct.	Freeman et al. (1968c)
37,600	20	24 h/day, 5 days/wk, 8 wk	Rat	Inflammation; edema; terminal bronchiolitis; hyperplasia of Type 2 cells; thickening of alveolar walls due to interstitial cell proliferation; degenerative changes seen 2-12 mo postexposure; increase in alveolar size.	Pickrell et al. (1981)
37,600	20	Continuous, 90 days	Rat	Terminal bronchiole: increase in thickness of collagen fibrils; increased thickness of basement membrane.	Stephens et al. (1971b)
47,000	25	Continuous, 7 wk	Rat	Increase in alveolar size (persistent after 36 wk recovery period).	Haydon et al. (1965)

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TABLE 13-13. EFFECTS OF CHRONIC EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
80	0.04	9-27 mo (24 h/day)	Rat	No significant change, but some tendency towards increase in arithmetic mean thickness of air-blood barrier.	Kubota et al. (1987)
380 (baseline) + 1,470 (spike) twice daily	0.2 (baseline) + 0.78 (spike) twice daily	Continuous, 52 wk, (23 h/day, 5 days/wk)	Mouse	No pathology.	Miller et al. (1987)
750	0.4	Continuous, 9-27 mo	Rat	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.	Kubota et al. (1987)
940	0.5	Continuous, 7 mo	Rat	Swelling of terminal bronchiolar cilia; hyperplasia of Type 2 cells.	Yamamoto and Takahasi (1984)
940	0.5	6-24 h/day, 3-12 mo	Mouse	3 mo: pneumonitis and alveolar size increase; loss of cilia in respiratory bronchioles and bronchiolar inflammation with 24 h/day; 6-12 mo: pneumonitis; cilia loss; bronchial and bronchiolar inflammation; alveolar size increase.	Blair et al. (1969)
940	0.5	Continuous, up to 19 mo	Rat	Type 2 cell hypertrophy and interstitial edema by 4 mo; increased thickness of alveolar septa by 6 mo; fibrous pleural thickening by 19 mo.	Hayashi et al. (1987)

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TABLE 13-13 (cont'd). EFFECTS OF CHRONIC EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
1,500	0.8	Continuous, lifetime (up to 33 mo)	Rat	Minimal changes: slight enlargement of alveoli and alveolar ducts; some rounding of bronchial and bronchiolar epithelial cells; increase in elastic fibers around alveolar ducts.	Freeman et al. (1966)
1,880	1.0	Continuous, 7 mo	Rat	Cilia loss in terminal bronchioles; hyperplasia of Type 2 cells; interstitial edema.	Yamamoto and Takahasi (1984)
1,880	1.0	Continuous, 16 mo	Squirrel monkey	No pathology.	Fenters et al. (1973)
1,880	1.0	6 h/day, 5 days/wk, up to 18 mo	Dog	6 mo: no pathology; 12 mo: dilated alveoli and alveolar ducts; 18 mo: dilated alveoli, edema, thickening alveolar septa due to inflammation.	Wagner et al. (1965)
1,880	1.0	6 h/day, 5 days/wk, 18 mo	Guinea pig	Mild thickening of alveolar septa due to inflammation; some alveolar dilatation.	Wagner et al. (1965)
1,880	1.0	7 h/day, 5 days/wk, 15 wk	Rat	No pathology.	Gregory et al. (1983)
3,760	2.0	Continuous, 2 yr	Rat	Loss of cilia in terminal bronchioles; abnormal ciliogenesis; crystalloid inclusions in bronchiolar epithelial cells; increased thickness of collagen fibrils and basement membrane in terminal bronchioles.	Stephens et al. (1971a,b)
3,760	2.0	Continuous, up to 12 mo	Rat	Hypertrophy of ciliated cells and cilia loss by 72 h; decreased number of ciliated cells by 7 days; normal ciliated cells from 21 days-12 mo.	Stephens et al. (1972)
3,760	2.0	Continuous, up to 360 days	Rat	No change in turnover terminal bronchiolar epithelial cells; increase in turnover of Type 2 cells in peripheral alveoli by 1 day, but normal by 7 days.	Evans et al. (1972)

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TABLE 13-13 (cont'd). EFFECTS OF CHRONIC EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Response	Reference
3,760	2.0	Continuous, 14 mo	Monkey (Macaca peciosa)	Bronchiolar epithelial hypertrophy, especially adjacent to alveolar ducts; change to cuboidal cells in proximal bronchiolar epithelium.	Furiosi et al. (1973)
3,760	2.0	Continuous, 14 mo	Rat	Minimal effect: some terminal bronchiolar epithelial hypertrophy.	Furiosi et al. (1973)
3,760	2.0	Continuous, lifetime (up to 763 days); (0.8 ppm for 1st 69 days, then 2 ppm)	Rat	Alveolar distension, especially near alveolar duct level; increased variability in alveolar size; loss of cilia and hypertrophy in terminal bronchiolar cells; no inflammation.	Freeman et al. (1968b)
7,520	4.0	Continuous, 7 mo	Rat	Cilia loss in terminal bronchioles; hyperplasia of Type 2 cells; interstitial edema; decrease in number of lamellar bodies in Type 2 cells; lysosomes with osmiophilic lamellar structure in ciliated cells of terminal bronchioles.	Yamamoto and Takahashi (1984)
7,520	4.0	Continuous, 9-27 mo	Rat	Hypertrophy and hyperplasia of bronchiolar epithelium and increase in arithmetic mean thickness of air-blood barrier by 9 mo; Clara cell hyperplasia by 27 mo; interstitial fibrosis; hypertrophy of Type 1 and Type 2 cells; some decline in arithmetic mean thickness of air-blood barrier.	Kubota et al. (1987)
7,520	4.0	Continuous, 16 mo	Rat	Bronchial epithelial hyperplasia.	Haydon et al. (1965)
9,400	5.0	6 h/day, 5 days/wk, 18 mo	Dog	6 mo: no pathology; 12 mo: dilated alveoli and alveolar ducts; 18 mo: edema, congestion, and thickened alveolar septa due to inflammatory cells.	Wagner et al. (1965)

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TABLE 13-13 (cont'd). EFFECTS OF CHRONIC EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
9,400	5.0	6 h/day, 5 days/wk, 14 mo	Mouse	No pathology.	Wagner et al. (1965)
9,400	5.0	4-7.5 h/day, 5 days/wk, 5.5 mo	Guinea pig	Some dilation of terminal bronchioles; tracheal inflammation; pneumonitis.	Balchum et al. (1965)
9,400	5.0	7 h/day, 5 days/wk, 15 wk	Rat	Focal hyperinflation and areas of subpleural accumulation of macrophages.	Gregory et al. (1983)
17,860	9.5	7 h/day, 5 days/wk, 6 mo	Rat (1-day and 6-mo old)	No significant pathology.	Mauderly et al. (1987)
22, 600	12	Continuous, up to 815 days	Rat	Hypertrophy and hyperplasia of bronchial and bronchiolar epithelium; increased variability in alveolar size.	Haydon et al. (1965)
28,200	15	Continuous, lifetime (up to 33 mo)	Rat	Hyperplasia of mucus secretory cells in trachea and bronchi and proliferation into smaller airways; respiratory bronchiolar epithelial hypertrophy; loss of cilia; and fibrosis at junction of bronchioles and alveolar duct; bronchiolar stenosis; thickening of alveolar septa and increase in septal connective tissue in proximal alveoli; enlarged alveoli; hypertrophy of epithelium in central alveoli; decrease in number of alveoli and destruction of septa in central alveoli (panlobular emphysema).	Freeman et al. (1972)
32,000	17	Continuous, with intervening recovery up to 610 days	Rat	Increased thickness of collagen fibrils in terminal bronchioles.	Stephens et al. (1971b)

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TABLE 13-13 (cont'd). EFFECTS OF CHRONIC EXPOSURE TO NO_2 ON LUNG MORPHOLOGY

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
33,800	18	Continuous, 20 wk	Rat	Increase in thickness and prevalence of collagen and elastic tissue, primarily at level of alveolar duct (persisted for 1 yr after exposure ended).	Freeman et al. (1969)
47,000	25	Continuous, up to 157 days	Rat	Hypertrophy and hyperplasia of bronchial and bronchiolar epithelium; increase in goblet cells in major bronchi; increase in connective tissue at junction of bronchiole and alveolar duct; increase in alveolar size.	Freeman and Haydon (1964)
47,000	25	0.5 h/day, 5 days/wk, 4.5 mo	Mouse	No pathology.	Boren (1964)
48,900	26	Continuous, 191 days	Dog	Emphysematous-like changes; increased collagen.	Lewis et al. (1973)

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levels $\leq$9,400 $\mu g/m^3$; although results of studies with higher concentrations are discussed if they reveal critical findings.

Examination of the tables shows variability in responses at similar exposure levels in different studies. This may be due to differences in animal species or strain, age, diet, microbiological status of the animals, or aspects of experimental protocol. This latter includes the methodology used to evaluate the morphologic response. For example, simple light microscopic examination may reveal no effect, while other techniques, such as quantitative morphologic (morphometric) procedures, can detect more subtle structural changes which may occur in the absence of overt lesions. It should also be kept in mind that conclusions as to resolution of lesions with continued exposure or after the end of exposures are highly dependent upon the method of morphologic assessment being used.

There is a large degree of interspecies variability in responsiveness to NO_2; this is clearly evident from those few studies (Table 13-11 and 13-12) where different species were exposed under identical conditions (Wagner et al., 1965; Furiosi et al., 1973; Foster et al., 1985; Azoulay-Dupuis et al., 1983). Such response differences, which may be due to differences in effective dose of NO_2 reaching target sites (due to anatomical and ventilatory differences) and/or to inherent differences in sensitivity of these sites, imply that the choice of experimental animal affects the end result observed. In direct comparisons, the guinea pig, hamster, and monkey all appear to be more severely affected by equivalent exposure to NO_2 than is the rat, which is the most commonly used experimental animal. However, in most cases, the types of histological lesions produced are similar when appropriate effective concentrations are used.

Sites Affected

The anatomic region most sensitive to NO_2, and within which injury is first noted, is the area that includes the terminal conducting airways (terminal bronchioles), and adjacent alveolar ducts and alveoli. Within this region, those cells that are most sensitive to NO_2-induced injury are the ciliated cells of the bronchiolar epithelium and the Type 1 cells of the alveolar epithelium.

In the alveolar region, short-term (acute) exposure to NO_2 results in hypertrophy of Type 1 cells, followed by death and desquamation of these cells, and proliferation of and

replacement by Type 2 cells. This may result in a thickened air-blood barrier. In the bronchioles, there is hypertrophy and hyperplasia of epithelial cells, Clara cells lose their secretory granules and surface protrusions, and there may also be loss of cilia or ciliated cells. These effects are generally seen, however, with exposure to levels >9,400 $\mu g/m^3$ (5 ppm) NO_2. With chronic exposure, many of these same changes are observed, but there is increased loss of cilia over larger areas of epithelium and in more proximal airways, and the structure of the remaining cilia may be altered. Furthermore, intraepithelial cilia have been noted between cells near the basement membrane, or elsewhere.

Permanent alterations in lung architecture resembling emphysema-like disease may result from chronic exposure. A detailed discussion of emphysema in relation to NO_2 exposure follows the discussion on susceptibility to NO_2-induced morphological changes.

The temporal progression of early events due to NO_2 exposure has best been described in the rat (e.g., Cabral-Anderson et al., 1977; Evans et al., 1972, 1973a,b, 1974, 1975, 1976, 1977; Freeman et al., 1966, 1968c, 1972; Stephens et al., 1971a, 1972). The earliest alterations resulting from exposure to concentrations of ≥3,760 $\mu g/m^3$ (2 ppm) are seen within 24 to 72 h of continuous exposure. These include increased macrophage aggregation, desquamation of the Type 1 cells and ciliated bronchiolar cells, and accumulation of fibrin in small airways. However, repair of injured tissue and replacement of destroyed cells can begin within 24 to 48 h of continuous exposure. The new cells in the bronchioli are derived from nonciliated cells, while in the alveoli the damaged Type 1 cells are replaced with Type 2 cells. These new cells are relatively resistant to effects of any continued NO_2 exposure. Incorporation of ^3H-thymidine by Type 2 cells is observed within 12 h after the initial NO_2 exposure, with the number of labeled cells becoming maximal by about 48 h and decreasing to preexposure levels by about 6 days, despite continued exposure (Evans et al., 1975). If exposure levels are very high (>18,800 $\mu g/m^3$ [10 ppm]), however, resolution and return to normal 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. On the other hand, some lesions may remain, depending upon exposure duration and concentration. For example, hamsters showed normal bronchiolar and alveolar epithelium by 6 days after 48 h of exposure to 22,600 $\mu g/m^3$ (12.0 ppm) or 32,000 $\mu g/m^3$

(17.0 ppm), or 19 days after 6 days of exposure to 41,400 $\mu g/m^3$ (22.0 ppm) NO_2 (DeNicola et al., 1981). Rombout et al. (1986) exposed rats to 20,000 $\mu g/m^3$ (10.6 ppm) continuously for 28 days. Bronchiolar epithelial hyperplasia was totally resolved beyond 4 days after cessation of exposure, while hypertrophy was totally resolved after 16 days of recovery; on the other hand, some abnormal cilia remained after 4 weeks. Finally, Kubota et al. (1987) examined the time course of alveolar 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 months. One phase, which lasted for 9 to 18 months of exposure, consisted of a decrease in number and an increase in cell volume of Type 1 epithelium, an increase in the relative ratio of Type 2 to Type 1 cells, and an increase in the number and volume of Type 2 cells. A second phase, which occurred at 18 to 27 months of exposure, showed some recovery of alveolar epithelium, but a decrease in total volume of interstitial tissue and an increase in collagen fibers in the interstitium. Thus, some lesions resolved with continued exposure, while others progressed. Furthermore, the temporal pattern of recovery may differ in different regions of the lungs (Kawakami et al., 1989). In general the largest percentage of NO_2-induced lesions will resolve following a recovery period. This period may be as short as 30 days for exposures at $\leq$9,400 $\mu g/m^3$ (5.0 ppm). With continuous exposure, morphologic damage induced early in the exposure regime may also be resolved. For example, rats exposed continuously for 7 months to 940 $\mu g/m^3$ (0.5 ppm) NO_2 showed resolution of epithelial lesions between 4 to 6 months after exposure began (Yamamoto and Takahashi, 1984).

In spite of the fact that there is a fairly extensive animal toxicologic data base concerning morphologic effects of NO_2, it is still quite difficult to establish a "no-observed-effect" level based upon these data. This is due to the great complexity of changes occurring with NO_2 exposure, as well as to the large interspecies differences in response. In general, morphologic alterations, some of which may be persistent, are found with chronic exposure to concentrations $<$1,880 $\mu g/m^3$ (1 ppm). However, long-term exposure to levels equal to or above 3,760 $\mu g/m^3$ (2 ppm) are needed for more extensive and permanent changes.

Effects of Nitrogen Dioxide as Function of Exposure Pattern

Although the extent and degree of morphologic alterations 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 or the effect of temporal patterns of exposure.

The relative roles of concentration and time in response to acute 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) 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 and concluded that concentration played a more important role in inducing lesions than did exposure duration, as long as the product of concentration $\times$ time was constant. These findings were consistent with Stavert and Lehnert (1988) who exposed rats to much higher concentrations of NO_2 (47,000 to 470,000 $\mu g/m^3$ [25 to 250 ppm] for 2 to 30 min). They also noted that the effect of concentration was stronger with intermittent exposure than with continuous exposure, and that the onset of response was delayed with intermittent compared to continuous exposure. Furthermore, continuous exposure resulted in an increase in AM number not seen with intermittent exposure at the same concentration (Rombout et al., 1986). These findings are similar to those using the infectivity model discussed in Section 13.2.3.1.

Most of the morphological studies with NO_2 involved a constant exposure level. However, actual ambient exposures are often characterized by transient peaks, which are superimposed upon a lower and relatively constant baseline level. The morphological effects of exposure patterns involving transient spikes was examined in a number of studies. The findings of these studies are equivocal in that it is not clear whether these transient peaks contribute to morphologic damage in excess of that due to exposure to a constant baseline level. In some cases, there was no group run at the constant level for comparison to those tested with transient peaks. Gregory et al. (1983) exposed rats (14 to 16 weeks old) for 7 h/day, 5 days/week for up to 15 weeks to atmospheres consisting of the following concentrations of NO_2: (1) 1,880 $\mu g/m^3$ (1 ppm); (2) 9,400 $\mu g/m^3$ (5 ppm); or (3) 1,880 $\mu g/m^3$ (1 ppm) with two, 1.5-h spikes of 9,400 $\mu g/m^3$ (5 ppm) per day (i.e., animals were exposed to NO_2 at 1,880 $\mu g/m^3$ for 1.5 h, 9,400 $\mu g/m^3$ for 1.5 h, 1,880 $\mu g/m^3$ for 3 h, 9,400 $\mu g/m^3$ for 1.5 h, and 1,880 $\mu g/m^3$ for 0.5 h). No change in lung weight was found in any exposure group. After 15 weeks of exposure, histopathology was minimal, 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 $\mu g/m^3$ (5 ppm), or to 1,880 $\mu g/m^3$ (1 ppm) with the 9,400 $\mu g/m^3$ (5 ppm) spikes.

Miller et al. (1987) exposed mice (6 to 8 weeks old) for 1 year (23 h/day, 7 days/week) either to a continuous baseline of 380 $\mu g/m^3$ (0.2 ppm) NO_2, or to this baseline onto which was superimposed, for 5 days/week, two, 1-h spikes (given in the morning and afternoon) of 1,470 $\mu g/m^3$ (0.78 ppm). Morphologic examination (light microscopy) performed after 32 and 52 weeks of exposure, and then 1 month after all exposures had ended, revealed no treatment-related lesions in either exposure group, although host defense and functional changes were noted (Sections 13.2.3.1 and 13.2.3.3). Furthermore, the type of fixation and analysis would not allow for detection of emphysema even if it were present.

Port et al. (1977) studied mice exposed to 190 $\mu g/m^3$ (0.1 ppm) NO_2 with daily, 2 h peaks to 1,880 $\mu g/m^3$ (1.0 ppm), for 6 months; dilated respiratory bronchioles and alveolar ducts were noted.

Crapo et al. (1984) and Chang et al. (1986) reported on the NO_2-induced changes in the proximal alveolar region of rats exposed for 6 weeks to a baseline concentration of 940 or 3,760 $\mu g/m^3$ (0.5 or 2.0 ppm), 23 h/day for 7 days/week into which was superimposed two daily 0.5-h spikes of three times the baseline concentration for 5 days/week. They used quantitative morphometric analyses. At the lower exposure level, the volumes of the Type 2 epithelium, interstitial matrix, and AMs increased, while the volume of the fibroblasts decreased. The surface area of Type 2 cells increased. The arithmetic mean of the interstitium increased. Most of these changes also occurred at the higher exposure level, and in some cases the change was greater than that at the lower level (i.e., increase in Type 1 and Type 2 epithelial volume). Other cellular changes occurred. At both levels exposures, the volume of Type 2 cells and interstitial fibroblasts increased, with no significant changes in their numbers. The number of Type 1 cells decreased and their average surface area increased in the highest exposure group. The number of AMs decreased at both exposure levels. Generally, there was a spreading and hypertrophy of Type 2 cells. 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. Pulmonary function changes in similiarily exposured were assessed by Stevens et al. (1988) (Section 13.2.3.3).

Susceptibility to Nitrogen Dioxide-Induced Morphological Changes

The morphologic effects of NO_2 may depend upon the age of the animals at the time of exposure; this could account for some of the variability in response seen for the same species exposed to comparable concentrations.

Azoulay-Dupuis et al. (1983) exposed both rats and guinea pigs aged 5 to ≥60 days old (adult) to 3,760 or 18,800 $\mu g/m^3$ (2 or 10 ppm) for 3 days. In both species, adult animals showed greater effects of exposure than did the newborns. At 3,760 $\mu g/m^3$ (2 ppm), rats showed no effects. Exposure of guinea pigs <45 days old to this level also produced no effect. The 45-day-old animals showed thickening of alveolar walls, alveolar edema, and inflammation, while animals older than 45 days showed similar, but more frequent, alterations which seemed to increase with age. Adults also had focal loss of cilia in bronchioli. At 18,800 $\mu g/m^3$ (10 ppm), only rats ≥45 days old showed any response, which included fibrinous deposits in alveoli and focal loss of cilia in bronchioli. On the other hand, guinea pigs of all ages were affected, although the adults showed the most severe alterations, namely focal emphysema-like changes and inflammation in the bronchi and bronchioles. All guinea pigs showed cilia loss in bronchioli and trachea, edema, increase in number of Type 2 cells, and alveolar inflammation. Similar findings were reported by Stephens et al. (1982) after exposing rats (adults and nursing pups) to high concentrations of NO_2. (See section on host defense mechanisms.)

Chang et al. (1986, 1988) exposed 1 day or 6-week-old rats for 6 weeks to a baseline of 940 $\mu g/m^3$ (0.5 ppm) NO_2 for 23 h/days, 7 days/week, with two, 1-h spikes (given in the morning and afternoon) of 2,820 $\mu g/m^3$ (1.5 ppm) 5 days/week, and examined the proximal alveolar region. Age clearly influenced morphometric response. The older animals showed an increase in the surface density of the alveolar basement membrane which was not found in the younger animals. Although both age groups showed an increase in the mean cellular volume of Type 2 cells, this increase was also greater in the 6-week-old animals. Thus, the responses differed with age during exposure, but the 6-week-old animals seemed to be

generally more susceptible to injury than were the 1-day-old animals. While there was no qualitative evidence of morphological injury in the terminal bronchioles of the juvenile rats, there a 19% increase in the average ciliated cell surface that was not evident in the adult rats. The authors also reported a 13% increase of the mean luminal surface area of Clara cells in the juvenile animals vs. control animals of the same age.

An extensive series of exposures designed to relate morphometric changes to age was performed by Kyono and Kawai (1982). Rats at 1, 3, 12 and 21 months of age were exposed continuously for 1 month to NO_2 at 207 $\mu g/m^3$ (0.11 ppm), 865 $\mu g/m^3$ (0.46 ppm), 5,264 $\mu g/m^3$ (2.8 ppm), or 16,544 $\mu g/m^3$ (8.8 ppm). Light and electron microscopic analyses followed exposure. 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. This study is difficult to evaluate, and quantitative estimations deliberately excluded the site of main damage. Analysis of individual results was complex, but depending upon the animal's age, exposure levels as low as 207 $\mu g/m^3$ did change specific morphometric parameters. There was a trend towards a concentration-dependent increase in air-blood barrier thickness in all age groups, with evidence of age-related differences in response. At any concentration, the response of this end point decreased from 1 to 12 months, but increased again in 21-month-old animals. 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. Thus, although age does play a role in modulating morphometric response, it may be a complex one.

In general, it seems that neonates, specifically prior to weaning, are relatively resistant to NO_2, and that responsiveness increases with age until weaning. Furthermore, the responsivenss 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 NO_2 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 and variable sensitivity of target cells during different growth phases (Stephens et al., 1982; Hahn, 1979) or perhaps dosimetry.

A possible concern in assessing NO_2 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) exposed developing rats (0 to 6 months of age) and adult animals (6 to 12 months of age) to 17,900 $\mu g/m^3$ (9.5 ppm) NO_2 for 7 h/day, 5 days/week for 6 months. Lung development, as determined at young adulthood, was not significantly affected by earlier exposures. There was no significant light microscopic evidence of lung injury in either group of animals, and there were no exposure-related differences in mean linear intercept of the alveoli or the internal surface area of the lungs. Thus, the available data base does not provide an answer to the question concerning a possible role of NO_2 exposure during development upon adult lung structure. The potential for early exposure to result in long-term effects was suggested by Lam et al. (1983) who reported finding emphysema-like changes in rats exposed to high concentrations of NO_2 as neonates.

Of importance in evaluating the effects of NO_2 is consideration of individuals with compromised lung function. One such group comprises those with respiratory disease. There is a very limited data base concerning morphologic effects of NO_2 in experimental animals with pre-existing chronic disease. Lafuma et al. (1987) exposed both normal and elastase-induced emphysematous hamsters (2 month old) to 3,760 $\mu g/m^3$ (2 ppm) NO_2 for 8 h/day, 5 days/week for 8 weeks and analyzed tissue with morphometric assays. Sacrifice was performed immediately after exposures had ended. The emphysematous lesions produced by elastase appeared to be aggravated by subsequent exposure to NO_2; those exposed to NO_2 showed 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, compared to those treated with elastase and exposed to clean air. The investigators suggested that these results may imply a role for NO_2 in enhancing pre-existing emphysema.

In contrast, Stavert et al. (1986) reported that NO_2 exposure did not potentiate pre-existing emphysema in the elastase-induced emphysema animal model. Normal and diseased

rats (12 week old) were exposed to 65,800 $\mu g/m^3$ (35 ppm) NO_2 for 6 h/day, 5 days/week for 5 weeks. The animals were allowed a 10-week recovery period prior to sacrifice. Morphologic analysis by light microscopy showed there to be no difference between animals treated with elastase and exposed to clean air or those treated with elastase and exposed to NO_2.

The above studies examined the effect of NO_2 exposure on pre-existing chronic lung disease. It is also possible that acute lung disease may affect morphologic response to NO_2. Fenters et al. (1973) challenged squirrel monkeys with an influenza virus at various times during continuous exposure to 1,880 $\mu g/m^3$ (1 ppm) NO_2 for 16 months, and compared the response to that seen in animals not challenged but exposed to NO_2. Only the virus-challenged animals showed a response to NO_2, which included thickening of bronchial and bronchiolar epithelium. This suggests that acute lung disease may affect NO_2 toxicity. The effects of NO_2 on infectivity due to challenges with microorganisms are discussed in Section 13.2.3.1.

Emphysema Following Nitrogen Dioxide Exposure

In evaluating the reports of emphysema following NO_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 which led them to the diagnosis of emphysema. Several professional groups have presented definitions of emphysema.

In 1959, a group of British physicians meet under the auspices of Ciba to clarify the terminology, definition, and classification of emphysema and related disorders (Fletcher et al., 1959). Their definition was: "Emphysema is a condition of the lung characterized by increase beyond the normal in size of air spaces distal to the terminal bronchiole either from dilatation or from destruction of their walls." They continued: "Emphysema can be diagnosed and classified consistently only on preparations from lungs distended and fixed before they are cut. The simplest technique is intrabronchial infusion of fixative." They also recognized the necessity of both appropriate aged controls and of morphometry to establish upper limits of normal size. One of the lasting benefits of their efforts 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).

In 1961, an expert committee of the World Health Organization defined a series of lung diseases including emphysema. They defined emphysema in anatomical terms as follows: "Emphysema is a condition of the lung characterized by increase beyond normal in the size of air spaces distal to the terminal bronchiole, with destructive changes in their walls."

In 1962, an American Thoracic Society committee developed the following definition: "Emphysema is an anatomic alteration of the lung characterized by abnormal enlargement of the air spaces distal to the terminal, nonrespiratory bronchiole, accompanied by destructive changes of the alveolar walls." The American Thoracic Society Committee also defined pulmonary overinflation as a "pathologic condition of the lung characterized by abnormal enlargement of the air spaces distal to the terminal, nonrespiratory bronchiole, without destructive changes in the alveolar walls". Following their definition of emphysema, the American Thoracic Committee also noted that "Determination of these characteristics in tissue obtained by surgical resection or at autopsy requires proper inflation prior to fixation and gross and microscopic examination. For this purpose, the use of the stereomicroscope is of real value." While the definitions of both the World Health Organization and the American Thoracic Society emphasized the presence of destruction of air space wall in emphysema, they did not define destruction.

The most recent definition is in the report of a National Heart, Lung and Blood Institute (NHLBI), Division of Lung Diseases Workshop (National Institutes of Health, 1985). This document 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 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 further indicates "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 qualitatively in appropriate specimens and quantitatively 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 American Thoracic Society Committee's requirement for tissue fixation. This document further 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." 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 NO_2, 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.

Thus, in reviewing reports of emphysema following experimental NO_2 exposure important considerations include: (1) whether the tissue was fixed in an inflated state, (2) whether air spaces distal to the terminal bronchiole were enlarged beyond normal and whether that enlargement was determined quantitatively by stereologic methods, and (3) whether or not air space wall destruction was present. Control animals of the identical age as the exposed animals should be used for stereologic studies to exclude the possibility that the airspace enlargement was due to age or the combination of age and environmental history. The importance of each of these factors must be weighed, depending upon the specific purpose of the study. Several of the papers reviewed in this section concerned the pathogenesis of emphysema or the sequence of events in the NO_2-injured respiratory systems rather than only addressing the presence or absence of emphysema following a specific NO_2 exposure regimen.

Presumably, both investigators who wrote manuscripts and reviewers for journals that published manuscripts concerning NO_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_2 inhalation relative to the dates of publication of the definitions of emphysema must be considered in this document. Because of these changes in the definitions of emphysema and in the methods used for evaluation of results of NO_2 exposures, the studies were reviewed chronologically. It is necessary to recall that many of the methods required by the National Institutes of Health workshop report were neither well developed nor widely used when these early manuscripts were written.

When reports of emphysema following NO_2 exposures of animals are to be extrapolated to potential hazards for humans, the definition of human emphysema, rather than that for emphysema in experimental animals, must be used. Thus, the presence of airspace wall destruction is critical. In published reports of emphysema following NO_2 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 1985 NHLBI definition of emphysema are quoted, and all published micrographs were examined for evidence of destruction.

Freeman et al. (1964) reported emphysema in rats exposed to 47,000 $\mu g/m^3$ (25 ppm) NO_2 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. While 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 which 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 $\mu g/m^3$ (5 ppm) NO_2 for up to 18 months. 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 22,560 $\mu g/m^3$ (12.0 ppm) NO_2, but not in other rats exposed to 7,520 or 1,504 $\mu g/m^3$ (4.0 or 0.8 ppm) NO_2. 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", but did not report destruction of airspace 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 per day) for 3 to 4 months for to 15,040 or 22,560 $\mu g/m^3$ (8 or 12 ppm) NO_2. The lungs were fixed via the trachea in an expanded state. They reported enlarged lungs which 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. While 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 NO_2, Freeman et al. (1968c) reported that rats exposed continuously (24 h/day) to 3,760 $\mu g/m^3$ (2 ppm) NO_2 for 112 to 763 days had only equivocal increases in lung weight and distension of airspaces. These lungs were fixed via the trachea in a distended state. These NO_2 exposures did not result in emphysema.

Freeman et al. (1968a) reviewed some of their earlier studies and new observations on rats exposed to 32,400 $\mu g/m^3$ (18 ppm) NO_2 24 h/day for 1 to 6 days or for 4 weeks. The authors did not present new data relative to enlarged airspaces or destruction of airspace walls, but rather appear to have based their diagnosis of emphysema on the gross size of the lungs. They again referred to the Ciba Symposium definition of emphysema. This review did not present relevant new information.

Freeman et al. (1968b) summarized many of their previously published NO_2 exposures in a manuscript resulting from a meeting (symposium) presentation. They again followed the definition of emphysema proposed by the Ciba Symposium and did not present new data relative to enlarged airspaces or destruction of alveolar walls. No new information relevant to emphysema following NO_2 exposure was presented.

At the same meeting (symposium), Kleinerman and Cowdrey (1968), reported results of exposures of hamsters to 84,600 to 103,400 $\mu g/m^3$ (45 to 55 ppm) NO_2, 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 statistically larger than similar-aged controls. While the size of alveolar spaces appeared enlarged in the exposed animals, as compared to the controls similarly fixed, there was no evidence of destruction of alveolar septal tissue. 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 NO_2, which results 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 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 NO_2 on control and pneumoconiotic lungs using hamsters and guinea pigs. Most exposures were for 2 h/day for 5 days/week. The NO_2 concentration appears to have been planned for 41,360 $\mu g/m^3$ (22 ppm), but varied with an initial range of 94,000 to 169,200 $\mu g/m^3$ (50 to 90 ppm) the first 4 weeks, which was

then reduced to ranges of 56,400 to 94,000 $\mu g/m^3$ (30 to 50 ppm) for a total exposure period of 12 months. The lungs were fixed via the trachea with formaldehyde fumes in a distended state. No morphometry of airspaces is 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 NO_2 it can also be concluded that long-term exposure of hamsters to NO_2 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. While 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 months. 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. It is possible that the lungs might have been distended with air, the trachea tied and then immersed in fixative which would diffuse into the lung through the thin pleura. Control unexposed mice had pneumonitis. While 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 type of lung fixation and of alveolar morphometry. They also mentioned alveolar "septal breakage", but not destruction, 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 Loosli (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 killed and the lungs 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 alveolar wall 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) NO_2 for varying periods up to 33 months. 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 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 NO_2-exposed rats. However, it is not entirely clear whether both experimental groups or only the group exposed to 28,200 $\mu g/m^3$ NO_2 had emphysema.

Ehrlich and Fenters (1973) exposed squirrel monkeys to 9,400 or 18,800 $\mu g/m^3$ (5 or 10 ppm) NO_2 for 3 months or to 1,880 $\mu g/m^3$ (1 ppm) NO_2 for 16 months 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 alveolar 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" in those exposed to 1,880 $\mu g/m^3$ NO_2 and to the virus. They also reported emphysema was not present in monkeys exposed to 1,880 $\mu g/m^3$ NO_2 without the viral challenge. The morphological methods used preclude useful information concerning emphysema following NO_2 exposure.

Stephens et al. (1976) in a long abstract of papers presented at that years Aspen conference, stated that "Rats exposed for long periods (3 to 5 months) to 28,200 $\mu g/m^3$ (15 ppm) NO_2 or 0.8 ppm O_3 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 the 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 NO_2. The mice were exposed

to 188 $\mu g/m^3$ (0.1 ppm) NO_2 with a 2-h peak of 1,880 $\mu g/m^3$ (1.0 ppm) NO_2 daily for 6 months. Control mice of the same age were also examined. Only one NO_2-exposed and one control rat were studied. The exposed rat breathed 28,200 $\mu g/m^3$ (15.0 ppm) NO_2 from 35 days to approximately 5 months of age, when clinical illness became apparent. The NO_2 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 via the trachea at a constant pressure in a distended condition. The lungs were examined by light and scanning electron microscopy. The NO_2-exposed rat had distended alveoli and evidence of alveolar wall destruction. Both dilated airspaces and evidence of alveolar wall destruction were reported in the NO_2-exposed mice lungs. While alveolar wall destruction was demonstrated, the small number of animals studied severely limits the value of this study.

Hyde et al. (1978) studied beagle dogs that had been exposed 16 h daily for 68 months to either filtered air or to 1,210 $\mu g/m^3$ (0.6 ppm) NO_2 with 310 $\mu g/m^3$ (0.16 ppm) NO or to 270 $\mu g/m^3$ (0.14 ppm) NO_2 with 2,050 $\mu g/m^3$ (1.1 ppm) NO. The dogs then breathed clean air during a 32- to 36-month postexposure period. The right lungs were fixed via the trachea at 30 cm fixative pressure in a distended state. Semiautomated image analysis was used for morphometry of air spaces. The dogs exposed to 1,210 $\mu g/m^3$ NO_2 with 310 $\mu g/m^3$ 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 $\mu g/m^3$ NO_2 with 2,050 $\mu g/m^3$ NO, implying a significant role of the NO_2 in the production of the lesions. The lesions in dogs exposed to the higher NO_2 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 $\mu g/m^3$ (30 to 35 ppm) NO_2, 23 hours/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. While 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. (1985b) describe the effects following exposure of hamsters to 56,400 $\mu g/m^3$ (30 ppm) NO_2, 22 hours/day for 12 months. The authors did not describe the methodology used in detail, for example, the methods of lung fixation and of morphometry. Mean linear intercept was statistically significantly longer in the exposed hamsters indicating larger alveoli. However, whether there was alveolar destruction was not indicated. The authors conclude 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 which had received a single intratracheal instillation of sterile normal saline to either 65,800 $\mu g/m^3$ (35 ppm) NO_2 6 h/day or to filtered air for 25 days. The rats were held an additional 10 weeks and then examined. The lungs were fixed by intratracheal formalin at 25 cm water pressure and morphometry 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 alveolar wall destruction nor is it evident in their published micrographs. They concluded that 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 $\mu g/m^3$ (30 ppm) NO_2, 24 h/day for up to 140 days. The primary objective of this study was to evaluate neutrophil recruitment and degranulation from NO_2-induced emphysema. The lungs were fixed via the trachea in a appropriate manner and mean linear intercept was determined by semiautomatic image analysis which was compared to manual methods. Exposed rats had statistically longer mean linear intercepts, indicating larger alveoli and reported 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. While 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 NO_2 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 $\mu g/m^3$ (30 ppm) NO_2, 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 report longer mean linear intercepts in the exposed rats, indicating larger alveoli. They mention that the lungs were examined for alveolar wall destruction, but do not clearly indicate whether destruction was present. They properly refer to the NO_2-induced lesions as "emphysema-like" rather than emphysema of the type seen in human lungs.

Lafuma et al. (1987) studied the effect of 3,760 $\mu g/m^3$ (2 ppm) NO_2, 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 NO_2 alone. They did not state whether alveolar wall destruction was or was not found. There is no evidence of alveolar wall destruction in the published micrographs of lungs from that group of hamsters. There is no evidence from this publication that NO_2 alone produced emphysema of the type seen in

human lungs. Table 13-14 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. Hypertrophy of Type 1 cells, followed by proliferation of and replacement by Type 2 cells, is the general lesion in the alveoli, while hypertrophy and hyperplasia of ciliated cells occurs in the bronchioli. Such effects are found with acute exposures to levels $>9{,}400$ $\mu g/m^3$ (5 ppm) NO_2. Chronic exposure results in similar changes, but in this case effective levels may be $<1{,}880$ $\mu g/m^3$ (1 ppm); in general, repeated exposures to levels $>3{,}760$ $\mu g/m^3$ (2 ppm) are needed for extensive and permanent morphologic changes. Both exposure concentration and duration are important, with concentration perhaps playing a more significant role. 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. Mature animals show somewhat of a decline in responsiveness with age, until an increase occurs at some point in senescence.

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 1985 NHLBI workgroup. 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". 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 of alveolar walls occurred. The latter group includes several studies testing specific hypotheses of the pathogenesis of emphysema which, as appropriate to their research objectives, used the 1985 NHLBI workgroup's definition of emphysema in experimental animals. That definition requires

TABLE 13-14. EFFECTS OF NO_2 ON THE DEVELOPMENT OF EMPHYSEMA

NO_2 Concentration		Exposure	Species	Emphysema*	Reference
$\mu g/m^3$	ppm				
188 with 2 h peaks to 1,880	0.1 with peaks to 1.0	Daily, 6 mo	Mouse	±	Port et al. (1977)
270 plus 2,050 $\mu g/m^3$ NO	0.14	16 h/day, 68 mo	Beagle dog	−	Hyde et al. (1978)
1,210 plus 310 $\mu g/m^3$ NO	0.6			+	
940	0.5	6, 18, or 24 h/day, 1-12 mo	Mouse	−	Blair et al. (1969)
1,504 7,520	0.8 4.0	51-813 days	Rat	−	Haydon et al. (1965)
1,880 (with and without viral challenge)	1.0	16 mo	Squirrel monkey	±	Ehrlich and Fenters (1973)
3,760	2.0	Continuous, 112-763 days	Rat	−	Freeman et al. (1968c)
3,760	2.0	8 h/day, 5 days/wk for 8 wk	Hamster	−	Lafuma et al. (1987)
9,400 18,800	5.0 10.0	3 mo	Squirrel monkey	±	Ehrlich and Fenters (1973)
9,400	5.0	Up to 18 mo	Dog Rabbit Guinea pig Rat Hamster Mouse	−	Wagner et al. (1965)
15,040 22,560	8.0 12.0	3-4 mo (presumably 24 h/day)	Rabbit	+	Haydon et al. (1967)
28,200	15.0	3-5 mo	Rat	±	Stephens et al. (1976)

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TABLE 13-14 (cont'd). EFFECTS OF NO_2 ON THE DEVELOPMENT OF EMPHYSEMA

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Emphysema*	Reference
28,200	15.0	Continuously for 35 days then intermittently for at least 2 years	Rat	±	Port et al. (1977)
32,400	18.0	24 h/day for 1-6 days or 4 wk	Rat	±	Freeman et al. (1968a)
37,600 reduced to either 28,200 or 18,800	20.0 reduced to 15.0 or 10.0	Up to 33 mo	Rat	+	Freeman et al. (1972)
47,000	25.0	32-65 days	Rat	−	Freeman et al. (1964)
56,400	30.0	22 h/day, 12 mo	Hamster	−	Kleinerman et al. (1985b)
56,400	30.0	Continuous, up to 140 days	Rat	±	Glasgow et al. (1987)
56,400	30.0	Continuous, up to 8 wk	Rat	−	Blank et al. (1978)
56,400-65,800	30.0-35.0	23 h/day for 7 days	Hamster	−	Lam et al. (1983)
65,800	35.0	6 h/day for 25 days	Rat	−	Stavert et al. (1986)
75,200	40.0	6 or 8 wk	Mouse	−	Buckley and Loosli (1969)
94,000-169,200 for 4 weeks; reduced 56,400-94,000	50-90 reduced to 30-50	2 h/day, 5 days/wk, 12 mo	Hamster Guinea pig	±	Gross et al. (1968)
84,600-103,400	45-55	22-23 h/day, 10 wk	Hamster	−	Kleinerman and Cowdrey (1968)

*+ = emphysema; − = no emphysema; ± = equivocal.

Emphysema is defined according to the 1985 NHLBI Workshop criteria for human emphysema. Although several of the papers reviewed reported finding emphysema, some of these studies (especially the early studies) were reported according to previous, different criteria; some reports did not fully describe the methods used; and/or the results obtained were not in sufficient detail to allow independent confirmation of the presence of emphysema. Thus, a "−" (i.e. no emphysema) should only be interpreted as lack of proof of emphysema, since it is conceivable that if the study were repeated with current methods and the current criteria applied, some might be positive.

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airspace enlargement but not alveolar wall destruction. Several studies were considered inconclusive due to problems with control animals or to insufficient numbers of animals.

13.2.4 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 it's reactive products penetrate the lung epithelial and endothelial layers to enter the blood and produce alterations in blood and various other organs.

13.2.4.1 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_2 on body weight can be found in Table 13-15.

The most comprehensive study was performed by Wagner et al. (1965) who exposed rabbits, guinea pigs, rats, hamsters, and 4 strains of mice to 1,880, 9,400, and 47,000 $\mu g/m^3$ (1, 5, and 25 ppm) and dogs to 1,880 and 9,400 $\mu g/m^3$ NO_2. For all species examined, no significant differences in body weight were observed after 6, 12, and 18 months of exposure.

The possibility that newborn mice were more sensitive to ambient levels of NO_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_2. Examination of the male offspring born during NO_2 exposure and exposed for 12 additional weeks showed less of an increase in body weight 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_2 fetally-exposed rats were not different. Neonates similarly exposed to 564 $\mu g/m^3$ (0.3 ppm) NO_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 1,504 $\mu g/m^3$ (0.17 to 0.8 ppm) NO_2 for periods ranging from 1 to 12 weeks showed no significant body weight changes.

TABLE 13-15. EXTRAPULMONARY EFFECTS OF NO_2: BODY WEIGHT

NO_2 Concentration		Exposure	Species	Effects	Reference
$\mu g/m^3$	ppm				
94	0.05	90 days	Rat	No effect.	Shalamberidze (1969)
320-1,504	0.17-0.8	8 h/day, 5 days/wk, 1-12 wk	Mouse	No effect.	Richters et al. (1987)
470-564	0.25-0.30	8 h/day, 5 days/wk, 3-12 wk	Mouse (newborn)	Reduced body weight gain compared to control at 3 and 12 wk, but not at 6 wk.	Richters et al. (1987)
658	0.35	8 h/day, 5 days/wk, 6 wk	Mouse	Reduced body weight gain in newborns but not adults.	Kuraitis et al. (1981)
940-3,760, twice daily 1 h spike of 3× baseline	0.5-2.0	Continuous, 1, 3, and 6 wk	Rat (1-day and 7-wk old)	Reduced body weight gain in young adult rats after 3 and 6 wk exposure to 1 or 2 ppm. No effects in juvenile rats.	Stevens et al. (1988)
1,300-1,500	0.7-0.8	30 days	Mouse	No effect.	Nakajima et al. (1969)
1,504	0.8	Continuous, lifetime	Rat	No effect.	Freeman et al. (1966)
1,880	1.0	6 mo	Guinea pig	Reduced body weight gain.	Kosmider et al. (1973)
1,900-47,000	1.0-25	18 mo	Dog Rabbit Guinea pig Rat Hamster Mouse	No effect.	Wagner et al. (1965)
2,400-5,680	1.3-3.0	2 h/day, 15-17 wk	Rabbit	Reduced body weight gain.	Mitina (1962)

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TABLE 13-15 (cont'd). EXTRAPULMONARY EFFECTS OF NO_2: BODY WEIGHT

NO_2 Concentration		Exposure	Species	Effects	Reference
$\mu g/m^3$	ppm				
3,760	2.0	Continuous, lifetime	Rat	No effect.	Freeman et al. (1968c)
18,800	10.0	Up to 62 days	Rat	Reduced body weight gain and length of pups.	Freeman et al. (1972)
24,000	12.5	Continuous, 213 days	Rat	Reduced body weight gain.	Freeman and Haydon (1964)

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Kuraitis et al. (1981) also reported that the body weights of newborn mice exposed to 658 $\mu g/m^3$ (0.35 ppm) NO_2 for 6 weeks were significantly less than age-matched controls. No decrease in body weight was observed when adult mice were exposed. Similarly, Nakajima et al. (1969) found no effects in adult female mice exposed 30 days to 1,316 or 1,504 $\mu g/m^3$ (0.7 or 0.8 ppm) NO_2.

Only very high exposure concentrations of NO_2 have been found to cause reduced body weights in rats. No exposure-related effects were found in rats exposed for 90 days to 94 $\mu g/m^3$ (0.05 ppm) NO_2 (Shalamberidze, 1969). Freeman et al. (1966, 1968c) found no effects on body weight after lifetime exposure to 1,504 or 3,760 $\mu g/m^3$ (0.8 or 2.0 ppm) NO_2. Freeman and Haydon (1964) did observe body weight changes after continuously exposing rats to 24,000 $\mu g/m^3$ (12.5 ppm) NO_2.

Although the data in mice suggest that newborns may be more sensitive to NO_2 exposure than adults, other evidence (see Section 13.2.3.4), 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 juvenile rats were less sensitive to NO_2 than young adult rats. Stevens and coauthors exposed young adult (7-week old) and juvenile (1-day old) Fischer-344 rats to 940, 1,880, and 3,760 $\mu g/m^3$ (0.5, 1.0, and 2.0 ppm) NO_2 for 6 weeks. To simulate ambient exposure patterns, twice daily the NO_2 exposure was increased to three times the baseline concentration for 1 h. The body weight of young adult rats were less than the control rats after 1 week and significantly less at 3 and 6 weeks when the baseline exposure concentration was 1,880 or 3,760 $\mu g/m^3$. Rats exposed to 940 $\mu g/m^3$ NO_2 showed a transitory decrease at 3 weeks that was no longer evident at 6 weeks. Juvenile rats, exposed within 36 h after birth to the same NO_2 concentrations, showed no changes in body weight after 1, 3, or 6 weeks of exposure.

Kosmider et al. (1973) reported a decline in body weight gain in guinea pigs exposed continuously to 1,880 $\mu g/m^3$ (1.0 ppm) NO_2 for 6 months. Mitina (1962) exposed rabbits to 2,400 and 5,680 $\mu g/m^3$ (1.3 and 3.0 ppm) NO_2 for 15 and 17 weeks and found significant decreases in body weight gain that persisted 5 to 7 weeks after the exposure ended.

13.2.4.2 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 NO_2^- and NO_3^-, 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 measured and shown effects on the number of erythrocytes (red blood cells [RBCs]) and hemoglobin concentration, although the results have been inconsistent. A summary of these studies can be found in Table 13-16. In some of these studies, leukocytes (white blood cells) and platelet counts were also examined. Table 13-17 separates these results for easy comparison.

Shalamberidze (1969) exposed rats continuously to 94 $\mu g/m^3$ (0.05 ppm) NO_2 for 90 days with no change in blood hemoglobin or RBC counts. Yakimchuk and Chelikanov (1972) reported that during a 3-month continuous exposure to 600 $\mu g/m^3$ (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 measured, but no significant effects were found.

Fenters et al. (1973) showed that exposing male squirrel monkeys to 1,880 $\mu g/m^3$ (1 ppm) NO_2 continuously for 493 days had no significant effect on hematocrit, hemoglobin, total protein, globulins, chloride, sodium, calcium, potassium, glucose, blood urea nitrogen, glutamicpyruvic transaminase, lactate dehydrogenase, and lactate dehydrogenase isoenzymes. Challenge with influenza A/PR/8/34 increased the leukocyte number in NO_2-exposed animals above the levels in similarly challenged controls.

Dogs exposed to 1,880 and 9,400 $\mu g/m^3$ (1 and 5 ppm) NO_2 for 18 months showed no significant change in hemoglobin, hematocrit, white cell count, or serum alkaline phosphatase and magnesium-activated phosphatase activity (Wagner et al., 1965). Rabbits exposed to 2,400 to 5,680 $\mu g/m^3$ (1.3 to 3.0 ppm) NO_2 for 15 and 17 weeks had a decrease in the number of RBCs and a significant increase in the number of leukocytes (Mitina, 1962).

TABLE 13-16. EFFECTS OF NO_2 ON RED BLOOD CELLS AND HEMOGLOBIN

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
94	0.05	Continuous, 90 days	Rat	No effect on blood hemoglobin or RBCs.	Shalamberidze (1969)
940-1,500 +	0.5-0.8 +	Continuous, 1 to 1.5 mo	Mouse	Addition of 50 ppm CO to NO_2 failed to affect carboxyhemoglobin.	Nakajima and Kusumoto (1970)
1,500	0.8	Continuous, 5 days	Mouse	No effect on methemoglobin.	Nakajima and Kusumoto (1968)
1,880	1.0	Continuous, 493 days	Squirrel monkey	No effect on hematocrit or hemoglobin with NO_2 and influenza exposure.	Fenters et al. (1973)
1,880 9,400	1.0 5.0	Continuous, 18 mo	Dog	No changes in hemoglobin or hematocrit.	Wagner et al. (1965)
1,880-56,400	1.0-30	18 h	Mouse	Concentration-related increase in methemoglobin and nitrosylhemoglobin.	Case et al. (1979)
2,400-5,640	1.3-3.0	2 h/day, 15 and 17 wk	Rabbit	Decreased RBCs.	Mitina (1962)
3,760 +	2.0 +	Continuous, 14 mo	Monkey Rat	With or without NaCl (330 $\mu g/m^3$): polycythemia with reduced mean corpuscular volume and normal mean corpuscular hemoglobin.	Furiosi et al. (1973)
9,400-75,200	5.0-40	1 h	Mouse	No increase in methemoglobin. Increased nitrite and especially nitrate.	Oda et al. (1981)

*RBC = Red blood cell.

TABLE 13-17. EFFECTS OF NO_2 ON LEUKOCYTES AND PLATELETS

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
600	0.32	Continuous, 3 mo	Rat	Increased leukocytes after 8-10 wk, no difference after 3 mo exposure.	Yakimchuk and Chelikanov (1972)
1,880	1.0	Continuous, 493 day followed by virus challenge	Squirrel monkey	Increased leukocyte count in viral-challenged, NO_2-exposed animals.	Fenters et al. (1973)
1,880 9,400	1.0 5.0	Continuous, 18 mo	Dog	No effect on leukocyte count.	Wagner et al. (1965)
2,400-5,640	1.3-3.0	2 h/day, 15 and 17 wk	Rabbit	Increased leukocytes followed by decreased phagocytic activity.	Mitina (1962)
3,760 +	2 +	Continuous, 14 mo	Monkey Rat	Neutrophil/lymphocyte ratio tendency to shift upwards in both animal species tested.	Furiosi et al. (1973)
18,800	10	Continuous, 14 day	Rat	Decreased platelets at 1 to 7 days of exposure, but not after 14 days.	Kobayashi et al. (1983)

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As discussed in the section on host defenses, a decrease in the phagocytic activity of the leukocytes was also reported.

Furiosi et al. (1973) exposed rats to 3,760 ± 1,880 $\mu g/m^3$ (2±1 ppm) NO_2 for between 12 and 21 months and found polycythemia with reduced mean corpuscular volume, but normal mean corpuscular hemoglobin concentrations. Exposure to NO_2 also increased the ratio of polymorphonuclear leukocytes to lymphocytes. Because exposures occurred while the rats were in plastic cages inside the exposure chamber, the actual concentration of NO_2 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.

Kobayashi et al. (1983) reported a reduction in rat platelets after 1, 3, 5, and 7, but not 14 days of continuous exposure to 18,800 $\mu g/m^3$ (10 ppm) NO_2. Because platelet thromboxane A_2 increased on Day 3, corresponding with a decrease in lung PGI_2, and the balance between these two arachidonic acid metabolites is thought to regulate platelet aggregation, the authors speculate that NO_2 may induce increased platelet aggregation.

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 $\mu g/m^3$ (0.35 ppm) NO_2 (Kuraitis et al., 1981). This finding could be interpreted as supporting the polycythemia that was observed in NO_2-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 NO_3^- or NO_2^- reacted with hemoglobin. Nakajima and Kusumoto (1968) found that the amount of methemoglobin was not increased when mice exposed to 1,500 $\mu g/m^3$ (0.8 ppm) NO_2 for 5 days. Oda et al. (1981) also found no increase in methemoglobin, but NO_3^- and especially NO_2^- were elevated in the blood of mice exposed for 1 h to between 9,400 and 75,200 $\mu g/m^3$ (5 to 40 ppm) NO_2. In contrast, Case et al. (1979) showed that mice exposed to 1,880 to 56,400 $\mu g/m^3$ NO_2 (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 NO_2 exposure (see Table 13-18). In a preliminary report, Mersch et al. (1973) showed that RBC D-2,3-diphosphoglycerate, a measure of tissue deoxygenation, was increased in all four guinea pigs continuously exposed to 677 $\mu g/m^3$ (0.36 ppm) NO_2 for 1 week.

Changes in the contents of RBC membranes were detected after exposure to 7,520 $\mu g/m^3$ (4 ppm) NO_2 (Kaya et al., 1980). Increased amounts of sialic acid were noted in rats exposed between 1 and 10 days to 7,520 $\mu g/m^3$ (4 ppm) NO_2. 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 NO_2 inhalation may have stimulated renewal of the RBC population (increased population of immature cells). An increased amount of lysophosphatidyl-ethanolamine, known to increase cell membrane fragility, was found on Days 5, 7, and 10 after exposure to 7,520 $\mu g/m^3$ and after 1, 5, and 7 days of exposure to 18,800 $\mu g/m^3$ (10 ppm) NO_2. Protein content of RCBs was slightly decreased at 18,800 $\mu g/m^3$ 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. (1984a) who showed that after 1 and 4 days exposure to 7,520 $\mu g/m^3$ NO_2, the activity and content of Na^+, K^+ -ATPase, and the amount of sialic acid were increased in RBC membranes. These changes have previously been associated with a younger population of RBCs (Cohen et al., 1976).

In contrast, Mochitate and Miura (1984) found that after 7 days of continuous exposure to 7,520 $\mu g/m^3$ (4 ppm) NO_2, there was a decreased population of younger RBCs. However, the activity of two glycolytic enzymes (pyruvate kinase and phosphofructokinase) was elevated in NO_2-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 NO_2-induced increase in the apparent aging of the RBCs.

Kaya and Miura (1982) investigated the effects of NO_2 on fatty acids in RBCs, sera, and liver. They found that in RBC membranes a net increase in unsaturated fatty acids (predominately arachidonic acid) occurred after exposure to 7,520 $\mu g/m^3$ (4 ppm) NO_2 for 10 days.

TABLE 13-18. EFFECTS OF NO_2 ON RED BLOOD CELL MEMBRANES

NO_2 Concentration		Exposure	Species	Effects*	Reference
$\mu g/m^3$	ppm				
677	0.36	Continuous, 7 days	Guinea pig	Increased RBC D-2,3-diphosphoglycerate.	Mersch et al. (1973)
940	0.5	8 h/day, 7 days	Guinea pig	Decrease in RBC GSH peroxidase ($p < 0.001$).	Menzel et al. (1976)
		8 h/day, 4 mo		No change in RBC GSH peroxidase.	
7,520	4.0	Continuous, 1-10 days	Rat	Increased sialic acid, Na^+, and K^+ -ATPase in RBC membranes associated with an increase proportion to younger RBCs.	Kunimoto et al. (1984a)
7,520	4.0	Continuous, 1-10 days	Rat	After 7 days exposure the fraction of young RBCs was reduced and the fraction of older RBCs increased. Activity of pyruvate kinase and phosphofructokinase were proportionally increased in the young RBCs.	Mochitate and Miura (1984)
7,520	4.0	Continuous, 10 days	Rat	Increased arachodonic acid in membranes and serum. Stearic palmatic acid decreased at 10 and 4 ppm in RBC membranes.	Kaya and Miura (1982)
18,800	10.0	Continuous, 7 days			
7,520-37,760	4.0-20	Continuous, 1-10 days	Rat	Decrease RBC membrane protein at Day 1, 5, and 7 but not 3. Lysophosphatidyl ethanolamine, sialic acid, and hexose were increased at 4 and 10 ppm.	Kaya et al. (1980)

*RBC = Red blood cell.
GSH = Glutathione.

Effects on Serum and Plasma

In the previous section, Kaya and Miura (1982) showed that arachidonic acid was increased in the RBC membrane after exposure to NO_2. Since *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 exposure to 7,520 $\mu g/m^3$ (4 ppm) NO_2 for 10 days, arachidonic acid was elevated in both the serum and in liver homogenates. However, exposure to 18,800 $\mu g/m^3$ (10 ppm) NO_2 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-$\mu g/m^3$ NO_2 exposure, but could metabolically compensate for the effects of exposure to 7,520 $\mu g/m^3$ NO_2.

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 months) continuous exposure to 940 $\mu g/m^3$ (0.5 ppm) NO_2. Plasma cholinesterase was significantly elevated after a 7-day exposure but was significantly decreased compared to control values with a long-term exposure (4 months). This depression in ChE suggests an hepatic lesion (Moore et al., 1957). A statistically significant depression in RBC GSH peroxidase activity was also initially observed, but the effect did not persist after 4 months of exposure. Similarly, several indices of nonspecific tissue damage (serum CPK, lactate dehydrogenase, SGOT, and SGPT) were also increased after 7 days, but were not altered on 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 $\mu g/m^3$ (1.05 ppm) NO_2, 8 h/day for 180 days. However, serum levels of α_1- and β-globulins were increased. These authors also found that serum alanine and aspartate aminotransferase activity was increased in the mitochondrial fraction but 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 cytoplasmic and mitochondrial fraction of serum is not clear from the translation of the article.

Kosmider et al. (1973) reported a general decrease in protein synthesis evidenced by decreased serum proteins in guinea pigs after continuous exposure to 1,880 $\mu g/m^3$ (1 ppm) NO_2 for 6 months. Following exposure to 1,000 $\mu g/m^3$ NO_x (mainly NO_2, ~0.5 ppm), 8 h/day for 120 days, Kosmider (1975) reported decreased serum cholesterol, total lipids, β and gamma lipoproteins and sodium, and increased serum α-lipoproteins in guinea pigs. Similarly, Mitina (1962), after exposing rabbits to 2,400 to 5,680 $\mu g/m^3$ (1.3 to 3.0 ppm) NO_2 for 15 and 17 weeks, found reduced amounts of albumin, but increased serum globulins. Table 13-19 summaries the data on NO_2-induced changes in serum proteins and clinical chemistries.

13.2.4.3 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 13.2.3.3).

Suzuki et al. (1981) exposed rats for up to 3 months to between 752 and 7,520 $\mu g/m^3$ (0.4 and 4.0 ppm) NO_2. After 3 months of exposure to 7,520 $\mu g/m^3$ NO_2, anesthetized rats, artificially ventilated at high frequencies, had a significant reduction in PaO_2. A reduction in heart rate was reported in unanesthetized mice exposed to 2,250 and 7,520 $\mu g/m^3$ (1.2 and 4.0 ppm) NO_2 for 1 month (Suzuki et al., 1984).

Tsubone et al. (1982) reported that rats developed bradycardia that progressed to significant electrocardiographic (ECG) abnormalities including atrioventricular blocks, premature beats, and wandering pacemakers at a 30 min exposure to 37,600 $\mu g/m^3$ (20.0 ppm) NO_2. Intravenously administered blood NO_2^- and NO_3^- did not affect the ECG, while injection of atropine sulfate during NO_2 exposure immediately reversed the bradycardia, suggesting that NO_2 alters parasympathetic activity. Electrocardiographic effects were not observed after exposure to 18,800 $\mu g/m^3$ (10.0 ppm) NO_2. Comparisons between tracheostomized and nontracheostomized rats suggested that irritation to the upper airways (trigeminal nerve) was not involved in the cardiovascular response (Tsubone et al., 1984).

TABLE 13-19. EFFECTS OF NO_2 ON SERUM PROTEINS AND CLINICAL CHEMISTRIES

NO_2 Concentration		Exposure	Species	Effects	Reference
$\mu g/m^3$	ppm				
600	0.32	Continuous, 3 mo	Rat	Cholinesterase was not affected.	Yakimchuk and Chelikanov (1972)
940	0.5	8 h/day, 7 days 8 h/day, 4 mo	Guinea pig	Serum lactate dehydrogenase, total creatinine phosphokinase, SGOT and SGPT, plasma cholinesterase, and lysozyme elevated. Lysozyme and plasma cholinesterase depressed.	Menzel et al. (1977)
1,000	(NO_x; mainly NO_2)	8 h/day, 180 days	Guinea pig	Serum cholesterol slightly elevated; total lipids depressed.	Kosmider (1975)
1,880	1.0	Continuous, 6 mo	Guinea pig	Protein synthesis inhibited. Total serum proteins and immunoglobulins decreased.	Kosmider et al. (1973)
1,880	1.0	Continuous, 493 days followed by viral challenge	Squirrel monkey	No effect on clinical biochemical parameters.	Fenters et al. (1973)
2,000	1.05	8 h/day, 180 days	Guinea pig	Plasma changes: Decreased albumin, seromucoid, cholinesterase, alanine, and aspartate transminases. Increased α_1 and β_2 globulins.	Drozdz et al. (1976)
1,880-56,400	1.0-30	18 h	Mouse	Decreased catalase and iron transferrin activity.	Case et al. (1979)
11,660	6.2	Continuous	Rat	No effect on serum lysozyme.	Chow et al. (1974)

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In a follow-up study, the cardiovascular effects of phenyl guanide were enhanced by preexposure to 18,800 $\mu g/m^3$ NO_2 for 24 h, but not by 7,520 $\mu g/m^3$ (4.0 ppm) for 1 week or 752 $\mu g/m^3$ (0.4 ppm) for 4 weeks (Tsubone and Suzuki, 1984).

Conscious sheep exposed to 28,200 $\mu g/m^3$ (15 ppm) for 4 h showed no significant changes in arterial blood gases, pulmonary vascular pressure or resistance, cardiac output, or pulmonary and capillary blood volumes (Abraham et al., 1980). This is in contrast to the findings of Dowell et al. (1971) who showed decreased cardiac output, blood pressure, PaO_2, and pH in dogs after a 1-h exposure to between 13,160 and 30,080 $\mu g/m^3$ (7 and 16 ppm) NO_2. 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.

13.2.4.4 Hepatic Function

As previously described (Section 13.2.4.2), changes in serum chemistries suggest that NO_2 exposure may affect the liver. Several studies have examined hepatic function either directly or by indirect means. These studies are cataloged in Table 13-20.

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. However, the effects reliably occurred after a 3-h exposure to concentrations as low as 470 $\mu g/m^3$ (0.25 ppm) NO_2. No significant effects were detected after 1 or 2 days exposure to 235 $\mu g/m^3$ (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 $\mu g/m^3$ (5.0 ppm) NO_2; however, no NO_2-related effects were found (Graham et al., 1982).

Increased hexobarbital-induced sleeping times were also reported in the progeny of maternally exposed rats (Tabacova et al., 1985). This effect was measured in the offspring

TABLE 13-20. EFFECTS OF NO_2 ON THE LIVER

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
470-9,400	0.25-5.0	3 h	Mouse	Increase in pentobarbital-induced sleeping time in female mice only. Repeated daily exposures caused no effect.	Miller et al. (1980)
235	0.125	1-2 days		No effect on pentobarbital-induced sleeping time.	
50-10,000	0.026-5.3	6 h/day, 7 days/wk to dams during gestation. Offspring evaluated up to 2 mo after birth	Rat	Increased hexobarbital-induced sleeping time at 0.5 and 5.0 ppm. Cyt. P-450 and aminopyrine N-demethylase were decreased, while lipid peroxides and O_2 consumption were increased at 5.0 ppm.	Tabacova et al. (1985)
752-7,520	0.4-4.0	Continuous, 14 wk	Rat	Cyt. P-450 decreased during first 8 wk of exposure; returned to control levels with continued exposure (4.0 ppm).	Takahashi et al. (1986)
7,520	4.0	Continuous, 1, 14, or 30 days	Rat	Cyt. P-450 and aminopyrine N-demethylase increased and aniline hydroxylase was decreased. No effect at Day 1.	Takano and Miyazaki (1984)
752-7,520	0.4-4.0	Continuous, 7 or 10 days	Rat	Succinate-cyt. C reductase was reduced at 10 ppm. Cyt. P-450 was reduced at 4.0 and 0.4 ppm but not at 1.2 ppm. NADPH-cyt. C reductase was reduced at 10 and 4.0 ppm.	Mochitate et al. (1984)

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TABLE 13-20 (cont'd). EFFECTS OF NO_2 ON THE LIVER

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
1,000 (mostly NO_2)	0.5	8 h/day, 3 mo	Guinea pig	Depleted liver magnesium and zinc. Swollen liver mitochondria.	Kosimider (1975)
2,000	1.05	8 h/day, 6 mo	Guinea pig	Decreased liver protein and cytoplasmic aspartate transaminase. Increased mitochondrial alanine and aspartate transaminase. Intracellular edema, inflammatory, and parenchymal changes observed.	Drodz et al. (1976)
9,400	5.0	3 h	Mouse	No decrease in Cyt. P-450 or mixed function oxidase enzymes.	Graham et al. (1982)

*Cyt. = Cytochrome.

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on Days 7, 14, and 21 postexposure (10,000 $\mu g/m^3$ [5.3 ppm] NO_2). Additionally, lipid peroxides increased and O_2 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 $\mu g/m^3$ (0.5 ppm) NO_2, increased hexobarbital-induced sleeping times occurred on Days 7 and 21, but not on Day 14. Liver lipid peroxides were 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 $\mu g/m^3$ (0.4 to 4.0 ppm) NO_2 during the first 12 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 $\mu g/m^3$ NO_2 for 1, 14, or 30 days. After 14 days, cytochrome P-450 levels and aminopyrine N-demethylase activity were increased, while 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.

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, $\sim$0.5 ppm), 8 h/day for 120 days. Swollen liver mitochondria were also observed. Significant reductions of succinate-cytochrome C reductase activity in rat liver homogenates were found during the third and fifth day of an 18,800 $\mu g/m^3$ (10.0 ppm) NO_2 exposure, but not during the first and seventh day, or with exposure to 7,520 $\mu g/m^3$ (4.0 ppm) NO_2 (Mochitate et al., 1984). Significant decreases in cytochrome P-450 from liver microsomes were also found after 7 days of exposure to 752 or 7,520 $\mu g/m^3$ (0.4 or 4.0 ppm) NO_2, but not after exposure to

2,256 $\mu g/m^3$ (1.2 ppm) NO_2. NADPH-cytochrome C reductase was reduced with 5 days of exposure to 7,520 and 18,800 $\mu g/m^3$ NO_2.

13.2.4.5 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 13-21 and discussed below.

Takahashi et al. (1986) found that continuous exposure to 2,256 and 9,400 $\mu g/m^3$ (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. Continued 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 discussed in Section 13.2.4.4 on hepatic effects.

Yakimchuk and Chelikanov (1972) reported that during a 3-month continuous exposure to 600 $\mu g/m^3$ (0.32 ppm) NO_2, rats showed a significant increase in the urinary excretion of 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, ~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 13.2.4.2, 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 750 $\mu g/m^3$ (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 NO_2^- and NO_3^-, Saul and Archer (1983) exposed rats for 24 h to 2,256 to 16,544 $\mu g/m^3$

TABLE 13-21. EFFECTS OF NO_2 ON THE KIDNEY AND ON URINE CONTENTS

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects	Reference
600	0.32	Continuous, 3 mo	Rat	Increased urinary coproporphyrins.	Yakimchuk and Chelikanov (1972)
940 750	0.5 0.4	Continuous, 8 h/day, 14 days	Guinea pig	Increased urinary protein and specific gravity. Proteins characteristic of the nephrotic syndrome.	Sherwin and Layfield (1974)
1,000	~0.5	8 h/day, 3 mo	Guinea pig	Increased urinary nitrite, nitrate, and coproporphyrin.	Kosmider (1975)
2,256 9,400	1.2 4.0	Continuous, 3 mo	Rat	Increased cytochrome P-450 and b_5 after 8 wk of exposure; less of an effect after 12 wk.	Takahashi et al. (1986)
2,256 16,544	1.2 8.8	24 h	Rat	Increased urinary nitrate; urinary nitrite appeared to be artifactual.	Saul and Archer (1983)

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(1.2 to 8.8 ppm) NO_2. They demonstrated that mostly NO_3^-, with very little NO_2^-, was excreted in the urine. The small amount of urinary NO_2^- 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 NO_3^- suggested that NO_2 does not form NO_3^- by reacting with respiratory water, but reacts with oxidizable tissue to form NO_2^-. Nitrite is then further oxidized in the blood by oxyhemoglobin (Kosaka et al., 1979) to form NO_3^- which is excreted in the urine. Nitrite and NO_3^- was also found in the urine of guinea pigs exposed to 1,000 $\mu g/m^3$ NO_x (mainly NO_2, ~0.5 ppm), 8 h/day for 120 days (Kosmider, 1975).

13.2.4.6 Effects on the Central Nervous System and Behavioral Effects

Information regarding the effects of NO_2 on development and animal behavior is limited to a few studies (see Table 13-22), most of which have uncertain relationships to humans. Shalamberidze (1969) exposed rats to 100 $\mu g/m^3$ (0.05 ppm) NO_2 for 3 months with no demonstrated effects on the central nervous system. Yakimchuk and Chelikanov (1972) reported that during 3 months continuous exposure to 600 $\mu g/m^3$ (0.32 ppm) NO_2, rats developed an increased latency of response to conditioned sound and light stimuli.

Exposure of guinea pigs to 1,000 $\mu g/m^3$ (0.53 ppm) NO_2, 8 h/day for 180 days affected brain enzyme activity levels (Drozdz et al., 1975). Decreased activity in brain protein metabolism enzymes were seen in brain malate dehydrogenase, alanine aminotransferase, sorbitol dehydrogenase, lactate dehydrogenase, adenosine triphosphatase, 5'-nucleotidase, and asparagine aminotransferase. Increases in brain glycolytic enzyme activity were seen in 1,6-diphosphofructose aldolase, isocitrate dehydrogenase, alpha-hydroxybutyrate dehydrogenase, phosphocreatine kinase, and cholinesterase.

A recent study (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 $\mu g/m^3$ (0.45 ppm) NO_2, 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 ratio of 5-HIAA:5-HT, 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 $\mu g/m^3$ (3.5 ppm) NO_2 for 1 or 2 months. The only significant

TABLE 13-22. EFFECTS OF NO_2 ON THE CENTRAL NERVOUS SYSTEM AND BEHAVIOR

NO_2 Concentration		Exposure	Species	Effects*	Reference
$\mu g/m^3$	ppm				
50-10,000	0.03-5.3	6 h/day, 7 days/wk	Rat	Significant defects in posture and gate were detected at 9 and 14 days at 0.05 ppm. Additional effects at higher levels. No monitoring method was described.	Tabacova et al. (1985)
100	0.05	Continuous, 3 mo	Rat	No effect on CNS.	Shalamberidze (1969)
600	0.32	Continuous, 3 mo	Rat	Decreased conditioned reflexes to sound and light.	Yakimchuk and Chelikanov (1972)
845	0.45	7 h/day, 4 wk	Mouse	Increased 5-HT, 5-HIAA, and turnover.	Sherwin et al. (1986)
1,000	0.53	8 h/day, 180 days	Guinea pig	Decreased malate, sorbitol, lactate dehydrogenase; alanine aminotransferase ATPase and 5'-nucleotidase homogenate. Increased 1,6-diphosphofructose aldolase, isocitrate, alpha-hydroxy-butyrate dehydrogenase, phosphocreatine kinase, and cholinesterase.	Drozdz et al. (1975)
1,880	1.0	20 min/day, to 6 mo	Rat	More or less constant swimming performance; only slight tendency to deterioration.	Tusl et al. (1975)
6,580 9,400 37,600	3.5 5.0 20.0	6 h/day, 8 wk		Decreased swimming performance at 3.5 ppm; decrease of 25% by 5th and 6th wk of 5 ppm exposure; declined from 1st mo at 20 ppm.	
9,400-75,200	5-40	24 h	Mouse	Decreased swimming performance at 10 ppm. Increased blood lactate compared to similarly exercised controls at 5 ppm.	Suzuki et al. (1982a)
14,000	7.7	6 h	Mouse	Decreased voluntary running activity; return to normal within 24 h postexposure.	Murphy et al. (1964)
15,040 37,600 75,000	8.0 20 40	19 days 1 day 5 h	Rat	Decrease in swimming endurance of 5, 10, and 20% at 8, 20, and 40 ppm, respectively.	Campbell (1976)

*CNS = Central nervous system.
5-HT = 5-hydroxytryptamine.
5-HIAA = 5-hydroxyindole acetic acid.

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effect reported was a decrease in the hypothalamic concentration of noradrenaline at both exposure durations.

As discussed in the section on reproductive, developmental, and heritable mutagenic effects (Section 13.2.4.7), Tabacova et al. (1985) also reported significant postnatal deficits in the onset of normal neuromotor development and reduced open field activity in the progeny of maternally exposed rats 2 months after the dams were exposed to 1,000 or 10,000 $\mu g/m^3$ (0.5 or 5.3 ppm) NO_2 for 6 h/day, 7 days/week. Postural and gait defects were also reported at 0.1 $\mu g/m^3$ (0.05 ppm). No monitoring method was specified.

Tusl et al. (1973) exposed rats to 9,400 $\mu g/m^3$ (5 ppm) NO_2 for 8 weeks. The influence of NO_2 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 $\mu g/m^3$ (1 ppm) NO_2, 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 to 75,200 $\mu g/m^3$ (5 to 40 ppm) NO_2 was reported by Suzuki et al. (1982a). Significant decrements in performance were reported at exposure concentrations as low as 18,000 $\mu g/m^3$ (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 $\mu g/m^3$ (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 $\mu g/m^3$ (5 ppm) both immediately and 24 h after exposure. These two findings suggest that lung edema prevented sufficient O_2 from entering the blood during exercise to meet aerobic demands. See section discussing NO_2-induced effects on pulmonary function.

13.2.4.7 Reproductive, Developmental, and Heritable Mutagenic Effects

As summarized in Table 13-23, few studies have examined the effects of NO_2 on reproduction and development or the heritable mutagenic potential of NO_2 in vivo. Exposure to 1,800 $\mu g/m^3$ (1 ppm) NO_2 for 7 h/day, 5 days/week for 21 days resulted in no alterations

TABLE 13-23. EFFECTS OF NO_2 ON REPRODUCTION, DEVELOPMENT, AND HERITABLE MUTAGENESIS

NO_2 Concentration		Exposure	Species	Effects	Reference
$\mu g/m^3$	ppm				
50 100 1,000 10,000	0.03 0.05 0.5 5.3	6 h/day, 7 days/wk, 21 days	Rat	Concentration-dependent delay in eye opening and incisor eruption in progeny of dams exposed to 0.5 or 5.3 ppm during gestation. Monitoring method not described.	Tabacova et al. (1985)
1,880	1.0	7 h/day, 5 days/wk, 21 days	Rat	No alterations in spermatogenesis, germina, or interstitial testicular cells. No effect on vitamin B_{12}.	Kripke and Sherwin (1984)
2,360	1.3	12 h/day, 3 mo	Rat	No effect on fertility. Decreased litter size and weight. No teratogenic effects.	Shalamberidze and Tsereteli (1971)
190 1,880 9,400 18,800	0.1 1.0 5.0 10	6 h	Mouse	Immediately and 1- to 2-wk postexposure, no increase in chromatid- or chromosome-type alterations in leukocytes or primary spermatocytes. No mutagenic effects.	Gooch et al. (1977)
18,800	10	Continuous, from pregnancy to 3 mo after delivery	Rat	Decreased litter size and mortality of neonates up to 15 days post-delivery. No teratogenic effects noted.	Freeman et al. (1974b)

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in spermatogenesis, germinal cells, or interstitial cells of the testes in 6 rats (Kripke and Sherwin, 1984). Additionally, the level of vitamin B_{12}, a coenzyme in folate metabolism that is used for DNA synthesis, was not affected by NO_2 exposure. Similarly, breeding studies by Shalamberidze and Tsereteli (1971) found that long-term NO_2 exposure had no effect on fertility. However, there was a statistically significant decrease in litter size and neonatal weight when male and female rats exposed to 2,360 $\mu g/m^3$ (1.3 ppm) NO_2, 12 h/day for 3 months were bred. In utero death due to NO_2 exposure resulted in smaller litter sizes, but no direct teratogenic effects were observed in the offspring. In fact, after several weeks, NO_2-exposed litters approached weights similar to controls.

In the only study that has examined post-natal 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 $\mu g/m^3$ (0.03, 0.05, 0.5, or 5.3 ppm) NO_2 for 6 h/day, 7 days/week throughout gestation and the offspring were studied for 2 months postexposure. Significant effects were detected in the offspring of dams exposed to 1,000 and 10,000 $\mu g/m^3$ NO_2. 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 NO_2 effects on the central nervous system and behavior (Section 13.2.4.6). The method of monitoring was not reported.

Balabaeva and Tabacova (1985) exposed pregnant and nonpregnant albino rats to 1,000 or 10,000 $\mu g/m^3$ (0.5 or 5.3 ppm) NO_2, 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, while the opposite was true in pregnant rats. Even more surprising was a four-fold increase in lipid peroxidation in the placenta of 10,000 $\mu g/m^3$ NO_2-exposed rats compared to unexposed pregnant controls. The authors then examined the offspring of the pregnant rats. The 1-month-old F_1 nonpregnant rats, exposed to air or the same concentrations of NO_2, showed similar changes as was observed in their mothers. However, pregnant F_1 rats, exposed to 1,000 or 10,000 $\mu g/m^3$, 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 F_1 rats could be due to decreased blood glutathione (no measurements presented) and that such levels of lipid peroxides could be fetotoxic. However, the method of monitoring NO_2 and lipid peroxides was 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 $\mu g/m^3$ (0.1, 1.0, 5.0, and 10.0 ppm) NO_2 for 6 h did not increase either chromatid- or chromosome-aberrations in the leukocytes of C3H 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, in these experiments the authors concluded that NO_2 exposure did not induce mutagenesis.

13.2.4.8 Potential Carcinogenic or Co-Carcinogenic Effects

No direct evidence indicates that tumors may be produced by NO_2 exposure alone. Several studies have evaluated the issue of carcinogenesis and co-carcinogenesis, 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 has recently been published (Witschi, 1988). Table 13-24 summarizes the available information on the carcinogenic or co-carcinogenic potential of NO_2.

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 co-carcinogenic effect of NO_2. However, the relatively frequent reports of hyperplasia, as discussed in the section on morphological effects, did not report 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 $\mu g/m^3$ (0.5 to 0.8 ppm) 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 INH, urethane, and 4-nitroquinoline-1-oxide. However, no adenomas were detected.

Rejthar and Rejthar (1975) exposed rats to 9,400 $\mu g/m^3$ (5 ppm) NO_2 continuously for periods of 3, 5, 7, 9, and 11 weeks. After 3 weeks of exposure, the bronchioles contained

TABLE 13-24. EFFECTS OF NO_2 ON CARCINOGENESIS OR CO-CARCINOGENESIS

NO_2 Concentration		Exposure	Species	Effects*	Reference
$\mu g/m^3$	ppm				
190-18,800	0.1-10	0.5-4 h	Mouse	Mice exposed to DMA had whole-body concentration-related increase in DMN.	Iqbal et al. (1981)
720 1,504	0.4 0.8	8 h/day, 5 days/wk, 10-12 wk	Mouse	Increased lung tumors in mice injected with melanoma cells after NO_2 exposure.	Richters and Kuraitis (1981)
940-1,504	0.5-0.8	Continuous, 30 days	Mouse	Hyperplastic foci identical to that observed after exposure to known carcinogens.	Nakajima et al. (1972)
1,504	0.8	8 h/day, 5 days/wk, 18 wk	Mouse	Enhanced retrovirus expression in two strains of mice.	Roy-Burman et al. (1982)
2,000 3,000	1.1 1.6	Continuous, lifetime	Rat	DMA plus NO_2 did not produce tumors. Nitroso-DMA, DMA, and NO_2 produced excess tumors.	Benemanskii et al. (1981)
9,400	5.0	Continuous, up to 11 wk	Rat	Hyperplastic foci at 3 wk. Decreased ciliated cells. Extensive hyperplasia, cuboidal metaplasia by 5 wk. Decreased bronchiolar lumen and polymorphous epithelium by 7 wk. Increased ciliated cells and decreased epithelial layers at 9 wk. By 11 wk return to 1 layer epithelium.	Rejthar and Rejthar (1975)
9,400-18,800	5.0-10	2 h/day, 5 days/wk, 50 wk	Mouse	Mice given 4-nitroquinoline-1-oxide during NO_2 exposure; NO_2 had no effect on tumor production.	Ide and Otsu (1973)

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TABLE 13-24 (cont'd). EFFECTS OF NO_2 ON CARCINOGENESIS OR CO-CARCINOGENESIS

NO_2 Concentration					
$\mu g/m^3$	ppm	Exposure	Species	Effects*	Reference
1,800 9,400 18,800	1 5 10	6 h/day, 5 days/wk, 6 wk	Mouse	No effect at 1 or 5 ppm. At 10 ppm, spontaneous adenomas in strain A/J mice increased only when compared to pooled control group.	Adkins et al. (1986)
18,800	10	2 h/day, 5 days/wk, 50 wk	Mouse	Mice given 4-nitroquinoline-1-oxide and NO_2. NO_2 decreased incidence of lung tumors.	Otsu and Ide (1975)
28,200-94,00	15-50	1-4 h	Mouse	Mice gavaged with morpholine had concentration-dependent increase in whole-body content of NMOR.	Iqbal et al. (1980)
31,020- 38,540	16.5-20.5	5-6 h/day, 4 days; plus 3 h on 5th day	Mouse	In vivo production of N-nitrosomorpholine when 1 g/mkg of morpholine was administered each day prior to exposure.	Van Stee et al. (1983)

*DMA = Dimethylamine.
NMOR = N-nitrosomorpholine.
DMN = Dimethylnitrosamine.

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uniform cuboidal one-layer epithelium composed of nonciliated cells. The cells showed vacuolization, and hyperplastic foci appeared in the bronchiolar epithelium. The foci were 2- to 4- 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 1,504 $\mu g/m^3$ (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, and 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 NO_2 Plus Known Carcinogens

Ide and Otsu (1973) showed tumor production in conventional mice receiving 5 weekly injections of 0.25 mg of 4-nitroquinoline-1-oxide (4NQO, a lung tumor-specific carcinogen) during exposure from birth to somewhere between 9,400 and 18,800 $\mu g/m^3$ (5 and 10 ppm) NO_2 for 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 NO_2 (6 of 13). Mice exposed to NO_2 alone had a similar number of tumors as the air controls. Thus, NO_2 did not facilitate the production of tumors.

One of the goals of a study by Benemanskii et al. (1981) was to evaluate the potential of NO_2 to influence the production of tumors during co-exposure 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/m^3 DMA + 2,000 $\mu g/m^3$ (1.1 ppm) NO_2. This suggested that NO_2 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/m^3), an excess of tumors, especially in males, was observed when DMA (0.05 mg/m^3) plus NO_2 (3,000 $\mu g/m^3$; 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 NO_2 were not reported, making the study difficult to evaluate.

Facilitation of Metastases

Richters and Kuraitis (1981) performed two experiments in which mice were exposed to either 720 or 1,504 $\mu g/m^3$ (0.4 or 0.8 ppm) NO_2 for 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 they 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 point to count tumors. The results indicated an increased number of tumors in the NO_2 group compared to filtered chamber and room air control groups. The authors concluded that NO_2 might facilitate the production of tumors; again, 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 $\mu g/m^3$ (0.3 or 0.4 ppm) NO_2 for 12 weeks (Richters and Kuraitis, 1983). However, at similar concentrations 940 $\mu g/m^3$ (0.5 ppm) for 8 weeks (Richters and Kuraitis, 1983) and 752 $\mu g/m^3$ (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 $\mu g/m^3$ (0.4 ppm) for 12 weeks lead to increased mortality in the mice. However, their post-hoc analysis of the data precludes this conclusion. Furthermore, the actual experimental design used in these studies probably did not evaluate metastases formation, as the term is generally understood,

but more correctly, colonization of the lung by tumor cells. Studies in a true tumor metastases model such as the Lobin Wistar rat, should be performed.

Recently, an abstract by Weinbaum et al. (1987) indicated that NO_2 could inhibit metastases formation if exposure occurred after injection of the the B16F10 tumor cell suspension. Thus suggests caution for the interpretation of NO_2 as a facilitator because NO_2 could inhibit metastases as well as facilitate their colonization or cause no change.

Studies in Animals With Spontaneously High Tumor Rates

A study by Wagner et al. (1965) suggested that NO_2 may accelerate the production of tumors in CAF_1/Jax mice (a strain that is genetically susceptible to pulmonary tumors) after continuous exposure to 9,400 $\mu g/m^3$ (5 ppm) NO_2. At the 12-month 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-month 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 $\mu g/m^3$ (10 ppm) NO_2 for 6 h/day, 5 days/week for 6 months (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 $\mu g/m^3$ (1 and 5 ppm) NO_2 did not increase the number of spontaneous adenomas in this in vivo short-term model for predicting carcinogenicity.

Production of N-Nitroso Compounds

Because of evidence that NO_2 could produce NO_2^- and NO_3^- in the blood, and NO_2^- is known to react with amines to produce animal carcinogens (nitrosamines), the possibility that NO_2 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 between the amount of N-nitrosomorpholine (NMOR) 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 $\mu g/m^3$ (15 to 50 ppm) NO_2. Thus, since NMOR (a nitrosamine) is an animal carcinogen, these studies are sometimes used to suggest that NO_2 exposure could theoretically react with amines in the body to produce tumors.

An extension of the previous study was performed (Iqbal et al., 1981) using DMA, an amine that is slowly nitrosated to dimethylnitrosamine (DMN). A concentration-related increase in biosynthesis of DMN was found to occur at concentrations as low as 190 $\mu g/m^3$ (0.1 ppm) NO_2; however, the rate was significantly greater at concentrations above 18,800 $\mu g/m^3$ (10 ppm) NO_2. 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. The Iqbal et al. method, which involved homogenization of the whole frozen mouse, did not use an adequate stopping solution to prevent in vitro production of nitrosamines. Mirvish et al. (1981) could verify the results of these researchers 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 NO_2 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 nitrosating agent (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 NO_2 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 NO_2 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) since 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 $\mu g/m^3$ (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 $\mu g/m^3$ (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 mice gavaged with 1 gm of morpholine/kg of body weight/day and then exposed 5 to 6 h for 5 days to 31,020 to 38,540 $\mu g/m^3$ (16.5 to 20.5 ppm) NO_2 revealed that NMOR could be produced in vivo. The single site containing the greatest amount of NMOR was in the gastrointestinal tract. Upon replicating this study in hamsters and rats at 18,800 $\mu g/m^3$ (10 ppm) NO_2 for 1 or 2 years, respectively, during chronic ingestion to various doses of morpholine in the drinking water, no excess incidence of cancer was found (Adkins, personal communication). Regardless of whether in vivo nitrosation can occur, the relative significance of NO_2^- from NO_2 compared to NO_2^- resulting from food, tobacco, and NO_3^--reducing oral bacteria is questionable (Murdia et al., 1982).

Aside from nitrosamines, recent 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 upon 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 epithelial layers 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 $\mu g/m^3$ (25 ppm) and dogs at 9,400 $\mu g/m^3$ (5 ppm) for up to 18 months. However, a decline in body weight in guinea pigs exposed continuously to 1,880 $\mu g/m^3$ (1 ppm) for 6 months, in rabbits exposed to 2,400 $\mu g/m^3$ (1.3 ppm), and in rats exposed to 24,000 $\mu g/m^3$ (12.5 ppm) NO_2 for 213 days have been reported. Newborn mice appear to be more sensitive to NO_2 exposure than adult mice but, based on limited data, juvenile rats appear to be less sensitive to the effects of NO_2 exposure than young adult rats.

Nitrogen dioxide-induced changes in blood constituents may result from the direct effect of NO_2, formation of NO_3^- and NO_2^-, or secondary effects emanating from other organs such as the lung, liver, hear, kidneys, and spleen. No effect on hematocrit and hemoglobin have been reported in squirrel monkeys exposed to 1,880 $\mu g/m^3$ (1 ppm) NO_2 for 493 days and in dogs exposed to up to 9,400 $\mu g/m^3$ (5 ppm) for 18 months. There was, however, polycythemia and an increase ratio of polymorphonuclear leukocytes to lymphocytes found in rats exposed to $3,760 \pm 1,880$ $\mu g/m^3$ (2 ± 1 ppm) NO_2 for 21 months. There have also been reported changes in RBC membranes of experimental animals following NO_2 exposure. Red blood cell D-2, 3-diphosphoglycerate was reportedly increased in guinea pigs exposed to 677 $\mu g/m^3$ (0.36 ppm) NO_2 for 1 week. An increase in RBC sialic acid, indicative of a younger population of RBC, was reported in rats exposed to 7,520 $\mu g/m^3$ (4 ppm) continuously for 1 to 10 days, but in another study, exposure to the same concentration of NO_2 produced a decrease in RBC.

Only limited information is available on the effect of NO_2 on the heart. However, cardiac performance may be affected by lung edema and changes in hemoglobin. A significant reduction in PaO_2 has been reported in rats exposed to 7,520 $\mu g/m^3$ (4 ppm) for 3 months. No affect on PaO_2 was reported in rats exposed to 752 $\mu g/m^3$ (0.4 ppm) over the same exposure period.

Decreases in serum proteins and lipoproteins and increases in serum globulins, indicating NO_2-induced hepatic damage have also been reported. Nitrogen dioxide increased pentobarbital-induced sleeping times in female mice after a 3 h exposure to 470 $\mu g/m^3$ (0.25 ppm), suggesting effects on hepatic xenobiotic metabolism. The effects only occurred

at specified time periods after exposure ended and did not exceed 1 day. Similar effects (increased hexobarital-induced sleeping time) were reported in the progeny of maternally exposed rats on postexposure Day 7 and 21 but not on Day 14 after being exposed to 1,000 $\mu g/m^3$ (0.5 ppm) NO_2. Decreases in cytochrome P-450 from liver microsomes have been found in rat liver homogenates after 7 days of exposure to 752 $\mu g/m^3$ (0.4 ppm) but not after exposure to 2,256 $\mu g/m^3$ (1.2 ppm) NO_2.

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 is fraught with methodological and interpretive problems. The evidence suggests that further study may be warranted.

13.3 EFFECTS OF MIXTURES CONTAINING NO_2

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 NO_2 involved exposures to only two pollutants, in which case 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 NO_2 (or NO_x) may have varied between exposure groups, or NO_2 was present in one group and not in another, so its relative influence could be assessed. This section discusses only those studies where the role of NO_2 (or NO_x) can be elucidated. In addition, a discussion of many of these studies addressing the effect of NO_2 alone on various organs and systems appears elsewhere in this chapter.

Table 13-25 outlines those studies in which experimental animals were exposed to constant levels of an atmosphere containing NO_2 with only one other material (binary mixtures). By far, the largest data base is for NO_2 plus O_3. Examination of these studies indicates that various degrees of interaction may occur. The morphologic response to an NO_2/O_3 mixture, as reported by Freeman et al. (1974b), was primarily that due to O_3 alone,

TABLE 13-25. TOXICOLOGIC INTERACTIONS TO SIMPLE MIXTURES CONTAINING NO_2

Pollutant Concentration ($\mu g/m^3$; ppm)	Exposure	Species	End Points	Response to Mixture	Interaction	Remarks	Reference
NO_2 (710-20,900 $\mu g/m^3$ [4.1-11.1 ppm]) + O_3 (11,000-23,300 $\mu g/m^3$ [5.6-11.9 ppm])	4 h	Rat	Mortality; dyspnea	Increase	Synergistic	Interaction due to production of nitrogen pentoxide.	Diggle and Gage (1955)
NO_2 (4,700 $\mu g/m^3$ [2.5 ppm]) + O_3 (490 $\mu g/m^3$ [0.25 ppm])	6 mo	Rat	Morphology	Hypertrophy of alveolar duct epithelium	None: lesion due primarily to O_3		Freeman et al. (1974a)
NO_2 (1,690 $\mu g/m^3$; 0.9 ppm) + O_3 (1,760 $\mu g/m^3$ [0.9 ppm])	60 days	Rat		Emphysema	None: lesion due primarily to O_3		
NO_2 (9,020 $\mu g/m^3$ [4.8 ppm]) + O_3 (880 $\mu g/m^3$ [0.45 ppm])	8 h/day, 7 days	Mouse	Lung weight rate of O_2 consumption (in lung homogenate), sulfhydryl metabolism in lung, activity of NADP reducing enzymes	Increase	Synergistic	No significant difference seen between effects of NO_2 and O_3; each alone produced no change.	Mustafa et al. (1984)
			Lung DNA content, lung protein content	No change	None	No effect with mixture or either alone.	
NO_2 (13,160 and 28,200 $\mu g/m^3$ [7 and 15 ppm]) + O_3 (980 and 1,960 $\mu g/m^3$ [0.5 and 1 ppm])	3 h/day, 7 days	Mouse	Level of reduced glutathione in lung	Increase	Additive		Watanabe et al. (1980)
NO_2 (5,640 $\mu g/m^3$ [3.0 ppm]) + O_3 (588 $\mu g/m^3$ [0.3 ppm])	2 h	Rabbit	Effects on arachidonic acid metabolites	Increase	Synergistic	Increases in PGE_2 and $PGE_{2\alpha}$.	Schlesinger et al. (1990)

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TABLE 13-25 (cont'd). TOXICOLOGIC INTERACTIONS TO SIMPLE MIXTURES CONTAINING NO_2

Pollutant Concentration ($\mu g/m^3$; ppm)	Exposure	Species	End Points	Response to Mixture	Interaction	Remarks	Reference
NO_2 (3,760-18,700 $\mu g/m^3$ [2-10 ppm]) + O_3 (980 and 1,960 $\mu g/m^3$ [0.5 and 1.0 ppm])	1-2 h	Mouse	Creatinine phosphokinase in plasma	Increase or decrease, depending on concentration	None: Effect due to O_3		Veninga et al. (1982)
NO_2 (2,820-9,400 $\mu g/m^3$ [1.5-5 ppm]) + 0_3 (100-980 $\mu g/m^3$ [0.05-0.5 ppm])	3 h	Mouse	Bacterial infectivity	Decreased survivial time with 0.5 ppm O_3 and any NO_2; and with 0.1 ppm O_3 and 3.5 ppm NO_2	Additive	Bacterial challenge after exposure.	Ehrlich et al. (1977)
NO_2 (3,760 $\mu g/m^3$ [2 ppm]) + O_3 (100 $\mu g/m^3$ [0.05 ppm])	3 h/day, 5 days/wk, 1-4 wk	Mouse	Bacterial infectivity	Excess mortality at all times	Synergistic	No effect with either alone, except for NO_2 at 2 wk.	Ehrlich et al. (1977)
NO_2 (2,820 $\mu g/m^3$ [1.5 ppm]) + O_3 (200 $\mu g/m^3$ [0.1 ppm])	4 h	Mouse	Bacterial infectivity	Decrease in bactericidal activity when $NO_2 > 4$ ppm + $O_3 > 0.36$ ppm		Bacterial challenge after exposure.	Goldstein et al. (1974)
NO_2 (2,800-7,860 $\mu g/m^3$ [1.49-4.18 ppm]) + O_3 (220-530 $\mu g/m^3$ [0.11-0.27 ppm])	17 h	Mouse	Bacterial infectivity	Decrease in bactericidal activity when $NO_2 \geq 1.8$ ppm + $O_3 \geq 0.2$ ppm	Additive	Bacterial challenge before exposure.	

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TABLE 13-25 (cont'd). TOXICOLOGIC INTERACTIONS TO SIMPLE MIXTURES CONTAINING NO_2

Pollutant Concentration ($\mu g/m^3$; ppm)	Exposure	Species	End Points	Response to Mixture	Interaction	Remarks	Reference
NO_2 (940 $\mu g/m^3$; 0.5 ppm with 1,880 $\mu g/m_3$; 1.0 ppm spike) + O_3 (98 $\mu g/m^3$; 0.05 ppm baseline with 196 $\mu g/m^3$; 0.1 ppm spike)	15 days baseline; twice daily 1-h spke	Mouse	Bacterial infectivity	Increased infectivity	Synergism		Graham et al. (1987)
NO_2 (94 $\mu g/m^3$; 0.05 ppm baseline with 188 $\mu g/m^3$; 0.1 ppm spike) + O_3 (98 $\mu g/m^3$; 0.05 ppm baseline with 196 $\mu g/m^3$; 0.1 ppm spike)					None		
NO_2 (3,760 $\mu g/m^3$; 2 ppm) + SO_2 (5,240 $\mu g/m^3$; 2 ppm)	Continuous, up to 13 wk	Rat	Morphology	No change	None	No effect of either SO_2 or NO_2.	Azoulay et al. (1980)
			Blood variables: oxyHb dissociation curve; red cell count; metHb; enzymes	No change	None		
NO_2 (8,000-11,000 $\mu g/m^3$; 4.2-5.8 ppm) + SO_2; (9,000-11,000 $\mu g/m^3$; 3.4-4.2 ppm)	24 h/day, 6 days/wk, 6 mo	Guinea pig	Respiratory mechanics (frequency, flow rate, min. vol.)	No change	None	No effect of either SO_2 or NO_2.	Antweiler and Brockhaus (1976)
NO_2 (3,760 $\mu g/m^3$; 2 ppm) + NaCl (330 $\mu g/m_3$)	Continuous, 14 mo	Monkey	Morphology	Respiratory bronchiolar epithelial hypertrophy	None: effect due to NO_2		Furiosi et al. (1973)

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TABLE 13-25 (cont'd). TOXICOLOGIC INTERACTIONS TO SIMPLE MIXTURES CONTAINING NO_2

Pollutant Concentration ($\mu g/m^3$; ppm)	Exposure	Species	End Points	Response to Mixture	Interaction	Remarks	Reference
NO_2 (3,760 $\mu g/m^3$; 2 ppm) + NaCl (330 $\mu g/m^3$)	Continuous, 18 mo	Monkey	Hematology	Polycythemia	None: effect due to NO_2		Furiosi et al. (1973)
NO_2 (9,400 $\mu g/m^3$; 2 ppm) + NaCl (330 $\mu g/m_3$)	Continuous, 6 mo	Rat	Hematology	Polycythemia	None: effect due to NO_2		
NO_2 (9,400 $\mu g/m^3$; 5 ppm) + NaCl (1,000 $\mu g/m^3$; 0.4 μm)	7 days	Rat	Rate of collagen synthesis by lung minces	Increase	Synergism		Last and Warren (1987)
NO_2 (9,400 $\mu g/m^3$; 5 ppm) + NaCl (1,000 $\mu g/m^3$; 0.4 μm)	1, 3 days	Rat	Protein content of lung lavage fluid	Increase at 1 and 3 days	Synergism at 3 days		
NO_2 (47,000-56,400 $\mu g/m^3$; 25-30 ppm) + carbon	6 h/day, 5 days/wk, 3 mo	Mouse	Morphology	Focal parenchymal lesions	Carbon acted as carrier for localized NO_2 deposition		Boren (1964)
NO_2 (9,400-47,000 $\mu g/m^3$; 5-25 ppm) + $(NH_4)_2SO_4$ (5,000 $\mu g/m^3$; 0.8-1 μm MMAD)	23.5 h/day, 7 days	Rat	Rate of collagen synthesis by lung minces	Increase	Synergism		Last et al. (1983)
NO_2 (3,760 and 9,400 $\mu g/m^3$; 2 and 5 ppm) + H_2SO_4 (890 $\mu g/m^3$; 0.4 μm MMAD)	23.5 h/day, 7 days	Rat	Rate of collagen synthesis by lung minces	Increase	Synergism		Last and Warren (1987) Last (1988)

TABLE 13-25 (cont'd). TOXICOLOGIC INTERACTIONS TO SIMPLE MIXTURES CONTAINING NO_2

Pollutant Concentration ($\mu g/m^3$; ppm)	Exposure	Species	End Points	Response to Mixture	Interaction	Remarks	Reference
NO_2 (9,400 $\mu g/m^3$; 5 ppm) + H_2SO_4 (500 $\mu g/m^3$; 0.4 μm MMAD)	2 h/day, 5 days/wk, 14 days	Rabbit	Particle clearance from respiratory region	Decrease	None at 0.3 ppm: response due to H_2SO_4; at 1 ppm: response different from both NO_2 and H_2SO_4		Schlesinger and Gearhart (1987)
NO_2 (560 and 1,880 $\mu g/m^3$; 0.3 and 1 ppm) + H_2SO_4 (500 $\mu g/m^3$; 0.3 μm MMAD)	2 h/day, 5 days/wk, 14 days	Rabbit	Alveolar macrophage function and numbers	Variable, depending on NO_2 concentration and end point	Additive, synergistic: depending on NO_2 concentration and end point		Schlesinger (1987)

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although in this study the level of NO_2 used would have produced only small changes that would easily be obscured by the more potent O_3. Acute lethality and some biochemical responses to NO_2/O_3 mixtures involve synergism (Diggle and Gage, 1955; Mustafa et al., 1984); some have ascribed this interaction to the production of new reaction products in the exposure atmosphere.

In terms of host antimicrobial defense (discussed in Section 13.2.3.1), toxicologic interactions involving NO_2 and O_3 are generally additive after acute exposures. It seems that each pollutant contributes to the observed response when its concentration reaches the threshold at which it would have affected bacterial resistance when administered alone (Goldstein et al., 1974). The subchronic exposure conducted by Ehrlich et al. (1977) did provide some evidence of synergism with 3,760 $\mu g/m^3$ (2.0 ppm) NO_2 and 97.5 $\mu g/m^3$ (0.05 ppm) O_3. Graham et al. (1987) exposed mice to various combinations and found synergism using bacterial infectivity as the end point.

Sagai et al. (1987) exposed mice, hamsters, guinea pigs, and rats to a mixture of 750 $\mu g/m^3$ (0.4 ppm) NO_2 and 780 $\mu g/m^3$ (0.4 ppm) O_3, 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 there to be 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 the most resistant.

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 10,340 $\mu g/m^3$ (5.5 ppm) and 2,120 $\mu g/m^3$ (1.1 ppm), respectively. The activity of phospholipase A_2 in the mitochondrial fraction was increased in those animals exposed to O_3-only, or to O_3 after NO_2, for 14 days; however, the response in the latter was significantly greater than that in the former. A decrease in activity

of lysolecithin acyltransferase in the supernatant fraction was found after 14 days only in those animals exposed sequentially to both NO_2 and O_3. In a second study, rats were exposed for 14 or 30 consecutive days to 10,150 $\mu g/m^3$ (5.4 ppm) NO_2 followed by 1,960 $\mu g/m^3$ (1.0 ppm) O_3. Pulmonary mechanics performed on the postmortem lung indicated a change in pulmonary resistance in the O_3- and sequential NO_2/O_3-exposed animals. 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 bronchi, not found with either NO_2 or O_3 alone, was seen in the animals exposed to both pollutants. In addition, damage at the bronchioloalveolar junction appeared to be somewhat more marked in animals exposed to both gases than in those exposed to O_3 alone. These studies suggest that sequential exposures produced responses which were, in most cases, not greatly different from those due to O_3 alone (i.e., NO_2 preexposure did not markedly alter the response to subsequent exposure to O_3).

Aside from sequential exposures, simulation of actual mixed pollutant exposure scenarios involving NO_2 and O_3 have also been performed by examining the effects, on bacterial resistance, of a continuous baseline exposure to one concentration, with superimposed short-term peaks to a higher level. Ehrlich et al. (1979) exposed mice for 1 to 6 months (24 h/day, 7 days/week) to a baseline concentration of 0 (air) or 190 $\mu g/m^3$ (0.1 ppm) NO_2, upon which was superimposed 3 h/day, 5 days/week peak exposures of 940 $\mu g/m^3$ (0.5 ppm) NO_2, or a combination of 940 $\mu g/m^3$ NO_2 and 200 $\mu g/m^3$ O_3; 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 months in all groups, with no evidence that exposure to the NO_2/O_3 peaks altered the response; there was also no change in survival time. In another experiment, mice were re-exposed for 14 days (after 1 to 3 months of initial pollutant exposure and bacterial challenge) to the same pollutant concentrations as above, and mortality was examined during this time. Animals pre-exposed for a least 2 months either to NO_2/O_3 peaks over the air baseline, or to NO_2/O_3 peaks over the 190 $\mu g/m^3$ NO_2 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. (1973) also examined the effect of the same baseline and peak exposures (for 1 to 3 months) on AMs. Cell viability was decreased after 3 months of exposure only when the NO_2/O_3 peaks were superimposed on continuous exposure to clean air. There was also a general increase in blood enzyme activity, but continuous exposure to 190 $\mu g/m^3$ (0.1 ppm) NO_2 with superimposed peaks of NO_2 and O_3 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 380 $\mu g/m^3$ (0.2 ppm) NO_2, with two daily peaks given 5 days/week as follows: 1,880 $\mu g/m^3$ (1.0 ppm) NO_2 for 1 h in the morning, and a mixture of 200 $\mu g/m^3$ (0.1 ppm) O_3 plus 1,880 $\mu g/m^3$ NO_2 given for 1 h in the afternoon. Exposures lasted for 9 months, followed by bacterial challenge. Other groups were exposed, continuously, to 380 $\mu g/m^3$ NO_2 with either no peak, or to 1,880 $\mu g/m^3$ given for 1 h in both the morning and afternoon. The only group that showed a significant increase in mortality was that exposed for 9 months to 380 $\mu g/m^3$ NO_2 with daily peaks of NO_2 in the morning, and NO_2 and O_3 in the afternoon. In addition, only this group showed a change (increase) in cellular ATP levels in AMs. By 8 months 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 NO_2/O_3 mixture with two daily 1-h peaks of the mixture, as follows: (1) high exposure level: 2,760 $\mu g/m^3$ (1.2 ppm) NO_2 plus 200 $\mu g/m^3$ (0.1 ppm) O_3 baseline; 4,700 $\mu g/m^3$ (2.5 ppm) NO_2 plus 590 $\mu g/m^3$ (0.3 ppm) O_3 peak; (2) intermediate exposure level: 940 $\mu g/m^3$ (0.5 ppm) NO_2 plus 100 $\mu g/m^3$ (0.05 ppm) O_3 baseline; 1,880 $\mu g/m^3$ (1.0 ppm) NO_2 plus 200 $\mu g/m^3$ (0.1 ppm) O_3 peak; or (3) low exposure level: 90 $\mu g/m^3$ (0.05 ppm) NO_2 plus 100 $\mu g/m^3$ (0.05 ppm) O_3 baseline; 190 $\mu g/m^3$ (0.1 ppm) NO_2 plus 200 $\mu g/m^3$ (0.1 ppm) O_3 peak. Animals were also exposed to the same baseline levels of either NO_2 or O_3 onto which were superimposed twice daily, 1-h peaks of either NO_2 or O_3 at concentrations as 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, while NO_2-alone

increased mortality and O_3 had no effect. At the high exposure level, the combined exposure was again synergistic, even though exposure to each given separately also increased mortality.

Some limited data exist for combinations of NO_2 with gases other than O_3 (Table 13-25). In the two reported studies with SO_2, neither SO_2 nor NO_2 given alone, or together, produced any response. The concentrations used by Azoulay et al. (1980) were quite low, while the respiratory mechanical end points assessed by Antweiler and Brockhaus (1976) were likely not very sensitive to pollutant-induced change.

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 months to carbon (38% of particles were <2 μm; 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, while no lesions were found due to carbon-only exposure. Thus, Boren 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.

Other aerosols, while not necessarily acting as carriers, may potentiate response to NO_2 by producing local changes in the lungs that enhance the toxic action of co-inhaled NO_2. Last et al. (1983) and Last and Warren (1987) have examined the effects of inhalation of acidic sulfate aerosols plus NO_2 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 $\mu g/m^3$ (5 to 25 ppm) NO_2 alone, or in combination with 5,000 $\mu g/m^3$ $(NH_4)_2SO_4$ (1 μm, 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 NO_2 indicated there to be an approximate doubling of synthesis rate when the mixture was employed compared to NO_2 alone; examination of responses at individual NO_2 concentrations showed that the mixture clearly began to increase

synthesis rate (above NO_2 alone) when NO_2 was $>18{,}800$ $\mu g/m^3$ (10 ppm). The investigators also noted that there was a tendency towards a reduction in lethal concentration for 75% of the animals (LC_{75}) when exposures were to $(NH_4)_2SO_4$ plus NO_2, compared to that for NO_2 alone. On the other hand, there was no difference in the level of pulmonary edema between animals exposed to NO_2 alone, or NO_2 in combination with $(NH_4)_2SO_4$. In a later study, Last and Warren (1987) exposed rats to 9,400 $\mu g/m^3$ (5 ppm) NO_2 alone, or in combination with either 1,000 $\mu g/m^3$ H_2SO_4 (0.4 μm) or NaCl (0.4 μm), for up to 7 days. A synergistic interaction for collagen synthesis rate was found when either aerosol was used with NO_2. Reduction of the NO_2 level to 3,760 $\mu g/m^3$ (2 ppm) also resulted in a synergistic increase in collagen synthesis rate when combined with 1,000 $\mu g/m^3$ H_2SO_4 (Last, 1989). Changes in protein content of the lavage fluid (an index of lung edema) showed evidence of synergism at 1 day with H_2SO_4, or 3 days with NaCl. The investigators suggested that the interaction with NaCl was due to the formation of acids (e.g., HCl, HNO_2, HNO_3) from nitrosyl chloride (NOCl) following its hydrolysis after deposition in the deep lung; the latter may be formed from a chemical reaction between NO_2 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 NO_2 and H_2SO_4 on lung clearance mechanisms have been examined by Schlesinger and Gearhart (1987) and Schlesinger (1987). In the former study, rabbits were exposed for 2 h/day for 14 days to either 560 $\mu g/m^3$ (0.3 ppm) or 1,880 $\mu g/m^3$ (1.0 ppm) NO_2, or 500 $\mu g/m^3$ H_2SO_4 (0.3 μm) alone, or to mixtures of the low and high NO_2 concentrations with 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 NO_2 accelerated clearance, while H_2SO_4 retarded clearance, compared to air-exposed controls. Exposure to the combination of 560 $\mu g/m^3$ NO_2 plus H_2SO_4 resulted in a response that was not different from that due to the acid alone. However, exposure to 1,800 $\mu g/m^3$ NO_2 plus H_2SO_4 resulted in a clearance pattern that differed from that of both NO_2 and H_2SO_4, but was more similar to that of the latter.

Schlesinger (1987) exposed rabbits to the same NO_2/H_2SO_4 atmospheres as above, but then sacrificed animals 24 h after 2, 6, or 13 exposures and recovered cells from the lungs by bronchopulmonary lavage. Exposure to 1,800 $\mu g/m^3$ (1 ppm) NO_2 with acid resulted in an increase in PMNs at all time points (not seen with either pollutant alone), and an increase in phagocytic capacity of AMs after two or six exposures. In contrast, exposure to 560 $\mu g/m^3$ (0.3 ppm) NO_2 with acid resulted in depressed phagocytic capacity and mobility. A comparison of responses due to exposure to the NO_2/H_2SO_4 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.

Furiosi et al. (1973) exposed rats and monkeys continuously to a combination of 3,760 $\mu g/m^3$ (2 ppm) NO_2 and 330 $\mu g/m^3$ NaCl. Histological response after 14 months of exposure in monkeys (respiratory bronchiolar epithelial hypertrophy) was similar in groups exposed to NO_2 alone, or with NaCl. Hematologic changes (polycythemia) in both monkeys, after 18 months, and rats, after 6 months, were similar for groups exposed to NO_2 with or without NaCl. Thus, in this study, the NaCl did not potentiate response to NO_2. Perhaps the end points were not sensitive to the effects of any reaction products between NO_2 and NaCl, or the concentration of NaCl was too low to allow production of significant amounts of such products.

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 $P388D_1$ cells exposed in vitro to the 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 μg/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 rats following adsorption of 0.36 μg NO_2/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, while dust was present in the lungs in the Shevchenko study for months, allowing a greater time for gas desorption.

Although many studies have examined the response to NO_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_2. But, as mentioned, in many cases, the exact role played by NO_2 in the observed responses is not always clear.

Kleinman et al. (1985a,b) exposed rats for 4 h to atmospheres consisting of various combinations of O_3 (1,180 $\mu g/m^3$ [0.6 ppm]), NO_2 (4,700 $\mu g/m^3$ [2.5 ppm]), SO_2 (13,100 $\mu g/m^3$ [5.0 ppm]), and particles. The particles consisted of 1 mg/m^3 (0.2 μm) of either H_2SO_4 or $(NH_4)_2SO_4$, 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 nitric acid (HNO_3) in atmospheres that contained O_3 and NO_2, and NO_3^- in those that contained O_3 and $(NH_4)_2SO_4$, but not NO_2. Nevertheless, a significant enhancement of tissue damage was produced by exposure to atmospheres containing H_2SO_4 or HNO_3, compared to those containing $(NH_4)_2SO_4$. In addition, there was a suggestion 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 (Kleinman et al., 1980).

One of the more common complex mixtures studied is that of automobile exhaust. In many cases, the exhaust is irradiated to produce a reactive mixture that is a model for photochemical smog. Coffin and Bloomer (1967) exposed mice for 4 h to irradiated automobile exhaust to assess effects on bacterial resistance. Levels of NO_x in the atmosphere were as follows: NO_2, 200 to 1,600 $\mu g/m^3$ (0.1 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_2 were less than those that were known to alter resistance when NO_2 was given alone and, thus, they suggested that the effect of the exhaust mixture was due to other oxidants, such as, O_3.

Stupfel et al. (1973) exposed rats for 6 h/day, 5 days/week for 2.5 months to 2 years to exhaust mixtures for morphologic analysis. The atmosphere contained carbon dioxide (CO_2), aldehydes, carbon monoxide (CO), and either 0.2 or 23 ppm NO_x. Only the mixture with the higher NO_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 auto exhaust atmospheres that differed in their component concentrations; all contained H_2SO_4, SO_2, and CO, as well as NO (8,700 to 13,300 $\mu g/m^3$ [7.1 to 10.8 ppm]) and NO_2 (560 to 9,600 $\mu g/m^3$ [0.3 to 5.1 ppm]). All exposures resulted in a significant depression of spontaneous locomotor activity not seen with exposure to either H_2SO_4 or CO alone; the investigators concluded that this response was due to either the hydrocarbon or NO_x components of the mixture.

The results of a long-term exposure of dogs to automobile exhaust have been described by several investigators (Stara et al., 1980). Animals were exposed for 68 months (16 h/day) to various atmospheres, which included raw auto exhaust, irradiated auto exhaust, or two mixtures of NO_x—one with a high NO_2 level and low NO level (1,210 $\mu g/m^3$ [0.64 ppm] NO_2, 310 $\mu g/m^3$ [0.25 ppm] NO), and one with low NO_2 and high NO (270 $\mu g/m^3$ [0.14 ppm] NO_2, 2,050 $\mu g/m^3$ [1.67 ppm] NO). 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; Block et al., 1973). Only results related to NO_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 months of exposure. However, by 36 months, a significant number of animals exposed to high NO_2/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 months of exposure; in the dogs breathing low NO_2/high NO or raw auto exhaust, residual volume was increased compared to animals exposed to control or high NO_2/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 NO_2/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 alveolo-capillary membrane. Block et al. (1973) reported no significant change in hematcrit, blood viscosity, or level of metHb due to any of the exposure atmospheres after 48 months 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 NO_2/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 auto exhaust or high NO_2/low NO, there was a rise in lung prolylhydroxylase, an enzyme involved in collagen synthesis. In addition, a correlation was found between lung weight and hydroxyproline content in animals exposed to the NO_x atmospheres.

Lung morphology of the dogs was evaluated by Hyde et al. (1978) 32 to 36 months after 68 months of exposure. In the high NO_2/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 NO_2 and high NO groups. In the high NO_2, but not the high NO group, there was cilia loss and hyperplasia of nonciliated bronchiolar cells. In the high NO group, there were lesions in the interalveolar pores. In the most severely affected dogs in the high NO_2 group, morphological changes considered to be analogous to centrilobular emphysema were present. Since these morphologic measurements were made after a 2.5 to 3 years 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.

Summary

Exposures to mixtures containing NO_2 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 NO_2 and O_3. Morphologic response to exposure to this mixture is generally due to O_3, but biochemical effects may involve synergism. Reactions of host defenses, specifically antibacterial activity, may be additive or synergistic. Mixtures of NO_2 and acid sulfates result in additive to synergistic effects. Although many studies examined response to simple mixtures of NO_2 with one other material, the atmosphere in most environments is a complex mix of more than two pollutants. Although effects of complex mixtures has been examined to some extent, the exact role played by NO_2 in the observed responses is not always clear.

13.4 NITRIC OXIDE

The toxicologic data base for nitric oxide (NO) is not extensive, except for its interaction with blood. One problem is that it is often difficult to obtain pure NO in air without some contamination with NO_2. Little is actually known about NO absorption in the respiratory tract, and nothing on its subsequent intrapulmonary distribution. Since 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 absorption 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 in through the nose and out through a tracheostomy tube; this compared to 90% removal for NO_2. Thus, respiratory tract absorption of NO is quite similar to that for 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 indicate that NO has a faster rate of diffusion through tissue than does NO_2 (Chiodi and Mohler, 1985).

High exposure levels of NO are apparently needed to be lethal. Pflesser (1935) reported that mice exposed to 380,400 $\mu g/m^3$ (310 ppm) NO for 8 h showed no mortality, while 50% mortality was seen during similar exposures to 392,600 $\mu g/m^3$ (320 ppm); however, possible NO_2 contamination was not accounted for. Greenbaum et al. (1967) reported that dogs exposed to 2% NO (24,540 mg/m^3 [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 $\mu g/m^3$ (10 ppm) NO for 6.5 months (Oda et al., 1976), or to 2,940 $\mu g/m^3$ (2.4 ppm) NO for their lifetime (23 to 29 months) (Oda et al., 1980a).

The few studies which have examined histologic response to nonlethal levels of NO are outlined in Table 13-26. With chronic exposure, the morphologic changes seen are similar to those with NO_2 (see section on morphological effects), except that 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 NO_2 (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 $\mu g/m^3$ (10.0 ppm) NO may be due to the presence of 1,880 to 2,820 $\mu g/m^3$ (1.0 to 1.5 ppm) NO_2 in the exposure atmosphere.

Data concerning physiological effects of inhaled NO are sparse. Murphy (1964) found no change in respiratory function of guinea pigs exposed for 4 h to NO at 19,600 $\mu g/m^3$ (16 ppm) or 61,300 $\mu g/m^3$ (50 ppm). Yoshida et al. (1980b) reported that guinea pigs exposed twice per week (30 min each) for 7 weeks to 5,898 $\mu g/m^3$ (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 those in guinea pigs exposed to 9,400 $\mu g/m^3$ (5.0 ppm) NO_2.

TABLE 13-26. EFFECT OF NO ON RESPIRATORY TRACT MORPHOLOGY

NO Concentration		Exposure	Species	Effects	References
$\mu g/m^3$	ppm				
2,460	2 (NO_2 = 0.08 ppm)[a]	Continuous, 6 wk	Rat	Slight emphysema-like alterations of alveoli.	Azoulay et al. (1977)
2,950	2.4 (NO_2 = 0.01-0.04 ppm)	Continuous, for lifetime (23-29 mo)	Mouse	No difference from control.	Oda et al. (1980b)
6,150	5 (NO_2 = $\leq$0.1 ppm)	Continuous, 14 days	Rabbit	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.	Hugod (1979)
12,300	10	2 h/day, 5 days/wk, up to 30 wk	Mouse	Enlarged air spaces in lung periphery; paraseptal emphysema; some hemorrhage; some congestion in alveolar septa; increased concentration of goblet cells in bronchi.	Holt et al. (1979)
12,300	10 (NO_2 = 1-1.5 ppm)	Continuous, 6.5 mo	Mouse	Bronchiolar epithelial hyperplasia; hyperemia; congestion; enlargement of alveolar septum; increase in ratio of lung to body weight.	Oda et al. (1976)

[a]This represents reported NO_2 levels measured during exposure.

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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 for 2 h/day, 5 days/week up to 30 weeks. Leukocytosis (i.e., an increased number of leukocytes) was evident by 5 weeks of exposure, while 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 sheep RBCs was seen at 10 weeks, but this was not evident at the end of the exposure series. Spleen cell phytohemagglutinin response (a measure of the incorporation of tritrated in T lymphocyte cultures) was decreased after 15 weeks of exposure, but levels then recovered and became greater than control. The ability of spleen cells to mount a graft vs. host reaction was stimulated by 20 weeks of exposure, but 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. A discussion of the effect of NO_2 on immunological end points appears in the section on host defense mechanisms.

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 ppm) NO for 6 h to 4 weeks, to assess the effect on resistance to infection induced by a bacterial aerosol administered after each NO exposure. Although there appeared to be somewhat of an increase in mortality in each group of females exposed to NO for at least 1 week compared to control (bacterial inoculation only), 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 effect 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 levels of reduced GSH, a co-factor for GSH peroxidase, in their lungs (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-GMP from GTP. 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. It is, however, not known whether NO would alter guanylate cyclase activity with in vivo exposure; this would suggest possible enzyme regulation by an environmental agent.

The bulk of the toxicologic data base for NO biochemistry concerns its reaction with hemoglobin (Hb). Inhaled NO that enters the bloodstream through the lungs binds to hemoglobin, producing nitrosylhemoglobin (NOHb) (Oda et al., 1975, 1980a, 1980b; Case et al., 1979; Nakajima et al., 1980). This may, in fact, be its major route of action, and studies in vitro have suggested that NO may severely reduce the ability of RBCs to carry O_2. These studies have shown that the affinity of Hb 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 measurement of O_2-dissociation curves for partially NO-liganded human Hb have shown that NO binding tends to reduce dissociation of bound O_2 on the molecule (Kon et al., 1977). Finally, NOHb is easily and rapidly oxidized to methemoglobin (MetHb) 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], 1 h) in mice and blood content of NOHb; however, levels of MetHb were found to increase exponentially with NO concentration, resulting in greater blood levels of MetHb than NOHb (Oda et al., 1980b). After exposure of mice to 49,100 $\mu g/m^3$ (40 ppm) for 1 h, concentrations of both MetHb and NOHb decreased rapidly, with half-times of only a few minutes (Oda et al., 1980b). Thus,

the steady-state concentration of NOHb during NO exposure would be fairly low, while that for MetHb would be somewhat higher (Maeda et al., 1987).

Studies of animals exposed to NO in vivo have shown that the amount of NOHb in blood was much less than would be expected from in vitro exposure data (Oda et al., 1980a, 1975), and that any reduction of O_2 transport was not lethal. Lifetime (23 to 29 months) exposures of mice to 2,940 $\mu g/m^3$ (2.4 ppm) NO resulted in the blood content of NOHb remaining relatively steady at 0.01%, while the maximum amount of MetHb was 0.3% (Oda et al., 1980b). Mice exposed to 12,300 $\mu g/m^3$ (10.0 ppm) NO for 6.5 months showed NOHb at 0.13% and MetHb at 0.2% (Oda et al., 1976). Thus, although the results of various studies have shown that the final product of NO reaction with hemoglobin is MetHb, with some persistent NOHb, 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 NOHb into MetHb, and the subsequent reduction of MetHb into ferrous Hb by MetHb reductase, an enzyme present in RBCs (Kon et al., 1980; Maeda et al., 1984b, 1987). As long as the activity of MetHb reductase is maintained, the conversion of NOHb to MetHb should mitigate any potentially toxic effect on Hb 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 10.0 ppm) NO_2, and after examination of organs sensitive to O_2 depletion (e.g., brain and heart) found no evidence of hypoxic damage.

Azoulay et al. (1977) exposed rats to 2,450 $\mu g/m^3$ (2 ppm) NO continuously for 6 weeks to examine various hematologic parameters, including blood-O_2 affinity. No exposure-related changes were found in Hb content, hematocrit, RBC count, red cell glucose metabolism, or in the oxyhemoglobin dissociation curve. In addition, no MetHb 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 investigation reported that in vitro studies had shown that blood-O_2 transport was altered by high levels of NO ($>$12,300 $\mu g/m^3$ [10 ppm]) in both human and rat blood.

In addition to interaction with Hb, exposure to NO may alter other aspects of blood. Case et al. (1979) exposed mice to 11,070 $\mu g/m^3$ (9 ppm) NO for 16 h and found a decrease in the level of iron transferrin, suggesting a reduction in the activity of this enzyme. Mice exposed to 12,300 $\mu g/m^3$ (10 ppm) NO for 6.5 months showed increased white blood cell

counts and an increase in the ratio of polymorphonuclear leukocytes to lymphocytes (Oda et al., 1976). These investigators noted that 11% of the RBCs obtained from NO-exposed mice contained Heinz bodies, while 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 $\mu g/m^3$ (2.4 ppm) NO for their lifetime (Oda et al., 1980a). In vitro, rat RBCs exposed to NO showed oxidative cross-linking between cell membrane proteins and hemoglobin (Maeda et al., 1984a), an alteration which could change the cells' rheological properties. However, in an in vivo exposure study (30.7 to 245.4 mg/m^3 [25 to 200 ppm] for 1 h) of rats, no cross-linking of membrane proteins was detected (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., 0.5-2.0% NO [5,000 to 20,000 ppm]) (Toothill, 1967; Greenbaum et al., 1967). Rats exposed to 2,450 $\mu g/m^3$ (2 ppm) NO continuously for 6 weeks showed no change in blood pH (Azoulay et al., 1977).

A few studies have examined the response to inhalation of mixtures of NO plus one other component. Watanabe et al. (1980) exposed mice for 3 h/day, for 7 days to NO (12,300 $\mu g/m^3$ [10 ppm]) plus O_3 (1,960 $\mu g/m^3$ [1 ppm]); they observed an increase in the level of lung GSH, but this was due solely to the O_3. Azoulay et al. (1980) exposed rats to NO (2,460 $\mu g/m^3$ [2 ppm]) with SO_2 (5,240 $\mu g/m^3$ [2 ppm]) for 13 weeks; no change in blood-O_2 affinity, MetHb level, RBC count or lung histology was seen with the mixture, or with either pollutant given above. Finally, 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 $P388D_1$ cells was found, compared to dust without adsorbed NO.

McFaul and McGrath (1985) examined the effect of inhalation exposure to NO (at levels of 18.4 to 78.6 mg/m^3 [15 to 64 ppm] for up to 38 h) on the reduction of MetHb produced initially in the blood of rats by injection of sodium nitrate. They found the MetHb 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 $\mu g/m^3$ (2 ppm) or greater, are needed for morphologic changes in the lungs following chronic inhalation. Inhaled NO that is absorbed into the bloodstream results in production of NOHb which, in turn, is oxidized to MetHb. This has the potential to reduce the ability of RBCs to transport O_2. But as with other effects, high exposure levels are needed for significant changes.

13.5 NITRIC ACID AND NITRATES

13.5.1 Nitric Acid

There are only a few toxicologic studies of nitric acid (HNO_3) 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 HNO_3 vapor at 63,000 $\mu g/m^3$ (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 HNO_3 (usually 1%), a procedure used in developing models of bronchiolitis obliterans in various animals, namely 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% HNO_3 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 in phase III of the single breath nitrogen washout curve. Histologically, there was widespread chronic inflammation of conducting airways, especially medium and small ones, peribronchiolar fibrosis, focal hemorrhage, edema, and hyperplasia of goblet cells in the trachea and bronchi.

Gardiner and Schanker (1976) examined the effect of HNO_3-induced damage on drug absorption from the lungs of rats. Instillation of 1% HNO_3 produced bronchiolitis and

alveolitis, and also 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.

The only study which was designed specifically to examine the pulmonary response to pure HNO_3 vapor was that of Abraham et al. (1982), who 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 $\mu g/m^3$ (1.6 ppm) HNO_3 vapor. The exposure, which was performed using a "head-only" chamber, resulted in a decrease in specific pulmonary flow resistance, compared to pre-exposure 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 HNO_3 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 HNO_3 exposure; according to the investigators, this suggested that some individuals in the normal population may be more sensitive than others.

13.5.2 Nitrates

The toxicologic data base for inhaled NO_3^- is quite sparse. Sackner et al. (1976) exposed anesthetized dogs to sodium nitrate (<1 μm diameter particles) at 740 or 4,000 $\mu g/m^3$ for 7.5 min. No effects on pulmonary function (i.e., static compliance, functional residual capacity, total respiratory resistance) were found. An injection of 100 mg $NaNO_3$ resulted in no change in pulmonary mechanics, arterial blood gases, cardiac output, or pulmonary blood pressure. Ehrlich (1979) examined the effect of NO_3^- on resistance to respiratory infection. Mice were exposed for 3 h to various NO_3^- salts at maximal concentrations as follows: lead nitrate ($Pb[NO_3]_2$), 2,000 $\mu g/m^3$; calcium nitrate ($Ca[NO_3]_2$), 2,800 $\mu g/m^3$; sodium nitrate ($NaNO_3$), 3,100 $\mu g/m^3$; potassium nitrate (KNO_3), 4,300 $\mu g/m^3$; ammonium nitrate (NH_4NO_3), 4,500 $\mu g/m^3$; and zinc nitrate ($Zn[NO_3]_2$), 1,250 $\mu g/m^3$. Following exposure, the animals were challenged with a bacterial aerosol, and mortality determined after 14 days. Only the $Zn(NO_3)_2$ exposure resulted in any significant mortality increase, the extent of which seemed to be exposure concentration related; the peak

exposure level increased mortality by ~20%. However, since the response was similar to that seen with zinc sulfate ($ZnSO_4$), the investigator ascribed the effect to the zinc ion (Zn^{+2}) rather than to the NO_3^-.

Busch et al. (1986) exposed rats and guinea pigs with either normal lungs or elastase-induced emphysema to NH_4NO_3 aerosols at 1 mg/m^3 for 6 h/day, 5 day/week for 4 weeks. Using both light and electron microscopy, the investigators concluded that there were no significant effects of exposure on lung structure due to nitrate exposure.

Charles and Menzel (1975) examined the effects of NO_3^- 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 20-200 mM NH_4NO_3. Histamine was released in proportion to the concentration of salt present. However, the response was not totally due to NO_3^-; NH_4^- was also a possible contributor.

Kunimoto et al. (1984b) studied the effects of NO_3^- upon components of rat RBC membranes. Cells were incubated with $NaNO_3$ for 1 h at levels up to 1 mM. A decrease in sialic acid content of the cell membrane was found at concentrations >0.1 mM, and a concentration dependent decrease in Ca^{2+}, Mg^{2+}-ATPase activity of the membrane, with little change in Na^+, K^+-ATPase activity, was noted. In vivo testing, injection of ≥50 mM of $NaNO_3$ also resulted in depressed Ca^{2+}, Mg^{2+}-ATPase activity. The investigators concluded that NO_3^- may affect RBCs by altering the transport of Ca^{2+} across the cell membrane.

Summary

Inhalation studies with nitric acid 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

A large number of experiments, designed to evaluate the health effects of NO_x (primarily NO_2) on various animal species, have been conducted; and NO_2 appears to be the most toxic. Many of the experiments were performed at very high concentrations of NO_2

(above 9,400 $\mu g/m^3$ [5.0 ppm] for days, weeks, or months) to ensure eliciting the effects being studied and thus provide only limited or no information directly applicable to standard setting. Therefore, this document focuses on studies conducted at less than 9,400 $\mu g/m^3$ (5.0 ppm). Even so, ambient levels are much lower. For example, except for the Los Angeles Basin, annual averages are less than the NAAQS of 100 $\mu g/m^3$ (0.053 ppm). How then are animal studies at levels considerably above ambient to be interpreted in terms of human health risk? Extrapolating animal data to humans has both qualitative and quantitative components. As has been discussed and will be summarized below, NO_2 causes a constellation of effects in several animal species, most notably, effects on host defenses against infectious pulmonary disease, lung metabolism/biochemistry, lung function, and lung structure. Because of basic physiological, metabolic, and structural similarities in all mammals (laboratory animals and humans), the commonality of the observations in several animal species leads to a reasonable conclusion that NO_2 could cause similar types of effects in humans. However, because of the differences between mammalian species, we do not know exactly what exposures would actually cause these effects in humans. That is the topic of quantitative extrapolation. Limited research on the dosimetric aspect (i.e., the dose to the target tissue/cell that actually causes toxicity) of quantitative extrapolation suggests that dose patterns within the respiratory tract of animals and humans are similar, without yet providing adequate values to use for extrapolation. Unfortunately, very little information is available on the other key aspect of extrapolation, species sensitivity (i.e., the response of the tissues of different species to a given dose). Thus, from currently available animal studies we know what human health effects NO_2 could cause, rather than what effects NO_2 actually causes. Such knowledge is still quite valuable because it can enable: (1) identification of potential hazards for humans that are either unmeasured or unmeasurable in humans; (2) estimation of the biological plausibility of effects observed in epidemiological studies, which, by their very nature, cannot provide definitive cause-effect relationships; (3) identification of mechanisms of effects that can enable enhanced interpretation of the potential severity of effects in human studies; and (4) generation of hypotheses for testing in human studies, thereby advancing the human data base.

With the above issues in mind, the animal toxicology data base for NO_2 will be summarized according to major classes of effects and topics of special interest. These

include: animal-to-human dosimetric estimates, biochemical and cellular mechanisms, effects on host defenses, effects of chronic exposure on the development of chronic lung disease, potential carcinogenic or co-carcinogenic effects, extrapulmonary effects, susceptibility of subpopulations, influence of exposure patterns, and interactions with other co-occurring pollutants. Several other topics or issues are not summarized here because of the paucity of the data or lack of current interpretability. For more information on these topics (e.g., mortality from NO_2 and effects of other NO_x compounds, such as NO and HNO_3), see the section summaries within this chapter.

13.6.1 Animal-to-Human Dosimetric Estimates

The number of experiments in which NO_2 uptake values is reported are few, two for the upper respiratory tract, one for the lower respiratory tract, and two for the total respiratory tract. The upper respiratory tract uptake efficiencies were 25 to 28% for rats and 45 to 85% for dogs, depending on the mode of breathing and ventilation. Thirty-six percent uptake was obtained in the lower respiratory tract of isolated ventilated rat lungs; however, because the low ventilation used is not representative of living rats, the measured uptake most probably is not representative. The two total respiratory tract uptake experiments involved normal humans and asthmatics and resulted in uptakes of 81 to 92% and 73 to 88%, respectively. In both experiments percent uptake increased as ventilation increased. Thus, the available data on in vivo uptake of NO_2 by various species indicate that a large percentage of inhaled gases is removed in the respiratory tract, depending on the species, mode of breathing, ventilation, and possibly just as important, the experimental procedures.

A comparison of the results from the one NO_2 dosimetry modeling study to morphological data that shows the centriacinar region to be most affected by NO_2 indicates qualitative agreement between predicted maximum tissue doses and observed effects in the pulmonary region. Comparisons in the tracheobronchial region, however, indicate that dose-effect correlations may be improved by considering other expressions of dose such as total absorption by an airway. Further research is needed to define toxic mechanisms, to refine our knowledge of important physical, chemical, and morphological parameters, to develop NO_2 dosimetry models using this information, and to perform dosimetry experiments with a variety of species.

13.6.2 Biochemical and Cellular Mechanisms

Acute exposure to NO_2 at concentrations at or below 9,400 $\mu g/m^3$ (5 ppm) can oxidize polyunsaturated 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). Such oxidation (free radical-mediated) reactions may well be the mechanisms by which NO_2 elicits direct toxicity on lung cells. Such a proposed mechanism of action suggests the importance of lung antioxidant defenses, both endogenous (e.g., maintenance of lung glutathione levels) and exogenous (e.g., dietary vitamin C and E), in identification of potential susceptible populations at risk of NO_2 inhalation. The direct cytotoxic effects of NO_2 on epithelial cell membranes may be the fundamental mechanism of edemagenesis in response to NO_2 exposure, while the direct cytotoxicity of NO_2 to membranes of alveolar macrophages could well be the mechanism underlying increased infectivity of bacteria and viruses in lungs of animals and humans exposed to NO_2. A large number of studies have suggested that various enzymes in the lung, including glutathione peroxidase, superoxide dismutase, and catalase, may also serve to defend the lung against oxidant attack. One may speculate that were there to be a threshold level for NO_2 toxicity to the lung, it would be that concentration of NO_2 that was able to overwhelm these endogenous defense systems of the lung.

13.6.3 Effects on Host Defenses

Although the primary function of the respiratory tract is to insure an efficient exchange of gases, this organ system must also provide the body with a first line protective barrier against inhaled viable and nonviable airborne agents. Thus, any breach in this defense system might increase the risk of disease in the host. During the past several years numerous studies that have contributed to our understanding of these various defense systems have been reported. From this literature it becomes readily evident that exposure to NO_2 can result in the dysfunction of these host defenses, increasing susceptibility to infectious respiratory disease. The typical host defense parameters affected by NO_2 include the rate of mucociliary clearance, functional and biochemical activity of alveolar macrophages, immunological competence, and susceptibility to experimentally induced respiratory infections.

The distribution of cellular injury from NO_2 extends throughout the lower respiratory tract. In the conducting airways, NO_2 reacts with the ciliated epithelium, causing a decrease in ciliary beating rates and, as a consequence, a significant reduction in mucus transport. The first of these effects begins to appear after several weeks of exposure to around 1,880 $\mu g/m^3$ (1 ppm) NO_2 and becomes clearly evident at 3,760 $\mu g/m^3$ (2 ppm). Shorter periods (several days) of exposure to levels as low as 3,760 $\mu g/m^3$ are also effective. Such impairment could render this physiological line of defense less effective in the process of lung clearance.

At the alveolar level the host defense system that receives direct exposure to the inhaled pollutant is the alveolar macrophage, which is responsible for maintaining sterility of the pulmonary region, clearing particles from this region, and participating in immunological functions. Morphological appearance of these defense cells changes after a 6-month exposure to as little as 940 $\mu g/m^3$ (0.5 ppm) with a 1-h spike to 3,760 $\mu g/m^3$ (ppm 2.0 ppm). Measurements of the functional integrity of macrophages isolated from the lungs of NO_2-exposed animals indicate that macrophages can be depressed to such a degree that the host can no longer effectively maintain pulmonary sterility. Functional changes that have been reported are: at 560 $\mu g/m^3$ (0.3 ppm), intermittent for 13 to 14 days, the suppression in phagocytic ability and stimulation of lung clearance; at 4,320 $\mu g/m^3$ (2.3 ppm) for 17 h, a decrease in bactericidal activity; and at 3,760 $\mu g/m^3$ (2.0 ppm) for 6 months, a decreased response to migration inhibition factor. Macrophages isolated from humans exposed for 3 h to 1,120 $\mu g/m^3$ (0.6 ppm) NO_2 were less able to inactivate influenza virus than controls.

The importance of these defenses in maintaining pulmonary sterility against invading microorganisms becomes evident when these "impaired" animals have to cope with a laboratory-induced pulmonary infection. Animals exposed to NO_2 succumb to the bacterial or viral infection in a concentration-response manner. Mortality also increases with increasing length of exposure to a given concentration of NO_2. After acute exposure, 3,760 $\mu g/m^3$ (2 ppm) is the lowest-observed-effect level. Exposure to concentrations as low as 940 $\mu g/m^3$ (0.5 ppm) will cause effects in the infectivity model after 6 months, but as the concentration increases, the effect becomes evident sooner and is more significant. Thus, evidence indicates that these cells are no longer capable of isolating, transporting, or detoxifying these deposited microbes due to their reduced level of phagocytosis, lytic

potential, ability to produce interferon, and other responses. Impacts of different exposure patterns on infectivity are described later (Section 13.6.8).

Since NO_2 has been shown to cause an increase in susceptibility to both bacterial and viral infections, a few investigators have examined the immune system to explain this change in susceptibility. In the cases in which lung humoral and cell-mediated immunity have been investigated, effects have been observed after acute exposure to concentrations >9,400 $\mu g/m^3$ (5 ppm). These findings illustrate the complexity of pulmonary immunotoxicity, since the direction of the effect (i.e., increase or decrease) was dependent upon NO_2 concentration and the length of exposure. Furthermore, systemic and pulmonary immune responses sometimes differed.

Subchronic exposure to NO_2 has also been found to affect the production of serum neutralizing antibody to viruses and humoral primary antibody responses at concentrations as low as 752 $\mu g/m^3$(0.4 ppm) and 940 $\mu g/m^3$ (0.5 ppm) with 1-h daily spikes to 3,760 $\mu g/m^3$ (2.0 ppm). Other significant immunological effects attributed to subchronic NO_2 exposure include alterations in serum immunoglobulin levels at 1,880 $\mu g/m^3$ (1.0 ppm).

Acute exposure to high concentrations of NO_2 significantly decreased the number of spleen and thymus cells; however, at a lower concentration, the number of spleen cells actually increased. Others have shown that the reduction in primary antibody response of spleen cells against sheep red blood cells was more influenced by a suppression of B cell function, while for the secondary antibody response, the T cells were more affected. Levels of NO_2 (intermittent exposure, 7 weeks) that affect the percentages of subpopulations of T and B cells have been as low as 470 $\mu g/m^3$ (0.25 ppm). Chronic intermittent exposure to high levels of NO_2 results in a biphasic response. Antibody titers to sheep red blood cells were increased after 10 weeks of exposure, unchanged at 20 weeks, and decreased at 30 weeks. There were no effects when a T-independent antigen was used. A similar biphasic response was observed for a graft versus host reaction. Response of lymphocytes to a T-cell mitogen (phytohemagglutin) was depressed at all exposure times tested.

13.6.4 Effects of Chronic Exposure on the Development of Chronic Lung Disease

Since humans can be exposed chronically to NO_2, such exposures in animals have been studied rather extensively, typically using morphologic and/or morphometric methods. Less focus has been on pulmonary function effects. Generally, this research has shown that a variety of structural and correlated functional alterations occur, many of which are reversible when exposure ceases; however, in some cases, emphysema has been observed.

Pulmonary function following NO_2 exposures in experimental animals showed consistent patterns among different treatment conditions and animal species. Exposures to diurnal spikes of NO_2 superimposed on a constant background level, simulating NO_2 patterns in the urban environment, produced a decrease in lung distensibility in both mice and rats. These changes were very subtle, occurring in mice after 1 year of exposure to very low concentrations, 380 $\mu g/m^3$ (0.2 ppm) NO_2 with spikes of 1,500 $\mu g/m^3$ (0.8 ppm) NO_2. The sensitivity of the lung to irritants was generally increased following NO_2 exposures, except when the irritant was higher concentrations of NO_2. Impaired gas exchange occurred following 3 months of exposure to 7,520 $\mu g/m^3$ (4.0 ppm) NO_2 and was reflected in decreased arterial O_2 tensions, impaired physical performance, and increased anaerobic metabolism. All these studies taken together demonstrate that NO_2 produces subtle to dramatic changes in pulmonary function depending on the concentration and duration of exposure. Lung distensibility and gas exchange are the parameters most consistently affected by exposure.

Although NO_2 produces morphological changes in the respiratory tract, the data base is sometimes confusing due to quantitative and qualitative variability in responsiveness between, and even within, species. Thus, for example, the rat appears to be relatively resistant to NO_2, 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 epithelial cells of the bronchioli and the Type 1 epithelial cells of the alveoli.

Acute exposures to concentrations of 9,400 $\mu g/m^3$ (5 ppm) or less generally produce minimal to no lesions in the rat; however, similar exposures in the guinea pig may result in

some epithelial damage. Higher exposure concentrations result in changes typified by hypertrophy and hyperplasia of bronchiolar and alveolar Type 1 epithelium and proliferation of Type 2 alveolar cells.

Longer-term exposures result in lesions in some species with concentrations as low as 560 to 940 $\mu g/m^3$ (0.3 to 0.5 ppm). These are characterized by epithelial damage similar to that described above but with the involvement of more proximal airways. Many of these changes, however, will resolve even with continued exposure, and long-term exposures to levels above about 3,760 $\mu g/m^3$ (2.0 ppm) are required for more extensive and permanent changes in the lungs. Some effects are relatively persistent, for example bronchiolitis; while others tend to be reversible and limiting even with continued exposure, for example, epithelial cell hyperplasia. In any case, it seems that for both acute or longer term exposure regimens, the response is more dependent upon concentration than exposure duration. Although ambient exposures are generally to a baseline level with superimposed spikes reaching some higher level, the contribution of these spikes to morphologic damage over that due to baseline exposure is not as yet certain.

There is very substantial evidence that long-term exposure of several species of laboratory animals to high concentrations of NO_2 results in morphologic lung lesions that meet the current NIH Workshop criteria for an animal model of emphysema. 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. While the lowest NO_2 concentration for the shortest exposure duration which will result in emphysematous lung lesions can not be determined from these published studies, the required NO_2 concentrations for such long-exposure durations are far greater than those currently reported in ambient air.

13.6.5 Potential Carcinogenic or Co-Carcinogenic Effects

Literature searches revealed no published reports on NO_2 studies using classical whole animal bioassays for carcinogenesis. Research with mice having spontaneously high tumor

rates was equivocal. Although several co-carcinogenesis investigations have been undertaken, methodological and interpretative problems prevent drawing adequate conclusions. Reports on whether NO_2 facilitates the metastases of tumors to the lung are also inadequate to form conclusions, since the most appropriate models and statistical methods were not used. Other investigations have centered on whether NO_2 could produce nitrates and nitrites that, by reacting with amines in the body, could produce animal carcinogens (nitrosamines). A few studies found that in animals treated with high doses of amines and exposed to NO_2, nitrosoamines were formed. However, in a related study in two species, a 1- or 2-year exposure to 18,800 $\mu g/m^3$ (10 ppm) did not cause an excess incidence of cancer. In summary, the evidence suggests that further study may be warranted.

13.6.6 Extrapulmonary Effects

Although it is clear that the effects of NO_2 exposure extend beyond the confines of the lung, the interpretation of these effects relative to potential human risk is not clear.

After acute exposure, there appears to be a transient rise in WBCs. Red blood cell count has been shown to increase and decrease, but changes in hematocrit and hemoglobin do not occur. Although NO_2^- and especially NO_3^- do appear in the blood, increased MetHb is not typically reported at near ambient levels of exposure. The evidence does suggest that there is a change in composition of the RBC membrane constituents; however, whether this indicates an increased younger population of erythrocytes or an apparent aging has not been resolved. Several of the traditional serum markers of tissue injury are elevated with acute near ambient exposure to concentrations as low as 470 $\mu g/m^3$ (0.25 ppm), but this effect is also transient. Chronic exposure (at least 3 months) seems to decrease serum proteins, lipoproteins, and plasma cholinesterase; all of which suggest hepatic damage. Although changes in the microsomal oxidative system are observed during acute exposure to concentrations as low as 470 $\mu g/m^3$ (0.25 ppm), little evidence of chronic hepatic damage has been reported. Swollen liver mitochondria, edema, and inflammatory parenchymal changes after chronic exposure to between 940 and 2,000 $\mu g/m^3$ (0.5 and 1.05 ppm) NO_2 have been reported in two studies. Studies of NO_2 effects on the kidney are too few to justify any conclusions.

Nitrogen dioxide can produce effects on the cardiovascular system, but these effects seem to occur only at levels 20 to 100 times ambient concentrations. The changes that have been observed in the central nervous system require further study before any kind of interpretation is possible. The behavioral changes seen, especially the changes in forced swim and running wheel performance, are reminiscent of the acute performance decrements seen in humans with ozone exposure.

13.6.7 Susceptibility of Subpopulations

Two subpopulations have been examined for susceptibility, young animals and animals with elastase-induced emphysema. Neither were more susceptible and in some studies were found to be more resistant than their appropriate NO_2-exposed controls.

Susceptibility to NO_2 exposures was not affected in animals treated intratracheally with elastase to produce a condition of experimental emphysema, but sensitivity was increased in mice with a genetic defect in connective-tissue metabolism that results in pulmonary emphysema. Newborn and mature animals are affected differently by NO_2 exposures, particularly rats exposed to continuous background NO_2 concentrations of 1,880 or 3,760 $\mu g/m^3$ (1 or 2 ppm) with diurnal spikes to three times the baseline. A 6-week exposure of the adult rats caused an increase in the thickness of the alveolar basement membrane and a greater increase in the volume of Type 2 cells, compared to the neonatally-exposed rats. Pulmonary function effects were correlated to these changes. Lung distensibility was increased transiently (after 3 but not 6 weeks of exposure) in rats exposed as newborns, but was decreased in rats exposed for 6 weeks as young adults. Hamsters exposed to high NO_2 concentrations as newborns had pulmonary function changes one year later indicative of mild pulmonary emphysema, but these changes were not found in hamsters exposed when older.

13.6.8 Influence of Exposure Patterns

Several animal toxicological studies have elucidated the relationships between concentration (C) and duration (T, time) of exposure, clearly indicating that the relationship is quite complex, rather than the simple formula, $C^1 \times T^1 = \text{Effect}$. Most of this research used the infectivity model in which air- and NO_2-exposed mice are exposed to bacteria, and

decreases in host defenses are measured as increases in mortality. Early C × T studies demonstrated that concentration had more impact on mortality than did exposure duration. More recent work evaluated ambient exposure patterns consisting of a continuous, low baseline of NO_2 on which are superimposed brief peaks of higher concentrations of NO_2. When components of this pattern were tested and compared to the entire exposure pattern, the decrease in host defenses was dependent upon the specific exposure pattern; that is, the outcome could not be predicted based upon knowledge of the components. Few studies of ambient patterns of NO_2 have been investigated for other classes of effects (e.g., pulmonary function, morphology); they generally support the conclusion that the pattern, rather than the simple product of C × T, is responsible for the results. Therefore, this body of work does not provide a mathematical model to relate various exposure patterns to specific effects. However, by illustrating the importance of concentration, duration, and exposure patterns, it does demonstrate the need for rather advanced exposure assessments for more precise estimates of health effects and the need to understand the C × T relationship far better to enable extrapolations of effects from one exposure scenario to another.

13.6.9 Interactions with Other Pollutants

Humans are exposed to complex pollutant mixtures, not NO_2 alone, making it important to understand the interaction of NO_2 with these other pollutants. Unfortunately, such studies are rare and most were conducted with binary mixtures containing O_3. For information on other interactions, see Section 13.3. Studies of O_3 plus NO_2, the focus of this summary, are especially pertinent because: (1) they both are photochemical oxidants, (2) NO_2, being a primary precursor of O_3, is temporally and spatially related to O_3, and (3) O_3 and NO_2 generally cause most of the same classes of effects, with O_3 being significantly more potent. Numerous studies of lung morphology, antioxidant metabolism, and host defenses against infection have found that the effects were primarily due to O_3 (typically in cases using low, noneffective levels of NO_2), were additive, or were synergistic, depending on the concentrations, exposure durations, exposure patterns, and end point examined. The findings of either additivity or synergism are of concern because of the ubiquitous, co-occurring nature of O_3 and NO_2 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 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, NO_2, O_3); there is some mixing between the peaks. Such a "real-world" pattern has not been tested under controlled conditions allowing estimations of the relative toxicological role of the compounds. Nevertheless, studies with NO_2 and NO_2-O_3 mixtures illustrate the importance of exposure patterns, so extrapolating the 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 oxides of nitrogen (NO_x) on human health. The major emphasis is on the effects of nitrogen dioxide (NO_2) because it is the NO_x compound measured in most epidemiological studies and because it is the NO_x compound currently of greatest concern from a public health perspective. Human health effects associated with exposure to NO_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 (1982), 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 text of the chapter are those studies which provide useful quantitative information on exposure-effect relationships for health effects associated with ambient air levels of NO_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 the health effects of NO_2. The studies discussed in the main text and additional studies, evaluated by the author but found to be of limited usefulness for present criteria development purposes, are summarized in Appendix 14A.

The chapter is organized as follows. First respiratory illness studies are discussed to include studies that meet criteria (see Section 14.5.3) for use in a quantitative analysis followed by studies that provide qualitative information. Next, studies are described that examine effects of ambient NO_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 (1982), a group of studies examining the relationship between respiratory illness and exposure in the home to gas combustion products (cooking fuel) were evaluated. At that time, those studies inferred the presence of NO_2 by the presence of gas combustion emission sources. Since then, new studies and updates of earlier ones have been conducted which provide data on NO_2 concentrations and estimates of exposure. Most studies of NO_2 exposure show more variation in indoor air than in outdoor air (see Chapter 7).

The studies assessed in this chapter are evaluated for several factors of importance for interpreting epidemiological studies of the health effects of NO_2. These factors are (1) measurement error in exposure, (2) misclassification of the health outcome, (3) selection bias, (4) adjustment for covariates, (5) publication bias, (6) internal consistency, and (7) plausibility of the effect based on other evidence. Because these factors are common to all studies, a brief discussion follows.

Measurement error in exposure is potentially one of the most important methodological problems in epidemiological studies of NO_2. 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. Instead, NO_2 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 use was related to health outcomes without any direct exposure estimates.

The effect of measurement error on estimation has been studied by several authors including Shy et al. (1978), Gladen and Rogan (1979), Stephanski and Carroll (1985), Walker and Blettner (1985), Fuller (1987), Lebret (1987), Schafer (1987), Whittemore and Keller (1988), Samet and Utell (1990), and Yoshimura (1990). In general, measurement error that is independent of the health outcome will result in estimated effects biased towards the null. 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 will result in an underestimate of the logistic regression coefficient by as much as 50%. 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 risks are not

extremely close to 0 or 1. The use of the presence of a gas stove as a surrogate for an actual NO_2 exposure introduces misclassification.

If the observed health effects (see Chapter 13 and Section 14.2) result from peaks generated during source use rather than long-term averages, then the use of estimated averages creates another source of measurement error. However, inadequate data are available to adequately evaluate the relative contributions of personal exposures of peak vs. average values to health effects studied in epidemiology studies. Peak levels in bedrooms and other locations probably are not as high as in kitchens (see Chapter 7), and most indoor activity probably occurs in locations other than the kitchens (see Chapter 8). Harlos et al. (1987) state that NO_2 concentrations in the kitchen are different for each cooking event in a 12- or 24-h period. To improve the measure of exposure, the NO_2 concentration during room occupancy is needed. The average bedroom NO_2 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.

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, illness, and infections are usually measured by a questionnaire. Obviously, questionnaire measurements that depend on recent recall are better than one 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. The effect of misclassification of the health outcome is an area in need of additional research.

The possibility of selection bias, although a concern of every study, seems very low for the epidemiologic studies of NO_2. Most studies are population-based with little selection by the individuals themselves. The selection of an area based on previous demonstration of an association of symptoms with gas stove use could potentially represent a serious flaw. However, we are unaware of the selection of any area for inclusion in the studies discussed based on existing health response data.

Most studies of respiratory disease and NO_2 exposures discussed here measured the important covariates of age, socioeconomic level of the parents, gender, and parental smoking habits. The estimated effect (regression coefficient of disease on NO_2 exposure) will be an overestimate when a missing covariate is either positively or negatively correlated with both the exposure variable and the health outcome. The estimated effect will be an underestimate in the other two situations. Ware et al. (1984) 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 to be left out of the analysis.

Publication bias 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.

Internal consistency is always a check on the validity of a study, but often the authors do not report sufficient detail to check for such consistency. For known risk factors for respiratory effects, a study should provide the anticipated associations (e.g., passive smoking with increased respiratory illness or with more wheeze in asthmatic children) and certain patterns of age or gender effects might be predicted. Results that differ dramatically by age or gender should be viewed with caution. If studies report results suggesting a significant beneficial effect of NO_2 exposure, then there may be a problem with the design or the analysis.

Finally, the health outcome should be an outcome for which there is a plausible basis to suspect that NO_2 exposure was associated with the effect. Two health outcome measures have been most extensively considered in the epidemiologic studies: 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). The human clinical and animal toxicological studies (see Chapters 13, 15 and Section 14.5.2) provide a data base demonstrating that NO_2 affects aspects of the respiratory host defense system—thus providing a biologically plausible basis for epidemiologic observations of associations between NO_2 exposure and an increase in respiratory symptoms and infections, especially in children.

Each study is subsequently reviewed with special attention given to the factors just discussed. Those studies that address these factors more appropriately provide a stronger basis for the conclusions that they draw. Consistency between studies indicates the level of the strength of the total data base.

14.2 STUDIES OF RESPIRATORY ILLNESS

Respiratory illness and the factors determining occurrence and severity are important public health concerns. This chapter discusses epidemiological findings relating NO_2 exposure to respiratory illness. This effect is of public health importance because of the potential for exposure to NO_2 and since childhood respiratory illness is common (Samet et al., 1983; Samet and Utell, 1990). This takes on added importance since 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).

A brief discussion of the epidemiology of lower respiratory illness (LRI) in children provides a background for studies examining NO_2 exposure in relation to LRI. 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) (see Section 14.5.1 for further discussion). In a study in Tucson during the first year of life the most common diagnosis was bronchiolitis, which accounts for 60% of all LRI identified (Wright et al., 1989). The

most common signs and symptoms associated with LRI were wet cough (85%), wheeze (77%), tachypnea (48%), fever (54%), and croupy cough (38%) (Wright et al., 1989). A few infectious agents are presumed to cause the majority of childhood LRI. Bacteria are not thought to be common causes of LRI in nonhospitalized infants in the United States (Wright et al., 1989). Seventy-five percent of the isolated microbes were one of four types: respiratory syncytial virus (RSV), para influenza virus type 1 and 3, and Mycoplasma pneumoniae (Glezen and Denny, 1973; McConnochie et al., 1988). Respiratory syncytial virus is particularly likely to cause LRI in 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). Denny et al. (1986) 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.

In summary, LRI 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 subsequential occurrence of respiratory symptoms. Special attention is directed at viral LRI in the first 2 years of life because the highest incidence and rate of hospitalization for LRI are found at this time and because of the risk of chronic sequelae from LRI in early childhood. The occurrence of LRI 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 impairment (Glezen, 1989; Gold et al., 1989). Britten et al. (1987) reported that the extension to age 36 of the earlier work of the Medical Research Council'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 to adult disease 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 NO_2, which increases the risk for LRI, are important because of the associated public health concern and the potential for increase in the development of chronic lung disease.

Various studies of LRI have reported rates based on visits to physicians ranging from about 20 to 30 illnesses per 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 LRI ranged from 24 per 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 12-14 years) to about 7.5 illnesses per 100 person-years. Several factors affect the rate of LRI in children, including: age, immunologic status, prior viral infections, level of health, socioeconomic status (Chanock et al., 1989), day care attendance, environmental tobacco smoke, NO_2 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 LRI is consistent in different geographic regions during the 2 decades covered by the studies, suggesting that diagnosis and infectious agents have changed little in that time period.

The biologic basis underlying increased susceptibility for respiratory illness reflects the status of functioning respiratory host defense mechanisms against the specific infectious organism (e.g., species, strain, virulence). The host defense system provides protection against inhaled infectious agents through physical and immunologic mechanisms. Additionally, 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 respiratory illness studies in this section are organized as follows. First, eleven studies that meet criteria (see Section 14.5.3) for use in a quantitative analysis are presented. Within these studies five related 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 four other quantitative studies are presented that were conducted by different authors in

different locations. The remainder of the section reviews several other studies that provide qualitative information concerning respiratory illness.

14.2.1 United Kingdom Studies

Results of five British studies have been reported by Melia et al. (1977; 1978; 1979; 1980; 1982; 1985), Goldstein et al. (1979; 1981), and Florey et al. (1979; 1982). Parts of these studies were reviewed previously (U.S. Environmental Protection Agency, 1982), but their importance requires a more complete discussion of them.

The initial study, reported by Melia 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 months. Colds going to the chest accounted for the majority of the symptoms reported. Information about cooking fuel (gas or electric), age, sex, and social class (manual vs. nonmanual labor) was obtained, but information on parental smoking was not. No measurements of NO_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.

A U.S. Environmental Protection Agency (EPA) reanalysis of the data in Table 14-1 using a multiple logistic model is given 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

TABLE 14-1. SYMPTOM RATES OF UNITED KINGDOM CHILDREN BY GENDER, SOCIAL CLASS, AND COOKING TYPE

	Social Classes I-III (Nonmanual)		Social Classes III-V (Manual)	
Age <8 years	Electric	Gas	Electric	Gas
Boys	25.6% (203)[a]	26.1% (88)	29.9% (375)	37.5% (309)
Girls	22.2% (171)	30.4% (112)	31.8% (393)	33.5% (337)
Age ≥8 years				
Boys	20.8% (365)	23.3% (189)	25.0% (675)	29.0% (654)
Girls	18.1% (303)	19.2% (187)	17.8% (674)	27.8% (623)

[a]Numbers in parentheses refer to number of subjects.

Source: Melia et al. (1977).

TABLE 14-2. U.S. ENVIRONMENTAL PROTECTION AGENCY MULTIPLE LOGISTIC ANALYSIS OF DATA FROM THE MELIA ET AL. (1977) STUDY

Factor[a]	Regression Coefficient	Standard Error	Likelihood Ratio Chi-Square	p-Value
SES and age by gender interactions (2 d.f.)			2.46	0.2922
Gas by gender interaction (1 d.f.)			0.72	0.3953
Gas cooker	0.2733	0.0616	19.78	<0.0001
Gender (female)	-0.1531	0.0612	6.29	0.0121
SES (Manual)	0.2730	0.0702	15.48	0.0001
Age (<8 years)	0.3864	0.0626	37.77	<0.0001

[a]SES = Socioeconomic status.
d.f. = Degrees of freedom.

(95% confidence limits of 1.16 to 1.48) and was statistically significant ($p < .0001$). The other main effects of gender, socioeconomic status, and age were all statistically significant. This reanalysis suggests that gas stove use in this study for children under age eight is associated with an estimated 31% increase in the odds of having respiratory illness symptoms.

Melia et al. (1979) report further results of the national survey covering two groups: (1) a new cohort of 4,827 boys and girls, aged 5 to 10 years, from 27 randomly selected areas that were examined in 1977, and (2) 2,408 children first examined in 1973 who were followed-up for at least 1 year and whose mothers reported the use of the same cooking fuel in each year the child was studied. 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 ($p \simeq 0.02$) 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 (1) none or (2) 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 ($p \simeq 0.01$ in boys, $p = 0.07$ in girls). The results of this analysis are presented in Table 14-3. The effect of gender, social class, use of pilot lights, and number of smokers in the house were examined.

An EPA reanalysis of the data in Table 14-3 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 to 1.42). This effect was statistically significant ($p < .0001$). The other main effects of gender, socioeconomic status, 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.

The longitudinal results seem to indicate that the relative risk may decline as the children grow older. The prevalence tended to be higher in 1973 than in 1977 for children of the same age, and the effect of gas cooking seemed to be smaller in 1977 than in 1973.

TABLE 14-3. UNADJUSTED RATES OF ONE OR MORE SYMPTOMS AMONG UNITED KINGDOM CHILDREN BY GENDER, SOCIAL CLASS, AND COOKING TYPE

	Social Classes I-III (Nonmanual)		Social Classes III-V (Manual)	
Age <8 years	Electric	Gas	Electric	Gas
Boys	27.4% (277)[a]	31.7% (145)	32.8% (485)	36.7% (313)
Girls	24.4% (291)	27.6% (134)	27.8% (497)	36.3% (336)
Age ≥8 years				
Boys	19.2% (286)	28.3% (113)	23.6% (501)	26.9% (338)
Girls	14.8% (243)	18.6% (118)	21.5% (437)	18.5% (313)

[a]Numbers in parentheses refer to number of subjects.

Source: Melia et al. (1979).

TABLE 14-4. U.S. ENVIRONMENTAL PROTECTION AGENCY MULTIPLE LOGISTIC ANALYSIS OF DATA FROM MELIA ET AL. (1979) STUDY

Factor[a]	Regression Coefficient	Standard Error	Likelihood Ratio Chi-Square	p-Value
SES and Age by gender interactions (2 d.f.)			1.11	.5749
Gas by gender interaction (1 d.f.)			.35	.5566
Gas cooker	.2183	.0674	10.43	.0012
Gender (female)	-.1970	.0664	8.81	.0030
SES (Manual)	.2225	.0764	8.60	.0034
Age (<8 years)	.5253	.0675	61.48	<.0001

[a]SES = Socioeconomic status.
d.f. = Degrees of freedom.

However, overall the study observed an association between respiratory illness and the use of gas for cooking in two separate groups of children, seen 4 years apart in this national study.

This study was followed by a study in 1978 of 808 schoolchildren (Melia et al., 1980), aged 6 to 7 years, in Middlesborough, 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 triethanlamine (TEA) 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 $\mu g/m^3$) with a mean of 0.112 ppm (211 $\mu g/m^3$), and levels in bedrooms ranged from 0.004 to 0.169 ppm (8 to 318 $\mu g/m^3$) with a mean of 0.031 ppm (56 $\mu g/m^3$). In homes with electric stoves, weekly levels of NO_2 in kitchens ranged from 0.006 to 0.188 ppm (11 to 353 $\mu g/m^3$) with a mean of 0.018 ppm (34 $\mu g/m^3$), and in bedrooms NO_2 levels ranged from 0.003 to 0.037 ppm (6 to 70 $\mu g/m^3$) with a mean of 0.014 ppm (26 $\mu g/m^3$). Outdoor levels of NO_2 were determined using diffusion tubes systematically located throughout the area, and the weekly average ranged from 26 to 45 $\mu g/m^3$ (0.014 to 0.024 ppm).

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 rates. Cooking fuel was found to be associated with respiratory illness, independent of social class, age, sex, 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.

Since no concentration-response estimates were given by the authors, a multiple logistic model was fitted by EPA to the data in Table 14-5 with 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

TABLE 14-5. UNADJUSTED RATES OF ONE OR MORE SYMPTOMS AMONG UNITED KINGDOM BOYS AND GIRLS BY BEDROOM LEVELS OF NO_2

	Bedroom Levels of NO_2 (ppm)			
	<0.020	0.020-0.039	>0.039	Total
Boys	43.5% (23)[a]	57.9% (19)	69.2% (13)	54.5% (55)
Girls	44.0% (25)	60.0% (15)	75.0% (8)	54.2% (48)
TOTAL	43.7% (48)	58.8% (34)	71.4% (21)	54.4% (103)

[a]Numbers in parentheses refer to number of subjects.

Source: Melia et al. (1980).

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 gave a chi-square of 4.94 with one degree of freedom, with a corresponding p-value of 0.03. This result suggests that an increase of 30 $\mu g/m^3$ (0.016 ppm) in bedroom NO_2 levels (well within the range of the data) would result in a 53% increase in the odds of respiratory illness. The 95% confidence limits for the odds ratio were 1.04 to 2.24.

The study was repeated in January to March of 1980 by Melia et al. (1982). 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 (Melia 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." An EPA reanalysis of the data in Table 14-6 was made using a multiple logistic model similar to the one used for the previous study (Melia 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

TABLE 14-6. UNADJUSTED RATES OF ONE OR MORE SYMPTOMS AMONG UNITED KINGDOM BOYS AND GIRLS BY BEDROOM LEVELS OF NO_2

	Bedroom Levels of NO_2 (ppm)			
	<0.020	0.020-0.039	>0.039	Total
Boys	56.4% (39)[a]	67.6% (37)	72.0% (25)	64.4% (101)
Girls	60.0% (25)	41.0% (39)	52.2% (23)	49.4% (87)

[a]Numbers in parentheses refer to number of subjects.

Source: Melia et al. (1982).

bedroom NO_2 data. The estimated logistic regression coefficient for NO_2 (in $\mu g/m^3$) was 0.004 with a standard error of 0.005. 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). This reanalysis suggests that an increase of 30 $\mu g/m^3$ (0.016 ppm) in bedroom NO_2 levels would result in a 11% increase in the odds of respiratory illness.

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 on colds going to the chest, and whether the child had experienced bronchitis, asthma, or pneumonia during the past 12 months. No relation 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 gave prevalence rates of children having at least one symptom by gas stove use and gender. The combined odds ratio for presence at symptoms by gas stove use was 0.63 with 95% confidence limits of 0.36 to 1.10.

14.2.2 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., 1989; Neas et al., 1990; Neas et al., 1991) have reported on a series of studies conducted in six different U.S. cities. The six cities were selected to represent a range of air quality based on their historic levels of outdoor pollution and included: Watertown, MA; Kingston and Harriman, TN; southeast St. Louis, MO; Steubenville, OH; Portage, WI; and Topeka, KS. Approximately 1,500 grade school children were enrolled in each community and were followed for several years. 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. Results of monitoring in Portage, WI, verify the fact that the presence of a gas stove contributes dramatically to the indoor NO_2 levels. Table 14-7 is taken from Quackenboss et al. (1986) based on data collected from 1981-82. These results clearly show the effect of a gas stove not only on the indoor concentrations but also on the personal exposure of the individual. The study was conducted very carefully with excellent quality control. It gives a good estimate of the exposure resulting from the use of gas stoves in the United States (see Chapter 7).

TABLE 14-7. NO_2 CONCENTRATIONS (ppm) BY SEASON AND STOVE TYPE IN PORTAGE, WI

		Indoor		Outdoor		Personal	
Season	Stove	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) reported results from the six cities studies based on 8,120 children, aged 6 to 10 years, who were followed from 1974-77. Health end points were measured by a standard respiratory questionnaire, which was completed by the parents of the children.

The authors used log-linear models to estimate the effect of current gas stoves vs. 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 to 1.26) for gas stove use. The results were adjusted for the presence of adult smokers, presence of air conditioning, and socioeconomic status of the family. Ware et al. (1984) reported results from the same six communities over a longer period (1974-79). 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, sex, city, and maternal smoking level gave estimated odds ratios for the effect of gas stoves ranging from 0.93 to 1.07 for bronchitis, cough, wheeze, LRI index, and illness for the last year. The index for LRI indicated the presence of either bronchitis, respiratory illness, or persistent cough during the past year. None of these 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 to 1.27 ($p=0.14$). Thus, the study suggests an increase in respiratory illness of about 11%, although the increase was not statistically significant at the 0.05 level. The end point in the Ware et al. study most similar to that of the Melia studies was the LRI index. The authors gave the unadjusted rates, and from those an estimated odds ratio of 1.08 with 95% confidence limits of 0.97 to 1.19 were calculated. Although this rate was not adjusted for other covariates, the effect of those adjustments on other end points was minimal.

Dockery et al. (1989) studied a different sample of 5,338 white children in the same six cities, but enrolled during the period 1983-86. The children were aged 7 to 11 years at the time of the study. The end points of chronic cough, bronchitis, chest illness, persistent wheeze, and nonrespiratory illness were not associated with gas stove use in the home. The health end point of doctor-diagnosed respiratory illness prior to age 2 was marginally significant. Multiple logistic regressions, including use of a gas stove for cooking, gave an odds ratio of 1.15 with 95% confidence limits of 0.96 to 1.37. The odds ratio was adjusted for age, sex, parental education, city of residence, and use of unvented kerosene heaters.

Neas et al. (1990, 1991) studied a cohort of 6,273 children from the same six cities. This cohort included children that were part of the Dockery et al. (1989) analysis, but was restricted to white children 7 to 11 years of age with complete covariate information and at least one valid indoor measurement of both NO_2 and respirable particles. This resulted in 1,286 children being included in the analysis. 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.

The analysis of the Neas et al. (1990, 1991) study was based on the third symptom questionnaire, which was completed by parents following the indoor measurements. The questionnaire reported symptoms during the previous year, including shortness of breath, chronic 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 17.3 ppb (31 $\mu g/m^3$) NO_2 exposure. This corresponded to the average effect of a gas stove with a pilot light, based on exposure information from the study. Table 14-8 shows the odds ratios for the five separate symptoms associated with the increase in NO_2 exposure.

TABLE 14-8. ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR THE EFFECT OF AN ADDITIONAL 17.3 ppb NO_2 ON THE SYMPTOM PREVALENCE

Symptom	Odds Ratio	95% Confidence Interval
Shortness of breath	1.27	0.92 to 1.73
Chronic wheeze	1.19	0.87 to 1.61
Chronic cough	1.21	0.86 to 1.71
Chronic phlegm	1.29	0.93 to 1.79
Bronchitis	1.05	0.71 to 1.56

Source: Neas et al. (1990).

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. The authors defined a combined symptom that was the presence of any of the symptoms just reported. A similar analysis of this combined lower respiratory symptom gave an estimated odds ratio of 1.47 with a 95% confidence interval of 1.17 to 1.86.

When split by gender, the odds ratio was higher in girls, and when split by smoking vs. nonsmoking homes, was higher in smoking homes. The odds ratio for the combined symptom score is slightly higher than the results of the other analyses, but is not inconsistent with those results.

14.2.3 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 14-9.

TABLE 14-9. REGRESSION COEFFICIENTS FOR MULTIPLE LOGISTIC ANALYSES OF RESPIRATORY ILLNESS IN TAYSIDE CHILDREN

Factor	Regres. Coeff.[a]	Odds Ratio	95% Confidence Limits
Parental smoking	0.429	1.54	
Age (in 5-year groups)	-0.094	(NA)[b]	
Presence of gas stove	0.130	1.14	(0.86 to 1.50)

[a]Regres. Coeff. = Regression coefficient.
[b]NA = Not available.

Source: Ogston et al. (1985).

Only the coefficient for parental smoking was statistically significant ($p<0.01$). The coefficient for gas stove use would have been significant only at the 0.14 level. The odds ratio of 1.14 suggests an increase in episodes of respiratory illness of about 14%. The study did not give measured NO_2 exposure values, but referenced the other Melia studies conducted elsewhere in the United Kingdom for exposure estimates.

14.2.4 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. Pulmonary function measurements were obtained from 89 children whose parents did not smoke and 94 children whose parents smoked. 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 a covariate in the analyses. The results of the analyses are presented in Table 14-10, and are based on 1,138 children. No measurements of NO_2 exposure, either inside or outside the homes, were reported. No differences in lung function were associated with gas stove use. The very small sample size used in the lung function study precludes any meaningful interpretation of the lung function results.

14.2.5 Dutch Studies

In the Netherlands, Houthuijs 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. Personal exposure to NO_2 and home concentrations were measured. An important NO_2 emission and exposure source in these homes are geysers, 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. Pulmonary function was measured at school. The study used Palmes diffusion

TABLE 14-10. ANALYSIS OF IOWA CITY SCHOOL CHILDREN RESPIRATORY SYMPTOMS BY GAS STOVE TYPE AND PARENTAL SMOKING

Factor	Hospitalization for Chest Illness before Age 2		Chest Congestion and Phlegm with Colds	
	Odds Ratio	SE[a]	Odds Ratio	SE[a]
Gas stove use	2.4[b]	0.684	1.1	0.188
Smoking effects				
Father alone smokes	2.3[b]	0.856	1.0	0.213
Mother alone smokes	2.9[b]	1.239	1.3	0.363
Both smoke	1.6	0.859	1.2	0.383

[a]SE = Standard error of the odds ratio.
[b]Indicates statistical significance at the 0.05 probability level.

Source: Ekwo et al. (1983).

tubes to measure a single weekly average personal NO_2 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 NO_2. Personal exposure was also estimated from time budgets and room monitoring. Estimated and measured exposures to NO_2 are given in Table 14-11.

TABLE 14-11. DUTCH STUDY ESTIMATED AND MEASURED PERSONAL NO_2 EXPOSURE ($\mu g/m^3$) FOR A SINGLE WEEKLY AVERAGE

NO_2 Source	Number	Estimated		Measured	
		Arith. Mean[a]	S.D.[b]	Arith. Mean	S.D.
No geyser	370	22	7	22	9
Vented geyser	112	29	9	31	12
Unvented geyser	111	40	9	42	11

[a]Arith. Mean = Arithmatic mean.
[b]S.D. = Standard deviation.

Source: Brunekreef et al. (1987).

Three measures of health were obtained from the questionnaire that 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 14-12. A logistic regression model was used to fit the combination variable. 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 30 $\mu g/m^3$ (0.016 ppm) in NO_2, with 95% confidence limits of 0.66 to 1.33. 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.

TABLE 14-12. FREQUENCY AND PREVALENCE OF REPORTED RESPIRATORY SYMPTOMS IN DIFFERENT CATEGORIES OF MEAN INDOOR NO_2 CONCENTRATIONS IN A POPULATION OF 775 DUTCH CHILDREN OF 6 TO 12 YEARS OLD

	Frequency and prevalence in category of indoor NO_2							
Symptom	0-20 $\mu g/m^3$ n=336		21-40 $\mu g/m^3$ n=267		41-60 $\mu g/m^3$ n=93		> 60 $\mu g/m^3$ n=79	
Cough	16	4.8%	12	4.5%	7	7.5%	3	3.8%
Wheeze	30	8.9%	18	6.7%	3	3.2%	7	8.9%
Asthma	22	6.6%	12	4.5%	2	2.2%	3	3.8%
One or more symptoms	36	10.7%	24	9.0%	8	8.6%	8	10.1%

Source: Dijkstra et al. (1990).

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 detect health effects may have been reduced by the smaller sample size of the measured NO_2

data compared to the categorical data (e.g., gas stove vs. electric stove use). They could not estimate whether they gained more precision by measured NO_2 than was lost by the reduction in the sample size.

14.2.6 Ohio Study

Keller et al. (1979a) and Mitchell et al. (1974) conducted a 12-month 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 one incident of illness per person per 2-week period was recorded.

The study measured NO_2 exposure, but it included both Jacobs-Hochheiser and continuous chemiluminescence methods. The electric stove users averaged 20 ppb (38 $\mu g/m^3$) NO_2 exposure, whereas the gas stove users averaged 50 ppb (94 $\mu g/m^3$). The paper does not report which rooms were measured in order to get this average. Thus, the estimated average difference between gas and electric stove use was 30 ppb (58 $\mu g/m^3$).

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 fathers 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 lower respiratory symptoms in homes with a gas stove was 53.2% (n=267) vs. 50.7% (n=286) in homes with electric stoves. Although the difference is not statistically significant, these rates give an estimated odds ratio of 1.10 with 95% confidence limits of 0.74 to 1.54. Neas (1990, personal communication) observed that Keller's model controls for a series of variables that specify the child's prior illness history and that if chronic exposure to NO_2 is a risk factor for prior illnesses, controlling for the child's illness history would substantially reduce the estimated effect of current NO_2 exposure.

14.2.7 Swiss Study

Braun-Fahrlaender et al. (1989) and Rutishauser et al. (1990a,b) studied the frequency of common airway symptoms in children up to 5-years old. The study was conducted for six periods in two rural and two urban areas of Switzerland. Nitrogen dioxide was measured weekly using Palmes tubes both inside and outside the home of the participants during a 6-week period. Additional air quality and meteorological data were obtained from local fixed monitoring stations. Parents recorded their child's respiratory symptoms daily by means of a diary. The symptoms recorded included cough during the day, cough at night, sore throat, running nose, fever, and earache. Physician visits and drug use also were recorded. Additionally, covariates, including respiratory symptoms of other family members, family social situation, size, living conditions, smoking habits of the family, age, and nationality, were recorded. A total of 1,225 families were included, of which 1,063 were Swiss nationals.

The incidence of upper respiratory illness, cough, breathing difficulties, and total respiratory illness was analyzed using Poisson regression. The model included age, social class, frequency of colds, and gas stove use as covariates, as well as a term for the number of days the individual was in the study. The adjusted annual symptom incidence rates and NO_2 exposure levels are given in Table 14-13.

The results show a pattern suggestive of increased incidence as a function of NO_2 exposure. Regression coefficients for NO_2 exposure were calculated by Schwartz (1990) but were not reported in the original paper. The incidence of any respiratory disease was used as the dependent variable, and annual indoor and outdoor NO_2 levels in micrograms per meter cubed were used as independent variables. The coefficient for indoor NO_2 was 0.0013 and

TABLE 14-13. ADJUSTED ANNUAL SYMPTOM INCIDENCE RATES AND NO_2 EXPOSURE LEVELS FOR FOUR COMMUNITIES IN SWITZERLAND[a]

Regions and NO_2 Concentrations in the Outside Air	Number of Symptoms per Child and Day (average)	Standard Error	Number of Children
Basel	0.40	0.02	507
Zürich	0.39	0.03	245
Wetzikon	0.38	0.03	219
Rafzerfeld	0.32	0.02	254
≤30 $\mu g/m^3$	0.31	0.02	310
30 - 49 $\mu g/m^3$	0.39	0.02	492
≥50 $\mu g/m^3$	0.41	0.02	412

[a]Statistics: t - test regions were Basel vs. Rafzerfeld, $t = 2.51$, $p<0.01$; Zürich vs. Rafzerfeld, $t = 1.99$, $p<0.05$; Variance analysis; NO_2 concentration: F-5.4, $p<0.05$.

Source: Braun-Fahrlaender et al. (1989).

for outdoor NO_2 it was 0.0002. The standard errors were 0.0064 and 0.0088, respectively. The indoor coefficient suggests that an increase of 30 $\mu g/m^3$ (0.016 ppm) in NO_2 would result in a 4% increase in the respiratory disease rate.

14.2.8 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 which did not. Ambient NO_2 levels ranged from 9 to 19 $\mu g/m^3$ (0.005 to 0.01 ppm) for the six 2-week time periods. Two-week average indoor NO_2 levels in homes of monitored children were highest for homes with kerosene heaters and gas stoves (91 $\mu g/m^3$; 0.05 ppm, n=8), second highest for kerosene only (36 $\mu g/m^3$; 0.02 ppm, n=45), third highest for gas stoves only (32 $\mu g/m^3$; 0.02 ppm, n=13), and lowest for no sources (6 $\mu g/m^3$; 0.003 ppm, n=43). Indoor levels did not fluctuate greatly over time, as indicated by the 2 wk averages.

A comparison of personal NO_2 exposures, as measured by Palmes' diffusion tubes, and NO_2 exposures measured in residences had a correlation of 0.94 for a subsample of 23 individuals. Results of this comparison are depicted in Figure 14-1 and show an excellent correlation between average household exposure and measured personal exposure.

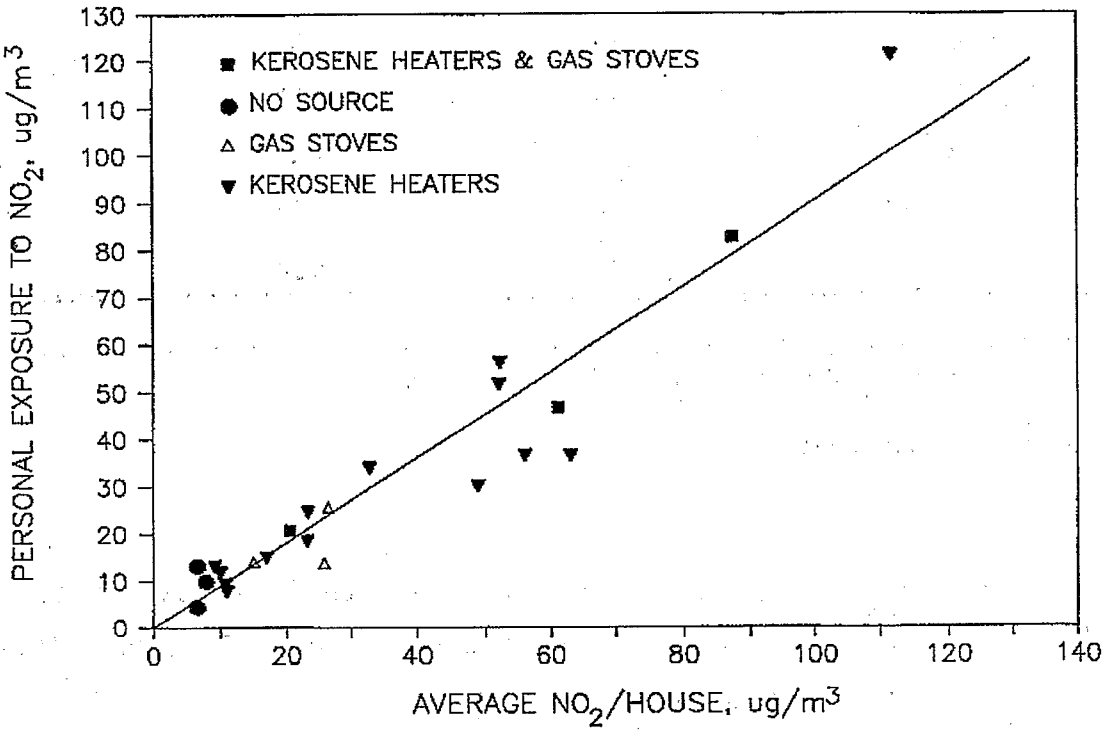

Figure 14-1. Total personal exposure to NO_2 vs. NO_2 levels in Connecticut residences.
Source: Leaderer et al. (1986).

The study defined LRI 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. This LRI definition may not be consistent with that defined by studies in Section 14.5. 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 of many potential covariates, which included socioeconomic status, age, gender, and exposure to environmental tobacco smoke. The ones having the largest effect were: age of the child, socioeconomic status of the family, and history of respiratory illness. Multiple logistic analysis was used to allow for the various factors.

When controlling for socioeconomic status and history of respiratory illness, children under the age of 7 exposed to 30 $\mu g/m^3$ (0.016 ppm) NO_2 or more were found to have an increased risk of lower respiratory symptoms 2.25 times children who were not exposed (95% confidence limits of 1.69 to 4.79). They also had an increased risk of upper respiratory symptoms of 1.33 (95% confidence limits of 1.19 to 1.49). Older children and adults showed no increased risk.

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 socioeconomic status, 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 (1990, personal communication) notes that the Berwick study controls for prior illnesses, as did the Keller study, which would reduce the estimated effect of current NO_2 exposure.

14.2.9 Chestnut Ridge Study

Vedal et al. (1987) conducted a panel study on 351 children selected from the 1979 Chestnut Ridge, Pennsylvania cross-sectional study of elementary school-aged children (mean age = 9.5 years). Parents completed a daily diary on respiratory symptoms. Most pollutants were measured at a single site, but sulfur dioxide (SO_2) was measured at 17 sites and then averaged. No air pollutant was strongly associated with respiratory symptoms. All pollutant levels were relatively low: maximum hourly levels for NO_2 levels ranged from 12 to 79 $\mu g/m^3$ (0.006 to 0.04 ppm). No indoor measurements were made nor were any surrogates for indoor pollution included in the analysis.

Schenker et al. (1983) reported a respiratory disease study of 4,071 children aged 5 to 14 in the same Pennsylvania community that utilized the ATS-DLD-78-C standardized questionnaire that was completed by the parents. The presence of a gas stove was reported to be used as a surrogate for NO_2 exposure. No significant association was found between use of gas stove use and any respiratory symptom or illness variable. No data were reported.

14.2.10 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 total suspended particulate (TSP), oxidants, SO_2, and NO_2. Symptoms were ascertained using the National Heart, Lung, and Blood Institute (NHLBI) questions 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 well as lifestyle characteristics relative to pollution exposure 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) SO_2 exposure above 0.04 ppm, ($p=0.03$), relative risk 1.18 for 13% of the study population with 500 h/year of exposure; (2) oxidants above 0.1 ppm, $p<0.004$, relative risk of 1.20 for 18% with 750 h/year; and (3) TSP above 200 $\mu g/m^3$, ($p<0.00001$), relative risk of 1.22 for 25% with 750 h/year. 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.

14.2.11 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, and chronic cough and phlegm, than those in households using electricity for cooking. The author noted that while 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.

14.2.12 Glendora, California Study

In another California study, Detels et al. (1979, 1981a, 1981b) 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 Azuza, about 5 km away. Pollutants measured included total oxidant (ultraviolet absorption method), NO_x (Saltzman method), carbon monoxide (nondispersive infrared spectroscopy method), SO_2 (conductimetric method), hydrocarbons (flame ionization detection method), and particles (high-volume method). The 5-year averages were computed for those pollutants with sufficient data. Comparing Lancaster to Glendora, the NO_2 levels were 3.2 vs. 11.4 $\mu g/m^3$ (0.002-0.006 ppm); the total oxidant levels were 6.5 vs. 11.6 $\mu g/m^3$, and the hydrocarbons were 2.9 vs. 4.8 $\mu g/m^3$. Comparable differences existed for SO_2, particles, and sulfate fraction of particles, but the data were only complete for the year 1977.

The authors looked at 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 authors also 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 forced expiratory volume (FEV_1) or forced vital capacity (FVC) below 50% of expected was also significantly higher in Glendora. Other lung function measurements showed less significant differences, but the trend was always towards lower values in Glendora.

The two primary weaknesses of the study are: (1) the two areas (Lancaster and Glendora) were measured at different times (1974 vs. 1977) and (2) the areas are quite different with respect to climate, commuting patterns, altitude, socioeconomic status, season, and general lifestyle. No specific analysis related to NO_2 levels were discussed. The effects of smoking habits were carefully controlled and should not be considered as a serious confounder. The authors did attempt to control variability in measurement methods and technicians, and results of this are reported by Tashkin et al. (1979).

14.2.13 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, 1982), 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. Second, much of the pollution may have been in the form of nitric acid (HNO_3), and possible health effects may be related to HNO_3 exposure rather than NO_2 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 NO_2 reached 286 $\mu g/m^3$ (0.152 ppm) near the arsenal (as measured by the Saltzman method), and nitrate fraction levels reached 4.1 $\mu g/m^3$ at the downtown post office.

It is likely that the elevated NO_2 levels were accompanied by elevated HNO_3 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, 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 HNO_3. Thus, it is possible that any adverse health effects seen in Chattanooga during this time period were associated with HNO_3 and NO_2 rather than with NO_2 alone. However, no conclusion is possible since the health effects of HNO_3 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 since the rate in the intermediate area was just as high as the high pollution area.

Shy and co-workers (Shy, 1970; Shy et al., 1970a,b; 1973) studied the effects of community exposure to NO_2 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-NO_2 exposure neighborhood than in the intermediate- and low-NO_2 areas. Although individual area pollution estimates are not available, one part of the high pollution area had an annual average NO_2 level of 286 $\mu g/m^3$ (0.152 ppm). Areas further from the major NO_2 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 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, NO_2 levels (as measured by the continuous chemiluminescent method) ranged from 60.2 $\mu g/m^3$

(0.032 ppm) in the high area to 28.9 $\mu g/m^3$ (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 NO_2 or its by-products on acute respiratory disease.

14.2.14 United States and Canadian Skating Rink Exposures

Hedberg et al. (1989) reported 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 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 were detected 2 days later with the ventilation system working, suggesting that levels during the games were higher. 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 months 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, suggesting much higher levels during the incident.

14.3 STUDIES OF PULMONARY FUNCTION

Pulmonary function studies are part of any comprehensive investigation of the possible effects of any air pollutant. The measurements can be done in the field, they are noninvasive, and their reproducibility has been well documented. Age, height, and gender are important determinants of lung function. In addition, changes in pulmonary function have been associated with environmental tobacco smoke (Hasselblad et al., 1981) and particulate matter in combination with SO_2 (Dockery et al., 1982 and Dassen et al., 1986) and other factors.

14.3.1 United States Six City Studies

Several of the pulmonary function studies also included respiratory disease symptoms as described earlier. Ware et al. (1984), reporting on the Six City study, described analysis 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 second (FEV_1) values were significantly lower for children of current smokers than for children of nonsmokers at both examinations and 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 FEV_1 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 FEV_1 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) give 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 study factors affecting pulmonary function growth. Children whose mothers smoked one pack of cigarettes per day had levels of FEV_1 at age 8 that were approximately 0.81% lower than children of nonsmoking mothers ($p<0.0001$), and FEV_1 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.

14.3.2 Tucson Study

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 ozone (O_3), TSP, carbon monoxide (CO), and NO_2 was done in or near the

clusters. Indoor sampling of O_3, TSP, respirable suspended particles (RSP), and CO was done 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 NO_x 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. In asthmatics, 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.

14.3.3 New York Study

Goldstein et al. (1987) reported preliminary data examining acute exposure to NO_2 and pulmonary function tests. Eleven asthmatic and 12 nonasthmatic women and children were monitored for 5 days with a portable continuous NO_2 monitoring instrument held at breathing level that provided 5-min average NO_2 levels. Pulmonary function (FEV_1, FEV_{25-75}) and peak flow as well as tracings of the entire flow curve were monitored at several different points during the exposure. While the data are limited, it seemed that at average NO_2 exposures below 0.3 ppm (564 $\mu g/m^3$) pulmonary function was as likely to be decreased as increased, whereas at exposure above 0.3 ppm there was mainly a decrease.

14.3.4 Chestnut Ridge Study

Vedal et al. (1987) conducted a panel study on 351 children selected from the 1979 Chestnut Ridge cross-sectional study of elementary school-aged children (mean age = 9.5 years). Peak expiratory flow rate (PEFR) was measured daily for 9 consecutive weeks. Most pollutants were measured at a single site, but SO_2 was measured at 17 sites and then averaged. No air pollutant was strongly associated with level of PEFR. All pollutant levels were relatively low; NO_2 levels ranged from 0.006 to 0.042 ppm (12 to 79 $\mu g/m^3$). No indoor measurements were made, nor were any surrogates for indoor pollution included in the analysis.

14.4 OUTCOMES RESULTING FROM OCCUPATIONAL EXPOSURES

Gamble et al. (1987) studied 232 workers in four diesel bus garages for the effects of NO_2 on acute respiratory illness and pulmonary function. Response was assessed by an acute respiratory questionnaire and before and after shift spirometry. Measurements over the shift of NO_2 (using passive Palme tube samplers) were made on each worker and collected on the same day as the pulmonary function tests and questionnaires. Other irritant gases were measured and were well below federal standards. Mean NO_2 levels over the shift ranged from 0.56 (standard deviation [SD] = 0.38) ppm NO_2 in the highest garage to 0.13 (SD = 0.06) ppm NO_2 in the lowest garage. Short-term NO_2 measurements indicated levels above 1 ppm as being not uncommon. 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.

Gamble et al. (1983) examined chronic respiratory effects in 259 sodium chloride (NaCl) miners. The Medical Research Council respiratory symptom questionnaire containing smoking history was administered by trained interviewers. A chest X-ray and spirometry was also conducted. Personal samples of NO_2 and respirable particles for jobs in each mine were used to estimate cumulative exposure. Mean exposure ranged from a low of 0.2 (SD = 0.1) ppm NO_2 to a high of 2.5 (SD = 1.3) ppm NO_2. Diesel emissions were the principal NO_2 source. The author reported that while cough was associated with age and smoking and dyspnea was associated with age, neither symptom was associated with exposure (i.e., years worked, estimated cumulative NO_2 or respiratory particle exposure.) Reduced pulmonary function showed no association with NO_2 exposure.

Robertson et al. (1984) reported on a 4-year study of lung function in 560 British coal miners. Overall average NO_2 levels at nine coal mine sites ranged from 0.02 to 0.06 ppm (38 to 113 $\mu g/m^3$), and NO levels ranged from 0.13 to 1.19 ppm. No relationship was found between exposure and decline in FEV_1 or respiratory symptoms. Jacobsen et al. (1988) conducted a more extensive investigation. Jacobsen et al. (1988) studied nearly 20,000 miners at the same nine British coal mines to examine whether long-term exposure to low concentration of NO_2 and NO were associated with increased susceptibility to respiratory infections. The NO_x source consisted of diesel emissions and blasting. Shift median levels were 0.2 ppm NO and 0.03 ppm NO_2. 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 1955-87 on 17 patients examined at the Mayo Clinic for Silo-Fillers disease shortly after exposure to silo gas (NO_2 levels from 200 to 2,000 ppm). Health outcome ranged from death to hypoxemia and transient obstruction of the airways. Epler (1989) notes that prevention is essential for elimination of silo-fillers disease. Other studies also examine high exposures (Lowry and Schuman, 1956; Grayson, 1956; Gregory et al., 1969; Yokey et al., 1980).

14.5 SYNTHESIS OF THE EVIDENCE

The weight of the evidence does not indicate that NO_2 exposure at the levels reported in the studies in this chapter has any effect on pulmonary function of biologically significant magnitude. Several of the studies, however, suggest an increase in respiratory morbidity in children from exposure to levels seen with gas stove use as compared to electric stove use. The effects in the majority of the studies do not reach statistical significance. The consistency of these studies is examined and the evidence synthesized in a quantitative analysis in the following section.

14.5.1 Health Outcome Measures

A concern in interpreting these studies (see Section 14.5.3 for criteria for including a study in the quantitative analysis) is that the LRI variable measured in these studies may represent differing outcomes that may have different mechanisms of causation related to NO_2 exposure. The origin of cough may be different than that of wheeze. The agents that cause them may be different. Different disease syndromes are being studied: croup, bronchitis, bronchiolitis, asthma, and pneumonia. The instruments measuring LRI in the studies are 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. Is LRI in 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 the outcome measures in the NO_2 studies, similarities in outcome measures in other LRI studies in children, the use of questionnaires as instruments for LRI, and, in general, the use of LRI as an outcome measure.

The studies in the quantitative analysis that follows use health outcome measures that provide an indication of the state of respiratory health of the various samples of children 12-years-old or younger. The NO_2 studies utilized standard questionnaires to evaluate LRI in children. As discussed below, some studies used a specific symptom while others used an index consisting of symptoms and disease. Diagnoses of specific respiratory diseases such as bronchiolitis or asthma were not made. The important factor is an attempt to measure some aspect of LRI. Table 14-14 lists the health outcome measures for each study considered. While 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 LRI, other measures use indexes, grouped responses, or combined indicators of LRI, some of which include measures such as colds going to chest.

In the Melia et al. (1977, 1979, 1980, 1982, 1983) 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 months. Respiratory symptoms and diseases surveyed include 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 reduced peak flow rates on a sample of the children examined in 1973. Since the answers to these questions were related to the reduced adjusted mean peak flow rates, an indicator of decreased respiratory function, this suggested that these questions may be indicators of LRI (while others such as earache or hospitalization for upper respiratory disease in the last 12 months may not). Thus, the more subjective instrument, the questionnaires, was supported to some extent by the 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

TABLE 14-14. HEALTH OUTCOME MEASURES IN SELECTED NO_2 EPIDEMIOLOGY STUDIES

Reference	Health Outcome Used in Meta Analysis	Method	NO_2 Exposure Measure Used in Analysis	Age (years)	Sample Size	Where/When
Melia et al. (1977)	Colds going to chest showed a prevalence of 26.8-19.8%.	Respiratory symptoms questionnaire completed by parent of child for the last 12 months.	Gas stove vs. electric stove.	6-11	5,658	28 Areas of England and Scotland (1973)
Melia et al. (1979)	Group response to respiratory questions into none or one or more symptoms or diseases. 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.	As above.	Gas stove vs. electric stove.	5-10	4,827	27 areas of England and Scotland (1977)
Melia et al. (1980) Florey et al. (1979) Goldstein et al. (1979)	Group response to respiratory questions as above.	As above.	NO_2 measured with Palmes Tubes. Gas stove homes only.	6-7	103	Middlesborough, England (1978)
Melia et al. (1982)	As above.	As above.	NO_2 measured with Palmes Tubes. Gas stove homes only.	5-6	188	Middlesborough, England (1980)
Melia et al. (1983)	Same respiratory questions as above with the addition of pneumonia during the last 12 months. The highest prevalence rating for wheeze (63.3-42.3%), followed by colds going to chest (50-36.9%).	As above.	Gas vs. electric.	<1	390	London (1975 to 1978)
Ware et al. (1984)	Lower respiratory illness index (index of respiratory health) indicating the pressure of either bronchitis, respiratory illness, or persistent cough.	Questionnaire (Ferris, 1978) completed by parent for symptoms during previous 12 months.	Gas vs. electric	6-10	8,240	Six U.S. cities (1974-1979)

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TABLE 14-14. (cont'd) HEALTH OUTCOME MEASURES, NO_2 EPIDEMIOLOGY STUDIES

Reference	Health Outcome Used in Meta Analysis	Method	NO_2 Exposure Measure Used in Analysis	Age (years)	Sample Size	Where/When
Neas et al. (1990, 1991)	Combined indicator of one or more lower respiratory symptoms as defined. The highest prevalences were for chronic phlegm and wheeze.	Symptom questionnaire completed by parent for the year during which measurements of NO_2 were taken.	NO_2 measured with Palmes Tubes. Gas and electric stoves.	7-11	1,286	Six U.S. cities (1983-1986)
Ogston et al. (1985)	Presence of any respiratory illness episode during previous year, including infection of upper and lower respiratory tracts.	Questionnaire form, recorded by health visitor, examined by a pediatrician.	Gas stove vs. electric stove.	<1	1,565	Tayside region, Scotland (1980)
Ekwo et al. (1983)	Chest congestion and phlegm with colds.	Questionnaire completed by parent (ATS).	Gas stove vs. electric stove.	6-12	1,138	Iowa City, Iowa
Dijkstra et al. (1990) Brunekreef et al. (1987)	Respiratory illness combination variable of presence of one or more of cough, wheeze, or asthma.	Questionnaire completed by parent (WHO).	NO_2 measured with Palmes Tubes. Gas and electric appliances.	6-12	775	Netherlands (1986)
Keller et al. (1979a,b)	Lower respiratory illness syndrome characterized by cough, wheezing, phlegm.	Telephone interview by nurse epidemiologist.	Gas stove vs. electric stove.	<12	553	Columbus, Ohio (1978)

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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 LRI. Disease indicators such as asthma and bronchitis episodes in the past year, while measures of lower respiratory disease, appear to play a substantially smaller role. The Melia et al. (1979) study provides data that allow development of graphs of the marginal likelihood functions (see Fig. 14-2) of the odds ratios for colds going to the chest 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 LRI for the other Melia studies.

Other NO_2 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; Ogston et al., 1985; Dijkstra et al., 1990; Keller et al., 1979b). 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 infection or illness in the lower respiratory tract. These symptoms are all indicators of lower respiratory illness and as such form a measure of 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 NO_2 studies and similar indexes in the other NO_2 studies provides consistency in the outcome measurements of the studies.

How do the outcome measures in the NO_2 studies compare with indicators of lower respiratory health status done in other studies? The studies of LRI (unrelated to NO_2 exposures) discussed in Section 14.2 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 NO_2 studies, that is, colds going to chest and wheeze. Thus, to an extent, the NO_2 respiratory studies are providing a measure of lower respiratory health

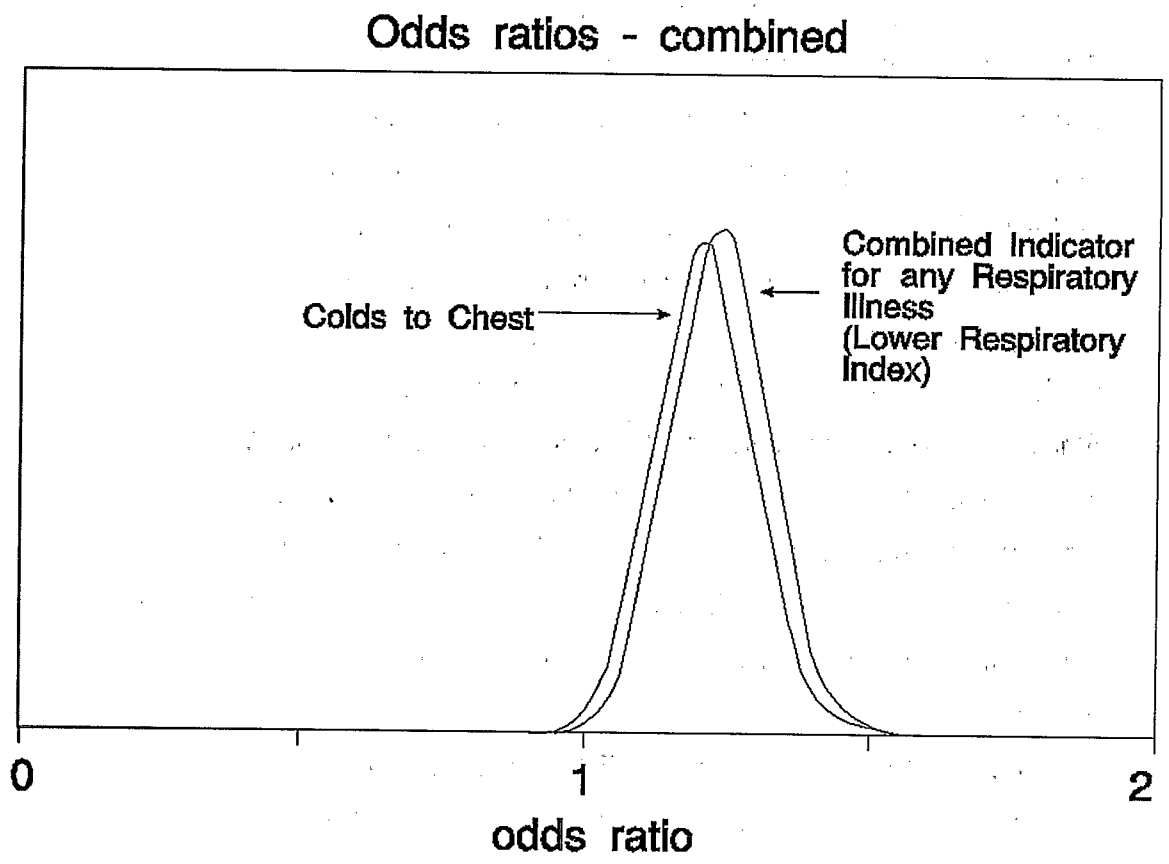

Figure 14-2. For the Melia et al. (1979) study, a graph of the marginal likelihood function of the odds ratios for combined gender (boys and girls) of the outcome measures colds to chest and any respiratory illness developed by EPA.

somewhat equivalent to these other studies. While specific pediatric diseases and agents such as bronchiolitis and RSV are investigated in these other studies and not in the NO_2 respiratory studies, the overall pattern and incidence of LRI are considered to be consistent in different geographic regions over time (Wright et al., 1989; Denny and Clyde 1986; McConnochie et al., 1988). As such, lack of direct evidence for disease and agents should not limit conclusions that could be drawn from the quantitative analysis of the NO_2 respiratory studies.

Lower respiratory illness is an entity commonly defined by criteria for clinical diagnosis (Wright et al., 1989). The instrument to determine LRI in these studies is substantially uniform—a standardized respiratory disease questionnaire mainly filled out by the parent for illness and symptoms during the past 12 months. How comparable are the measures of LRI in the studies? As background, consider the classification of lower respiratory disease and surveillance methods. Samet (1990) reviews key studies that include Monto et al. (1971), Taussig et al. (1989), Glezen et al. (1971), Gardner et al. (1984), and Maletzky et al. (1971). Standardized and uniformly accepted clinical criteria have not been developed for the illnesses considered to make up LRI; that is, for croup, tracheobronchitis, bronchiolitis, and pneumonia. The extent to which the diagnostic criteria for LRI were documented varies in these studies (see Table 14-15, Samet, 1990). Diagnosis of pneumonia usually requires evidence on chest X-rays. Characteristic signs and symptoms are wheeze, phlegm from chest, and deep cough. These are basic respiratory symptoms indicative of lower respiratory involvement.

For the purpose of analysis of illness rates, children with LRI may be considered to belong to a single population with a similar illness. While different diseases are grouped together as LRI (such as croup, bronchiolitis, and pneumonia), the LRI 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 NO_2 studies determined the presence of LRI in the subjects by tabulating responses to questions on respiratory symptoms such as cough and wheeze, and respiratory illnesses such as asthma and bronchitis. Symptom reporting provides subjective evidence of respiratory infection and diseases such as bronchiolitis. Diseases are characterized by groups of symptoms. Disease end points such as asthma and bronchiolitis may have similar defining symptoms. Lower respiratory illness reflects a broader grouping, within which the lack of the ability to differentiate between the wheeze of asthma and wheeze related to other infections in the pediatric age group may not be important. Wright et al. (1989) note that wheeze is recorded as occurring for several

TABLE 14-15. CLASSIFICATION OF ILLNESSES IN SURVEILLANCE STUDIES

Study	Reference	Classification
Tecumseh	Monto et al. (1971)	Lower respiratory illness (LRI) = Pain on breathing, wheezy breathing, or phlegm from chest Upper respiratory illness (URI) = Coryza with/without other symptoms Laryngopharyngeal illness = Sore throat or hoarseness Cough = Cough with/without earache Earache = Earache
Seattle	Maletzky et al. (1971)	Pneumonia = Fine inspiratory rales and/or infiltrate on chest X-ray Croup = Presence of inspiratory stridor Bronchiolitis = Presence of wheezing and air-trapping on exam or chest X-ray
North Carolina	Glezen et al. (1971)	Epiglottitis = Marked edema and erythema of the epiglottis Croup = Hoarseness and barking cough with/without inspiratory stridor Tracheobronchitis = Deep cough and ronchi audible in larger air passages Bronchiolitis = Expiratory wheezing, often with evidence of air trapping Pneumonia = Fine rales or evidence of pulmonary consolidation
Houston	Gardner et al. (1984)	Not given
Tucson	Taussig et al. (1989)	Criteria for LRI: (1) a history of acute cough and/or wheeze and positive physical findings, or (2) acute inspiratory stridor, or (3) positive chest X-ray findings including hyperaeration, and (4) for infants under 6 months, a history of prolonged cough and/or wheeze and positive findings as in 1-3. No specific criteria for individual syndromes.

Source: Samet (1990).

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. 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.

The studies of LRI are implemented by use of standardized questionnaires. The purpose of a respiratory symptom questionnaire is to compare prevalence of symptoms (Fairbairn 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).

In perspective, a question of similarity exists for other potential outcomes usually considered similar. FEV_1 changes may be caused by different diseases and may have different mechanisms of causation. But a study of FEV_1 changes in children would probably be considered as a homogenous outcome. Thus, LRI may be a comparatively similar end point.

Childhood LRI is a grouping of symptoms and diseases that reflect changes located anatomically in the lower respiratory tract. This characterization represents an indication of severity, that has been used to characterize the respiratory health status of children. The LRI syndrome designation presents a means of grouping illnesses of similar patterns. Lower respiratory illness is a multifaceted approach to respiratory health in a population living under natural conditions. The classification is the combination of different respiratory effects that have in common an evaluation of the morbidity status of the lower respiratory tract. While the use of identical health outcome measures would be most desirable, the level of similarity and common elements between the outcome measures in the NO_2 studies provide confidence in their use in the qualitative analysis. Also, the similarity to other non-NO_2 studies of LRI, while again less than perfect, provide confidence as to LRI use as an outcome measure.

14.5.2 Biological Basis

The biological basis relating NO_2 exposure to respiratory symptoms and infection shown in epidemiological studies are presented in discussions in the animal toxicology and clinical studies (Chapters 13 and 15 respectively) and in a recent review (Kotchmar et al., 1990). A brief summary is presented here.

The lung is one of the sites of microbial infection. While 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, while 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. Specific T cell-mediated immune mechanisms are important in augmenting host defenses of the lung (Kaltreider, 1986). T lymphocytes augment resistance to intracellular microbial organisms and lyse virally infected cells. To an 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 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. Meulenbelt and Sangster (1990) note that NO_2 may cause direct epithelial damage that could increase susceptibility to infection.

Based on available information, exposure to NO_2 impairs one or more defense mechanisms, leaving the host potentially more susceptible to respiratory infections and diseases. One of the first lines of defense against foreign agents entering the respiratory tract is the mucociliary clearance system, responsible for the removal of particles from the conducting airways of the lungs (Proctor et al., 1977). Studies on the effects of NO_2 exposure on mucociliary transport show a reduction in the number and activity of the cilia, morphological changes in the cilia, and decreased mucociliary velocity (Stephens et al., 1972; Azoulay et al., 1978; Schiff, 1977; Abraham et al., 1980; Yamamoto and Takahashi, 1984). As a foreign agent moves beyond the mucociliary region, and enters the gaseous exchange region of the lung, host defenses are provided by the alveolar macrophage, which acts to remove or kill viable particles and thereby maintain sterility against infectious agents and remove damaged and dead cells. Exposure to NO_2 has produced a variety of effects on alveolar macrophages including decreased viability (Rombout et al., 1986; Suzuki et al., 1986; Chang et al., 1986; Mochitate et al., 1986). Decreases in the phagocytic ability of

alveolar macrophages have been reported in rats, hamsters, and rabbits (Suzuki et al., 1986; Gardner et al., 1969; Acton and Myrvik, 1972; Foster et al., 1985; Schlesinger et al., 1987). In addition to functional changes to alveolar macrophages, NO_2 exposure has also been reported to produce structural changes in the macrophages (Aranyi et al., 1976). Controlled human exposure studies have examined alveolar macrophage function and show that alveolar macrophages exposed to NO_2 tended to inactivate influenza virus in vitro less effectively than cells collected after air exposure (Frampton et al., 1989a).

Few studies are available that evaluate the effects of NO_2 on the immune system; however, there is some indication that exposure to NO_2 suppresses the immune response and that the effect is concentration- and time-dependent. For example, a significant suppression of serum humoral antibody response has been reported in experimental animals acutely exposed to NO_2 (Lefkowitz et al., 1986; Fujimaki and Shimizu, 1981). Exposure to NO_2 suppresses the number of mature splenic helper T and cytotoxic T lymphocytes (Richters and Damji, 1988). The cause of the suppression is not clear. Exposure to NO_2 resulted in decreased numbers of T lymphocyte, T-helper/induced lymphocytes, and T-cytotoxic/suppressor lymphocytes in mice (Damji and Richters, 1989).

Recent controlled human exposure studies examining the effects of NO_2 on pulmonary host defense systems report a trend toward an elevated rate of infection, though not statistically significant, in a study examining the effect of NO_2 exposure on the infectivity rate of live attenuated influenza A/Korea/reassortment virus (Goings et al., 1989). Also, exposure to NO_2 may transiently increase levels of antiprotease alpha-2-macroglobulin (α_2M) in bronchoalveolar lavage (BAL) fluids presumably through local release. While serving as an indicator of changes in protease-antiprotease balance, alterations in α_2M in the alveolus may have significance for local immunoregulation. Changes in α_2M may alter alveolar macrophage defenses against infection (Frampton et al., 1989a,b). These findings suggest, but do not prove, that NO_2 may play a role in increasing the susceptibility of adults to respiratory virus infections.

Animal infectivity studies present key data relating exposure to NO_2 and effects on host defense mechanisms. In these studies, animals were exposed to varying concentrations and durations of NO_2 followed by exposure to an aerosol of an infectious agent. Mortality was used as the end point. Exposure to NO_2 increased bacterial-induced mortality and

influenza-induced mortality. The studies indicate that NO_2 increased microbial-induced mortality by impairing the host's ability to defend the respiratory tract from infections agents (Ehrlich and Henry, 1968; Ito, 1971; Ehrlich et al., 1977). Recently, an infectivity model designed to evaluate the effects of NO_2 on nonfatal respiratory disease indicated that NO_2 decreased the intrapulmonary removal (killing) of the microorganisms in mice in a concentration-related manner without a decrease in the number of alveolar macrophages. Exposure to NO_2 was found to increase the severity of the mycoplasma-induced lesions but not found to increase the susceptibility of the mice to the infection (Parker et al., 1989). These animal studies clearly demonstrate that acute and chronic exposures to NO_2 increase susceptibility to viral, mycoplasma, and bacterial infections and the effects were concentration- and time-dependent, species-dependent, and infectious agent-dependent (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).

The evidence from animal toxicological and human clinical studies of host defenses provide a rationale for investigating the relation between exposure to NO_2 and an increase in frequency and severity of respiratory symptoms and/or infections in humans. The strength of various parts of the data base help substantiate the biological basis for the relationship seen in epidemiology studies between respiratory disease and exposure to NO_2. The above discussion provides a plausible biological basis for similarity in the pattern of biological mechanisms for the various outcome measures studied in the epidemiology studies. When microorganisms attack a lung that has been exposed to NO_2, host defense mechanisms altered by the NO_2, exposure may result in increased severity or rate of respiratory illness. While 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 be reported or objectively discerned as a general outcome such as a chest cold, a cough, or an incident of asthma or bronchitis.

14.5.3 Quantitative Analysis

In order to compare available studies on respiratory effects of NO_2, a common end point was defined, and then each study was compared with this standard end point. For purposes of discussion, the end point chosen was the presence of LRI in children aged 12 or under. Assume that the relative odds of developing LRI is similar across this age range as a function of NO_2 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 an increase in the household average of 30 $\mu g/m^3$ (0.016 ppm) in NO_2 exposure for a chronic exposure such as a year. This is approximately the increase seen as a result of gas stove use as compared with electric stove use in both the United States and England. Studies with NO_2 exposure measures used 1 to 2 week integrated indoor measurements by Palmes passive diffusion tubes that provide an estimate for chronic exposure such as a year.

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

As discussed earlier in this chapter, the exposure estimates used in these studies are either a surrogate (gas vs. electric) or a 2-week integrated NO_2 average measured by Palmes tubes. 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 measurement error. These studies can not distinguish between the relative contributions of peak and average exposures and their relationship with the observed health effects.

The British studies give us several estimates of this odds ratio. Melia et al. (1977) studied children aged 6 to 11 years old, 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 the majority of the time was colds going to the chest, which was used as an indicator of LRI. This study did not measure NO_2 exposure, and so the assumption was made that the increase in NO_2 exposure from gas stove use in England was reasonably similar to that in the other British studies which measured NO_2. The average increment in NO_2

resulting from the presence of a gas stove was 31 $\mu g/m^3$ (0.016 ppm) (see Chapter 7). This value is extremely close to the specified value of 30 $\mu g/m^3$ (0.016 ppm), and so no adjustment to the estimated odds ratio was made. The estimated odds ratio was 1.31, with 95% confidence limits of 1.16 to 1.48. 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 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 to 1.42.

Melia et al. (1980) studied children aged 6 to 7 and measured bedroom NO_2 levels for the exposure estimate. This study applied the same combined health end point as the previous study. The estimated odds ratio for an increase of 30 $\mu g/m^3$ (0.0159 ppm) was 1.53, with 95% confidence limits of 1.04 to 2.24.

Melia et al. (1982) studied children aged 5 to 6 and also measured NO_2 exposure in the bedroom and also applied the same combined health end point. The estimated odds ratio for an increase of 30 $\mu g/m^3$ (0.0159 ppm) was 1.11, with 95% confidence limits of 0.83 to 1.49.

Melia et al. (1983) studied children aged 1 or less and used the presence or absence of a gas stove as a surrogate for NO_2 exposure, as in the other Melia studies. The same respiratory index was applied as in the other Melia studies, with the addition of episodes of pneumonia during the last 12 months. The estimated odds ratio for an increase of 30 $\mu g/m^2$ (0.0159 ppm) was 0.63, with 95% confidence limits of 0.36 to 1.10.

In the first Six City study cohort, Ware et al. (1984) reported an index of respiratory illness. Exposure to NO_2 with and without gas stove use was measured for only one of the six cities (Portage, WI). The analysis was based on the presence or absence of a gas stove. The estimated odds ratio was 1.08 with 95% confidence limits of 0.97 to 1.19.

A second cohort of subjects in the Six City study was initially reported by Dockery et al. (1989). 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 estimated odds ratio for an increase in the presence of any respiratory symptom resulting from an increase in exposure of 31 $\mu g/m^3$ was 1.47, with 95% confidence limits of 1.17 to 1.86.

Ogston et al. (1985) studied the presence of any respiratory disease in children of age one in Tayside, Scotland. No NO_2 exposure estimates were obtained, and exposure classification was based on the presence or absence of a gas stove. The estimated odds ratio was 1.14 with 95% confidence limits of 0.86 to 1.50.

Ekwo et al. (1983) studied several respiratory illness end points from children surveyed at aged 6 to 12. No exposure measurements were obtained, and the exposure was based on the presence or absence of a gas stove. None of the end points matched the end point of interest closely. The two most similar 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 1.10, with 95% confidence limits of 0.79 to 1.53. 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 30 $\mu g/m^3$ (0.0159 ppm) in NO_2 exposure. The 95% confidence limits were 0.66 to 1.33. The study had measured NO_2 exposure data, but the EPA analysis did not adjust for covariates since 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 LRI was 1.10, with 95% confidence limits of 0.74 to 1.54.

Only two studies with sufficient information for analysis were excluded in the synthesis. The Schwartz (1990) analysis of the Swiss study gave Poisson regression coefficients that could have been converted to odds ratios, but these computations were not published and available for peer review. 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. For these reasons, the above two studies were not included in the synthesis.

Graphs of the odds ratio from each study are depicted in Figure 14-3. 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

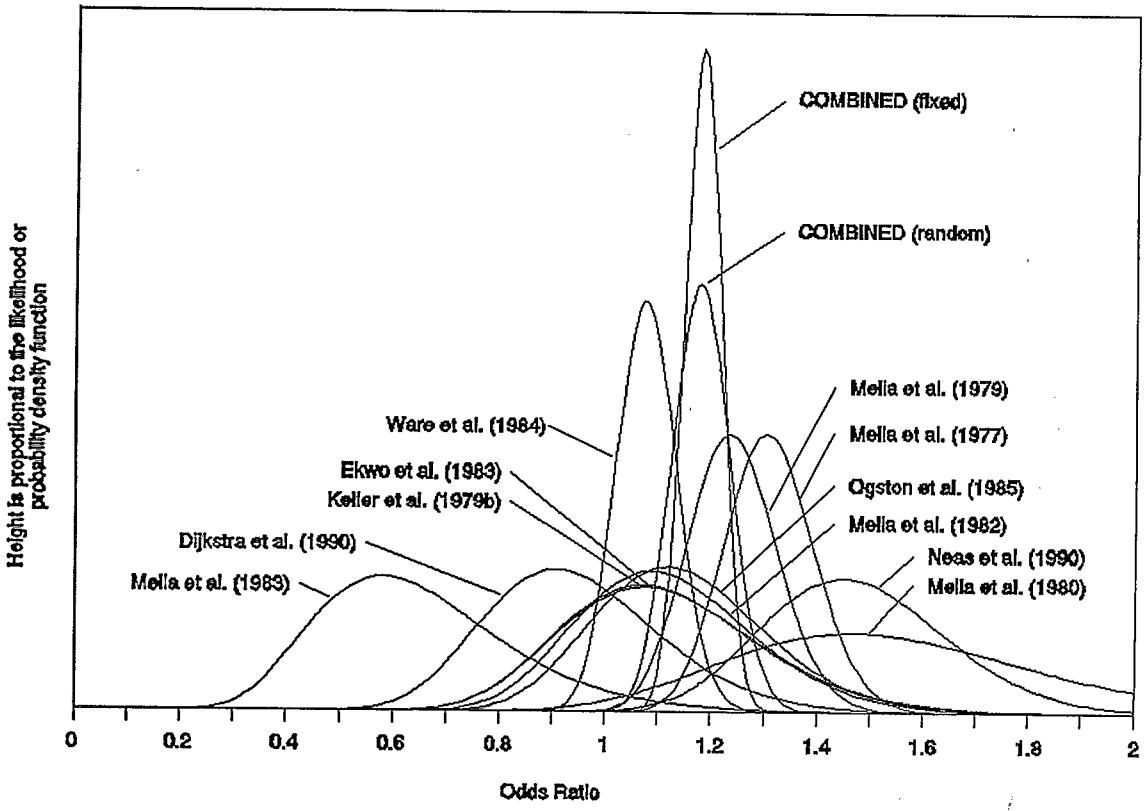

Figure 14-3. U.S. Environmental Protection Agency meta-analysis of epidemiologic studies of NO_2 exposure effects on respiratory disease in children ≤12 years old. Each curve can be treated as a likelihood function or posterior probability distribution. If treated as a likelihood function, the 95% confidence 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.

prior on the log-odds ratio. The basic information for each curve are provided in Table 14-16.

Methods for combining or synthesizing evidence, often referred to as meta-analyses, are being used more frequently as indicated by Mann (1990). 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). Several methods for combining clinical

TABLE 14-16. SUMMARY OF ODDS RATIOS FROM STUDIES OF THE EFFECTS OF NITROGEN DIOXIDE

Author	Estimated Odds Ratio	2.5 and 97.5 Percentiles (Confidence Interval)
Melia et al. (1977)	1.31	(1.16, 1.48)
Melia et al. (1979)	1.24	(1.09, 1.42)
Melia et al. (1980)	1.53	(1.04, 2.24)
Melia et al. (1982)	1.11	(0.83, 1.49)
Melia et al. (1983)	0.63	(0.36, 1.10)
Ware et al. (1984)	1.08	(0.97, 1.19)
Neas et al. (1990)	1.47	(1.17, 1.86)
Ogston et al. (1985)	1.14	(0.86, 1.50)
Ekwo et al. (1983)	1.10	(0.79, 1.53)
Dijkstra et al. (1990)	0.94	(0.66, 1.33)
Keller et al. (1979b)	1.10	(0.79, 1.54)

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.

Two basic models are employed in order to combine evidence. 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 classical methods was the variance weighted method (Hald, 1962). This 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 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 14-16, 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. 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 vary different parameters, then the variances of the estimates will more closely reflect the variance of the unknown distribution being sampled.

The 11 studies described earlier (Tables 14-14, 14-16) 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 14-3). Two different estimates of random effects models were used. The first was estimated using the noniterative estimates assuming unequal variances as described by DerSimonian and Laird (1986) and is labeled "D & L." The second is a hierarchical Bayesian model using a flat prior for the unknown variance and this model is labeled "H-B". The results of the analyses are provided in Table 14-17.

The first line of Table 14-17 shows the results of combining all 11 studies using a fixed model. The estimated odds ratio is 1.18 and the 95% confidence limits are 1.11 and 1.25. The analysis was made assuming that the parameters were the same (homogeneous), and this can be tested. The chi-square for homogeneity for the 11 studies was 18.75 for 10 degrees of freedom, which has a p-value of 0.0436. Thus, there is some evidence that the parameters from each study are not identical. The estimates for the two random effects models give similar estimates to the fixed model, but the confidence limits are slightly broader. The conclusion from all three models is the same, namely that the odds ratio is estimated to be about 1.2 with 95% confidence limits ranging from about 1.1 to 1.3.

TABLE 14-17. U.S. ENVIRONMENTAL PROTECTION AGENCY COMBINED ANALYSES OF STUDIES ON RESPIRATORY ILLNESS EFFECTS OF NITROGEN DIOXIDE

Study group	Number of Studies	Model[a]	Odds ratio	Percentiles (Conf. Int.)
All	11	fixed	1.18	(1.11, 1.25)
All	11	H-B	1.18	(1.08, 1.29)
All	11	D&L	1.18	(1.08, 1.30)
Children >1	9	fixed	1.19	(1.12, 1.27)
Children >1	9	H-B	1.20	(1.10, 1.31)
Children >1	9	D&L	1.19	(1.09, 1.30)
Children <1	2	fixed	1.01	(0.79, 1.30)
Measured NO_2, age >1	4	fixed	1.27	(1.09, 1.47)
Gas stove, age >1	5	fixed	1.18	(1.10, 1.26)
British, age >1	4	fixed	1.27	(1.17, 1.39)
Six City	2	fixed	1.13	(1.03, 1.24)
Other studies	3	fixed	1.05	(0.86, 1.27)

[a]Fixed = Fixed-effects model; H-B = Hierarchial Bayesian model; D-L = DerSimonian and Laird model.

Two of the 11 studies were on children under 1 year of age, whereas the others were on children of elementary school age. Since the end point of wheeze is more predominant in the children less than 1 year old as opposed to the older children and the outcome measure in Ogston et al. (1985) includes upper respiratory illness, making it dissimilar to the others, there may be reason to separate these studies from the others. These analyses are shown on lines four through seven of Table 14-17. Note that the odds ratio for the two studies of children under age 1 gives an odds ratio of 1.01, which is well below the estimate of 1.19 for the older children. The estimate for the younger children has much greater uncertainty due to the fewer number of studies. The nine studies of older children have a chi-square for homogeneity of 13.55 for 8 degrees of freedom ($p=0.0942$). Although this value is no longer statistically significant at the 0.05 level, it still suggests some randomness in the parameter values. In spite of this, the estimates from all three models are reasonably similar to each other and are similar to the results of all 11 studies combined.

Four of the nine studies of older children used measured NO_2 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 NO_2 studies gave an estimated odds ratio of 1.27, whereas the others gave an estimate of 1.18, which is consistent with a measurement error effect. The chi-square tests for homogeneity were not significant at the 0.1 level for either group of studies.

Finally, the studies were compared by similarity of subjects. Four of them were done in Great Britain (Melia studies), two were based on the Harvard Six City studies (Ware et al. and Neas et al.), and the other three had little in common. The British studies provide the highest estimated odds ratio, 1.27, the Six City Studies give a combined estimate of 1.13, and the other three studies give an estimate of 1.05. The estimates from the British and Six City studies have confidence intervals that do not include 1.0.

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, especially those of elementary school age. The estimates are generally centered about an odds ratio of 1.2 with 95% confidence limits of 1.1 to 1.3, although the studies using measured NO_2 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 hierarchical analyses. Thus, any lack of homogeneity is not a major concern.

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 Schwartz (1990) 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. It is also possible to delete any one study from the analysis, and still obtain nearly the same results. In fact, any two studies can be deleted from the analysis, and the estimated odds ratio will have a confidence interval that does not include 1.0.

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, since these are the studies most likely to get published. This is referred to as "publication bias." There are two reasons to be less concerned with publication bias in this particular situation. First, epidemiological

studies are very expensive and require the work of many individuals. The 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. In spite of this, it is of interest to contemplate an undiscovered study with results so negative that, when combined with the other studies, produces a confidence interval for the odds ratio that includes the value 1. If we assume that the hypothetical study would be the size of the Ware et al. study, then its odds ratio for increased respiratory symptoms as the result of a 30 $\mu g/m^3$ exposure would have to be 0.766.

14.6 CONCLUSIONS

The evidence from individual studies of the effect of NO_2 on respiratory disease is somewhat mixed. All but two of the 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 studies giving quantitative estimates of effects tend to show increases of respiratory illness in children associated with long-term exposure to NO_2. Combining the studies as if the end points were similar gives an estimated odds ratio of 1.20 (95% confidence limits of 1.1 and 1.3) for the effect of gas stove use on respiratory illness. When combined, the studies indicated that an increase of 30 $\mu g/m^3$ in NO_2 exposure would result in an increase of about 20% in the odds of respiratory disease, subject to the assumptions made for the synthesis. Although several assumptions were made to combine the studies, the consistency between the individual studies is demonstrated, indicating greater strength to the data base that suggests that the effect is real. To the extent that health effects depend on peak exposures rather than average exposures, the exposure estimates used introduce measurement error. These studies can not distinguish between the relative contributions of peak and average exposures and their relationship with the observed health effects. However, the estimated effect is almost surely an underestimate, given the problems of misclassification of exposures and outcomes. The effect was not dependent on any one or

two studies. Thus, the combined evidence is supportive for the effects of exposure to NO_2 on respiratory disease in children under 12 years of age.

Only the Harvard Six City study attempted to relate some measure of indoor and outdoor NO_2 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 NO_2 on pulmonary function.

14.7 SUMMARY

This chapter discusses the epidemiological evidence for the effects of NO_x on human health. The major emphasis is on the effects of NO_2 because it is the NO_x compound studied in most epidemiological studies and because it is the NO_x compound currently of greatest concern from a public health perspective. The results from the various epidemiological studies of effect of NO_2 exposure 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) selection bias, (4) adjustment for covariates, (5) publication bias, (6) internal consistency, and (7) plausibility of the effect based on other evidence. The health outcome should be an outcome for which there is good reason to suspect that NO_2 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. Those studies which address these factors more appropriately provide a stronger basis for the conclusions that they draw. Consistency between studies indicates the level of the strength of the total data base.

Respiratory illness and factors that affect its rate and/or severity are important public health concerns. This chapter discussed epidemiological findings relating NO_2 exposure to

respiratory illness. This effect is of public health importance because of the potential for exposure to NO_2 and since childhood respiratory illness is common (Samet et al., 1983; Samet and Utell, 1990). This takes on added importance since recurrent childhood respiratory illness may be a risk factor for later susceptibility to damage to the lungs (Glezen, 1989).

A biologic basis for an increased susceptibility for respiratory illness with exposure to NO_2 is found in studies of the respiratory host defense system. The host defense system provides protection against inhaled infectious and chemical agents. The available information, discussed in the animal toxicology and clinical studies chapter, indicates that exposure to NO_2 impairs one or more defense mechanisms, leaving the host susceptible to respiratory illness.

Several of the epidemiological studies gave some evidence that repeated NO_2 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 NO_2 exposure. A reanalysis of that 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 to 1.42. Melia et al. (1980) described on the results of a third study of respiratory symptoms in children aged six to seven 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 NO_2 levels. Melia et al. (1982) reported on a fourth study of children in England. Multiple logistic regression analysis of this data was not statistically significant, although the symptoms were positively related to NO_2 exposure. An EPA reanalysis suggests that an increase of 30 $\mu g/m^3$ in bedroom NO_2 levels yields an 11% increase in the odds of respiratory illness. In a final Melia et al. (1983) study, 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.

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 to 1.19) for a LRI 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 cohort enrolled later, used a different symptom questionnaire and made indoor NO_2 measurements for all subjects. They found increased respiratory disease which gave an estimated odds ratio of 1.47 (95% confidence limits of 1.17 to 1.86) at an exposure of 31 $\mu g/m^3$.

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 odds ratio of 1.14, with 95% confidence limits of 0.86 to 1.50. Ekwo et al. (1983) studied respiratory symptoms in relation to gas stove use in Iowa City, IA. Gas stove use provided in 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 yielded an odds ratio of 0.94 with 95% confidence limits of 0.66 to 1.33, thus showing no evidence of an increase in respiratory disease with increasing 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 1.10, with 95% confidence limits of 0.74 to 1.54.

Other studies did not provide sufficient information to derive any quantitative estimates of the effect of NO_2 or gas stove use on respiratory disease. Several other studies contain information about the effects of NO_2 on respiratory illness, but most of the studies either used very different health end points or did not provide quantitative estimates of the effects. The study of Berwick (1987) showed increased relative risk of respiratory disease in some age groups, but not in others. Euler et al. (1988) did not find increases in chronic respiratory symptoms associated with changes in NO_2 levels. Vedal et al. (1987) studied children in the Chestnut Ridge in Pennsylvania, but did not obtain any relationship between daily respiratory symptoms and NO_2 levels.

Several of the studies suggest an increase in respiratory symptoms in children from exposure to levels seen with gas stove use as compared to electric stove use. The associations in the majority of the studies do not reach statistical significance. The consistency of these studies was examined and the evidence synthesized in the following quantitative analysis. The studies described used different indicators to study health endpoints. In order to compare these studies, a standard end point was defined, and then each study will be compared with

this standard end point. For purposes of discussion, the end point chosen was the presence of lower respiratory disease in children aged 12 or under. It was assumed that the relative odds of developing lower respiratory disease is similar across this age range as a function of NO_2 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 an increase of 30 $\mu g/m^3$ in NO_2 exposure. This is approximately the increase seen as a result of gas stove use as compared with electric stove use in both the U.S and England.

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

Graphs of the odds ratio from each study are presented in Figure 14-3. Each curve can be given one of three interpretations: (1) the normal approximation to the likelihood of the logarithm of the odds ratio, (2) a distribution such that the 0.025 percentile and the 0.975 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.

Two methods of combining evidence are employed here. The first of these is a Bayesian method, and is described by Eddy (1989) and Eddy et al. (1990a,b) as it is used here. The result of the analysis is a distribution for our belief about the location of the true value of the odds ratio. This can be done separately for each study or for the combination of all studies, and the results are shown in Figure 14-3. The second basic model assumed that the parameter of interest is not fixed, but is itself a random variable from a distribution. These models are 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 effect model.

The conclusion from the several models was the same, namely that the odds ratio is estimated to be about 1.2 with 95% confidence limits ranging from about 1.1 to 1.3. The

measured NO_2 studies gave an estimated odds ratio of 1.27, whereas the others yield an estimate of 1.18, which is consistent with a measurement error effect.

The individual evidence of the effect of NO_2 on respiratory disease is somewhat mixed. All but one of the 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 studies giving quantitative estimates of effects tend to show increases of respiratory illness in children associated with long-term exposure to NO_2. When combined, the studies indicated that of increase of 30 $\mu g/m^3$ in NO_2 exposure would result in an increase of about 20% in respiratory disease, subject to the assumptions made for the synthesis. Although several assumptions were made to combine the studies, the consistency between the individual studies is demonstrated, indicating greater strength to the data base which suggests that the effect is real. To the extent that health effects depend on peak exposures rather than average exposures, the exposure estimates used introduce measurement error. These studies can not distinguish between the relative contributions of peak and average exposures and their relationship with the observed health effects. However, the estimated effect is almost surely an underestimate, given the problems of misclassification of exposures and outcomes. The effect was not dependent on any one or two studies. The results of this analysis are not sensitive to the inclusion (or exclusion) of any one study. In fact, any two studies can be eliminated, and the 95% confidence limits will exclude the no effect odds ratio of 1.0. Thus, the combined evidence is supportive for the effects of exposure to NO_2 on respiratory disease in children under 12 years of age.

Only the Harvard Six City study attempted to relate some measure of indoor and outdoor NO_2 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 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 NO_2 on pulmonary function.

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APPENDIX 14A. SUMMARY OF HEALTH EFFECTS OF NO_2

TABLE 14A-1. SUMMARY OF THE EFFECTS OF NO_2 ON RESPIRATORY ILLNESS[a]

Study	NO_2 Exposure	Effects Seen	Discussion	References
Children aged 6 to 11 with and without gas stoves in the house in 28 areas of England and Scotland.	Gas stove use.	Respiratory symptoms (bronchitis, cough, wheeze) higher with gas stoves present.	Indoor NO_2 not measured at time of study.	Melia et al. (1977)
4827 Children aged 5 to 10 years in a second British Cohort.	Gas stove use.	Respiratory symtoms higher in children with gas stoves. Smoking in home, and use of pilot lights examined.	Result significant for boys but not girls.	Melia et al. (1979)
808 Children aged 6 to 7 in Middlesborough, England.	NO_2 7 to 318 $\mu g/m^3$ (0.004-0.169 ppm) in bedroom with gas stoves, 6 to 70 $\mu g/m^3$ (0.004-0.0371 ppm) without gas stoves.	Incidence of respiratory illness higher in homes with gas stoves ($p=0.10$).	Marginally significant results. Subset data with gas stove only.	Melia et al. (1980) Goldstein et al. (1979) and Florey et al. (1979)
Children aged 5 to 6 in Middlesborough, England.	NO_2 levels ranged from 16 to 530 $\mu g/m^3$ (0.009-0.281 ppm). Gas stoves only.	Slight trend for increase in respiratory disease rates.	Results not even marginally significant.	Melia et al. (1982)
Six City study of 9,000 grade school children in the United States.	7 to 49 $\mu g/m^3$ (0.004-0.26 ppm) estimate of total personal exposure gas stove use used as a surrogate.	Marginally significant association of gas stove use with respiratory illness rate in children under 2.	Differences marginally significant. Incomplete indoor exposure.	Speizer et al. (1980) Ware et al. (1984)
Six City study of 6,273 children aged 7 to 11 in the United States.	Measured indoor exposure average 31 $\mu g/m^3$ (0.0164 ppm) higher in homes with gas stoves and pilot lights.	Increases in individual symptoms of 5-29%, combined symptom odds ratio of 1.47.	Larger increase in combined symptoms than in individual symptoms.	Dockery et al. (1989) Neas et al. (1990, 1991)
Respiratory illness study of 1,565 infants in the Tayside region of Scotland.	Gas stove use.	Increase of 14% in respiratory disease in homes with gas stove use.	Indoor NO_2 not measured at time of study. Results not statistically significant.	Ogston et al. (1985)
Respiratory symptom study of 1,355 children aged 6 to 12 in the Iowa City Schools.	Gas stove use.	Increase in chest congestion of 10% and increase of hospitalization of more than 100%.	Only hospitalization was statistically significant. End points different from other studies. No indoor air measurements.	Ekwo et al. (1983)

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TABLE 14A-1. (cont'd) SUMMARY OF THE EFFECTS OF NO_2 ON RESPIRATORY ILLNESS

Study	NO_2 Exposure	Effects Seen	Discussion	References
Respiratory symptom study of 6 to 9-year-old children in the SE region of the Netherlands.	Weekly average NO_2 ranged from 22 to 42 $\mu g/m^3$ (0.012-0.022 ppm). Gas appliance.	No evidence of any increase in respiratory disease.	Wide confidence intervals for effects measured.	Dijkstra et al. (1990) Houthuijs et al. (1987) Brunekreef et al. (1987)
Respiratory illness study of children under age 12 in Columbus, OH.	Annual average NO_2 ranged from 38 to 94 $\mu g/m^3$ (0.020-0.050 ppm).	No difference found in children's respiratory illness.	Adjustments for previous illness may have lost any effect. Analysis model different from other studies.	Keller et al. (1979a,b) Mitchell et al. (1974)
Respiratory symptom study of children aged 0 to 5 in four areas of Switzerland.	Outdoor mean NO_2 levels ranged from 25 to 52 $\mu g/m^3$. Indoor levels ranged from 11 to 34 $\mu g/m^3$.	Increase symptom scores in children exposed to outdoor NO_2 levels of 30 $\mu g/m^3$ (0.0159 ppm).	End points different from other studies. Effect of indoor NO_2 marginally significant.	Braun-Fahrlaender et al. (1989)
Twelve-week study of lower and upper respiratory symptoms in women and children.	Outdoor NO_2 levels ranged from 9 to 19 $\mu g/m^3$. Indoor NO_2 ranged from 6 to 91 $\mu g/m^3$ (0.003-0.048 ppm).	Children under age 7 with exposure to more than 30 $\mu g/m^3$ (0.0159 ppm) had 2.17 times the LRI.	Inconsistent results by age group, possibly due to very small sample sizes available.	Berwick et al. (1984, 1987, 1989) Berwick (1987) Leaderer et al. (1986)
Study of chronic respiratory disease symptoms in 8,572 Southern California Seventh-Day Adventist.	Hours of SO_2 exposure above 4 pphm, oxidants above 10 pphm, and TSP above 200 $\mu g/m^3$. NO_2 exposure levels were not linked to health effects.	Relative risks of about 1.2 for the pollutants listed.	No relationship with measured NO_2.	Euler et al. (1988)
Study of 5,561 adults aged 25 to 39 living in Lancaster and Glendora, CA.	Annual means ranged from 2.8 to 3.9 ppm in Lancaster and from 9.5 to 12.0 ppm in Glendora (Azuza station).	Decreased FEV_1 and FVC and increased cough, phlegm, and wheezing found in Glendora when compared to Lancaster.	Areas measured at different times, other differences impossible to adjust for.	Detels et al. (1981a,b)

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TABLE 14A-1. (cont'd) SUMMARY OF THE EFFECTS OF NO_2 ON RESPIRATORY ILLNESS

Study	NO_2 Exposure	Effects Seen	Discussion	References
Respiratory disease study of approximately 2,800 adults and children for three different 6-month periods in Chattanooga, TN.	Annual averages of daily NO_2 levels ranged from 43 to 91 in 1972, and from 22 to 40 $\mu g/m^3$ in 1973.	Increased respiratory illness in all age and sex groups found in 1972, but not found in 1973.	No indoor measurements. NO_2 measured by Jacobs-Hochhesiser method for some periods.	Love et al. (1982)
Study of respiratory symptoms in four French cities.	NO_2 12 to 16 $\mu g/m^3$ (0.006-0.008 ppm) daily means; NO 7 to 140 $\mu g/m^3$ daily means.	No relationship of respiratory symptoms with NO_2.	No indoor measurements. Low NO_2 exposure.	PAARC (1982a,b)
Respiratory symptom and pulmonary function study of 4th - 6th graders in Akron, OH.	NO_2 averaged 54 $\mu g/m^3$ (0.029 ppm) in polluted area, 37 $\mu g/m^3$ (0.019 ppm) in cleaner area.	Higher rates of acute respiratory disease in polluted area.	No indoor measurements. Small differences seen. Relatively low NO_2 exposure.	Mostardi et al. (1981a)
Study of asthma emergency room visits and hospitalizations for children at the Children's Hospital of Los Angeles.	Monthly average NO_2 levels ranged from 12 to 18 pphm, and NO levels ranged from 13 to 59 pphm.	Increased asthma emergency room visits and hospitalizations correlated significantly with NO and NO_2, as well as coefficients of haze and hydrocarbons.	No indoor measurements. Pollutants could not be separated.	Richards et al. (1981)
Study of acute illnesses in elderly patients in long-term care.	Mean values of nitrogen oxides indoors was 0.062 ppm and outdoors was 0.055 ppm.	Respiratory diseases correlated with NO_2.	No adjustment for covariates. Details of pollution monitoring not given.	Loewenstein et al. (1985)
Study of COPD rates in COPD and normal subjects in Helsinki, Finland.	NO_2, 5 to 70 $\mu g/m^3$ (0.003-0.0371 ppm).	COPD correlated with NO_2.	No indoor measurements. No adjustments for covariates other than temperature. Small sample sizes.	Pershagen et al. (1984)

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TABLE 14A-1. (cont'd) SUMMARY OF THE EFFECTS OF NO_2 ON RESPIRATORY ILLNESS

Study	NO_2 Exposure	Effects Seen	Discussion	References
Los Angeles Student Nurse Data originally collected daily from 1962-64, reanalyzed by Schwartz. Symptoms included eye irritation, cough, phlegm, sore throat, headache, chest discomfort.	Daily outdoor NO_2 exposure ranged from 1 to 45 ppm over a 3-year period. Sites located within 2½ miles of the subject.	NO_2 exposure related to phlegm, sore throat, and eye irritation.	No indoor NO_2 exposure measurements, and only one outdoor station .	Schwartz et al. (1988); Schwartz and Zeger, 1990
Respiratory disease study of 4,071 children aged 5 to 14 in Pennsylvania. Results based on parents questionnaire.	Presence of gas stove used as a surrogate for NO_2 exposure.	No significant association between use of gas stove and any symptom or illness variable.	No indoor NO_2 exposure. No significant results.	Schenker et al. (1983)
The data reported by Love et al. 1982 were analyzed for short-term effects.	Daily NO_2 levels were split into three categories using the cut-offs of 75 and 150 $\mu g/m^3$ (0.04-0.08 ppm).	No consistent short-term effect of NO_2 on acute respiratory disease was found.	No indoor measurements. No biological explanation for results found.	Harrington and Krupnick (1985)
Study of influenza cases over a 2-month period in Sofia, Bulgaria.	NO_x had means of 21 and 37 $\mu g/m^3$ (0.014-0.45 ppm) for the two epidemics.	NO_x related to NO_2. Two-day lag in illnesses.	No indoor measurements. No measurement method for NO_x given.	Kalpazanov et al. (1976)
Acute symptom study of 35 asthmatics in Barcelona, Spain .	Mean daily maximum hourly NO_2 levels ranged from 271 to 846 $\mu g/m^3$ (0.14-0.45 ppm) for two weeks in December, 1985.	Symptoms in asthmatics not related to NO_2 levels. Second study relates symptoms to soybean dust.	Effects related to soybean dust in definitive study.	Anto et al. (1986) Anto et al. (1989)
Study of asthma in 20 children seen in two Japanese hospitals.	Quarterly mean NO_2 levels ranged from 3.0 to 5.7 pphm.	No relationship found between frequency of attacks and NO_2 levels.	No indoor measurements. Attack rates are generally longest when NO_2 is highest.	Watanabe et al. (1984)
Ecological study attempting to relate nitrates and oxides of nitrogen to mortality.	County-wide NO_x estimates.	Two million death certificates examined.	Both positive and negative coefficients found in the multiple regression, which were almost always not statistically significant.	Mendelsohn and Orcult (1979)

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TABLE 14A-1. (cont'd) SUMMARY OF THE EFFECTS OF NO_2 ON RESPIRATORY ILLNESS

Study	NO_2 Exposure	Effects Seen	Discussion	References
Ecological analysis of mortality rates and air pollution.	NO_2 data from the National Air Sampling Network.	Mortality rate associated with various disease end points.	Quality and applicability of both the mortality and monitoring data preclude considering the results as anything but speculative.	Hickey et al. (1970)
Study of variations in daily mortality in relation to daily air pollution in several U.S. Cities from 1962-65.	Nonspecific NO_x levels.	Multiple regression analysis showed significant negative association between winter mortality in New York City and daily NO_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.	The results suggest that other factors are causing the relationship.	Lebowitz (1971)
A study of the prevalence of respiratory symptoms in school children in two Israeli cities with different NO_2 levels.	Maximum NO_x levels in the cities of 62 and 23 $\mu g/m^3$, respectively.	Various respiratory symptom rates were higher in Ashdad, the more heavily polluted city. Confounding factors controlled.	Effects related to both SO_2 and NO_x.	Goren and Hellmann (1988)
Case-control study of 231 children in the Netherlands for respiratory symptoms vs. NO_2 levels with different NO_2 levels.	Indoor NO_2 levels.	Respiratory symptoms determined by questionnaire and school health survey data.	No difference in levels between case and controls.	Hoek et al. (1984)
Study of changes in respiratory symptom rates in relation to NO_2 levels in a rural district of Japan.	NO_2 measurements from 1974-79.	Significant correlation between daily maximum NO_2 and subacute phlegm, cough, and wheezing for prick skin test (house dust extract)-positive children.	Similar results for SO_2 during this period. The fact that SO_2 and NO_2 were correlated and that no covariates were included in the analysis means the study can only be considered suggestive.	Kagamimori et al. (1986)

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TABLE 14A-1. (cont'd) SUMMARY OF THE EFFECTS OF NO_2 ON RESPIRATORY ILLNESS

Study	NO_2 Exposure	Effects Seen	Discussion	References
Study of respiratory illness in 2,900 school children vs. levels of NO_2 in Oska Prefecture, Japan.	Annual outdoor NO_2 levels.	Children attending schools in the highest polluted area had the highest rates of bronchial asthma and recurring respiratory infections.	No indoor NO_2 data. NO_2 levels analyzed in relation to distance between children's homes and distance from highways.	Nagira et al. (1981)
Study of incidence of mediastinal and subcutaneous emphysema in young bronchial asthma patients in relation to NO_2 levels in Japan.	Daily average outdoor NO_2 levels.	Report correlation between NO_2 levels and occurrence at mediastinal and subcutaneous emphysema.	No indoor NO_2 data. Other factors that cause mediastinal and subcutaneous emphysema not evaluated.	Odajima and Baba (1987)
Study of respiratory symptoms in over 90,000 school children in Japan in relation to NO_2 levels.	Outdoor mean NO_2 value 3 years prior to the survey.	Asthma-like symptoms was higher in district with the highest NO_2 concentration.	No indoor NO_2 data, confounding with other pollutants. Confounding due to smoking and socio-economic factors.	Tsunetoshi et al. (1987)
Study of respiratory symptoms in the district of Pisa, Italy.	Gas stove use.	High prevalence rate for respiratory symptoms for those using tank gas for cooking.	No NO_2 measurements.	Viegi et al. (1990)
Study respiratory infection in children and adults and absenteeism in Helsinki, Finland.	Annual average of NO_2 was 47 $\mu g/m^3$ (0.025 ppm).	The level of NO_2 has little effect on the frequency of disease. The effect of low temperature is highly significant.	No indoor NO_2 measurements. Possible confounding with temperature.	Pönkä (1990)
Study of respiratory illnesses in children and mothers in Hong Kong.	Mean levels of NO_2 estimated by personal samplers.	Among 312 mothers, statistically significant allergic rhinitis for NO_2 levels of 22.6 vs. 19.0 ppb, while among the children there was no statistically significant difference in presence vs. absence of the respiratory symptoms by NO_2 levels.	Difference in mean levels of NO_2 for children for different symptoms were very small, ranging from 0.00 to 1.79 ppb (0 to 3.4 $\mu g/m^3$).	Koo et al. (1990)

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TABLE 14A-1. (cont'd) SUMMARY OF THE EFFECTS OF NO_2 ON RESPIRATORY ILLNESS

Study	NO_2 Exposure	Effects Seen	Discussion	References
Study of croup incidence and NO_2 levels in Bochum, Germany.	Outdoor NO_2 levels, daily averages.	Positive correlation between NO_2 levels and incidence of croup as determined by hospital admissions.	No indoor NO_2 data. No discussion of confounding factors.	Severien and Mietens (1987)
Study of croup incidence and NO_2 levels in Mannheim and Darmstadt, Germany.	Outdoor NO_2 levels, annual and monthly average.	Monthly NO_2 average in Mannheim but not in Darmstadt showed linear correlation with monthly cases of croup.	No indoor NO_2 data. No discussion of confounding factors.	Wemmer (1984)
Study on the influence of various factors to include NO_2 on the incidence of croup in Innsbruck, Austria.	NO_2 levels were measured by a chemolumicescence detector at a measuring station in the inner city area.	Pseudocroup has a multifactor pathogenesis. Rapid changes in air pollutants (especially NO and NO_2) are followed by increased occurence of croup.	No indoor NO_2 data. Weather conditions cannot by this statistical evaluation be eliminated as an important factor whether directly or indirectly.	Guggenbichler et al. (1990)
A pilot study of 130 third graders of NO_2 levels and hematological, clinical and immunological end points in Rostock, Germany.	Indoor monitors for NO_2 and other pollutants.	Selected results of questionnaires and smoking history.	Limited findings presented in the preliminary study.	Thielebeule and Huelsse (1989)
Study of NO_2 exposure and respiratory disease function and symptoms in Japan.	Indoor NO_2 measurements, urine hydroxproline measurements.	Respiratory disease questionnaires, pulmonary function tests in 1 thousand 3 year-old children.	Ongoing study expected completion in 1989-90 with analysis at a later date.	Tominaga and Ono (1985)
Study of acute illness symptoms and pulmonary function in New York in relation to NO_2 exposure.	Measures of personal exposure to NO_2.	Asthmatic and nonasthmatic women and children are evaluated for PFT and respiratory symptoms.	Preliminary data.	Goldstein et al. (1987)
An ongoing longitudinal study of respiratory illness during the first year of life in relationship to NO_2 exposure in Albuquerque, New Mexico.	Personal exposure estimate based on Palmes tube and activity data.	Careful standardized ascertainment of illness, assessment of potential confounding factors.	Study in progress.	Samet and Spengler (1989)

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TABLE 14A-2. SUMMARY OF EFFECTS OF NO_2 ON PULMONARY FUNCTION

Study	NO_2 Exposure	Effects Seen	Discussion	References
Six City study of 9,000 grade school children.	7 to 49 $\mu g/m^3$ (0.004-0.026 ppm) estimate of total personal exposure gas stove use used as a surrogate.	FEV_1, and FVC decreases ranged from 0.2 to 0.6%, depending on examination and adjustment.	Differences marginally significant, incomplete indoor exposure.	Speizer et al. (1980)
Six City study analyzed for effects on pulmonary function growth.	7 to 49 $\mu g/m^3$ (0.006-0.04 ppm) estimate of total personal exposure gas stove use used as a surrogate.	No effect of gas stove use on growth of pulmonary.	Differences marginally significant, incomplete indoor exposure.	Berkey et al. (1986)
Lung function panel study of 351 children at the Chestnut Ridge Elementary School.	NO_2 ranged from 12 to 79 $\mu g/m^3$ (0.006-0.04 ppm).	Peak flow not affected by pollutant levels.	No indoor pollutant measurements made, outdoor levels were low.	Vedal et al. (1987)
Lung function study of 117 middle class households in Tucson, AZ.	Gas stove use.	Peak flow was marginally related to gas stove use ($p=0.066$).	No indoor NO_2 measurements made.	Lebowitz et al. (1985)
Study of traffic policemen in urban Boston with nearby suburban areas.	75 to 103 $\mu g/m^3$ (0.04-0.055 ppm) annual mean daily concentrations.	No differences in various pulmonary function tests.	No indoor measurements, small sample sizes.	Speizer and Ferris (1973a,b)
Study of $FEV_{.75}$ in school children in Chattanooga, TN.	Ambient annual NO_2 levels as high as 286 $\mu g/m^3$ (0.15 ppm) (Saltzman method).	Suggestion of lower $FEV_{.75}$ means in high NO_2 area.	No indoor measurements, much of the NO_2 measured by Jacob-Hochheiser method, no strong differences found.	Shy et al. (1970a,b)
Study of nonsmoking adults in Los Angeles and San Diego.	43 to 96 $\mu g/m^3$ (0.023-0.051 ppm) annual mean daily concentrations 113 to 118 $\mu g/m^3$ 90th percentile.	No difference in several ventilatory measurements including spirometry and flow volume curves.	No indoor exposures, complicated outdoor exposures.	Cohen et al. (1972)

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TABLE 14A-2. (cont'd) SUMMARY OF THE EFFECTS OF NO_2 ON PULMONARY FUNCTION

Study	NO_2 Exposure	Effects Seen	Discussion	References
Lung function study of 12 asthmatic children living in the Sunair Home in California.	Maximum hourly value of 22 pphm.	Morning peak flow was reduced, but afternoon peak flow was not.	Effects of other pollutants could not be separated. Seasonal factors not adjusted for. No indoor pollutant measurements.	Richards et al. (1981)
Office workers in Los Angeles and San Francisco.	65 to 130 $\mu g/m^3$ (0.034-0.07 ppm) median NO_2 110 to 250 90th percentile.	No difference in most pulmonary tests.	No indoor measurements. Small sample size.	Linn et al. (1976)
Respiratory symptom and pulmonary function study of 4th through 6th graders in Akron, OH.	NO_2 averaged 54 $\mu g/m^3$ (0.029 ppm) in polluted area.	Small decrease seen in ratio of FEV_1 to FVC.	No indoor measurements. Small differences seen. Relatively low NO_2 exposure.	Mostardi et al. (1981a, b)
Study of pulmonary function in French cities.	NO_2 12 to 16 $\mu g/m^3$ (0.006-0.008 ppm) daily means. NO 7 to 140 $\mu g/m^3$ daily means.	No decrease in pulmonary function related to NO_2 exposure.	No indoor measurements. Low NO_2 exposure.	PAARC (1982a, b)
Study of 5,561 adults aged 25 to 39 living in Lancaster and Glendora, CA.	Annual means ranged from 2.8 to 3.9 ppm in Lancaster and from 9.5 to 12.0 ppm in Glendora (Azyza station).	Decreased FEV_1 and FVC and increased cough, phlegm, and wheezing found in Glendora when compared to Lancaster.	Areas measured at different times. Other differences between areas impossible to adjust for.	Detels et al. (1981a,b)
Japanese study of school children in Japan, aged 11 years.	40 to 360 $\mu g/m^3$ (0.02-0.19 ppm) 1-h values at time of testing.	Correlation of peak flow and 25% and 50% FVC with NO_2, NO, SO_2, and TSP.	Correlations not adjusted for other pollutants.	Kagawa and Toyama (1975)
Case control study of 213 nonsmoking women in the Tecumseh Community Health study evaluated Fev values vs. various cooking fuels.	Cooking fuels, duration of exposure to cooking fuels, and exhaust fan use.	Association of low FEV and gas cooking was marginally significant ($p=0.07$).	No indoor NO_2 data. Small sample size reduces power, especially the limited number of gas stoves in sample.	Jones et al. (1983)

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TABLE 14A-2. (cont'd) SUMMARY OF THE EFFECTS OF NO_2 ON PULMONARY FUNCTION

Study	NO_2 Exposure	Effects Seen	Discussion	References
NHANES II lung function data coupled with EPA's SAROAD aerometric data. Lung function measurements included FVC, FEV_1, and PEF.	NO_2 exposures ranged from less than .01 to .08 ppm. Individual estimates based on average of all stations within 10 miles of census tract.	Highly significant regression coefficients showing a decrease in FVC, FEV_1 and PEF with increasing NO_2 exposure.	No indoor NO_2 exposure measurements. Outdoor estimates over a wide area.	Schwartz (1989)
In a sample of over 16,000 children, PFT were compared to gas stove use.	Gas stove use.	Marginally significant decrease ($p=0.052$) in lung function in girls 9 to 13 years of age. PFT numbers were adjusted for parental smoking habits.	No indoor measurements.	Hasselblad et al. (1981)
A study of PFT in women in relation to NO_2 levels in the Netherlands.	Weekly personal exposure estimates ranged from 15 to 300 $\mu g/m^3$ (0.008-0.159 ppm).	Statistically significant negative associations between various pulmonary function measures and NO_2 exposure in nonsmoking women.	No association with longitudinal pulmonary function decline.	Fischer et al. (1985, 1986) Remijn et al. (1985)
A study of the effect of domestic air pollution on respiratory function in a group of housewives in Lebanon, CT.	Gas stove use.	Some decreases observed in lung function in homes with gas stoves.	Sample sizes too small to give meaningful information.	Hosein and Corey (1986)
Study of lung function and respiratory function in relation to homes with coal or gas cooking fuels.	NO_2 levels of over 400 $\mu g/m^3$ (0.21 ppm) in kitchens.	Lung function measures in women were reduced by elevated levels of pollutants. No respiratory symptoms were related to gas.	Method of analysis and adjustments for covariate were not given.	Liu and Wang (1987)

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TABLE 14A-2. (cont'd) SUMMARY OF THE EFFECTS OF NO_2 ON PULMONARY FUNCTION

Study	NO_2 Exposure	Effects Seen	Discussion	References
Study of children's pulmonary function in relation to NO_2 exposure in the Netherlands.	NO_2 levels measured by Palmes Tubes in homes, mean concentration ranged from 23 to 72 $\mu g/m^3$ (0.012-0.038 ppm) NO_2.	PFT were evaluated in over 800 children aged 6 to 12 years. No relationship was found. The power of the study to detect small effects on lung function was considered adequate.	Exposure measure may not have captured short-term peak concentrations.	Brunekreef et al. (1990)
Study of NO_2 exposure on pulmonary function and symptoms in Japan.	Indoor NO_2 measurements, urine hydroxyproline measurements.	Respiratory disease questionnaires, pulmonary function tests in 1 thousand 3 year-old children.	Ongoing study expected completion in 1989-90 with analysis at a later date.	Tominaga and Ono (1985)
Study of children's bronchial reactivity in relation to NO_2 exposure in Switzerland.	NO_2 levels measured by passive samplers.	Bronchial reaction to carbachol measured in 312 school children in two exposure groups.	Urban NO_2 levels of 36.2 $\mu g/mg^3$ vs. 26.2 $\mu g/m^3$ in rural areal. 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.	Gschwend-Eigenmann et al. (1989)
Study of SO_x, NO_2, hydrocarbons and particulate exposure on pulmonary function in two cities in Southern California.	Outdoor daily peak hourly levels for NO_2 range from 0.03 to 0.11 ppm.	Chronic exposure to the pollutant mix results in less rapid growth of lung function in children and a greater rate of deterioration in adulthood.	The proportion of participants retested in the study was low (45 to 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.	Detels et al. (1991)

[a]Abbreviations: NO_2 = Nitrogen dioxide; SO_2 = Sulfur dioxide; TSP = Total suspended particulate; FEV = Forced expiratory volume; FVC = Forced vital capacity; NO = Nitric oxide; COPD = Chronic obstructive pulmonary disease; PFT = Pulmonary function tests; PEF = Peak expiratory flow.

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15. CONTROLLED HUMAN EXPOSURE STUDIES OF OXIDES OF NITROGEN

15.1 INTRODUCTION

This chapter discusses the effects of nitrogen oxides (NO_x) on human volunteers exposed under controlled conditions. The NO_x species of major concern is nitrogen dioxide (NO_2). Nitric oxide (NO), and nitrates (XNO_3) have also been evaluated in controlled human exposures and nitric acid (HNO_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 provides summaries and critiques of studies conducted since then, while also including the earlier literature.

Controlled human exposure studies deal with relatively brief 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 <4h), NO_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 prevalence of NO_2 in both outdoor and indoor environments, there are major concerns regarding the potential impact of NO_2 exposure on human health particularly with regard to the lung. Dosimetry modeling and animal histological studies indicate that NO_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_2. These concerns are presented in the following list of critical questions. Some of these questions are of a "generic" nature, applying to many ambient air pollutants and some are specific to NO_2 inhalation. Several of the questions may be answered only partially by controlled human exposure studies.

NO_2 Exposure and Human Health: Critical Questions.

1. Does short-term NO_2 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 who exhibit greater responses to NO_2 exposure than the average healthy subject? The possible groups may include young children, adolescents, elderly subjects, 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 NO_2 first)?

3. Does NO_2 cause an inflammatory response in the lungs of healthy individuals or patients with lung disease? Specifically, does NO_2 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 NO_2 exposure cause increased responses of the lung (including airways responsiveness, lung function, inflammation, cell damage, etc.) to other pollutants such as ozone (O_3), sulfur dioxide (SO_2), acid aerosols; to bronchoconstrictors such as histamine or methacholine; to other agents such as cold-dry air or exercise; or to specific antigenic substances?

5. Does NO_2 exposure alter respiratory tract host defenses? Does NO_2 alter airway epithelial permeability or mucociliary clearance? Does NO_2 exposure alter local or systemic immune response to infection? Is killing or removal of microorganisms impaired by NO_2 exposure? Is inflammatory response or tissue injury caused by microorganisms worsened by coincident NO_2 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_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, may include 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. Characterization of asthma is discussed in more detail in Appendix A. 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 (increased airways resistance, restriction of lung volume) has a greater physiological/pathological consequence. 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 NO_2 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) and are 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 involve primarily "noninvasive" procedures. Lung 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), breathing pattern (frequency and depth of breathing), and a variety of other "breathing" tests have been utilized (Bouhuys, 1974). These tests provide information about some of the basic physiological functions of the lung. Dynamic spirometry tests (forced expiratory tests such as forced expiratory volume in 1 second [FEV_1], maximal and partial flow-volume curves [including those using gases of different densities such as helium], peak flow measurements, etc.) and specific airway resistance/conductance measurements (SR_{aw}, SG_{aw}) provide information primarily about large airway function. 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) (for FEV_1, the CV is about 3% and for SR_{aw} the CV is about 10 to 20%). However, because NO_2 deposits primarily in peripheral airways, many of the above tests may not provide the necessary information to fully evaluate the effects of NO_2. 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 been more utilized in recent years to determine response to pollutants 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 (AR) may change as a result of alterations in a disease state, such as inflammation associated with asthma, 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; Chai 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 the two bronchoconstrictors are well correlated ($r=0.70$) (Chatham et al., 1982). Unfortunately,

because of the large variety of methods and provocative agents used in the airway challenges and the absence of adequate standardization, 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 (^{99m}Tc)-labeled diethylene triamine penta-acetate or pentetate (DTPA). 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. In contrast to many animal models, humans have a wide 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 NO_2 exposure) if the sample size (i.e., the number of subjects) is too small to statistically detect an effect, if present. 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 NO_2 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 NO_2. 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, NO_2-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). Since controlled exposure studies of humans deal exclusively with acute or subchronic exposures, the applicability of these data are limited to short-term exposure effects and of limited usefulness in the evaluation of the effects of chronic NO_2 exposure.

More than 25 additional studies have become available since the 1982 Air Quality Criteria for Oxides of Nitrogen Document (U.S. Environmental Protection Agency, 1982a) on the effects of NO_2 on healthy, normal subjects. Several new studies of the effects of NO_2 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 NO_2 effects in sensitive subjects still requires concentration-response studies in moderately sensitive asthmatics, information concerning the inflammatory response to NO_2 inhalation, further examination of the effects of NO_2 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 NO_2 in humans.

One of the more important observations in studies of NO_2-exposed animals is that NO_2 exposure is associated with increased susceptibility to viral and bacterial infections due to impairment of host-defense mechanisms (see Section 13.2.2.1). Epidemiology studies clearly suggest a link between NO_2 exposure and increased rates of respiratory illness (Section 14.2.1). These studies have provided a basis for several recent investigations of human immune host defenses after NO_2 exposure. Studies have utilized both in vitro exposure of cultured human cells (e.g., macrophages) and in vivo exposures of human subjects.

Recently published reports generally support prior conclusions regarding the effects of NO_2 exposure on healthy young adults. There were several new studies that examined the specific effect of NO_2 on cardiopulmonary function in normal adults (see Section 15.2). The NO_2 concentrations ranged from 0.2 to 4.0 ppm. In another group of studies, the effects of pollutant mixtures or ambient air, of which NO_2 was a component, were examined. These studies are summarized in Section 15.2.3.

Studies examining the specific effects of NO_2 in normal subjects supported the conclusions 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 NO_2 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) were published in which NO_2 was a component of an ambient oxidant air mixture. The effects of ambient air exposures, if any, were attributed to O_3. There was no apparent influence of the very low (0.04 to 0.07 ppm) concentrations of NO_2 present in the ambient air.

In addition, several controlled exposure studies used pollutant mixtures containing NO_2 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; Kleinman 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 NO_2 in pollutant mixtures, which, in addition to NO_2, contained O_3, SO_2, and/or particles.

15.2 EFFECTS OF NO_x IN HEALTHY NORMAL SUBJECTS

Early studies indicated that the effect of NO_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_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). Also discussed are studies on NO and NO_2-mixtures.

15.2.1 Lung Function Effects of NO_2

This section is divided according to the concentrations of NO_2 used in the study. The first subsection deals with subjects exposed to greater than 1.0 ppm, the second with exposures to less than 1.0 ppm. All studies dealing with NO_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, etc. 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_2 levels greater than 1.0 ppm have been examined in several studies. In two studies, von Nieding and Wagner (1977) and von Nieding et al. (1979) studied 11 males exposed to 5.0 ppm NO_2 for 2 h while performing light, intermittent exercise. Airway resistance increased from 1.51 to 2.41 cm H_2O/L/s after 2 h of exposure. There was also an apparent decrease of arterial oxygen partial pressure (PaO_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_2 data prior to testing for significance. von Nieding and Wagner, 1977 state: "To increase the power of the tests PaO_2-differences $\leq$5 mm Hg and R_t-increases $\leq$0.5 cm H_2O/L/s were regarded as zero." This transformation would increase the likelihood of finding a significant effect.

TABLE 15-1. RESPONSES OF HEALTHY NORMAL SUBJECTS TO NO_2 EXPOSURE[a]

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects Gender (M/F)	Subject Characteristics	Notes on Effects
Abe (1967)	4.0-5.0	10	--	--	--	--			Bag exposure technique. Airway resistance increased 30 min after end of exposure. No change in spirometry.
Adams et al. (1987)	0.6	60	60	70	21-25	45-60	20 M	Normal	No effect of NO_2 on spirometry or airway resistance.
				50			20 F		
Beil and Ulmer (1976)	1.0	120	--	--			16	Healthy	After 7.5 ppm (120 min.) and 5.0 ppm (14 h), responsiveness to acetylcholine increased. Resistance increased after all but the 1.0-ppm exposure.
	2.5	120	--	--			16	11 S	
	5.0	120	--	--			16	5 NS	
	7.5	120	--	--			16		
	5.0	840	--	--			8	8 S	
Boushey et al. (1988) (part 2)	0.6	120/day for 4 days	60	~30-40	21.0	56	4 M/1 F	Healthy, NS, 21-36 yr, FEV_1/FVC% range 73-83%, "normal" methacholine responsiveness	No effects of repeated NO_2 exposure on respiratory function (SR_{aw}, FVC, FEV_1) or symptoms. Slight increase in circulating (venous) lymphocytes 1,792 ± 544 mm^3 (post-NO_2) vs. 1,598 ± 549/mm^3 (baseline). No change in BAL lymphocytes except an increase in natural killer cells 7.2 ± 3.1% (post-NO_2) vs. 4.2 ± 2.4% (baseline). No change observed in IL-1 or TNF.

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TABLE 15-1 (cont'd). RESPONSES OF HEALTHY NORMAL SUBJECTS TO NO_2 EXPOSURE[a]

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity Percent	Number of Subjects Gender (M/F)	Subject Characteristics	Notes on Effects
Bylin et al. (1985)	0.0 0.12 0.25 0.5	20	--	--	22	35	5 M/4 F	20-36 yr, NS	Suggestion of change in SR_{aw} in normals: SR_{aw} 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.
Chaney et al. (1981)	0.2	120	0	--	22	40	19 M (15)	Young adult, normal	Increase in blood glutathione levels after NO_2 exposure.
Drechsler-Parks et al. (1987)	0.60	120	60	25	24	54	8 M/8 F	51-76 yr	No statistically significant changes in lung function due to NO_2 exposure.
Drechsler-Parks (1987)	0.60	120	60	25	24	55	8 M/8 F	18-26 yr, NS	No significant changes in spirometry attributable to NO_2.
Folinsbee et al. (1978)	0.62	120	15 30	33 33	25	45	5 M 5 M	Healthy	No significant pulmonary function responses attributed to NO_2 exposure.
Frampton et al. (1989a)	0.6	180	60 (6x10)	~40	22	30	7 M/2 F	Healthy, NS	No change in spirometry, R_{aw}, or carbachol reactivity. No change in cell recovery or differential counts. Possible decrease in macrophage inactivation of virus in vitro. Possible sensitive subgroup.
	0.05 with 2.0 spikes	135 min 3x15 min	60 (6x10)				11 M/4F	Nonreactive (carbachol), no recent upper respiratory infection	

TABLE 15-1 (cont'd). RESPONSES OF HEALTHY NORMAL SUBJECTS TO NO_2 EXPOSURE[a]

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects Gender (M/F)	Subject Characteristics	Notes on Effects
Frampton et al. (1989b)	(1) 0.6	180	60	39	22.0	30	6 M/2 F	30.3 ± 1.4 yr	Total NO_2 uptake (1) 3.4 mg (2) 5.6 mg, (3) ~3.3 mg (4) 8.1 mg. BAL fluid analysis showed no significant effect on total protein or albumin. 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. No changes in percentage of lymphocytes or neutrophils. Concluded that NO_2 at these concentrations neither altered epithelial permeability nor caused inflammatory cell influx.
	(2) VAR (0.05 background with 3 x 15 min at 2.0 ppm)	180	60	43	22.0	30	11 M/4 F	25.3 ± 1.2 yr 32.6 ± 1.6 yr	
	(3) 0.6	180	60	~40	22.0	30	5 M/3 F	23.5 ± 0.7 yr	
	(4) 1.5	180	60	39	22.0	30	12 M/3 F		
								Healthy, NS	
Frampton et al. (1991)	See groups 1,2,4 above								There were no changes in airway mechanics (FVC, FEV_1, SG_{aw}). Responsiveness to carbachol was significantly increased after 1.5 ppm NO_2 (group 4) but not after the other exposures (groups 1,2). Degree of baseline responsiveness to carbachol was not related to response after 1.5 ppm.

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TABLE 15-1 (cont'd). RESPONSES OF HEALTHY NORMAL SUBJECTS TO NO_2 EXPOSURE[a]

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects Gender (M/F)	Subject Characteristics	Notes on Effects
Goings et al. (1989) (see Kulle and Clements, 1988)	1.0 2.0 3.0 0.0	120/day 3 days	--	--	22.2	60	21 22 22 23, 21 or 22	Healthy, NS, seronegative	Overall trend for a slight decrement in FEV_1 with NO_2 exposure (≤1%). Methacholine response decreased with consecutive tests but not as a result of NO_2 exposure or infection status. Study conducted over 3-year period. NO_2 did not significantly increase viral infectivity although a trend was observed. This study had a low power to detect small differences in infection rate.
Hackney et al. (1978)	1.0	120 (2 consecutive days)	60	Light	31	35	16	Healthy	Air-NO_2-NO_2-fixed exposure sequence. 1.5% decrease in FVC after second day of NO_2. Not clear that the decreased FVC is an NO_2 effect or an order effect. No other effects.
Hazucha et al. (1982)	0.1	60	--	--	21	40	15 M	23-39 yr, NS	No symptoms; no odor detection; no effect of on SR_{aw}.
Hazucha et al. (1983)	0.1	60	--	--	21	40	15 M	23-39 yr, NS	No symptoms; no odor detection; no effect of on SR_{aw}.
Kagawa and Tsuru (1979)	0.15	120	60	50W	27-29	50-60	6 M	19-24 yr	No symptoms; no pulmonary function effects. Suggested individual changes in SG_{aw}.

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TABLE 15-1 (cont'd). RESPONSES OF HEALTHY NORMAL SUBJECTS TO NO_2 EXPOSURE[a]

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects Gender (M/F)	Subject Characteristics	Notes on Effects
Kagawa (1982)	1.0 ppm NO	120	60	50W	28	55	8 M	19-24 yr	Suggested change in density dependance of expired flow.
Kagawa (1986)	0.30	120	60	50W	28	50-60	6	19-25 yr	No effect on SG_{aw}; other NO_2 mixtures studied but effect of NO_2 cannot be ascertained.
Kerr et al. (1979)	0.5	120	15	Light/ moderate	24	45	10	Healthy; three exsmokers in group	Decreased quasistatic compliance. Nonrandom exposure sequence air-NO_2. No change in spirometry or resistance. Apparent compliance change may be due to exposure order.
Kim et al. (1991)	0.18 0.30	30	10L 16H	L~25 H~72	22	45	9M	18-23 yr, "collegiate athletes"	No change in lung function.
Koenig et al. (1985)	0.12	60	--	--	22	75+	4 M/6 F	13-18 yr	No effects on lung function.
Koenig et al. (1987a,b)	0.12 0.18	40 40	10 --	32.5 --	22	75	3 M/7 F 4 M/6 F	14-19 yr 15-19 yr	No effects of either 0.12 or 0.18 ppm NO_2 on R_T or spirometry.
Kulle (1982)	0.50	120	15	--	24	45	10	Normal adults	Decreased static lung compliance.

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TABLE 15-1 (cont'd). RESPONSES OF HEALTHY NORMAL SUBJECTS TO NO_2 EXPOSURE[a]

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects Gender (M/F)	Subject Characteristics	Notes on Effects
Linn and Hackney (1983) and Linn et al. (1985b)	4.0	75	15-L/ 15-H	L 20-29 H 44-57	21	50	16 M/ 9 F	18-45 yr, NS	No change in SR_{aw} associated with NO_2. Small but significant decrease in blood pressure; some mild increase in symptoms.
Mohsenin (1987b)	2.0	60	--	--	21	50	8 M/3 F	18-36 yr, NS	Vitamin C blocked NO_2-induced increase in airway reactivity to methacholine.
Mohsenin (1988)	2.0	120	--	--	21	50	13 M/5 F	Normal, NS 18-33 yr	No symptoms; no lung function changes. Increased methacholine reactivity.
Mohsenin and Gee (1987)	3.0 (n = 6) 4.0 (n = 4) 0.0 (n = 7)	180	60	38	21	Dewpoint 10	16 M/1 F	25 yr, NS	45% decrease in alpha-1-protease inhibitor in BAL fluid.
Morrow and Utell (1989)	0.3	225	30 (3x10)	~40	21	40	10M/10F	Healthy, young, 20-48 yr, FEV_1/FVC%	No symptom, lung function or airway reactivity responses for the group.
		225	21 (3x7)	30-40	21	40	10M/10F	Elderly, normal, 49-69 yr, FEV_1/FVC 72-84%, 3 male S 4 female S	No symptom, lung function or airway reactivity responses.
Muelenaer et al. (1987)	0.6	240 min x3 day	60 (4x15)	~15	~22	~40	14 M	NS, Nonatopic, no residential NO_2 exposure	No change in urinary hydroxy-proline excretion.
Rehn et al. (1982)	0 0.27 1.06	60	--	--	22-27°C	25-45%		Healthy, young M	Possible small increase in R_{aw} at 0.27 ppm. No change in nasal or tracheobronchial clearance.

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TABLE 15-1 (cont'd). RESPONSES OF HEALTHY NORMAL SUBJECTS TO NO_2 EXPOSURE[a]

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects Gender (M/F)	Subject Characteristics	Notes on Effects
Sackner et al. (1980)	0.1 0.3 0.5 1.0	240	--	--	--	--	6	Normal adults	No effects of NO_2 in resting adults
Sandström et al. (1989)	2.25 4.0 5.5	20	20	~35	--	--	8 8 8 Total n = 18	Healthy, NS	Increased levels of mast cells in BAL fluid at all concentrations. Increased numbers of lymphocytes at 4.0 and 5.5 ppm. (BAL 24 h postexposure).
Sandström et al. (1990a)	4.0	20 min(?) alternate days for 12 days	20(?)	~35(?)	--	--	8	Healthy, NS	Total cell counts were reduced. Alveolar macrophages had enhanced phagocytic activity but fewer were present. Decreased numbers of mast cells, T and B lymphocytes and natural killer cells. BAL 24 h postexposure.
Stacy et al. (1983)	0.5	240	30	55	30	60	10 M	26.4 yr	No significant effects on spirometry or R_{aw}.
Smeglin et al. (1985)	0.30	240	30 min	~30	--	--	20	21-48 yr, NS	No change in lung function or airway reactivity.
Suzuki and Ishikawa (1965)	0.7-2.0	10					10		Increased resistance 10 min after exposure.
Toyama et al. (1981)	0.7	60	--	--			5	3 were investigators, 22-19 yr	No effects on airway conductance or responsiveness.
von Nieding et al. (1973a)	5.0	15	--	--	--	--	16	Healthy	Decreased D_LCO 18%.

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TABLE 15-1 (cont'd). RESPONSES OF HEALTHY NORMAL SUBJECTS TO NO_2 EXPOSURE[a]

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects Gender (M/F)	Subject Characteristics	Notes on Effects
von Nieding et al. (1977)	5.0	120	Inter-mittent	Light	22	55	11 M	Healthy	Increased resistance 60%. Remained elevated for 60 min. Possible decrease in PaO_2.
von Nieding et al. (1979)	5.0	120	60 (4x15)	220	22	55	11 M	Healthy	Resistance increased 60%. Remained elevated 60 min after exposure. Possible decrease in earlobe PO_2.

[a]Abbreviations:

M = Male
F = Female
S = Active smoker
NS = Nonsmoker
FEV_1 = Forced expiratory volume in 1 second
FVC = Forced vital capacity
SR_{aw} = Specific airway resistance
BAL = Bronchoalveolar lavage
IL-1 = Interleukin-1
TNF = Tumor necrosis factor
R_{aw} = Airway resistance
SG_{aw} = Specific airway conductance
W = Watts
R_T = Total respiratory resistance
L = Light
H = Heavy
D_LCO = Diffusing capacity for carbon monoxide
P_aO_2 = Arterial partial pressure of oxygen

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 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_2O/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.

Beil 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_2O/L/s above a baseline of 2.6 cm H_2O/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. This study was cited in the 1982 Air Quality Criteria for Nitrogen Oxides Document 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 $\mu g/m^3$ (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, a total of four exposures. The authors reported that approximately 11 $\mu g/m^3$ 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 (see Section 13.2.1). Blood pressure was obtained while the subject was seated quietly in the body plethysmograph using an automated procedure.

Mohsenin (1987b) studied the responses to 1 h resting exposure to 2.0 ppm NO_2 on 11 normal subjects to determine the effect of ascorbic acid administration prior to NO_2 exposure. The author hypothesized that the antioxidant properties of ascorbic acid would modify the effect of NO_2. There were a total of four exposures. In the first set of clean air and NO_2 exposures, the subjects received a placebo for 3 days prior to the exposures. In the second air/NO_2 exposure pair, the subjects received vitamin C. In both cases, the order of the NO_2 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 NO_2 in these subjects under placebo or vitamin C conditions. There was a significant increase in airway responsiveness to methacholine (bromide) after NO_2 exposure. Responsiveness to methacholine was quantified by the dose required to reduce SG_{aw} by 40% (PD40); this corresponds to a 67% increase in SR_{aw}. (A 50% decrease in SG_{aw} corresponds to a doubling of SR_{aw}.) After the two air exposures, PD40 averaged about 64 mg/mL but was reduced to 53 mg/mL after NO_2 exposure (placebo). When the subjects were given ascorbic acid prior to exposure, methacholine responsiveness after NO_2 exposure was unchanged. Ascorbic acid pretreatment apparently blocked the airway responsiveness increase, which had previously been observed with NO_2 exposure, although it had no effect on baseline methacholine responsiveness. Ascorbic acid has been previously 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 this apparent block of NO_2 effects could be explained by a decreased methacholine response after ascorbic acid supplementation.

Mohsenin (1988) studied the response of 18 normal adults exposed to 2 ppm NO_2 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 specific airway conductance. However, airway responsiveness to methacholine was increased following exposure in 13 of 18 subjects and decreased in only 2 of the

18 ($p=0.003$). The dose of methacholine needed to cause a 40% reduction in SG_{aw} was 101 $\pm$ 44 mg/mL after air and 81 $\pm$ 45 mg/mL after NO_2.

Kulle and Clements (1988) studied the effects of NO_2 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 NO_2 (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 NO_2 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 25-75% of VC ($FEF_{25-75\%}$) during the last of four consecutive clean air exposures. Methacholine airway responsiveness 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 NO_2-exposed subjects. Influenza virus infection did not alter airway responsiveness in either air or NO_2 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 airway mechanics (FVC, FEV, SG_{aw}) after any of these exposures. However, there was a small statistically significant increase in FVC 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 FEV after the "peaks" NO_2 exposure than after the air exposure. This observation suggests the possibility that some subjects may be affected by NO_2 to a considerably 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 vital capacity (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.

Kagawa (1983a) reported the results of exposing an additional 7 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 (i.e., Kagawa and Tsuru, 1979) in this new data set, there were no statistically significant mean changes in any of the plethysmographic or spirometric tests associated with NO_2 exposure.

Toyama and colleagues (1981) exposed five healthy subjects (two were smokers; three were investigators) to 0.7 ppm NO_2. The exposures lasted 60 min 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 NO_2 exposure or was a statistical artifact, as the authors (Kerr et al., 1979) suggested.

Rather than compare the data across the postexposure measurements obtained with clean air and NO_2 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 NO_2 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 pre-exposure value on the NO_2 exposure day, an effect that could not be attributed to NO_2. Quasistatic lung compliance was decreased after NO_2 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 NO_2 exposure. With the exception of the apparently artifactual change in "closing volume", no new conclusions can be drawn from the reanalysis of this data.

Stacy et al. (1983), as part of a large multipollutant exposure study, exposed a group of 10 men to 0.5 ppm NO_2 for 4 h, including two 15-min periods of moderately heavy exercise. None of the plethysmographic or spirometric tests showed a significant effect of NO_2. 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 NO_2.

Hazucha et al. (1982, 1983) studied a group of 15 healthy adult males exposed to either air or 0.1 ppm NO_2 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 NO_2 nor was there an increase of symptoms related to the NO_2 exposure. There were no effects of NO_2 on spirometry, airway resistance (SR_{aw} or R_T), or methacholine responsiveness.

Rehn et al. (1982) reported a small (17%) increase in SR_{aw} after exposure of eight healthy men to 0.27 ppm (500 $\mu g/m^3$) for 1 h. However, no response was seen at 1.06 ppm (2,000 $\mu g/m^3$). This was reported in a technical paper (in Swedish) and has not yet been published in a peer-reviewed journal.

Bylin et al. (1985) exposed eight normal subjects to 230, 460, and 910 $\mu g/m^3$ (0.12, 0.24, and 0.48 ppm) for 20 min. An analysis of variance did not reveal any significant

effects of NO_2 on changes in SR_{aw}, but specific comparisons indicated a significant 11% increase in SR_{aw} at 0.24 ppm and a 9% decrease in SR_{aw} at 0.48 ppm. Even though statistically significant, such small changes ($\pm$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 NO_2 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. In the (1) rest exposures, there were no significant changes in lung function, symptoms, or arterial oxygen saturation. In the mild exercise exposures to 0.12 (2) and 0.18 (3) ppm NO_2, there were no significant effects related to NO_2 exposure.

Morrow and Utell (1989) studied both young (20 to 48) healthy subjects and elderly (49-69) healthy subjects exposed to 0.3 ppm NO_2 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 NO_2 exposure for symptom responses, changes in lung function, or in airway responsiveness to carbachol in either young or older subjects.

Adams et al. (1987) studied the effects of 0.60 ppm NO_2 on young men and women during 1 h of continuous heavy exercise. There was no significant effect of NO_2 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 NO_2 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 minutes were spent walking. Overall ventilation is estimated to have averaged about 50 L/min. There were no significant changes in respiratory symptoms, FEV_1, R_T, peak expiratory flow rate (PEFR), or ventilation ($\dot{V}_{50\%VC}$) as a result of NO_2 exposure in this group of athletic male subjects.

Young (18 to 26) and older (51 to 76) men and women were exposed to 0.60 ppm NO_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_2 (as compared to air)

for FVC or FEV_1 were outside of the normal range (i.e., there were no individual subjects who appeared reactive to NO_2).

15.2.1.3 Respiratory Symptom and Sensory Effects of NO_2 Exposure

Several studies reported in the previous section examined symptomatic responses of subjects exposed to NO_2. None of the studies of NO_2 exposure in normal subjects, including exposure for as long as 75 min to 4.0 ppm NO_2 resulted in a significant increase in respiratory symptoms. Sensory effects were examined in at least two studies (Bylin et al., 1985; Hazucha et al., 1983). Hazucha et al.'s (1983) subjects were unable to detect the odor of 0.1 ppm NO_2. Bylin et al. (1985) reported an odor threshold of 0.04 ppm for normals and 0.08 for asthmatics.

15.2.1.4 Mucociliary Clearance after NO_2 Exposure

Rehn et al. (1982) examined the effects of NO_2 exposure on mucociliary clearance in both the nose and lung. Nasal clearance was determined using the rate of saccharin transport. 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 $\mu g/m^3$) NO_2, there were no changes in either nasal or tracheobronchial clearance rates.

15.2.2 Effects of Nitric Oxide

In addition to NO_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 pre-exposure 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 $He\dot{V}_{50}/air/\dot{V}_{50}$. 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 this 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}], $\dot{V}_{50\%VC}$, 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 (~10 to 12%) after exposure to ≥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 NO_2-Gas or Gas/Aerosol Mixtures on Lung Function in Normal Subjects

Several studies of NO_2-containing pollutant mixtures were included in Air Quality Criteria for Oxides of Nitrogen Document (U.S. Environmental Protection Agency, 1982a). The general finding in 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). 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 while the effect of NO_2 was delayed and more persistent. The effect of mixed gas 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 NO_2-containing pollutant mixtures.

TABLE 15-2. EXPOSURE OF HEALTHY NORMAL SUBJECTS TO NO_2 MIXTURES[a]

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects/ Gender	Subject Characteristics	Notes on Effects
Adams et al. (1987)	0.60 NO_2 + 0.30 O_3	60	60	70 50	21-25	45-60	20 M 20 F	Normal	No additional effect of NO_2 over and above effect of O_3.
Avol et al. (1983)	0.04 (Amb)	60	60	56	32	43	42 M/8 F	Normal	No apparent effect over and above that of O_3 alone.
Avol et al. (1985a) and Avol et al. (1987)	0.04 (Amb)	60	60	22.4	32.7	43	33 M/33 F	Children, 8-11 yr	No effects of ambient air exposures.
Avol et al. (1985b)	0.055 (Amb)	60	60	32	32	45	46 M/13 F	Adolescents, 12-15 yr	Ambient air exposures effect attributed to O_3.
Drechsler-Parks (1987)	0.60 NO_2 +0.45 O_3	120	60	25	24	55	8 M/8 F	18-26 yr, NS	No significant changes attributable to NO_2.
	0.60 NO_2 (+0.45 ppm O_3)	120	60	25	24	55	8 M/8 F 8 M/8 F	51-76 yr 51-76 yr	Tendency ($p>0.05$) for NO_2 + O_3 to be greater than O_3 alone.
Folinsbee et al. (1981)	0.50 (+0.5 ppm O_3)	120	30	40	25 30 35 40	45% 85 40 50	10 M 10 M 10 M 10 M	Young adults, NS Young adults, NS Young adults, NS Young adults, NS	FEV_1, decreased from 8-14%. No differences between O_3 + NO_2 and O_3 alone.
Hackney et al. (1975b)	(a) 0.50 O_3 + 0.29 NO_2 (b) 0.50 O_3 + 0.29 NO_2 + 30.00 CO	240 (2 consecutive days of exposure to each mixture)	120	~20	31	35	4	Healthy	With each group minimal alterations in pulmonary function caused by O_3 exposure. Effects were not increased by addition of NO_2 or NO_2 and CO to test atmospheres.

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TABLE 15-2 (cont'd). EXPOSURE OF HEALTHY NORMAL SUBJECTS TO NO_2 MIXTURES

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects/ Gender	Subject Characteristics	Notes on Effects
Hackney et al. (1975c)	(a) 0.25 O_3 + 0.29 NO_2 (b) 0.25 O_3 + 0.29 NO_2 + 30.00 CO	120 (2 consecutive days of exposure)	60	~20	31	35	7	Healthy	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.
Islam and Ulmer (1979a)	5.0 NO_2 + 0.1 ppm O_3 + 5.0 ppm SO_2	120	60	?	22	60	8 M 8 M 8 M	<30 yr 30-40 yr >49 yr	FVC (-5%), $FEV_{1.0}$ (-11.7%), decreased with exercise exposure to this mixture in <30 yr group.
Islam and Ulmer (1979b)	0.16 NO_2 0.34 SO_2 0.08 O_3	480	0	--	22	60	15	16-26 yr	No change in FVC, acetyl-choline airway reactivity.
Kagawa (1983b)	0.15 NO_2 0.15 O_3 0.15 SO_2	120	60	~25	--	--	7 M	19-23	No significant enhancement of the effects of O_3 and SO_2 by presence of NO_2.
Kagawa (1986)	0.30 NO_2 + 0.30 O_3 + 200. $\mu g/m^3$ H_2SO_4	120	20	~25	28-29	50-60	6	(Some smokers), Japanese men	Possible small decrease in SG_{aw}.
	0.15 NO_2 + 0.15 O_3 + 200 $\mu g/m^3$ H_2SO_4	120	60	~25	28-29		6	(Some smokers), Japanese men	Possible small decrease in SG_{aw}.
	0.15 NO_2 + 0.15 O_3 + 0.15 SO_2 + 200 $\mu g/m^3$ H_2SO_4	120	60	~25	28	59-60	3	(Some smokers), Japanese men	Possible small decrease in FEV_1.

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TABLE 15-2 (cont'd). EXPOSURE OF HEALTHY NORMAL SUBJECTS TO NO_2 MIXTURES

Reference	NO_2 (ppm)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Vent (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects/ Gender	Subject Characteristics	Notes on Effects
Kleinman et al. (1985)	0.50 NO_2 + 0.5 SO_2 + $ZnSO_4$ $(NH_4)_2$ SO_4 + NaCl	135	60	~20	20	85	11 M/9 F	20-53 yr	No effects on function; possible symptom responses. NO_2 effects not discernible from mixture.
Linn et al. (1980b)	0.07 Ambient and other pollutants	120	60	~20	33	32	14 M/20 F	29 yr	Small decreases in FVC, FEV_1, in ambient air mostly attributable to O_3. No association of NO_2 levels with lung function change.
Linn et al. (1980a)	0.50 NO_2 + 0.50 SO_2	120	60	~20	31	40	10 M/14 F	26 ± 4 yr, 21 NS, 3 S	No significant effect on lung function in normals. Trend for a slight decrease in FVC after combined exposure.
von Nieding et al. (1979)	5.0 + 5.0 SO_2 + 0.1 O_3	120	60	~20 (70W)	22	55		23-38 yr, two atopic	R_T increased from 1.5 to 2.4 ($p<.01$); questionable decrease in PaO_2 (8 torr).
	0.1 (NO_2) 0.3 (SO_2)	120	60	~20	22	55		23-38 yr, two atopic	No effects at all.

[a]Abbreviations:

O_3 = Ozone
M = Male
F = Female
NS = Nonsmoker
FEV_1 = Forced expiratory volume in 1 second
CO = Carbon monoxide
FVC = Forced vital capacity
SG_{aw} = Specific airway conductance
SO_2 = Sulfur dioxide
H_2SO_4=Sulfuric acid
$ZnSO_4$=Zinc sulfate
$(NH_4)_2SO_4$=Ammonium sulfate
NaCl =Sodium chloride
S =Active smoker
R_T =Total respiratory resistance
W =Watts
PaO_2 =Arterial partial pressure of oxygen

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The pollutant mixture of greatest interest, in terms of research effort, was the combination of NO_2 and ozone (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 NO_2-SO_2 combination exposures(Kagawa, 1983a,b; Kleinman et al., 1985; Linn et al., 1980a). Other pollutant mixtures containing NO_2 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 O_3 and NO_2 in combination. Three studies investigated the effects of 0.5 to 0.6 ppm NO_2 in combination with 0.3 to 0.5 ppm O_3 (Adams et al., 1987; Folinsbee et al., 1981; Drechsler-Parks, 1987). In these studies, NO_2 alone had no effect on measured health end points and the significant effects of O_3 on lung function were not altered by the presence of NO_2. Kagawa and Tsuru (1979) and Kagawa (1983a) reported conflicting results. In their first study, it was suggested that the effects of NO_2 and O_3 were "more than additive." However, in the second study, they reported no significant enhancement of the effect of O_3 by the mixture of other pollutants. (All pollutants—NO_2, O_3, and SO_2—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 NO_2-O_3 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 NO_2 and O_3 that would result in enhancement of acute O_3-induced changes in lung function in normal subjects following short duration exposures at NO_2 concentrations less than 1 ppm. However, this conclusion is not necessarily applicable to other measured end points.

Linn et al. (1980a) exposed a group of normal subjects to a mixture of 0.5 ppm NO_2 and 0.5 ppm SO_2 during which light, intermittent exercise was performed for the 2-h period. There were no lung function (spirometry, closing volume, R_T) responses. There was an overall increase in symptoms as a result of the exposure but no significant increase in any specific symptom category. Using the same 2-h intermittent, light exercise exposure protocol, Kleinman et al. (1985) examined the effects of similar NO_2-SO_2 levels in combination with sodium chloride (NaCl) aerosol (330 $\mu g/m^3$) and zinc ammonium sulfate

(20 $\mu g/m^3$). 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 SO_2-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 NO_2-SO_2 mixture.

Islam and Ulmer (1979a) examined the effects of a mixture of 5 ppm NO_2, 5 ppm SO_2 and 0.1 ppm 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%, FVC decreased 5%, and FEV_1 decreased 11.7%; P_aO_2, determined from ear lobe blood samples, was unchanged. Similar effects occurred in the older subjects, but the changes were of a smaller magnitude. P_aO_2 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 SO_2, 0.16 ppm NO_2, and 0.08 ppm O_3 for 8 h at rest on 4 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 NO_2 (0.5 ppm) and aerosols of either sulfuric acid (H_2SO_4, 100 $\mu g/m^3$), ammonium sulfate ($(NH_4)_2SO_4$, 133 $\mu g/m^3$), ammonium bisulfate (NH_4HSO_4, 116 $\mu g/m^3$), ammonium nitrate (NH_4NO_3, 80 $\mu g/m^3$). There were no effects of any of the pollutant mixtures on spirometry, plethysmography, or symptoms. Kagawa (1986) studied the effects of a mixture of (A) NO_2 (0.30 ppm), O_3 (0.30 ppm), and H_2SO_4 (200 $\mu g/m^3$); (B) NO_2 (0.15 ppm), O_3 (0.15 ppm), and H_2SO_4 (200 $\mu g/m^3$); or (C) NO_2 (0.15 ppm), O_3 (0.15 ppm), SO_2 (0.15 ppm), and H_2SO_4 (200 $\mu g/m^3$). Exposure A included 20 min of exercise (total) and B and C included 60 min of exercise over 2 h. Symptoms were attributed to O_3 exposure. Small, possibly

significant decreases (≤-10%) in G_{aw} were observed after exposure to mixtures A and B. A possible decrease in FEV_1 (unknown magnitude) was observed after exposure C. The differences observed with these mixtures were no different than responses observed with O_3 alone, indicating no enhanced response due to the presence of NO_2 in the mixture.

Several reports of exposure to NO_2-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 NO_2 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. In the Linn et al. (1980b) study, there was no association between NO_2 levels and symptom or lung function effects either in normal or asthmatic subjects. In the Linn et al. (1982) study, the O_3 levels were only 0.03 ppm, and there were no significant effects associated with ambient exposure in normals or asthmatics. There was a relationship between O_3 concentration and change in FEV_1 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 NO_2 level. Ambient air exposure of adolescents (Avol et al., 1985a) was associated with decreased FEV_1 in male and female adolescents with a somewhat larger response in female subjects (-7.5% [female] vs. -3.4% [male]). The responses tended to be associated with the O_3 levels. A similar study (Avol et al., 1987) was conducted with exercising children who showed a trend to larger pulmonary function decrements with increasing O_3 levels. Although these ambient air exposure studies were not designed to test for the interaction of NO_2 with other pollutants, even though Los Angeles has the highest NO_2 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 O_3.

There has been one study of the effects of HNO_3 vapor in combination with O_3 (Aris et al., 1991). Ten healthy men were exposed to 500 $\mu g/m^3$ HNO_3 vapor or clean air. Another six subjects were exposed to either 0.20 ppm O_3 or the combination of HNO_3 vapor and O_3. Exposures lasted 4 h and were accompanied by moderate exercise (ventilation during exposure [$\dot{V}_E$] = 40 L/min). No changes were observed in FVC, FEV_1, or SR_{aw} after HNO_3 exposure. Ozone exposure caused increased SR_{aw} and decreased FVC and FEV_1, which was increased slightly by the presence of HNO_3 vapor.

There appears to be no obvious synergistic or more than additive interactions between NO_2 and other pollutant gases or particles that have been evaluated to date. Pulmonary function responses to O_3, for example, do not appear to be increased by the addition of low levels (<0.6 ppm) of NO_2. 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 NO_2 exposure. Increases in R_{aw} were reported at concentrations of 5 to 8 ppm from both short (5 min) and longer (1 to 2 h) exposures (1-2 h) (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, and Frampton et al. (1991) reported increased carbachol response after exposure to 1.5 ppm. None of the seven studies of exposure to less than 1.0 ppm in normal subjects demonstrated clear responses to NO_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 NO_x EXPOSURE IN SENSITIVE SUBJECTS

Certain groups within the population may be more responsive to the effects of NO_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_2-induced responses or exacerbation of disease may be much higher than in healthy young adults. Studies on other NO_x gases and NO_x-mixtures are also discussed.

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 NO_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 NO_2-induced increase in airway response to the already heightened responsiveness to other substances raises the possibility of exacerbation of this pulmonary disease by NO_2. One of the potential mechanisms by which NO_2 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 NO_2 on Asthmatics

Studies of the exposure of asthmatics to NO_2 are summarized in two tables that describe characteristics of the asthmatic subjects (Table 15-3) and the exposure conditions and responses to NO_2 (Table 15-4).

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) (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. However, in all studies summarized in this section, a control exposure to clean air was performed including exercise when appropriate.

A major issue in the evaluation of human clinical studies involving asthmatics is the variability in response between and even within laboratories. In the absence of a clear reason

TABLE 15-3. SUBJECT CHARACTERISTICS OF ASTHMATICS EXPOSED TO NO_2[a]

Reference	Number	Age (yrs)	FEV_1[b] FVC%	SR_{aw} ($L \cdot cmH_2O/L/s$)	IgE[c] Elevated	Reversibility[d]	Allergy[e]	Medications[f]	Medication Witholding[g]	Airway Reactivity[h]	Notes
Ahmed et al. (1983b)	20 M/34 F	18-39	58-97	3.4-20.1	–	–	10/20	–	–	MET HIST EIB COLD	–
Ahmed et al. (1983a)	9	20-51	53-96	4.3-14.9	–	–	Ragweed	–	24-48 h	Ragweed antigen	"History of bronchial asthma."
Avol et al. (1988)	27 M/32 F	18-50	43-98	3.8-21.2	Y	Y	Y	14 OB 30 IB/OB 15 IB	48 h AH 8 h IB, OB (Some noncompliance)	MET	Some moderate asthmatics.
Avol et al. (1989)	24 M/10 F	10-16	61-98	3-15		History	33/34	10 IB/OB 7 IB 6 OB 10 None[1]	48 h AH 24 h OB 12 h IB	COLD MET EIB	No cromolyn; No steroid.
Bauer et al. (1986a)	15	20-45	49-83	4.39	–	–	–	13 IB 7 OB	12 h IB 24 h OB	COLD	Mild asymptomatic.
Bylin et al. (1985)	6 M/2 F	17-45	–	6.99	Y	Y	4/8	5 Occasional IB/OB 3 OB-daily	6 h	HIST	Very mild asymptomatic.
Bylin et al. (1988)	8 M/12 F	17-56	–	3.76 ± 0.33	Some	Y	12/20	2 IB, OB (occasional)	12 h OB 8 h IB	HIST	Very mild asymptomatic.
Hazucha et al. (1983, 1982)	15 M	21-46	–	6.73	–	History	–	None daily	48 h	MET	Mild or inactive diease.
Jörres and Magnussen (1990)	10 M/4 F	20-55	38-83	2.8-18.2	–	–	12/14	11/14 IB 5 OB 1 ST 6 ICS 2 CROM 4 IPB	10 h IB 10 h IPB 10 h CROM 16 h OB 10 h ICS	Histamine SO_2	"Mild asthma"; all subjects asymptomatic except one (FEV_1/FVC ratio 38%).
Kerr et al. (1979) also Kulle (1982)	9 M/4 F	19-50	~71	~7.4	–	–	–	–	–	–	3 smokers.
Kleinman et al. (1983)	12 M/19 F	18-55	~74	Rt = 2.62	–	–	16/31	8 None 21 IB, OB 8 ST, 1 CROM	4 h	MET	Wide range of clinical severity.
Jorres and Magnussen (1991)	9 M/2F	18-55	67-96	3.9-10.7			7/11	6 None 5 OB	8 h	MET	Mild asymptomatic asthmatics.

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TABLE 15-3 (cont'd). SUBJECT CHARACTERISTICS OF ASTHMATICS EXPOSED TO NO_2

Reference	Number	Age (yrs)	FEV_1[b] FVC%	SR_{aw} ($L \cdot cmH_2O/L/s$)	IgE[c] Elevated	Reversibility[d]	Allergy[e]	Medications[f]	Medication Witholding[g]	Airway Reactivity[h]	Notes
Koenig et al. (1985)	I 4 M/6 F	12-18	FEV_1 1.95-4.10	Rt = 4.57	Y	Y	Y	8 IB 6 OB	4 h	EIB MET	Asymptomatic extrinsic allergic asthmatics.
Koenig et al. (1987a,b)	II 4 M/6 F	11-19	2.3-4.2	Rt = 5.07	Y	Y	Y	7 IB 5 OB	~4 h	EIB MET	
	III 7 M/3 F	12-18	1.7-4.6	Rt = 4.70	Y	Y	Y	6 IB 7 OB	~4 h	EIB MET	
Koenig et al. (1989a)	6 M/3 F	12-18	2.4-5.4 (FEV_1)	--	--	--	5/9	--	--	MET EIB	
Koenig et al. (1989b)	5 M/4 F	12-17	2.24-4.0	--	--	--	9/9	5 IB 1 NONE 3 CROM 3 OB 3 AH		MET EIB	
Linn et al. (1980a) (mixed NO_2 + SO_2)	7 M/12 F 6 Ex-smokers	33	2.32 (FEV_1)	3.45 Rt = 3.45	--	--	Y	--	~4 h	--	Physician diagnosed asthma.
Linn and Hackney (1984)	12 M/11 F	18-34	67-100	5.48±2.33	--	--	--	--	--	SO_2	Physician diagnosed asthma.
Linn et al. (1985b)	12 M/11 F	18-34	67-100	5.48±2.33	--	--	--	--	~4 h	SO_2	
Linn et al. (1986)	15 M/6 F	20-34	3.16 (FEV_1)	4.21	13/21 >180	--	--	10 IB 5 None 8 OB 1 ST	~4 h	COLD 19/21 EIB 10/21 SO_2 17/21	Mild asthmatics.
Mohsenin (1987b)	10	22-44	60-93	3.8-13.2	--	Y	Y	No steroids No caffeine No vitamin C	24 h	MET	Mild asthmatics.
Morrow and Utell (1989)	10 M/10 F	19-54	42-86	(50.) 3.95-14.5 x = (11.3) 9.29	--	Y	14/20	4 IB 5 OB 10 IB, OB		CARB	
Orehek et al. (1976)	13 M/7 F	15-44		6.8±0.6	--	--	16/20	No steroids	24 h	CARB	Duration asthma (1-24 yr), 6 smokers. 13 mild/7 moderate.

TABLE 15-3 (cont'd). SUBJECT CHARACTERISTICS OF ASTHMATICS EXPOSED TO NO_2[a]

Reference	Number	Age (yrs)	FEV_1[b] FVC%	SR_{aw} ($L \cdot cmH_2O/L/s$)	IgE[c] Elevated	Reversibility[d]	Allergy[e]	Medications[f]	Medication Witholding[g]	Airway Reactivity[h]	Notes
Orehek et al. (1981)	6 M/1 F	31.1		5.4±1.1	--	--	Y	--	--	Grass Pollen	1 smoker, 3 asthmatics, 4 allergic.
Roger et al. (1990)	A. 13 M	19-35	55-81	2.6-13.5	80-2,040	Y	12/13	IB, OB	48 h, OB	MET	Mild asthmatics, no cromolyn, no steroids.
	B. 21	19-30	59-85	3.2-10.6	38-2,040	Y	18/21	IB, OB	12 h, IB		
Rubinstein et al. (1990)	5 M/4 F	23-34	51-85	4.8-12.0	--	--	--	9 IB	8 h, IB	MET	No respiratory illness within 4 weeks.
								4 0B	12 h, OB, AH		
								4 AH	4 h, CAF		

[a]Abbreviations:

FEV_1	=	Forced expiratory volume in 1 second	OB	=	Oral theophyline
FVC	=	Forced vital capacity	AH	=	Antihistamine
SR_{aw}	=	Specific airway resistance	IB	=	Inhaled β agonist
IgE	=	Immunoglobulin E	ST	=	Oral corticosteroid
M	=	Male	ICS	=	Inhaled corticosteroid
F	=	Female	CROM	=	Cromolyn sodium
MET	=	Methacholine	IPB	=	Ipratroprium bromide
HIST	=	Histamine	SO_2	=	Sulfur dioxide
EIB	=	Exercise-induced bronchospasm	CARB	=	Carbachol
COLD	=	Cold air			

[b]Ratio of FEV_1/FVC in percent

[c]Elevation of IgE levels above those reported for the normal population

[d]Reversibility of bronchoconstriction tested with bronchodilator

[e]Allergy—number of subjects with one or more known allergies

[f]Standard medications and number of subjects using them (see list); none indicates no regular use of medication during the study period

[g]Period for which specific types of medication were withheld prior to the study (in hours)

[h]Airway reactivity was tested using one or more standard methods (see list)

TABLE 15-4. EXPOSURE CONDITIONS AND RESPONSES IN ASTHMATICS EXPOSED TO NO_2[a]

Reference	NO_2 (ppm)	Exposure Duration (min)	Exercise Duration (min)	Exercise Ventilation (L/min)	Temp. (°C)	Relative Humidity (Percent)	Effects
Ahmed et al. (1983b)	0.1	60	--	--	--	--	No significant effect on SG_{aw}, FEV_1, V_{ISOV} variable effect on carbachol reactivity. No information on controlled exposure.
Ahmed et al. (1983a)	0.1	60	--	--	--	--	No effect of NO_2 on FEV_1, SG_{aw}, or on bronchial reactivity to ragweed antigen, either immediately or 24 h postexposure.
Avol et al. (1988)	0.3	120	60	40	22	46	Exercise-related increases in symptoms. Possible NO_2 related decrease in FEV_1, PEFR. Increased cold air response after 0.3 ppm.
	0.6	120	60	41	--	--	More consistent increases in SR_{aw} at 0.6 ppm but not significantly different from air and 0.3 ppm.
Avol et al. (1989)	0.3	180	90	30	24	45	After 60 min of exposure, FEV, FVC, and PEFR (-3.4, -4.0, and -5.6%, respectively) were significantly reduced. No change in airways responsiveness to cold air challenge. SR_{aw} increased 17% after NO_2 exposure. After 180 min of exposure, the responses had returned to baseline levels.
Bauer et al. (1986a)	0.3	30	10	30	20	9-14	Resting 20 min exposures produced no effects. Slight excess decrease in FEV_1 and PEFR in NO_2 plus exercise above that caused by exercise alone. PEFR, -16% (air), -28% (NO_2); FEV_1 -5.5% (air) - 9.3% (NO_2). Significantly increased response to cold air after NO_2 exposure.
Bylin et al. (1985)	0.12	20	--	--	22	35	No significant change in SR_{aw} at any NO_2 levels. Histamine reactivity tended to increase.
	0.25	20	--	--	--	--	
	0.5	20	--	--	--	--	

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TABLE 15-4 (cont'd). EXPOSURE CONDITIONS AND RESPONSES IN ASTHMATICS EXPOSED TO NO_2

Reference	NO_2 (ppm)	Exposure Duration (min)	Exercise Duration (min)	Exercise Ventilation (L/min)	Temp. (°C)	Relative Humidity (Percent)	Effects
Bylin et al. (1988)	0.14 0.27 0.54	30	--	--	25.9	43	Overall trend for SR_{aw} to decline during exposure period, not related to NO_2 concentration. Histamine bronchial reactivity tended to increase after 0.14 and 0.27 ppm NO_2 exposure.
Hazucha et al. (1982) Hazucha et al. (1983)	0.10	60	--	--	21	40	No significant changes associated with NO_2 exposure.
Jörres and Magnussen (1990)	0.25	30 min	--	--	24	50	After resting breathing of 0.25 ppm NO_2 responsiveness to inhaled SO_2 was increased. No effect of NO_2 alone on SR_{aw}.
Kleinman et al. (1983)	0.20	120	60	~20	22	50	No effects on spirometry or airway resistance. Airway reactivity to methacholine results variable - tended to increase with exposure.
Koenig et al. (1985)	0.12	60	--	--	22	75	No significant responses in pulmonary function due to NO_2. Increased symptoms after NO_2 exposures.
Koenig et al. (1987a,b)	I. 0.12 II. 0.12 III. 0.18	60 40 40	-- 10 10	-- 33 39	22 22 22	75 75 75	(Same as Koenig et al., 1985). No change in FEV_1, R_T increased 10.4% (NS), 3% decrease in FEV_1 ($P<.06$).
Koenig et al. (1989b)	50 ppb HNO_3	40	10	~25-30	25	65	FEV_1 decreased -4.4% after HNO_3 and -1.7% after HNO_3 air exposure. R_T increased +22.5% after HNO_3 and +7.4% after air exposure.
Koenig et al. (1989a)	57 ppb HNO_3	45	30	25-30	22	65	FEV_1 decreased -3.3% after NO_2 exposure and -1.7% after air exposure (difference NS). Reduction of oral ammonia did not increase response (-1.7%).
Kulle (1982) (same as Kerr et al., 1979)	0.50	120	15	--	24	45	Increased respiratory symptoms in 4/13 subjects. Increased static lung compliance. Impossible to determine amount of effect due to NO_2.
Jorres and Magnussen (1991)	0.25	30	10	30	26	20	Mouthpiece exposure system. No changes in methacholine responsiveness were observed after NO_2 exposure in these mild asthmatics.

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TABLE 15-4 (cont'd). EXPOSURE CONDITIONS AND RESPONSES IN ASTHMATICS EXPOSED TO NO_2

Reference	NO_2 (ppm)	Exposure Duration (min)	Exercise Duration (min)	Exercise Ventilation (L/min)	Temp. (°C)	Relative Humidity (Percent)	Effects
Linn et al. (1980a)	0.5 + 0.3 ppm SO_2	120	60	~20	31	40	No significant effect on spirometry, R_T.
Linn and Hackney (1984) Linn et al. (1985b)	4.0	75	a. 15 b. 15	a. 25 b. 49	21	50	No NO_2 effects on SR_{aw}, symptoms, heart rate skin conductance. Small decrease in systolic blood pressure.
Linn et al. (1986)	0.3	60	30	41	22	50	No effect of NO_2. Exercise related increase in SR_{aw} under all conditions.
	1.0	60	30	41	22	50	
	3.0	60	30	41	22	50	
Mohsenin et al. (1987b)	0.5	60	--	--	21	50	No change in symptoms. Significant group mean increase in responsiveness to methacholine after NO_2 exposure. No other function changes.
Morrow and Utell (1989)	0.30	225	30 (3x10)	30-40	21.0	40	Group findings indicated no significant responses. No change in lung function, symptoms, carbachol reactivity. Subjects previously studied (Bauer et al., 1986a) showed possible responses to NO_2. New subject subgroup showed significantly greater response in air exposures.
Orehek et al. (1976)	0.11 (n=20) (.09-0.13)	60	--	--	--	--	13/20 subjects had enhanced responses to carbachol after 0.11 ppm NO_2.
	0.26 (n=4)						1/4 subjects had enhanced responses to carbachol after 0.26 ppm NO_2.

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TABLE 15-4 (cont'd). EXPOSURE CONDITIONS AND RESPONSES IN ASTHMATICS EXPOSED TO NO_2

Reference	NO_2 (ppm)	Exposure Duration (min)	Exercise Duration (min)	Exercise Ventilation (L/min)	Temp. (°C)	Relative Humidity (Percent)	Effects
Orehek et al. (1981)	0.11 (0.07-0.16)	60	--	--	--	--	No change in SR_{aw} or in responsiveness to grass pollen in 3 allergic asthmatics and 4 allergic subjects.
Roger et al. (1990)	A. 0.3	110	60	42	20	40	FEV_1 decreased 11% in NO_2 but only 7% in air, after first 10 min of exercise. Smaller changes later in exposure.
	B. 0.15	75					
	0.30		30	42	20	40	
	0.60						No increase in airway reactivity to methacholine 2 h after exposure. No change in FEV_1 or SR_{aw} as a result at NO_2 exposure.
Rubinstein et al. (1990)	0.30	30	20	3x (~30)	22	55	No changes in SR_{aw}, FVC, FEV_1, SBN_2 or symptoms after NO_2 exposure. NO_2 exposure did not increase airways responsiveness to SO_2.

[a]Abbreviations:

SG_{aw} = Specific airway conductance
FEV_1 = Forced expiratory volume in one second
V_{ISOV} = Volume of isoflow
PEFR = Peak expiratory flow
SR_{aw} = Specific airway resistance
NS = Not significant
FVC = Forced vital capacity
R_T = Total respiratory resistance
HNO_3 = Nitric acid
SO_2 = Sulfur dioxide
SBN_2 = Single breath nitrogen washout

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based on exposure protocol or exposure regimen, the explanation most often invoked to explain differences in response is that the severity of disease in one subject group may have been different than the other. Appendix 15-A (Asthma Severity) discusses a possible approach that can be used to evaluate physiological and clinical differences among asthmatics. These "asthma severity" descriptors will be applied to the key asthma studies discussed insofar as possible based on the data available. Where the authors' evaluation of asthma severity disagrees with the scheme in Appendix 15-A, this will be noted.

Symptomatic effects were observed in asthmatics exposed to 0.5 ppm for 2 h (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."

Avol et al. (1988) studied a group of 59 moderate-to-severe asthmatics exposed to clean air, 0.3, and 0.6 ppm NO_2 for 2 h while performing moderate ($\dot{V}_E$ = 41 L/min), intermittent (6 × 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 FEV_1 as a function of exposure duration for all exposure conditions, but there was no significant effect of NO_2 exposure on these measures of pulmonary function. Cold air bronchial reactivity (assessed by the decrease in FEV_1 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 post and 24 h post), 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 NO_2 exposure. A post hoc analysis of a subgroup of subjects with the most abnormal lung function (i.e., FEV_1/FVC ratios <0.65) revealed no statistically significant effects of NO_2. In addition to the controlled exposures, 36 subjects also were exposed to ambient air containing 0.09 ppm NO_2 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 NO_2 in a group of 15 asthmatics exposed to 0.3 ppm NO_2 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. NO_2 deposition studies indicated that 72%, at rest, and 87%, during

exercise, of the inhaled NO_2 was deposited within the respiratory tract. According to the authors, the measurements of NO_2 deposition were in general agreement with the model predictions of Miller et al. (1982) (see also Section 13.2.1). After NO_2 exposure, 9 of 15 asthmatics had a decrease in FEV_1 relative to their postexercise FEV_1 in clean air. The postexercise FEV_1 was 4.1% lower after NO_2 (mean = 2,788 mL) than after air (mean = 2,906 mL) exposure; the pre-post exposure difference on the NO_2 day (10.1%) and the pre-post NO_2 minus the pre-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 (MEF) 60% total lung capacity (TLC) (PEFV curve) was also decreased more after NO_2 exposure than after air exposure. Changes in FVC and SG_{aw} were not different between air and NO_2 exposures. 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 vs. 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, 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 nitric or nitrous acid (HNO_2 or HNO_3) 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 Bylin 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 SR_{aw}, during the four exposures averaged +3%, +9%, -2%, and -14%, respectively; the coefficient of variation for the SR_{aw} measurements was 19% for these subjects. A three-way analysis of variance (ANOVA) revealed no significant differences in SR_{aw} due to NO_2 exposure. There was a tendency for the pre-post exposure 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 pre-exposure values for the 0.1 - and the 0.5-ppm NO_2 exposures; such an effect, if real, should not be attributed to NO_2. 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 NO_2 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 since 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 NO_2 exposures. This paper suggested possible increased histamine reactivity after 0.50 ppm NO_2 exposure of asthmatics but no direct effect of NO_2 on R_{aw} at concentrations up to 0.5 ppm for 20 min.

Bylin et al. (1988) also reported the effects of 260, 510, and 1,000 $\mu g/m^3$ (0.14, 0.27, 0.53 ppm) on a group of 20 mild asthmatics. There were no significant changes in SR_{aw}, although there was a general trend for SR_{aw} 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 $\mu g/m^3$) 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) that was not adjusted for multiple comparisons, suggests that these results should be interpreted with some reservation. This observation contrasts with the earlier observation (Bylin et al., 1985) that suggested a possible increased responsiveness after exposure to 910 $\mu g/m^3$. (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 NO_2 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 NO_2 exposure on the bronchial sensitivity of minimal to mild asthmatic patients to carbachol, a bronchoconstricting agent. Exposures took place in an airtight room. Nitrogen dioxide concentration started at 246 $\mu g/m^3$ (0.13 ppm) and declined to 169 $\mu g/m^3$ (.09 ppm) over 60 min; the average concentration was 210 $\mu g/m^3$ (0.11 ppm). For 20 asthmatics, dose-response curves were developed for changes in SR_{aw} as a result of the subjects inhaling carbachol after a 1-h exposure to either clean air or NO_2. Following NO_2 exposure, increases in SR_{aw} were observed in only 3 of 20 asthmatic test subjects. However, NO_2 exposure was associated with increased airway responsiveness in 13 of 20 subjects. The mean dose of carbachol producing a twofold (100%) increase in SR_{aw} in the 13 sensitive subjects was significantly decreased from 0.66 mg to 0.36 mg as a result of NO_2 exposure. Seven of the asthmatic subjects showed neither an increase in R_{aw} in response to the exposure to NO_2 alone nor an enhanced effect of NO_2 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 NO_2. However, the mean of measurements of SR_{aw} in 13 responders to the carbachol treatment was significantly higher after the NO_2 exposure than it had been prior to exposure. 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 NO_2 exposure, but after the fact, following the carbachol 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 NO_2 for 1 h. The major hypothesis to be tested was that NO_2 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_2 was added to an exposure room with a starting concentration of 0.16 ppm, which was allowed to decay over the exposure to a concentration of 0.07 ppm. There was no change in R_{aw} or symptoms as a result of the NO_2 exposure. There was also no difference in the R_{aw} response to allergen challenge in these subjects (i.e., NO_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 parasympathomimetic drug) following 1 h resting exposure to 0.1 ppm NO_2 in a well characterized group of 20 methacholine-reactive mild asthmatics. A small (8%) increase in SR_{aw} ($p=0.23$) was observed after NO_2 exposure. Three of the 15 subjects had a greater than 20% decrease in the dose of methacholine required to double SR_{aw} (PD_{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_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_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_2 for 2 h while performing light, intermittent exercise. There were no significant effects of NO_2 exposure on forced expired spirometry. Total R_T (forced oscillation) tended to increase (9%) after NO_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_2 exposure. The determination of the dose which would cause a 10% decrease in FEV_1 (D10) was the most "conventional" approach (O'Connor et al., 1987) to assessing methacholine responsiveness. In the 21 subjects in which this dose could be ascertained, D10 was 8.6 ± 16.2 μg on the air day and 3.0 ± 6.2 μg on the NO_2 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 exposure to 0.20 ppm NO_2 for 2 h.

Koenig et al. (1985) have studied the effects of 1 h resting exposure of asthmatic adolescents to 0.12 ppm NO_2. There were no "consistent significant changes in pulmonary functional parameters" after NO_2 exposure. Although symptom data were not presented, the authors indicated that subjects had more symptoms after NO_2 exposure but that the trend was not significant.

Subsequent studies by Koenig et al. (1987a,b) of mouthpiece exposures to 0.12 ppm NO_2, which incorporated exercise (30 min rest followed by 10 min exercise), indicated increases in R_T and decreases in FEV_1 after both air and NO_2 exposure. These changes were apparently due to exercise alone (R_T increased 8.1% with air and 10.4% with NO_2; postexercise FEV_1 was decreased 7.4% with air and 4.1% with NO_2). In the final phase of the study, subjects were exposed to 0.18 ppm NO_2 using the same exercise protocol. In this case, no differences in R_T were seen and FEV_1 decreases were -1.3 and -3.3% for air and NO_2, respectively. This 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 NO_2 exercise exposure studies.

Morrow and Utell (1989) studied a group of 20 asthmatics exposed to 0.30 ppm NO_2 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 NO_2 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 FEV_1 decrements in NO_2 than in air), the new subjects had significantly greater FEV_1 decrements during the air exposures.

Linn et al. (1985b) and Linn and Hackney (1984) exposed a group of 23 mild asthmatics to 4 ppm NO_2. Subjects completed a total of four exposures (two each to NO_2 and clean air) separated by 1 week. 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 SO_2 during exercise. Mean baseline preexposure SR_{aw} measurements varied from 5.48 and 5.59 on the air exposure days to 6.14 and 6.44 on the NO_2 exposure days, although it is unlikely that the slightly higher baseline values on the NO_2 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 NO_2; if anything, SR_{aw} tended to be slightly lower with the NO_2 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 NO_2 exposure. The authors suggested the possibility that NO_2 deposited in the respiratory tract may form a vasoactive substance such as an organic or inorganic nitrate. Nitrate formation after NO_2 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 which could be attributed to NO_2 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

NO_2 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 NO_2 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 NO_2 exposure but is not known for either group. Secondly, the asthmatics in the Linn et al. study, although reactive to SO_2, tended to have milder disease; none used regular asthma medications and all but three subjects had an FEV_1/FVC ratio in excess of 75% (i.e., minimal asthma as per Appendix 15-A). 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_1/FVC ratio less than 75% (i.e., mild to moderate asthma). It is not clear whether the effects of NO_2 could have been confounded by exposure to an ambient aeroallergen. All subjects in the Linn et al. study were exposed in March at a time when outdoor aeroallergens would have tended to be minimal for several months (because of the mild climate, allergens are present during the winter months). Also, increased bronchial reactivity to cold air was an important finding in the Bauer et al. study; it was not measured by Linn et al.

Further studies were conducted by Linn et al. (1986) on 21 (minimal to mild) asthmatics exposed to 0, 0.30, 1.0, and 3.0 ppm NO_2 for 1 h. The exposures included intermittent, moderate exercise ($\dot{V}_E$= 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_2 exposures, there were no significant effects of NO_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_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_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_2 exposure. The previously observed decrease in systolic pressure, associated with 4.0 ppm NO_2 exposure, was not examined in these subjects.

Mohsenin (1987a) studied 10 mild asthmatics exposed to 0.5 ppm NO_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_2 exposure. The response to methacholine was evaluated with partial expiratory flow at 40% VC (PEF40%VC), rather than changes in SR_{aw} or FEV_1, to test for "small airway abnormality" without the influence of prior deep breaths. There was a significant increase in airway responsiveness to methacholine (bromide) after the NO_2 exposure. The dose of methacholine required to decrease PEF40%VC by 40% was 9.2 $\pm$ 15 after air and 4.6 $\pm$ 8.2 after NO_2 ($p=0.042$).

Roger et al. (1990) reported the results of NO_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_2, they found an 11% decrement in FEV_1, which was significantly larger than the 7% decrease seen after the clean air exposure. These differences between air and NO_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_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_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_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_2. Exposures lasted 120 min and included 10 min of exercise. There were no significant changes in lung function (SR_{aw}, FEV_1) or airway responsiveness to histamine resulting from NO_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 (Jörres and Magnussen, 1990; Rubinstein et al., 1990). Jörres and Magnussen (1990) exposed 14 mild-to-moderate asthmatic subjects to 0.30 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 such. 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 vs. 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, $FEV_{1.0}$) 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) at a constant, isocapnic ventilation of 20 L/min, maintained for 4 min. SR_{aw} was measured after each step increase in SO_2 concentration. The concentration of SO_2 required to increase SR_{aw} by 8 units ($PD_{8u}SO_2$) was interpolated from a dose-response curve of SO_2 concentration vs. SR_{aw}. The $PD_{8u}SO_2$ was 1.25 $\pm$ 0.70 ppm after air exposure and 1.31 $\pm$ 0.75 after NO_2 exposure, indicating no mean change in responsiveness to SO_2. Only one subject showed a tendency toward increased responsiveness to SO_2 after NO_2 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 NO_2 concentration and exercised during exposure. However, Jörres and Magnussen's subjects appeared to have had slightly more severe asthma and were somewhat older. The modest increase in SR_{aw} induced by exercise in the Rubinstein et al. study may have interfered with the response to SO_2 (i.e., the subjects may have been in a refractory state). Finally, the different method of administering the SO_2 bronchoprovocation test (i.e., increased $\dot{V}_E$ at constant SO_2 vs. increasing SO_2 at constant $\dot{V}_E$) may produce a different response, since hyperventilation alone could contribute to the increase in SR_{aw} (Deal et al., 1979; Eschenbacher and Sheppard, 1985). Thus, although similar, the two SO_2 challenges are not necessarily comparable.

15.3.1.1 Effects of HNO_3 Vapor on Asthmatics

Koenig and associates (1988; 1989a,b) have recently reported preliminary results of a study of nine adolescent asthmatics exposed to HNO_3 vapor. Subjects were exposed to 50 and 100 ppb HNO_3 and to 50 ppb HNO_3 plus 68 $\mu g/m^3$ H_2SO_4. FEV_1 decreased following exposure (30 min rest followed by 10 min mild exercise) under all three conditions.

Koenig et al. (1989a) and Koenig (1989) have examined the responses of adolescent asthmatics to a 40-min exposure to 50 ppb (2 $\mu M/m^3$) HNO_3 vapor exposure via a mouthpiece exposure system. In the initial study, after 30 min of rest and 10 min of exercise while breathing HNO_3 vapor, there was a 4.4% decrease in FEV_1 compared to 1.7% decrease after air breathing. A 22.5% increase in total respiratory resistance was also observed after HNO_3 compared to a 7.4% increase after air.

In the second study (Koenig et al., 1989a), subjects were exposed for 45 min including two 15-min mild ($\dot{V}_E = \sim 25$ 1/min) exercise periods. The subjects were exposed to air and 57 ± 16 ppb HNO_3 twice: once without and once with a preliminary gargle of lemonade, intended to reduce oral ammonia (NH_3) levels. Baseline oral NH_3 of 318 ± 84 ppb was reduced to 113 ± 98 ppb after lemonade gargle. There were small, but not statistically significant, decrements in FEV_1 after all exposures, -3.3% after HNO_3 alone, and -1.7% after both air and HNO_3 plus lemonade. Similar trends (-9.4%, HNO_3; -5.5%, HNO_3 plus lemonade; -5.1%, air) were observed for $\dot{V}_{50\%VC}$. The author suggested that the HNO_3 vapor was likely to have been neutralized by oral NH_3 and that this potential reaction may minimize the entry of HNO_3 to the respiratory tract. This led to the interesting suggestion that, in mixtures of HNO_3 vapor and H_2SO_4 aerosol, NH_3 may react preferentially with the gaseous HNO_3, thus reducing the potential neutralization of H_2SO_4 aerosol.

15.3.2 Effects of NO_2 on Patients with Chronic Obstructive Lung Disease

Patients with COPD represent an important potentially sensitive population group. Many 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 COPD may lead to a greater delivery of NO_2 to the segment of the lung which is well ventilated, thus resulting in a greater regional tissue dose. Tables 15-5 and 15-6 summarize these studies.

In a review of their studies, von Nieding and Wagner (1979) summarized previously reported findings. The main observations were that R_{aw} increased in chronic bronchitics exposed to 2.0 ppm NO_2 or greater and that, after exposure to 4 to 5 ppm NO_2, arterial partial pressure of oxygen (PO_2) was decreased and the alveolar-arterial O_2 gradient was widened.

TABLE 15-5. SUBJECT CHARACTERISTICS FOR COPD PATIENTS EXPOSED TO NO_2[a]

Reference	Subjects (Number)	Age	FEV_1/FVC (Percent)	SR_{aw} or SG_{aw} ($L \cdot cmH_2O/L/s$)	Reversibility	Dyspnea	Diagnosis	Medications	Witholding	Medication Reactivity	Airway Notes
Kerr et al. (1979)	7	24-53	75	5.10	--	--	CBR	--	--	--	Daily cough for three consecutive months
Linn et al. (1985a)	13 M/9 F	48-69	29-67	16.6	--	19/22	6 CBR 21 EM 4 AS	11 IB 12 OB 2 ST	4 h IB	--	
Morrow and Utell (1989)	13 M/7 F	47-70	43-75	*(0.30-) (2.87) 3.48-33.3	N	Y	COPD	--	--	--	8 mild 12 moderate
von Nieding and Wagner (1979)	116	25-74		Chronic nonspecific lung disease	--	--					
	14		$R_T = 5.93$	CBR							
von Nieding et al. (1973a)	84 M	30-72	--	3.5-10	--	--	COPD	--	--	--	Chronic nonspecific lung disease
von Nieding et al. (1971, 1970)	88	--	--	--	--	--	CBR	--	--	--	

[a]Abbreviations:
CBR = Chronic bronchitis
M = Male
F = Female
IB = Inhaled β agonist
EM = Emphysema
OB = Oral bronchodilator (theophylline)
AS = Asthma
ST = Oral corticosteroid
COPD = Chronic obstructive pulmonary disease
R_T = Total respiratory resistance

*SG_{aw} = Specific airway conductance

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TABLE 15-6. EXPOSURE CONDITIONS AND RESPONSES IN COPD PATIENTS EXPOSED TO NO_2[a]

Reference	Conc. (ppm)	Exposure Duration (min)	Exercise Duration (min)	Exercise Ventilation (L/min)	Temp. (°C)	Relative Humidity (Percent)	Responses
Kerr et al. (1979)	0.5	120	15	25	24	45	No effects in bronchitics alone. Possible decrease in quasistatic compliance.
Linn et al. (1985a)	0.5 1.0 2.0	60	30	16	22.5	49	No change in FVC, FEV_1, etc. at any NO_2 level. SR_{aw} tended to increase after first exercise period. Possible decrease in peak flow at 2 ppm. No symptom changes. No change in SaO_2.
Morrow and Utell (1989)	0.30	225	21 (3x7)	25	21	40	Total NO_2 inhaled dose 1.215 mg. Decrease in FVC after exposure -9.6%. 5.2% decline in FEV_1 significant after ~4-h exposure.
von Nieding and Wagner (1979)	1-8 ppm	5-60	--	--	--	--	At 4-5 ppm for 15 min, PaO_2 decreased (arterialized capillary blood). R_{aw} increased with exposure to 1.6 ppm or greater.
von Nieding et al. (1973a)	(a) 1-5	30 breaths (15 min)	--	--	--	--	Increase in R_{aw} related to NO_2 concentration. No effect on R_{aw} below 1.5 ppm.
	(b) 5	60	--	--	--	--	Changes in PO_2 of earlobe capillary blood. Change occurred in first 15 min., effect did not increase with further exposure.
von Nieding et al. (1971, 1970)	0.5-5	15	--	--	--	--	Decrease in earlobe blood PO_2 at 4.0 ppm and above. Increased R_{aw} at concentrations of 1.6 ppm and above.

[a]Abbreviations:
FVC = Forced vital capacity
FEV_1 = Forced expiratory volume in 1 second
SR_{aw} = Specific airway resistance
SaO_2 = Arterial oxygen saturation
PaO_2 = Arterial partial pressure of oxygen
PO_2 = Partial pressure of oxygen
R_{aw} = Airway resistance

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The results of two NO2 exposure studies were discussed in von Nieding et al. (1980). In the first study, 14 normal and 14 bronchitic patients were exposed to 5 to 8 ppm NO_2 for up to 5 min on 4 separate days. The mean increase in R_{aw} was 1.07 cm H_2O/L/s. Except for three subjects with an increase in R_{aw} of greater than 2.0 cm H_2O/L/s, the responses of the bronchitics were similar to the normal subjects.

In the second study (von Nieding et al., 1980), 30 normal subjects and 40 bronchitic subjects were exposed to 5 ppm NO_2 for 5 min. The subjects were divided into clusters according to their pre-exposure R_{aw}'s, which ranged from less than 1 cm H_2O/L/s to greater than 4.0 cm H_2O/L/s. There was a tendency for the response to NO_2 to be greater in the subjects with the highest baseline R_{aw}. In subjects with baseline R_{aw} >4.0 cm H_2O/L/s, the increase in R_{aw} averaged just less than 1.5 cm H_2O/L/s; it was less than 0.5 cm H_2O/L/s in subjects with baseline R_{aw} <1cm H_2O/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 emphysematics and chronic bronchitics, exposed, while exercising intermittently, for 1 h to 0.5, 1.0, and 2.0 ppm NO_2. 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 NO_2. 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 NO_2 for 3.75 h during which time they performed mild exercise for three 7-min periods. FVC showed progressive and significant decreases during and following NO_2 exposure with the largest change of -9.6% occurring after 3.75 h of exposure. Smaller decrements were seen in FEV_1 (-5.2%) at the end of exposure. There was no effect on SG_{aw} or diffusing capacity as a result of NO_2 exposure. The differences between subjects with more severe disease (FEV_1 <60% predicted) and those with milder disease (FEV_1 ≥60%) in terms of their relative responses to NO_2 were generally not significant, except for a possible slightly greater decrease in FEV_1 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 NO_2; the COPD patients showed a decrement while the healthy nonsmokers showed an improvement in FEV_1. There were also apparent differences in NO_2 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 NO_2 because of their current or previous smoking habit or because of some predisposition to NO_2 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 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 Linn et al.'s subjects had similar or worse lung function than Morrow and Utell's subjects. Note also that Morrow and Utell's mild COPD subjects had greater responses.

15.3.3 Summary

Although findings in asthmatics have been mixed, the pulmonary function responses to NO_2, within the ambient range, are relatively small when compared to SO_2 exposure (see Table 15-7 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 NO_2 concentrations, indicative of 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 exposed 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 15-7. FEV_1 AND RESISTANCE CHANGES IN ASTHMATICS EXPOSED TO NO_2[a]

Reference	NO_2	Percent Δ FEV_1		Percent Δ SR_{aw} or SG_{aw}[b] or ΔR_T[c,d]		Notes
		Air	NO_2	Air	NO_2	
Avol et al. (1988)	0.3	-10	-11	+34	40.5	SG_{aw} is mean for last two measurement.
	0.6	-11	-12	+34	78	
Bauer et al. (1986a)	0.3	-4.1	-10.1[e] (-7.0)	NS[b]	NS[b]	Air (10-15+), NO_2 (15-). Excludes two anomalous subjects.
Bylin et al. (1985)	0.12			+3.0	+4.1	Data given for 20 min of exposure.
	0.25			+3.0	-3.9	
	0.50			+3.0	-23.0	
Bylin et al. (1988)	0.14			-12	-7.5	
	0.27			-12	-8.8	
	0.53			-12	-13.5	
Hazucha et al. (1982, 1983)	0.1			-1.9	+6.6	
Kleinman et al. (1983)	0.2	=3.3	=2.61	1[e]	+9[e]	
Koenig et al. (1985)	0.12	0	-3.0	-5.3[e]	-4.4[e]	Greater % Δ FEV_1 due to higher baseline on NO_2 day.
Koenig et al. (1987a,b)	0.12	-6.3	-6.1	-4.3[e]	+10.3[e]	
	0.18	-1.3	-3.3	+5.1[e]	+11.6[e]	
Linn et al. (1985b)	4 ppm (twice)	--	--	+37	+15	Average of two exposures.
		--	--	+17	+22	

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TABLE 15-7 (cont'd). FEV_1 AND RESISTANCE CHANGES IN ASTHMATICS EXPOSED TO NO_2[a]

Reference	NO_2	Percent Δ FEV_1		Percent Δ SR_{aw} or SG_{aw}[b] or ΔR_T[c,d]		Notes
		Air	NO_2	Air	NO_2	
Linn et al. (1986)	0.3	-2.5	-0.3	+63	+64	
		1.0		-1.9		+35
		3.0		+0.6		+29
Roger et al. (1990)	0.3	-6	-12	--	--	
		0.15	--	--	--	--
		0.30	--	--	--	--
		0.60	--	--	--	--

[a]Abbreviations:
FEV_1 = Forced expiratory volume in 1 second
SR_{aw} = Specific airway resistance
SG_{aw} = Specific airway conductance
R_T = Total respiratory resistance
[b]A decrease in SG_{aw} indicates an increase in airway resistance.
[c]A decrease in SR_{aw} indicates a reduction in airway resistance.
[d]A decrease in R_T indicates a reduction in airway resistance.
[e]Significant difference.

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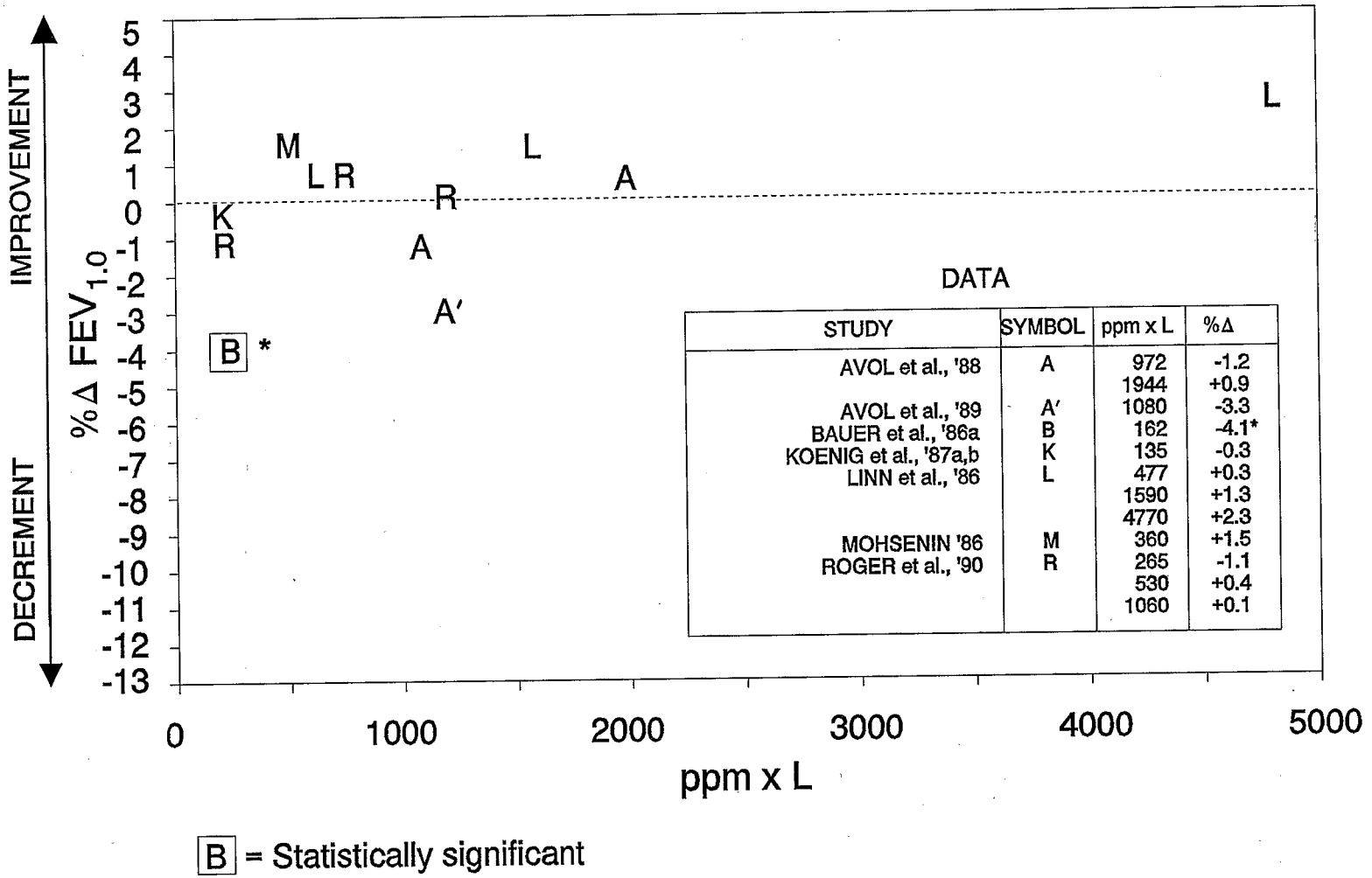

STUDY	SYMBOL	ppm x L	%Δ
AVOL et al., '88	A	972	-1.2
		1944	+0.9
AVOL et al., '89	A′	1080	-3.3
BAUER et al., '86a	B	162	-4.1*
KOENIG et al., '87a,b	K	135	-0.3
LINN et al., '86	L	477	+0.3
		1590	+1.3
		4770	+2.3
MOHSENIN '86	M	360	+1.5
ROGER et al., '90	R	265	-1.1
		530	+0.4
		1060	+0.1

Figure 15-1. Percent change (post-air vs. post-NO_2) in FEV_1 vs. NO_2 dose in ppm x liters in asthmatics.

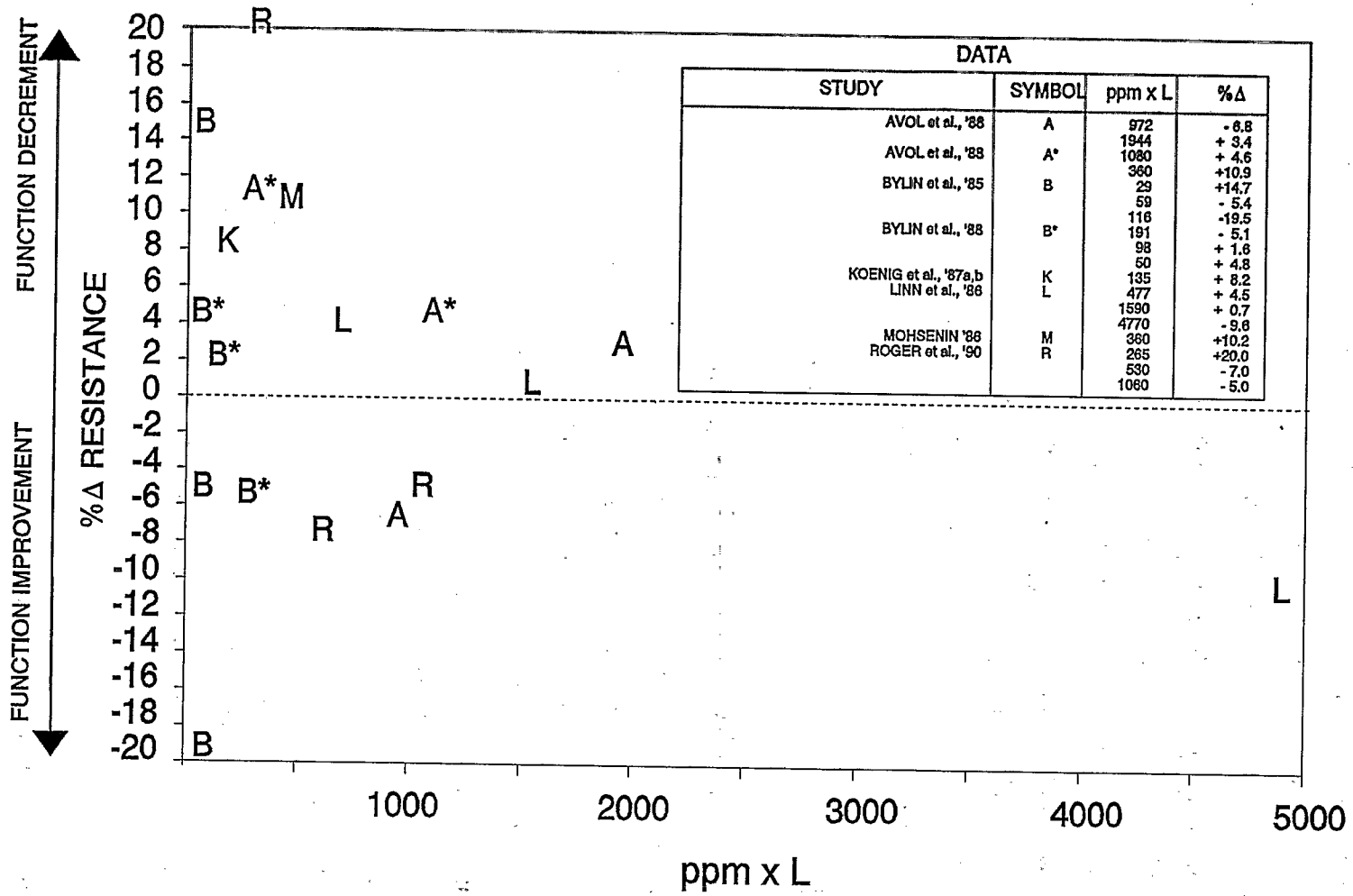

Figure 15-2. Percent change (post-NO_2 – post air/post-air) in resistance (R_{aw}, SR_{aw}, or R_T) vs. NO_2 dose in ppm x liters in asthmatics.

15.4 EFFECTS OF NO_2 EXPOSURE ON AIRWAYS RESPONSIVENESS

Physiological changes in the airways induced by a variety of inhaled substances have been used to assess their "responsiveness" or "reactivity." Comparing results across studies is difficult because of the variety of types of airway challenges, methods used to administer the tests, physiological end points used to quantify the responses, 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 SO_2; or (4) specific antigenic substances such as ragweed or grass pollen. In this section, the effects of NO_2 on measures of airway responsiveness are discussed. A more detailed discussion of many 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 airways hyperresponsiveness (BHR) in some asthmatics and the presence of BHR 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 histamine (Hamielec et al., 1988), hypertonic saline (Belcher et al., 1987), or 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, while the responses to a number of airway challenges may be correlated (Chatham et al., 1982), they cannot be considered equivalent.

15.4.1 Healthy Normal Subjects

In a small number of recent studies, the effects of NO_2 on airway responsiveness in healthy, normal subjects have been reported. Airway responsiveness has been shown to

increase in normal subjects after exposure to NO_2 concentrations in excess of 1.0 ppm. Beil 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-NO_2 exposure could be blocked by elevation of serum ascorbate levels through vitamin C pretreatment. 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 NO_2 on 3 consecutive days. Nasal inoculation with influenza virus occurred on the second day of NO_2 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 NO_2 concentration. Responses were not altered after virus inoculation. In two studies, NO_2 concentrations in excess of 1.5 ppm NO_2 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 NO_2 exposure in asthmatics. The findings from the various studies reported here are summarized in Table 15-8. See Section 15.3.1 for a more detailed description of the exposure and measurement methodology.

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

TABLE 15-8. CHANGES IN AIRWAY RESPONSIVENESS ASSOCIATED WITH NO_2 EXPOSURE[a]

	Number	NO_2 (ppm)	Duration of Exp.	Challenge Type	Endpoint	Time Post-ex.	Exercise	Change in AR[b] +	Change in AR[b] -	Average PD ± SD[c] Air	Average PD ± SD[c] NO_2	Notes
ASTHMATICS												
Ahmed et al. (1983b)	20	0.1	60	CARB	SGaw	-	N	13	7	6.04	2.67	
Ahmed et al. (1983a)	19	0.1	60	RAG	SGaw	-	N	10	8	8.97±24.7	3.36±4.63	
Hazucha et al. (1983)	15	0.1	60	METH	SRaw	20	N	6	7	1.9±(0.4)	2.0±(1.0)	
Orehek et al. (1976)	20	0.1	60	CARB	SRaw	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)		
Orehek et al. (1981) (4 allergic + 3 asthmatic)	7	0.11	60	GRASS	SRaw	IM	N	-	-	1.2±0.3	1.3±0.3	
Bylin et al. (1988)	20	0.14	30	HIST	SRaw	25	N	14	6	-	-	
Roger et al. (1990)	19	0.15	80	METH	SRaw	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)		
Jörres and Magnussen (1990)	14	0.25	30	SO_2	SRaw	27	N	11	2	46.5±(5.1)	37.7±(3.5)	
Jörres and Magnussen (1991)	11	0.25	30	METH	SRaw	60	Y	7	4	0.41±(1.6)	0.41±(1.6)	
Bylin et al. (1988)	20	0.27	30	HIST	SRaw	25	N	14	6	-	-	
Avol et al. (1988)	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
Bauer et al. (1986a)	12	0.30	30	COLD	FEV_1	60	Y	9	3	0.83±0.42	0.54±0.33	PD_{10}RHE[d]
Linn et al. (1986)	21	0.30	120	COLD	FEV_1	IM	Y	-	-	(-11.4	-12.1)	Delta FEV_1
Morrow and Utell (1989)	20	0.30	225	CARB	SGaw	-	Y	-	-			
Roger et al. (1990)	19	0.30	80	METH	SRaw	60	Y	8	9	3.3±0.7	3.3±0.8	
Rubinstein et al. (1990)	9	0.30	30	SO_2	SRaw	60	Y	4	5	1.3±0.7	1.3±0.8	

TABLE 15-8 (cont'd). CHANGES IN AIRWAY RESPONSIVENESS ASSOCIATED WITH NO_2 EXPOSURE[a]

	Number	NO_2 (ppm)	Duration of Exp.	Challenge Type	Endpoint	Time Post-ex.	Exercise	Change in AR[b] +	Change in AR[b] -	Average PD ± SD[c] Air	Average PD ± SD[c] NO_2	Notes
Bylin et al. (1985)	8	0.48	20	HIST	SRaw	20	N	5	-	-	-	
Mohsenin et al. (1987a)	10	0.50	60	METH	SGaw	IM	N	7	2	9.2±1.5	4.6±8.0	
Bylin et al. (1988)	20	0.53	30	HIST	SRaw	25	N	12	7	-	-	
Avol et al. (1988)	37	0.60	120	COLD	FEV_1	60	Y	13	16	-8.4±11	-10.4±14	Delta FEV_1
Roger et al. (1990)	19	0.60	80	METH	SRaw	60	Y	11	8	3.3±0.7	3.7±1.1	
Rasmussen et al. (1990)	20	0.80	120	METH	FEV_1	IM	Y	-	-	(AIR-NO_2 = -0.06)		
Linn et al. (1986)	21	1.00	120	COLD	FEV_1	IM	Y	-	-	-11.4	-11.2	Delta FEV_1
HEALTHY NORMAL SUBJECTS												
Ahmed et al. (1983b)	20	0.1	60	CARB	SGaw	-	N	10	10	20.7	19.6	
Hazucha et al. (1983)	15	0.1	60	METH	SRaw	20	N	6	7	16.2±(2.7)	18.3±(3.0)	
Bylin et al. (1985)	8	0.48	20	HIST	SRaw	20	N	1	2	-	-	
Beil and Ulmer (1976)	16	1.0	120	ACH	R_T	IM	N			0.6	0.9	
Frampton et al. (1991)	15	1.5	180	CARB	FEV_1	30	Y	11	4	(-4.8±1	-7.5+1)	Delta FEV_1
Kulle and Clements (1988)	21	2.0	120	METH	FEV_1	IM	N			-10.7[e]	-12.7	Delta FEV_1
Kulle and Clements (1988)	21	3.0	120	METH	FEV_1	IM	N			-7.0	-9.9	Delta FEV_1
Mohsenin (1988)	18	2.0	60	METH	SGaw	IM	N	12	2	101±44	81±45	
Beil and Ulmer (1976)	16	2.5	120	ACH	R_T	IM	N			0.8	0.6	
Beil and Ulmer (1976)	16	5.0	120	ACH	R_T	IM	N			0.9	0.9	
Beil and Ulmer (1976)	16	7.5	120	ACH	R_T	IM	N			0.7	1.0	

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[a] Abbreviations:
CARB = Carbachol
SGaw = Specific airway conductance
N = Rest
Y = Included exercise
RAG = Ragweed
METH = Methacholine
SRaw = Specific airway resistance
GRASS = Grass pollen
HIST = Histamine
FEV_1 = Forced expiratory volume in 1 second
IM = Immediately after exposure
SO_2 = Sulfur dioxide
COLD = Cold-dry air
ACH = Acetylcholine
R_T = Total respiratory resistance
[b] Change in AR = + = increased airway responsiveness (AR) after NO_2 compared to air; - = decreased AR after NO_2 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] **PD_{10} RHE** = Respiratory heat exchange (loss) for 10% drop in FEV_1
[e] Separate control and exposure groups. First day of three consecutive daily exposures.

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. Kleinman 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 PD40 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, since 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 20 to 30 min resting NO_2 exposure. In the first study, 5 of 8 subjects showed an increase in response after 0.48 ppm exposure and in the second study 14 of 20 subjects showed an increase in response after 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 a decrease in cold air airway responsiveness after a 30-min exposure to 0.30 ppm NO_2. The airway responsiveness was expressed as the quanitity of respiratory heat loss reuired to produce a 10% drop in FEV_1; this 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 since 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 15-8 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 SO_2 has been evaluated after 30 min of exposure to 0.25 to 0.30 ppm NO_2 in two studies. Jörres and Magnussen (1990) found an increased airway responsiveness to SO_2 after a resting exposure to 0.25 ppm NO_2, but Rubinstein et al. (1990) found no change in airway responsiveness to SO_2 after a 0.30-ppm NO_2 exposure of similar duration that included 20 min of exercise. The SO_2 challenges were administered by different techniques; Jörres and Magnussen used a series of increasing levels of ventilation at

a constant SO_2 concentration whereas Rubinstein et al. (1990) used increasing concentrations of SO_2 at a constant ventilation.

The effects of NO_2 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 NO_2. 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 NO_2.

For 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 15-8. Tabulation of data from this table provided information regarding the direction of the change (i.e., increase or decrease) in airway responsiveness following NO_2 exposure. One of the problems in this kind of analysis is that it is often difficult to distinguish between a negative and no-change situation (i.e., it is less likely that airway responsiveness would decrease from its baseline level than increase).

For studies of exposure to <0.20 ppm, the overall data indicated 67 subjects with airway responsiveness increased and 38 with airway responsiveness decreased. Further breakdown of this data into exercise/ no-exercise or exposure duration was not fruitful.

For the studies ($n=11$) 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. 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 47 increases and 33 decreases in airway responsiveness. For resting studies, 24 subjects had increased airway

responsiveness while 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 15-9. The studies in which the change in airway responsiveness was assessed after NO_2 exposure are presented in three concentration ranges and 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% (106 increased and 48 decreased) since, in the exercising studies, only 52% (104 increased and 96 decreased) had an increase in airway responsiveness (i.e., no effect). There was a trend 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; Jörres 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 in unclear since 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 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 NO_2 causing increased airway responsiveness at a low exposure doses (for example causing mast cell degranulation, vis a vis Sandström et al. (1990a), with a reversal of this response at higher exposure doses

TABLE 15-9. FRACTION OF NO_2-EXPOSED SUBJECTS WITH INCREASED AIRWAY RESPONSIVENESS

NO_2 (ppm)	All Exposures	Exposures with Exercise	Exposure at Rest
ASTHMATICS			
$0.05 < [NO_2] < 0.20$	0.64 (105)[a]	0.59 (17)	0.65 (88)[a]
$0.20 \leq [NO_2] \leq 0.30$	0.57 (169)	0.52 (136)	0.76 (33)[a]
$0.30 < [NO_2]$	0.59 (80)	0.49 (47)	0.73 (33)[b]
All $[NO_2]$	0.59 (354)[a]	0.52 (200)	0.69 (154)[a]
HEALTHY			
$[NO_2] < 1.0$	0.45 (31)		0.45 (31)
$[NO_2] > 1.0$	0.79 (29)[a]	0.73 (15)	0.86 (14)[b]
All $[NO_2]$	0.62 (60)	0.73 (15)	0.60 (45)

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.

[a] $p<.01$ two-tailed sign test.
[b] $p<.05$ two-tailed sign test.

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possibly through a direct relaxing effect on airway smooth muscle? For example, nitrites formed in the lungs of NO_2-exposed animals (Postlethwait and Mustafa, 1981) may have a direct relaxing effect on smooth muscle, including bronchial smooth muscle.

In healthy normal subjects, an increase in airway responsiveness clearly occurs at higher NO_2 exposure doses (Beil and Ulmer, 1976; Frampton et al., 1991; Mohsenin, 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.

15.5 EFFECTS OF NO_2 OR HNO_3 EXPOSURE ON BLOOD, URINE, AND BRONCHOALVEOLAR LAVAGE FLUID BIOCHEMISTRY

The effects of NO_2 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 NO_2-induced alterations in body fluids that could potentially result in systemic effects. Investigations have been aimed at determing the effects of NO_2 on levels of serum enzymes and antioxidants as well as direct effects on red blood cells and hemoglobin. Studies of the effects of NO_2 on airway lining fluids have focused on changes in $alpha_1$-antitrypsin levels. Potential effects of NO_2 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 NO_2 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 disphosphoglycerate, 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. The average baseline level of GSH was 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 NO_2 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 NO_2. However, Gohil 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 NO_2 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 NO_2 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 NO_2 (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 NO_2.

15.5.2 Bronchoalveolar Lavage Fluid Biochemistry

Mohsenin and Gee (1987) have reported that subjects exposed to 3 to 4 ppm NO_2 for 3 h had a 45% decrease in the activity of alpha-1-protease inhibitor, 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 NO_2-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 alpha-1-protease inhibitor 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 α-1-antitrypsin deficiency.

Johnson et al. (1990) also examined the response of alpha-1 protease inhibitor (α_1PI) to in vivo NO_2 exposure in a group of 24 healthy nonsmokers. The subjects were exposed to either 1.5 ppm NO_2 for 3 h or to a variable concentration consisting of a baseline level of 0.05 ppm NO_2 with three 15-min "peaks" of 2.0 ppm. The details of the exposure protocol and the subject characteristics are provided in Frampton et al. (1989b); (Table 15-1, Section 15.2). BAL was performed 3.5 h after exposure and the fluid was frozen for subsequent analysis. The functional activity of α_1PI was taken to be the elastase inhibitory activity corrected for the concentration of α_1PI determined by immunoassay. Neither the levels of α_1PI, as determined by immunoreactivity, or its functional activity were significantly changed by NO_2 exposure.

The different findings by Johnson et al. (1990) and Mohsenin and Gee (1987) with regard to α_1PI 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 α_1PI concentrations in the two studies. This issue is also discussed in Section 13.2.2.2.6. As discussed by Mohsenin and Gee (1987), there appears to be a large range of α_1PI activity that is compatible with lung health, and there is broad range of activity of α_1PI in relation to its concentration. The importance of small changes in α_1PI is not clearly established, and therefore, the usefulness of changes in α_1PI 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 3 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 NO_2 OR HNO_3 VAPOR EXPOSURE ON HUMAN PULMONARY HOST DEFENSE RESPONSES

From the epidemiological (Sections 14.2.1 and 14.3.1) 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) (see also Section 13.2.2.1.3). In human volunteers, the effects of NO_2 exposure on viral infectivity have been studied. The effects of NO_2 on macrophage functions has been examined both 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 is discussed in Section 15.2.1.4.

Kulle and Clements (1988) and Goings et al (1989) [two reports of the same study] examined the effect of NO_2 exposure on 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 ppm (n=22), 2.0 ppm (n=21;22), or 3.0 ppm (n=22). The exposures consisted of one preliminary day of clean air exposure and then 3 consecutive days of the treatment (i.e., either NO_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%, and in the 3.0, 2.0, 2.0, and 1.0 ppm NO_2 exposure groups were 77%, 57%, 91%, and 91%. Although the rates of infection were elevated after NO_2 exposure in three of four NO_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; this implies that the hypothesis that NO_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_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_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 while 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_2 exposure, and to examine the possibility that a series of peak exposures would cause more impairment than a constant concentration (see also 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_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. IL-1 is an important proinflammatory protein produced by macrophages which performs a number of functions, including induction of fibroblast proliferation, 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_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_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_2 may influence host defense mechanisms in humans.

Frampton et al. (1989b) also analyzed the protein content of BAL fluid obtained from NO_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_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_2. Exposures included 10 min of exercise during each half-hour of exposure. There were no significant changes in pulmonary function, airways reactivity, or respiratory symptoms observed after NO_2 exposure. 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 0.60 ppm exposure indicated an increase in alpha-2- macroglobulin (α_2-M), a regulatory protein that has antiprotease activity and immunoregulatory effects. The observed increase in α_2-M appears to be transient (no change was seen at 18 h postexposure) and was not observed at a higher NO_2 concentration (1.5 ppm). Further information appears to be necessary to establish the implications of this finding.

The effects of NO_2 on macrophage function in NO_2-exposed animals is discussed in Section 13.2.2.1.2. NO_2-induced changes have been noted in macrophages harvested from animals exposed to NO_2 concentrations less than 1.0 ppm. These studies are discussed in detail in the animal toxicology chapter.

The effect of in vitro exposure to NO_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 18 h incubation, the cells were exposed to 5, 10, or 15 ppm NO_2 or 5% CO_2 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_2 concentrations. There were no changes in release of NCF in any of the NO_2-exposed cell cultures. Furthermore, NO_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_2-exposed and air-exposed macrophage cultures. Therefore, NO_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_2 exposure did not impair the ability of macrophages to produce either IL-1 or NCF, in response to conventional stimuli.

Sandström 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. BAL 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_2 exposures. Increased levels of lymphocytes were observed only at the two higher concentrations.

In order to determine the time course of this response, Sandström et al. (1990a) exposed 32 subjects to 4 ppm NO_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 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_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; other investigators (Frampton et al., 1989a,b) did not, apparently, observe changes in mast cell numbers. The authors considered the increased numbers of mast cells and lymphocytes to represent an unspecific inflammatory response.

Boushey et al. (1988) studied five healthy volunteers exposed to 0.60 ppm NO_2 on 4 days over a 6-day period. Exposures each lasted 2 h and included alternating 15-min periods of rest and exercise ($\dot{V}_E$ ~30-40 L/min). On the final (fourth) day of NO_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_2 exposure on pulmonary function (SR_{aw}, FVC, $FEV_{1.0}$) or respiratory symptoms. Following the fourth day of NO_2 exposure, a slight increase in circulating (venous blood) lymphocytes was observed (1792 $\pm$ 544/mm^3 post-NO_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_2). The authors expressed reservations that the apparent increase in NK cells may have been an artifact of the cell separation process. IL-1 and tumor necrosis factor (TNF) levels in BAL fluid were not detectable. TNF is another proinflammatory protein that, among other activities, promotes aherence of polymorphonuclear leukocytes to endothelial cells and enhances their phagocytic activity.

Sandström et al. (1990b) studied a group of eight healthy nonsmokers exposed to 4.0 ppm NO_2 for 20 min per day (moderate exercise, $\dot{V}_E$ ~35 L/min) on alternate days over a 12-day period (seven exposures total). BAL 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_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 lavage fluid. In addition, there were reduced numbers of T-suppressor, B lymphocytes, and NK cells in the alveolar portion of the BAL fluid compared to the baseline lavage. These observations contrast with those seen by Sandström et al. (1989) after single NO_2 exposures, suggesting some alteration in bronchial and alveolar cell populations after repeated NO_2 exposure. The most obvious difference between Sandström et al. (1990b) and Boushey et al. (1988) is the higher NO_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_2 exposure dose necessary to invoke modification of bronchoalveolar cell populations.

The effects of HNO_3 vapor exposure (in vivo) have been examined in two recent studies. Becker et al. (1991) exposed five healthy subjects (4 males/1 female) to 200 $\mu g/m^3$ of HNO_3 vapor for 120 min, including 100 min of moderate exercise. Bronchoalveolar lavage performed 18 h postexposure indicated an increase in number of lymphocytes, an increased concentration of protein in the lavage fluid, and increased phagocytic activity of macrophages harvested from the exposed lung. These results suggest that HNO_3 may cause lymphocyte infiltration into the lung, stimulate macrophage phagocytosis, and increase pulmonary epithelial permeability. The absence of markers of tissue damage, (lactate dehydrogenase [LDH]) suggest that under these exposure conditions HNO_3 did not cause frank tissue damage.

Aris et al. (1991) exposed 10 healthy subjects to 500 $\mu g/m^3$ HNO_3 vapor for 4 h, including moderate exercise. Lavage fluid was obtained from bronchial as well as broncho alveolar washings. No change in LDH levels were observed as a result of HNO_3 exposure. These investigators found no differences in differential cell counts in the lavage fluid. They also exposed a different group of subjects to 500 $\mu g/m^3$ HNO_3 plus 0.20 ppm O_3 and found no potentiation of the O_3-induced inflammatory response by the addition of HNO_3 vapor to the exposure. Their data suggest that HNO_3 does not cause tissue injury, nor does HNO_3 alter the inflammatory response typical of O_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 15-10). These studies have been discussed in the Acid Aerosol Issues Paper (U.S. 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.

TABLE 15-10. EXPOSURE CONDITIONS AND RESPONSES IN SUBJECTS EXPOSED TO NITRATES[a]

Reference	NO_3 ($\mu g/m^3$)	Exposure Duration (Min)	Exercise Duration (Min)	Exercise Ventilation (L/min)	Temp. (°C)	Relative Humidity (Percent)	Number of Subjects	Subject Char.	Aerosol MMAD	Effects
Kleinman et al. (1980)	NH_4NO_3 200	120	60	~20	31	40	20	Normal	1.1	No significant changes due to NO_3 in normals or asthmatics except possible decrease in R_T. No symptom effects.
							19	Asthma		
Sackner et al. (1979)	$NaNO_3$ 10,100,100	10	--	--	--	--	5	Normal	0.2	No changes.
							5	Asthma		
	1000						6	Normal		
							6	Asthma		
	1000						6	Normal		
							6	Asthma		
Stacy et al. (1983)	80 (NH_4NO_3)	240	30	55	30	60	12	Normal	0.55	No effects.
	80 (NH_4NO_3) +0.5 ppm NO_2						12	Normal		
Utell et al. (1979)	$NaNO_3$ 7000	16 (x2) 32 (Tot.)	--	--	--	25	10	Normal	0.46	No effects.
							11	Mild Asthmatics		
Utell et al. (1980)	$NaNO_3$	16 (x2)	--	--		25	11	Influenza	0.49	No symptoms. SG_{aw} decrease 17% and $\dot{V}_E$ max 40% TLC decreased 12% after nitrate, within 2 days of onset of illness. Similar effects 1 week later but not 3 weeks later.
	7000	32 (Tot.)						Patients		

[a] Abbreviations:
MMAD = Mass median aerodynamic diameter
NH_4NO_3 = Ammonium nitrate
R_T = Total respiratory resistance
$NaNO_3$ = Sodium nitrate
SGaw = Specific airway conductance
TLC = Total lung capacity
$\dot{V}_E$max40%TLC = maximum expiratory flow at 40% of TLC on a PEFV curve

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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 μm $NaNO_3$ 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 in 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 re-exposed 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 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 $NaNO_3$ aerosols, ammonium nitrate (NH_4NO_3) exposure has been studied by Kleinman and associates (1980). Twenty normal and 19 asthmatic subjects were exposed to a nominal 200 $\mu g/m^3$ of 1.1 μm NH_4NO_3 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 NH_4NO_3 exposure in either subject group.

Stacy et al. (1983) also studied the effects of 80 $\mu g/m^3$ of NH_4NO_3 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 NO_2 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.

NO_2 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 NO_2 concentrations exceeding 1 ppm (Beil and Ulmer, 1976; von Nieding et al., 1979; von Nieding and Wagner, 1977; von Nieding et al., 1980). However, at concentrations of NO_2 between 2 and 4 ppm, some investigators have not observed any NO_2-induced changes in airway resistance or spirometry (Linn et al., 1985b; Mohsenin, 1987b; Mohsenin, 1988; Sandström et al., 1990a). At NO_2 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 NO_2 concentrations should not be viewed as evidence that NO_2 has no effects on lung function. Further developments will be necessary to permit sensitive, reproducible, noninvasive evaluation of small airways, the primary site of NO_2 deposition in the lung.

Symptoms associated with NO_2 exposure in healthy subjects have been limited to detection of the odor of NO_2, 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. Sandström et al. (1990a) noted mild nasopharyngeal irritation after exposure to 4 ppm for 20 min.

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. Increased airway responsiveness may be associated with airway inflammation. 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 NO_2 for other reasons. There are insufficient data on children, adolescents or older adults, either healthy or with asthma, to determine their NO_2 responsiveness relative to healthy young adults.

At the concentrations that may fall within the ambient range (e.g., <1.0 ppm), the effects of NO_2 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 FEV_1 in asthmatics exposed to 0.3 ppm NO_2 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 NO_2. 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 NO_2 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 NO_2 (von Nieding et al., 1970, 1971, 1973a) (see Table 4.3-54). 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 NO_2 (>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 NO_2-induced pulmonary responses in asthmatics that have been reported at low but not at high NO_2 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 NO_2 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 NO_2 may be attributed to differences in the severity of asthma. This is a complex issue and is discussed in Appendix A. In patients with chronic obstructive lung disease, Bauer et al. (1987) and Morrow and Utell (1989) have observed decreased lung function (FVC, FEV_1) 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 below 2.0 ppm for short duration exposures. It appears that further work will be necessary to provide enough information to estimate the concentration-response relationships for NO_2 exposure of asthmatics and COPD patients, who appear to be the sensitive subpopulations.

In several studies of asthmatics exposed to NO_2, 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; the approach is described in Section 15.4. Without regard to the type of airway challenge, NO_2 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 NO_2 exposure.

Increased airway responsiveness could potentially lead to temporary exacerbation of asthma leading possibly 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 NO_2 could induce a pulmonary inflammatory response and/or alter immune system host defenses. These studies typically include collection of cells and airways fluids washing from the lung using BAL. In contrast to O_3 exposure, NO_2 does not, at the concentrations studied, induce an increase in neutrophils or eosinophils, the typical markers of inflammation following O_3 exposure. However, Sandström et al. (1990a) have observed an increase in mast cells and lymphocytes in BAL fluid, which they attribute to an unspecific inflammatory response. Boushey et al. (1988) have reported an increase in natural killer lymphocytes in BAL fluid. Macrophage numbers have not been increased by NO_2 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. At present there is no evidence of increased pulmonary epithelial permeability, although this possibility has not been examined systematically. Mucociliary clearance was not altered after NO_2 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 NO_2 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 NO_2 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 NO_2 exposure and increased respiratory tract infections.

The effects of repeated NO_2 exposure have been examined in two studies (Sandström 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. Sandström 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_2 have been examined, primarily using spirometry and airway resistance measurements as end points. In general, NO_2 does not cause responses to other pollutants, such as O_3, SO_2, or particulate matter, to be increased significantly. In other words, there is no more than an additive response when NO_2 is included in the pollutant mixture. However, further investigation of NO_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_2 exposure (see previous discussion). In one study, asthmatics were also more responsive to SO_2 after a previous exposure to NO_2 (Jörres and Magnussen, 1990). In addition to other pollutants, NO_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_2 but no significant difference in the response after air and NO_2 exposures was observed. Given the increase in responsiveness to nonantigenic substances such as methacholine, histamine, SO_2, or cold air discussed previously, it may be worthwhile to re-examine this hypothesis using higher NO_2 concentrations or more prolonged exposures.

Responses to other NO_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_2 (Kagawa, 1990). von Nieding et al., (1973b) reported increased airways resistance in subjects exposed to excessively high concentrations (>20 ppm). Responses to HNO_3 vapor have been studied in adolescent asthmatics (Koenig et al., 1989a,b). The results are suggestive of small changes in lung function but further investigation is needed to confirm these apparent responses to HNO_3 vapor. Nitrates (e.g., sodium nitrate) have not been found to cause any deleterious effects (Utell et al., 1979, 1980; Kleinman et al., 1980; Stacy et al., 1983) at levels that might be expected in the atmosphere.

Conclusions:

1. Nitrogen dioxide causes decrements in lung function, particularly increased airway resistance in healthy subjects at concentrations exceeding 1.0 ppm.

2. Nitrogen dioxide exposure results in increased airway responsiveness in healthy subjects exposed to concentrations exceeding 1.0 ppm for exposure durations of 1 h or longer.

3. Nitrogen dioxide exposure is associated with cellular inflammatory responses in the airways that may include increased levels of mast cells and lymphocytes but not neutrophils and eosinophils. Changes in some biochemical mediators of inflammation or enzymes may be altered by NO_2 exposure.

4. Nitrogen dioxide exposure of asthmatics causes in some subjects increased airway responsiveness to a variety of provocative mediators, including cholinergic and histaminergic chemicals, SO_2, 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. However, NO_2 concentration-response relationships are not evident.

5. Modest decrements in spirometric measures of lung function (3 to 8%) may occur in asthmatics and COPD patients exposed to NO_2 concentrations (0.30 ppm).

6. Nitric acid exposure may cause some pulmonary function responses in asthmatics but other commonly occurring nitrogen oxide species do not appear to cause any function responses at concentrations expected, even at higher levels than in worst-case scenarios, in the ambient environment.

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APPENDIX 15A. SEVERITY OF ASTHMA

A major issue in the evaluation of human clinical studies involving asthmatics is the variability in response between, and even within, laboratories. In the absence of significant differences in exposure protocol or exposure dose, the explanation most often invoked to explain differences in response is that the severity of disease and/or disease characteristics in one subject group may have been different than the other. There are a large number of physiological tests which may be used to characterize the severity of asthma, although no single test appears to be definitive. There are also numerous clinical distinctions between the various categories of asthma that are discussed below. The premise presented here suggests that since asthma is a clinically defined disease, the factors used to characterize subjects should be based on clinical and physiological information.

Asthma is a disease "characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli" (American Thoracic Society, 1987; McFadden, 1988, 1991). A characteristic feature of asthma is widespread narrowing of the airways that changes in severity either spontaneously or with treatment. There is a broad range of severity, from "mild and almost undetectable to severe and unremitting", and considerable heterogeneity of clinical features that include, most commonly, wheezing, dyspnea, and cough. Fatal asthma is also characterized by mucosal edema, inflammation of the airways, and mucus plugging of the peripheral airways. Even mild asthma is associated with eosinophilic inflammation, epithelial damage, degranulation of mast cells, and thickening of the subepithelial basement membrane (Beasley et al., 1989; Laitinen et al., 1985; Wardlaw et al., 1988). Although there have been numerous attempts to precisely define asthma, Bates (1990) suggests that "endless attempts to refine linguistic definition are misplaced."

Prevalence: The prevalence of asthma in the U.S. adult population ranges from 2 to 4% (McWhorter et al., 1989); a point prevalence rate of 2.6% was estimated from the National Health and Nutrition Examination Survey I (NHANES) survey. Based on data from National Health and Nutrition Examination Survey II, Schwartz et al. (1990) reported a prevalence rate for asthma in children less than 12 years of age at 4%; 3% among whites and 7.2% among blacks. Prevalence was higher among males, older children (age 8-11), and urban residents.

Categorization: Scadding (1985) discussed extensively the categorization and degree of severity of asthma. He defined two basic classes of asthmatics which he referred to as *extrinsic* and *cryptogenic*. The term extrinsic, or allergic, implies that an external agent (such as ragweed pollen) is responsible for the antigen-antibody reaction which triggers the cascade of events responsible for airway narrowing. The term cryptogenic, or intrinsic, implies there is no *known* environmental or antigen-antibody reaction that has been identified to have precipitated the disease. Scadding (1985) proposed further subdivision of these two basic qualitative classifications into extrinsic atopic (the largest category), extrinsic nonatopic, cryptogenic intrinsic, and cryptogenic unspecified. Diagnostic features of the various categories of asthma suggested by Scadding (1985) are presented in Table 15A-1.

Classes of asthmatics:

a. Extrinsic
   i. Atopic (IgE)
   ii. Nonatopic (non-IgE)
b. Cryptogenic
   i. Intrinsic
   ii. Unspecified

Severity of Disease: Each qualitative class is then graded in severity (Table 15A-2) according to the clinical course of the disease: mild (controlled by bronchodilators and avoidance of precipitating factors, does not interfere with normal activities); moderate (requires steroids, occasionally interferes with activities); and severe (history of life-threatening episodes, seriously interferes with activities). The temporal course of the asthma is also described (Table 15A-3) as episodic (episodes of asthma interspersed with symptom-free periods) or persistent (persistent symptoms with periodic exacerbations).

There is a group of mild extrinsic atopic asthmatics (often participants in clinical exposure studies) who rarely use medication; have infrequent episodes of mild bronchospasm that usually do not require medical intervention or medication, although they may occasionally use a bronchodilator; and have normal lung function. Perhaps these individuals with near normal function should be classified as *minimal* asthmatics. There appears also to be some difference of opinion as to what precise criteria are required before an individual is classified as asthmatic. For example, many (about 40%) individuals with allergic rhinitis

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TABLE 15A-1. DIAGNOSTIC FEATURES OF CLINICAL CATEGORIES OF ASTHMA

	Extrinsic		Cryptogenic	
	Atopic	Nonatopic	Intrinsic	Unspecified
Resistance to flow in intrapulmonary airways				
Lability	+	+	+	+
Increased by				
Nonspecific factors	+	+	+	+
Exercise	+	+ ±	±	+ ±
Allergens	+	±	0	0
Other specific factors	0	±	0	0
Diminished by				
Bronchodilators	+	+	+ ±	+
Corticosteroids	+	+	+ ±	+
Family history (asthma, hay fever, eczema)	±	-	-	-
Associated disease				
Eczema, especially infantile	±	-	-	-
Rhinitis, hay fever	±	-	±	-
Systemic vasculitis	0	0	±	0
Aspirin sensitivity	0	0	±	0
Eosinophilic lung infiltrations				
Owing to *Aspergillus* hypersensitivity	±	0	0	0
Of unknown cause	0	0	±	0
Skin tests to inhalant allergens				
Type I (immediate)	+	as part of dual reaction	0	0
Dual	-	±	0	0
Eosinophilia	+ ±	±	+ or +	±
Antibodies			+	
IgE	+	±	0	0
STS IgG	±	?	0	0
Precipitating	-	±	0	0

++ = prominent
\+ = present
± = in some cases
\- = may occur
0 = not found
+± = usually
? = unknown

TABLE 15A-2. CLINICAL SEVERITY OF ASTHMA

Mild	Controlled by bronchodilators and avoidance of known precipitating factors; does not interfere with normal activities
Moderate	Occasionally interferes with normal activities; requires use of systemic corticosteroids in treatment
Severe	Seriously interferes with normal activities; life-threatening episodes (status asthmaticus)

TABLE 15A-3. TEMPORAL COURSE OF ASTHMA

Episodic	Episodes of wheezy dyspnea with symptom-free intervals; frequency and severity of episodes may be indicated
Persistent	Persistent symptoms with episodic exacerbations; severity both of persistent symptoms and of episodes may be indicated

Other forms of chronic bronchopulmonary disease may be present, notably bronchial hypersecretion, persistent airways obstruction, and emphysema and may be partly responsible for persistent symptoms; if so, additional diagnostic terms, as appropriate, should be added

may experience exercise-induced bronchospasm (Voy, 1986). Furthermore, allergic subjects may be more sensitive than normal subjects to SO_2-induced bronchoconstriction (Koenig et al., 1987). However, the nonspecific airway reactivity of these individuals may be within the normal range (McDonnell et al., 1987). (Note, however, that McDonnell et al. [1987] screened their subjects so that only subjects with allergic rhinitis were selected; none of the subjects had exercise-induced bronchospasm.)

In general, the asthmatics who participate in human clinical studies come from the group of asthmatics who would fall into the category of mild extrinsic atopic asthma with an episodic time course. In most cases the disease is sufficiently mild or outside the normal allergy season that the patient may go without medication altogether or can discontinue it for brief periods.

Two clinical features which can easily be ascertained are the temporal nature of the disease (i.e., frequency of episodes of bronchospasm) and the type, dosage and frequency of medication use (i.e., frequency of use of inhaled or oral bronchodilators or steroids). These could be documented by a diary and intermittent measurement of peak flow. In order to differentiate between the minimal and mild severity group, several criteria could be used including frequency of episodes (bronchospasm and/or medication) and normality of function (FEV_1/FVC ratio, SR_{aw}, Peak Flow). An alternative solution may be to provide a detailed clinical and physiological characterization of individual subjects and allow the reader to judge.

From the standpoint of interpreting responses to a specific pollutant, it may be useful to work with a more homogeneous group of asthmatics, defined as indicated above. Though this might result in increased sensitivity to detection of adverse responses, it would, of course, reduce the size of the population to which the findings could be extrapolated. Nevertheless, more precise definition of study populations would facilitate comparisons between different studies.

Certain basic anthropomorphic information is necessary to characterize any subject population, minimally including height, weight, age, and gender. However, in the case of asthmatics, other useful quantitative information can be valuable in comparing subjects in various studies.

In addition to evaluation of baseline lung function and frequency of episodes (as medication use, frequency of attacks, age at onset of disease, family history of atopy, frequency of emergency treatment, hospitalization, or all of the above, possibly using a diary and peak flow meter), the following information may be useful in evaluating studies of asthmatics exposed to inhaled materials:

1. Nonspecific bronchial reactivity (methacholine or histamine)
2. Evaluation of reversibility of obstruction with beta agonist
3. General classes of medication and frequency of use
4. Levels of serum Immunoglobulin E (IgE)
5. Number of positive skin tests to regionally/seasonally common allergens
6. Known precipitating factors; seasonal variability
7. Response to standard exercise challenge
8. Duration of disease

Many of these factors and suggested categories to quantitate them are included in a questionnaire and survey (Table 15A-4) developed by Howard Kehrl, M.D., of the Clinical Research Branch, Health Effects Research Laboratory of the U.S. Environmental Protection Agency. It is recognized that it may, at times, be difficult to persuade journal editors to include an extensive list of subject (patient) characteristics; but that does not preclude obtaining the information and making it available to interested parties or submitting the data to a data repository.

Many of the more recently published studies have carefully evaluated the clinical and physiological characteristics of asthmatics (and others) used as subjects. However, the subjects have not been adequately characterized in some studies and the basis of the patient classifications is unclear. Table 15-A-4 provides a format for collecting some of the important clinical information. The absence of adequate subject characterization significantly inhibits cross-study comparisons that are needed to evaluate differences between studies.

TABLE 15A-4.

ASTHMA SYMPTOMS

1. Spontaneous Wheezing or Chest Tightness or Shortness of Breath
   a. never - none in last year
   b. rare - less than once a month
   c. infrequent - less than 5 times a month
   d. frequent - more than once a week but not every day
   e. daily
   f. nocturnal or early a.m.

2. Cough - frequency
   a. never
   b. with chest infections
   c. usually only with wheezing or chest tightness
   d. occasional, usually upon awakening
   e. frequent, often nocturnal and interferes with sleep
   f. daily, interferes with sleep and activities of everyday living

3. Cough - productive of sputum
   a. never
   b. only with chest infection
   c. rare - 1 to 5 times per month
   d. occasionally - 6 to 15 times per month
   e. frequently or almost daily

FACTOR PROVOKED ASTHMA

1. Exercise-induced Wheezing or Chest Tightness
   a. all seasons with
      1. mild exercise
      2. moderate exercise
      3. heavy exercise
   b. only with cold air
      1. mild exercise
      2. modest exercise
      3. rigorous exercise
   c. abates with sustained exercise
      yes ___ no ___

2. Avoidance of
   a. aspirin - (i) rhinitis
      (ii) facial flushing
      (iii) wheezing
   b. NSAID's
   c. sulfites
   d. pets
   e. environmental tobacco smoke
   f. other _____ (foods, perfumes, odors, etc.)

3. Seasonal or Allergen Provoked Asthma
   yes ___ no ___
   a. concommittant allergen-induced hay fever
   yes ___ no ___
   b. percent component of symptoms due to allergen exposure
      1. 100%
      2. 50 to 100%
      3. 20 to 49%
      4. <20%
   c. allergen exposure increases asthma activity
      yes ___ no ___
   d. allergen exposure increases susceptibility to chest colds
      yes ___ no ___

4. Infection Induced Asthma Flare
   a. colds/URI - nasal congestion or discharge, sore throat sinusitis
      1. cause wheezing
      2. frequency
         a. almost never; less than once a year
         b. infrequent; no more than twice a year
         c. occasional; 3-6 times per year
         d. frequent; more than 6 times a year
   b. LRI - chest cold, bronchitis, pneumonia
      1. usually preceded by URI
         yes ___ no ___
      2. cause wheezing
         yes ___ no ___
      3. frequency
         a. none in last 5 years
         b. almost never; less than once a year
         c. infrequent; no more than twice a year
         d. occasional; 3 to 6 times per year
         e. frequent; more than 6 times a year

TABLE 15A-4. (cont'd)

MEDICATION

1. Steroid Burst and Taper within Past 3 Years (oral corticosteroids)
   a. short, tapering course
   b. chronic use

2. Theophyline Usage
   a. daily
   b. intermittent

3. Beta Agonist Inhaler
   a. routine daily usage
   b. usage more than 5 times per week
   c. from 1 to 5 times per week
   d. occasional usage
   e. only for EIB (exercise-induced bronchoconstriction)

4. Inhaled Corticosteroids
   a. intermittent use
   b. chronic use

5. Other Medications (e.g., chromolyn sodium)

PHYSICIAN DIRECTED TREATMENT

1. Insured or Student Health
   yes ___ no ___

2. Hospitalization within Last 2 Years

3. Emergency Treatments (ER or doctor's office)
   a. more than once a month
   b. more than once a year but less than once a month
   c. less than once a year
   d. none in last 3 years

4. Routine physician contacts (doctor's office)
   a. visits more than once a month
   b. more than once a year but less than once a month
   c. less than once a year
   d. none in last 3 years

TABLE 15A-4. (cont'd)

IMMUNOLOGY PARAMETERS

1. Eosinophil Count (cells x 10^4/nl)
   a. less than 200
   b. 200-400
   c. 401-600
   d. 600-900
   e. more than 900
   f. Note: Allergy season? (Y/N) Recent steroid use? (Y/N)

2. Serum IgE (IU/ml)
   a. less than 10
   b. 10-100
   c. 101-400
   d. 401-800
   e. more than 800

3. Skin Tests (number positive)
   a. less than 3
   b. 3-5
   c. 6-10
   d. more than 10

PULMONARY FUNCTION

1. Spirometry - FEV_1/FVC
   a. more than 80%
   b. 72-79%
   c. 64-71%
   d. 55-63%
   e. less than 55%

2. Plethysmography - SRaw (cm $H_2O \bullet L^{-1} \bullet s$)

	Male	Female
a.	<4.0	<5.0
b.	4.0 to 7.5	5.0 to 8.0
c.	7.5 to 12.0	8.0 to 12.0
d.	>12.0	>12.0

TABLE 15A-4. (cont'd)

AIRWAY RESPONSIVENESS

1. Airway Narrowing Triggered by (Note provocative dose, if known)
   a. Methacholine
   b. Histamine
   c. Cold/Dry Air
   d. Exercise
   e. Sulfur Dioxide

2. Nonspecific Airway Reactivity
   a. *Normal range*
   b. < 10th percentile of *normal range*
   c. 75th-100th percentile for ***asthmatic range***
   d. 25th-50th percentile for ***asthmatic range***
   e. < 25th percentile for ***asthmatic range***

NSAID = Non-steroidal anti-inflammatory drugs.
URI = Upper respiratory infection.
LRI = Lower respiratory infection.

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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 NO_2 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) ambient and indoor NO_2 levels and related exposure aspects; (2) qualitative and quantitative characterization of key health effects of NO_2 and their biological bases; and (3) identification of population groups potentially at enhanced risk for health effects associated with NO_2 exposure.

16.2 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 NO_2) are often characterized by short episodes at relatively high concentrations.

The highest hourly and annual ambient NO_2 levels are reported from 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 U.S. usually have less prominent seasonal patterns and may peak in the winter, in the summer, or contain little discernable variation. One-hour NO_2 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. Since 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.

Since 1980, the U.S. nationwide mean annual-average level among reporting NO_2 stations has been consistently below 0.03 ppm, with no significant trend evident. For 103 Metropolitan Statistical Areas reporting a valid year's data for at least one station in 1988 and/or 1989, annual averages ranged from 0.007 to 0.061 ppm. The only recently measured exceedances of the current annual standard, 0.053 ppm, have occurred at stations in southern California.

Most people, however, spend a significant portion of their time indoors. This can result in increased 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 which affect the degree of penetration of outdoor NO_2 into buildings. Although the data base on peak hourly concentrations in homes with and without indoor sources is much smaller than for 24-h and weekly averages, several studies (Wade et al., 1975; Hollowell and Traynor, 1978; Hollowell et al., 1980) report peak hourly NO_2 values in gas stove kitchens in the range of 0.1 to 1.2 ppm. Modeling efforts suggest that 1-h NO_2 concentrations are commonly 0.15 ppm or greater during cooking periods in gas stove homes (Sexton et al., 1983).

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; and 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 that 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. found relatively high correlations between measurements of indoor NO_2 concentrations and total personal exposure in gas stove homes ($r = 0.85$ summer and 0.87 winter) and to a lesser extent in electric stove homes ($r = 0.68$ summer and 0.61 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).

In contrast, 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 account 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.

In most regions of the country, except southern California, total personal exposure to elevated NO_2 concentrations appears to be dominated by the existence of indoor sources of NO_2 (i.e., gas stoves, kerosene heaters, etc.). Total personal exposure for residents of electric stove homes (approximately 50% of the U.S. population) is expected to be significantly lower than levels observed at outdoor fixed monitoring stations (Sexton et al., 1983).

16.3 KEY HEALTH EFFECTS OF NO_2

This section concisely discusses below two key types of health effects that are of most concern at ambient or near-ambient concentrations of NO_2: (1) increases in airway responsiveness in response to acute, short-term exposures of asthmatic individuals; and (2) increased occurrence of respiratory illness among children associated with longer-term exposures to NO_2. A third category of NO_2 effects, emphysema, is also noted below but appears to be of major concern with exposures to much higher than ambient levels of NO_2.

16.3.1 Airway Reactivity in Asthmatics and Short-Term (1-3 h) Exposure to NO_2

Subjects with asthma who as a group have airway hyperresponsiveness to a variety of chemical and physical stimuli are considered one of the potentially most NO_2-responsive groups in the population. The physiological end point which, to date, appears to be the most sensitive indicator of response to NO_2 in asthmatics is a change in airway responsiveness or reactivity. Airway inhalation challenge tests are used to evaluate the "responsiveness" of a

subject's airways to inhaled materials. To test for the degree of airway responsiveness, a pharmacologically active chemical (such as histamine, methachloine, 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.

Asthma is defined as a disease "characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli" (American Thoracic Society, 1987; McFadden, 1988). A characteristic feature of asthma is widespread narrowing of the airways that changes in severity either spontaneously or with treatment. There is a broad range of severity, from "mild and almost undetectable to severe and unremitting," and considerable heterogeneity of clinical features that most commonly include wheezing, dyspnea, and cough. Even mild asthma is associated with eosinophilic inflammation, epithelial damage, degranulation of mast cells, and thickening of the subepithelial basement membrane (Beasley et al., 1989; Laitinen et al., 1985; Wardlaw et al., 1988). 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 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 non-asthmatics (Pattemore et al., 1990), there is a correlation between increased asthma symptoms or increased medication usage and increased airways responsiveness (Britton et al., 1988).

The prevalence of asthma in the U.S. adult population ranges from 2 to 4% (McWhorter et al., 1989); a point prevalence rate of 2.6% was estimated from the NHANES I survey. Based on data from the more recent NHANES II survey, Schwartz et al. (1990) reported a prevalence rate for asthma in children less than 12 years of age at 4%; 3% among whites and 7.2% among blacks. Prevalence was higher among males, older children (age 8-11), and urban residents.

At concentrations below 1.0 ppm NO_2, there is little (if any) convincing evidence of lung function decrements or changes in airway responsiveness in healthy individuals. There

is some evidence that acute exposure to NO_2 may cause an increase in airway responsiveness in asthmatics. This response has been observed only at relatively low NO_2 concentrations, mostly within the range of 0.20 to 0.30 ppm NO_2, the concentration range of concern within the ambient environment. A meta-analysis of data on more than 300 asthmatics experimentally exposed to NO_2 indicates a slight excess increase of airway responsiveness following NO_2 exposure (see Chapter 15). In the concentration range between 0.20 and 0.30 ppm, the excess increase in airway responsiveness was attributable to subjects exposed to NO_2 at rest. The change in nonspecific (i.e., not to specific allergens) airway responsiveness may reflect increased NO_2-induced permeability of the airway epithelium leading to increased access of the provocative agent to airway receptors, release of local mediators of inflammation, or alterations in airway smooth muscle tone. Since NO_2 does not appear to cause airway inflammation at these levels 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 NO_2 could lead to increased responses to a specific antigen, there is presently no plausible evidence to support this hypothesis. It is also 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 NO_2-induced pulmonary responses in asthmatics that have been reported at low, but not at high, NO_2 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 NO_2 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 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 for both airway responsiveness and pulmonary function changes.

Controlled human exposure studies are limited to acute 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 one to two weeks and rarely longer than 8 h/day. These data, therefore, are primarily useful in evaluation of short-term NO_2-induced health effects.

16.3.2 Respiratory Morbidity in Children Associated with Exposure to NO_2

The effects of NO_2 on respiratory illness and the factors determining occurrence and severity are important public health concerns because of the potential for exposure to NO_2 and because childhood respiratory illness is common (Samet et al., 1983; Samet and Utell, 1990). This takes on added importance since 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 for the effects of exposure to NO_2 on respiratory disease in children under 12 years of age. The studies were evaluated for several key factors, including: (1) measurement error in exposure, (2) misclassification of the health outcome, (3) selection bias, (4) adjustment for covariates, (5) publication bias, (6) internal consistency, and (7) plausibility of the effect based on other evidence. The health outcome should be an outcome for which there is good reason to suspect that NO_2 exposure has an effect. The health outcome measure considered was lower respiratory illness, which is typically attributed to infectious disease, probably of viral origin. Symptoms evaluated commonly consisted of cough, wheeze, colds going to chest, chronic phlegm and bronchitis. Each study was reviewed with special attention given to the factors just discussed. Those studies which most appropriately address these factors provide stronger bases for conclusions to be drawn. Consistency between studies indicates the level of the strength of the total data base.

The epidemiological studies generally provide some evidence that repeated NO_2 exposure increases respiratory illness in children, although many reported non-statistically significant results. 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 NO_2 exposure. An EPA reanalysis of that data yields an estimated odds ratio of 1.31 for the presence of symptoms of respiratory illness. 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 to 1.42. Melia et al.

(1980) described the results of a third study of respiratory symptoms in children aged six to seven 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 NO_2 levels. Melia et al. (1982), reporting on a fourth study of children in England, found that a multiple logistic regression analysis of these data was not statistically significant, although the symptoms were positively related to NO_2 exposure. However, an EPA reanalysis of the Melia et al. (1982) results suggest that an increase of 30 $\mu g/m^3$ (0.016 ppm) in bedroom NO_2 levels yields an 11% increase in the odds of respiratory illness. In a final Melia et al. (1983) study, infants under 1 year of age were examined, but no relationship was found between type of fuel used for cooking and the prevalence of respiratory symptoms.

An analysis of the Six City studies reported by Ware et al. (1984) estimated an unadjusted odds ratio of 1.08 (95% confidence limits of 0.97 to 1.19) for an index of lower respiratory illness 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 cohort enrolled later in the Six City studies, used a different symptom questionnaire, and made indoor NO_2 measurements for all subjects. They found evidence for increased respiratory disease, with an estimated odds ratio of 1.47 (95% confidence limits of 1.17 to 1.86) at an exposure of 31 $\mu g/m^3$ (0.016 ppm).

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 odds ratio of 1.14, with 95% confidence limits of 0.86 to 1.50. 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 effects of indoor factors on respiratory health of children in The Netherlands. A logistic regression analysis yielded an odds ratio of 0.94 with 95% confidence limits of 0.66 to 1.33, thus showing no evidence of an increase in respiratory disease with increasing NO_2 exposure. Keller et al. (1979) also 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 1.10, with 95% confidence limits of 0.74 to 1.54.

Many of the above studies suggest an increase in respiratory symptoms in children from exposure to levels of NO_2 occurring in homes with gas stoves as compared to homes with electric stoves, but the reported associations in the majority of the studies did not reach statistical significance. The overall consistency of these studies was examined and the evidence synthesized in a quantitative meta-analysis carried out be EPA (Hasselblad and Kotchmar, submitted).

The studies used in the EPA meta-analysis employed different health end point indicators. In order to compare these studies, a standard end point was defined, and then each study was compared with this standard end point. For purposes of discussion, the end point chosen was the presence of lower respiratory illness in children aged 12 or under. It was assumed that the relative odds of developing lower respiratory illness is similar across this age range as a function of NO_2 exposure, even though the actual rates may not be (a common assumption in many analyses). The goal was to estimate the odds ratio corresponding to an increase of 30 $\mu g/m^3$ (0.016 ppm) in NO_2 exposure. This is approximately the increase seen as a result of gas stove use as compared with electric stove use in both the United States and England.

An attempt was made to include as many studies as possible. The criteria for inclusion were: (1) the health end point measured must be reasonably close to the standard end point; (2) exposure differences must exist and some estimate of exposure must be available; and (3) an odds ratio for a specified exposure must have been calculated, or data presented so that it can be calculated.

Graphs of the odds ratio from each study are presented in Figure 16-1. Each curve can be given one of three interpretations: (1) the normal approximation to the likelihood of the logarithm of the odds ratio; (2) a distribution such that the 0.025 percentile and the 0.975 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.

Two methods of combining evidence were employed in the EPA meta-analysis, one being the Bayesian method described by Eddy (1989) and Eddy et al. (1990a,b). The result of the analysis is a distribution for estimates of the location of the true value of the odds ratio. This can be done separately for each study or for the combination of all studies, and

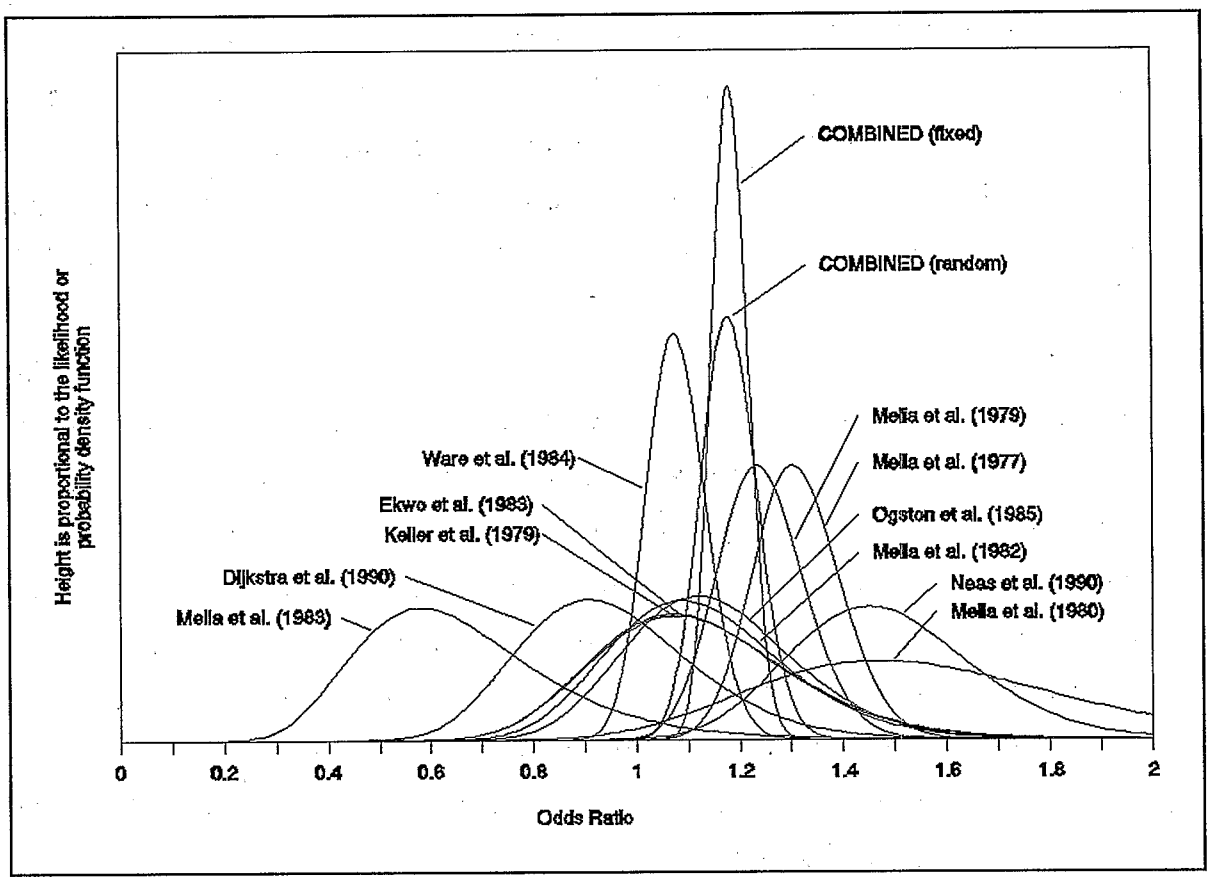

Figure 16-1. U.S. Environmental Protection Agency meta-analysis of epidemiologic studies of NO_2 exposure effects on respiratory disease in children ≤12 years old. Each curve can be treated as a likelihood function or posterior probability distribution. If treated as a likelihood function, the 95% confidence 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.

the results are shown in Figure 16-1. The second basic model assumed that the parameter of interest is not fixed, but is itself a random variable from a distribution. Such models are 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. The results from using the different models were basically the same, namely that the

odds ratio is estimated to be about 1.2 with 95% confidence limits ranging from about 1.1 to 1.3. The studies in which NO_2 was actually measured gave an estimated odds ratio of 1.27, whereas the others yielded an estimate of 1.18 (which is consistent with a measurement error effect).

The individual evidence for an effect of NO_2 on respiratory disease is somewhat mixed. All but one of the 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 eleven studies giving quantitative estimates of effects tend to show increases of respiratory illness in children associated with exposure to NO_2. The EPA meta-analysis indicates that, when combined, the studies collectively provide evidence for an increase of 30 $\mu g/m^3$ (0.016 ppm) in NO_2 exposure being associated with an increase of about 20% in the odds of respiratory illness, subject to the assumptions made for the synthesis. Although several assumptions were made to combine the studies, the consistency between the individual studies is demonstrated, indicating greater collective strength in the data base and suggesting that the effect is real.

The exposure estimates used in these studies are either a surrogate (gas vs. electric) or a 2-week integrated NO_2 average measured by Palmes tubes. The effects studied may be related to peak exposures, average exposures, or a combination of the two. To the extent that the observed health effects depend on peak exposures rather than average exposures, the above exposure estimates introduce measurement error. These studies can not distinguish between the relative contributions of short-term peak exposures and longer-term average exposures to the observed health effects. However, the estimated effect is almost surely an underestimate, given the problems of misclassification of exposures and outcomes. The effect was not dependent on any one or two studies. The results of this analysis are not sensitive to the inclusion (or exclusion) of any one study. In fact, any two studies can be eliminated, and the 95% confidence limits will exclude the no effect odds ratio of 1.0. Thus, the combined evidence is supportive for the effects of exposure to NO_2 on respiratory disease in children under 12 years of age.

Several of the epidemiology studies in the quantitative analysis in Chapter 14 used a single 2-week NO_2 average or used two 2-week NO_2 averages to characterize chronic exposure. The representativeness of such estimates of chronic 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 ten 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 16-1). 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 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. Since 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 for possibly cities with very low means such as Dallas. 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 analysis may not be representative of actual exposure situations. These selected data and analyses are offered only to describe potential relationships between ambient NO_2 annual averages and 2-week data periods.

The plausibility of respiratory illness in children being increased by NO_2 exposure (as demonstrated by the epidemiologic studies and EPA meta-analysis discussed above) is supported by findings from numerous animal studies evaluating NO_2 impacts on host defense mechanisms. Key findings from such studies are summarized next.

TABLE 16-1. U.S. ENVIRONMENTAL PROTECTION AGENCY ANALYSIS OF VARIABILITY IN 2-WEEK AMBIENT AVERAGES OF 1-H NO_2 DATA AT TEN SELECTED LOCATIONS

			Fraction of Annual Average			
			2-week Average		Two 2-week Average	
City	Year	Annual Average (ppm)	10th Percentile	90th Percentile	10th Percentile	90th Percentile
Los Angeles, CA	1988	0.061	0.77	1.27	0.87	1.20
Azusa, CA	1989	0.051	0.81	1.27	0.83	1.17
Upland, CA	1988	0.047	0.79	1.38	0.87	1.22
Anaheim, CA	1988	0.046	0.71	1.23	0.86	1.10
New York, NY	1988	0.041	0.84	1.19	0.91	1.16
Chicago, IL	1989	0.034	0.86	1.18	0.93	1.10
Cincinnati, OH	1989	0.030	0.84	1.23	0.88	1.11
Worchester, MA	1988	0.029	0.81	1.19	0.83	1.23
Miami, FL	1989	0.018	0.77	1.26	0.90	1.18
Dallas, TX	1989	0.011	0.45	1.46	0.75	1.31

16.3.3 Biological Bases Relating NO_2 Exposure to Respiratory Morbidity: Effects of NO_2 on the Respiratory Host Defense System

The lung is one of the common sites of attack of microorganisms. While 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 in the body. Two major approaches have been used to demonstrate the effects of NO_2 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. These two approaches are discussed below.

Nitrogen dioxide exposure can impair one or more components of this important defense system, resulting in the host being more susceptible to respiratory infection. Epidemiological studies have reported an association between an increase in symptoms of respiratory disease of infectious origin and NO_2 exposure (Chapter 14). Animal studies provide important

evidence indicating that several defense systems are a target organ for inhaled NO_2. Nitrogen dioxide affects all of the host defenses studied (e.g., mucociliary clearance, alveolar macrophages, and the humoral and cell-mediated immune system.) The biological basis relating NO_2 exposure to respiratory symptoms and infection shown in epidemiological studies (Chapter 14) are presented in extensive discussions in Chapter 13 (animal toxicology).

Studies on the effects of NO_2 exposure on mucociliary transport in the conducting airways show a reduction in the number and activity of the cilia, morphological changes in the cilia, and decreased mucociliary velocity at concentrations as low as 0.5 ppm for 7 mo of exposure (Yamamoto and Takahashi, 1984). Others have observed similar effects at higher levels for shorter exposure durations. Several short-term (days to weeks) studies with concentrations at or above 2 ppm demonstrated structural changes in cilia and ciliated cells, decreases in numbers of ciliated cells, and decreases in ciliary beating. As a foreign agent deposits below the mucociliary region, in the gaseous exchange region of the lung, host defenses are provided by the alveolar macrophage 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 NO_2 has produced a variety of effects on alveolar macrophages in several animal species after several weeks of exposure to levels as low as 0.3 ppm; however, most effects were observed at higher levels. These effects included decreased phagocytosis and 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; Suzuki et al., 1986; Schlesinger et al., 1987; Mochitate et al., 1986). Decreases in the ability of alveolar macrophages to engulf foreign particles (phagocytosis) and bactericidal activity are likely highly related to increased susceptibility to pulmonary infections. Controlled human exposure studies have also examined macrophage function and show that these cells, when exposed to NO_2, tended to inactivate influenza virus in vitro less effectively than cells collected after air exposure (Frampton et al., 1989a).

An example of the alteration of host defenses against tumor cells following exposure to NO_2 is seen in studies examining the colonization of the lung by B16 melanoma tumor cells. Richters and Kuraitis (1981, 1983) and Richters et al. (1985) reported that when mice were exposed to 0.4 or 0.8 ppm NO_2 for 10-12 weeks and then injected with transplantable tumor

cells, colonization of these cells increased in the lung, indicating decreased resistance to such challenge. Questions have been raised regarding the appropriateness of the statistical analysis used in evaluating these data and the interpretation of the model used. Although the authors attribute effects to increased metastases, an alternative interpretation is an NO_2-induced change in lung permeability to cells. Another study reported that NO_2 inhibits formation of B16F10 tumors in the lung (Weinbaum et al., 1987).

The humoral and cell-mediated immune systems together are essential for antibody production and the secretion of cellular products that are lethal to certain invading organisms and also regulate the normal host's defense response. There is some indication that exposure to NO_2 suppresses some of these specific immune responses and that the effect is 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 week to 1 mo to NO_2 concentrations as low as 0.4 - 0.5 ppm (Lefkowitz et al., 1986; Fujimaki et al., 1982). Subchronic exposure to NO_2 also resulted in decreased numbers of circulating T lymphocytes, T-helper/inducer lymphocytes, and T-cytotoxic/suppressor lymphocytes in mice (Damji and Richters, 1989). The cause of this suppression is not clear.

Animal infectivity studies present key data relating exposure to NO_2 and effects on the overall functioning of host defense mechanisms. In these studies, animals were exposed to varying concentrations and durations of NO_2 followed by exposure to an aerosol of an infectious agent. Microbial-induced mortality was used as the end point. Exposure to NO_2 increased both bacterial- and influenza-induced mortality after subchronic exposures to levels as low as 0.5 - 1.0 ppm NO_2 (Ehrlich and Henry, 1968; Ito, 1971; Ehrlich et al., 1977). After acute (2 h) exposure, 2.0 ppm NO_2 is the lowest effective concentration measured (Ehrlich et al., 1977). Nitrogen dioxide increases microbial-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, 1987; Motomiya et al., 1973; Miller et al., 1987). Using an animal model designed to evaluate the effects of NO_2 on non-fatal respiratory infection, NO_2 decreased the intrapulmonary bactericidal activity in mice in a concentration-related manner, without a decrease in the number of alveolar macrophages (Goldstein et al., 1973). Exposure

to NO_2 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 NO_2 exposure (Ito, 1971). In studies with cytomegalovirus and paramyxovirus (Jacob, 1987; Rose et al., 1988), the pathogenesis of these infections are enhanced.

The toxicology literature also provides evidence that the host's response to inhaled NO_2 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 NO_2 than by the duration of the exposure (Gardner et al., 1977a,b). The exposure pattern of NO_2 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 NO_2 superimposed on a lower continuous background level of NO_2, 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-NO_2-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 NO_2 exposure patters. 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 represent the causative exposure.

It is of interest that morphological studies have also indicated that the NO_2 concentration played a more important role in inducing lung lesions than did exposure duration when the product of C×T was constant. The measured effect of concentration was greater with intermittent exposure than with continuous (Chapter 13).

Recent controlled human exposure studies examining the effects of NO_2 on pulmonary host defense systems report a trend (not statistically significant) toward an elevated rate of infection to a laboratory-induced live attenuated influenza, A/Korea/reassortment virus

(Goings et al., 1989). Frampton et al. (1989a) report a trend ($p<0.07$) for less effective inactivation of virus by macrophages obtained from subjects exposed continuously to 0.60 ppm NO_2, but no effects of virus inactivation were seen in subjects exposed to 2.0 ppm spikes. Exposure to NO_2 may transiently increase levels of antiprotease alpha-2-macroglobulin (α_2M) in lavaged fluids isolated from the respiratory system through local release. While serving as an indicator of changes in protease-antiprotease balance, alterations in α_2M in alveoli may have significance for local immunoregulation and may alter alveolar macrophage defenses against infection (Frampton et al., 1989b). These findings suggest, but do not prove, that NO_2 may play a role in increasing the susceptibility of adults to respiratory virus infections.

The strength of the animal toxicological, human clinical, and epidemiological studies on host defenses provides the rationale and plausible biological basis to explain the relationship seen in population studies showing increased frequency and severity of respiratory symptoms and/or infections in humans exposed to NO_2. The human body must be able to defend itself against a wide variety of inhaled foreign substances. When these defenses are overcome, serious consequences can occur. The significance of the observations from relevant animal models is clear. With minor variations, the mammalian species, including humans, share in common an array of defensive mechanisms that are anatomically, functionally, and physiologically integrated in the respiratory tract to prevent and control infectious disease. All information available to date would indicate that qualitative extrapolation of data observed in animals to humans is valid. The accumulated evidence that NO_2 causes dysfunction in several defenses provides the plausible biological mechanism necessary to link NO_2 exposure to increased morbidity and respiratory symptomatology in children indicative of respiratory infection.

16.3.4 Emphysema and Exposure to NO_2

Studies in several species of animals have shown that chronic exposure to high levels of NO_2 (relative to ambient) can cause emphysema. Since emphysema is an irreversible disease, representing important public health concerns, whether NO_2 creates a risk for this disease in humans is a major question. Although this question cannot be definitely answered yet, the potential for risk requires discussion. The definition of emphysema as used in the United

States is an anatomic one best characterized by National Institutes of Health (NIH) (1985) criteria. These criteria are: "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., 1977; Port et al., 1977) relate long-term (1 to over 30 months) exposure of rats and rabbits to high concentrations of NO_2 (> 8 ppm, much greater than ambient levels) with morphologic lung lesions which 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 NO_2 and 0.25 ppm NO for 68 months. Upon examination 32 to 36 months 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 post-exposure. 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" NO_2 (0.14 ppm) and "high" NO (1.1 ppm), but emphysema was not observed. Since the study did not include an NO_2-only group, it is not possible to discern the effects of NO_2 in the mixture. However, the presence of emphysema in the "high" NO_2-"low" NO group and its absence in the "low" NO_2 - "high" NO group implies that NO_2 was a significant etiologic factor.

Emphysema was reportedly observed in numerous other NO_2 studies with several species of animals, but either the reports lacked sufficient detail for independent conclusions 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 may also play a role in the outcome of studies. Potential differences may relate to 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 difference in response, and inadequate description of methods and findings in some published reports. Qualitatively, it is clear that NO_2 can cause emphysema in animals. While the lowest NO_2 concentration for the shortest exposure duration that will result in emphysematous lung lesions can not be reliably determined from the published studies, the exposures that did cause emphysema, according to the NIH criteria, are far higher than those currently reported in ambient air.

16.3.5 Subpopulations Potentially Susceptible to NO_2 Exposure

Certain groups within the population may be more susceptible to the effects of NO_2 exposure, including persons with preexisting respiratory disease, children, and the elderly. The reasons for paying special attention to these groups is that they may be affected by lower levels of NO_2 than other subpopulations or the impact of a given response may be greater. Some causes of heightened susceptibility are better understood than others. Subpopulations that already have reduced lung functions and reserves (e.g., the elderly, asthmatics, emphysemics, chronic bronchitics) will be more impacted than other groups by decrements in pulmonary function. For example, a young healthy 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 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 NO_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 NO_2-induced increase in airway response to the already heightened responsiveness to other substances raises the possibility of exacerbation of this pulmonary disease by NO_2. This is discussed in Section 15.4.

Other potentially susceptible groups include patients with chronic obstructive pulmonary disease (COPD), such as emphysema and chronic bronchitis. Many of these patients have

airway hyperresponsiveness to physical and chemical stimuli. A major concern with COPD patients is the absence of an adequate pulmonary reserve, a susceptibility factor described above. The poor distribution of 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.

Since over 2 million Americans have emphysema, it would be important to know whether NO_2 has the potential to excaberate the disease. Lafuma et al. (1987) exposed both normal hamsters and hamsters with laboratory-induced (with elastase) emphysema to 3,760 $\mu g/m^3$ (2 ppm) NO_2 for 8 h/day, 5 days/week for 8 weeks. The emphysematous lesions produced by elastase and NO_2 showed increases in mean linear intercepts and pulmonary volumes and a decrease in internal alveolar surface areas, compared to those treated with elastase and exposed to clean air. The NO_2-exposed animals developed a different type of emphysematous lesions than those caused by elastase. The investigators suggested that the results may imply a role for NO_2 in enhancing preexisting emphysema. Stavert et al. (1986) state that whereas NO_2 inhalation alone may not cause emphysema, it is conceivable that this oxidant gas may act as a co-determinant to allow the progression or amplification of emphysema caused by other means. However, it is not clear what the potential would be for exacaberation of emphysema in humans at ambient concentrations.

Based upon epidemiology studies, children 12 years or younger constitute a subpopulation potentially susceptible to an increase in respiratory morbidity associated with NO_2 exposure (Section 14.5). Children may be more susceptible due to an immature host defense system. Data on the resident population of the United States provides information on the numbers of children in various age ranges (Table 16-2). Approximately 37 million children are in the age group 9 years and younger, while around 54 million children are in the age group 14 years and younger. It is also possible that the increase in respiratory morbidity may be more detectible in this age group compared to adults due to the greater frequency of lower respiratory illness in this age group of children (Glezen and Denny, 1973).

TABLE 16-2. ESTIMATES OF THE RESIDENT POPULATION OF CHILDREN AND YOUNG ADULTS OF THE UNITED STATES, BY AGE AND SEX, JULY 1, 1989

Age	Population		
	Total	Male	Female
All Ages	248,239,000	120,982,000	127,258,000
<1	3,945,000	2,020,000	1,925,000
1-4	14,808,000	7,578,000	7,229,000
5-9	18,212,000	9,321,000	8,891,000
10-14	16,950,000	8,689,000	8,260,000
15-19	17,812,000	9,091,000	8,721,000

Source: Centers for Disease Control (1990).

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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
$AaDO_2$	Difference between alveolar and arterialized partial pressure of oxygen
AAS	Atomic absorption spectroscopy
AATCC	American Association of Textile Chemists and Colorists
Ad	A particular strain of laboratory mouse
AICHE	American Institute of Chemical Engineers
AM	Alveolar macrophage
AMP	Adenosine monophosphate; adenosine 5′ phosphate
ANSA	8-anilino-1-naphthalene-sulfonic acid
APCD	Air Pollution Control District
APHA	American Public Health Association
A/PR/8	A particular strain of influenza virus
A/PR/8/34	A particular strain of influenza virus
AQCR	Air Quality Control Region
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
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BP	Blood pressure

b_{scat}	Extinction coefficient due to scatter by aerosols
C3H	A particular strain of laboratory mouse
C57BL	A particular strain of laboratory mouse
C57BL/6	A particular strain of laboratory mouse
cAMP	Cyclic adenosine monophosphate; adenosine 5′-phosphate
CAMP	Community Air Monitoring Program
CD-1	A particular strain of laboratory mouse
cGMP	Cyclic guanosine monophosphate; guanosine 5′-phosphate
°C	Degrees Celsius (Centigrade)
^{14}C	A radioactive form of carbon
CHE	Cholinesterase
C_L	Lung compliance
C_{Ldyn}	Dynamic lung compliance
C_{Lstat}	Static lung compliance
cm	Centimeter
CNS	Central nervous system; the brain and spinal cord
CO	Carbon monoxide
CO_2	Carbon dioxide
CoA	Coenzyme A
COH	Coefficient of haze
CPK	Creatine phosphokinase
CR-1	A particular strain of laboratory mouse
CRD	Chronic respiratory disease
CV	Closing volume
C x T	Exposure concentration in ppm multiplied by time of exposure in hours or other time measurement
d	Day
DEN	Diethylnitrosamine (also DENA)
DIFKIN	Diffusion Kinetics Model
DL_{CO}	Diffusion capacity of the lung for carbon monoxide
DMN	Dimethylnitrosamine
DNA	Deoxyribonucleic acid

D = CT	Dose equals concentration multiplied by time
DPPD	N,N diphenylphenylenediamine
EC	Prefix of International Commission on Enzymes' identification numbers
EKG	Electrocardiogram
EPA	U.S. Environmental Protection Agency
°F	Degrees Fahrenheit
FEF	Forced expiratory flow
FET	First-edge time
FEV	Forced expiratory volume
$FEV_{1.0}$	One-second forced expiratory volume
$FEV_{0.75}$	0.75-second forced expiratory volume
FRM	Federal Reference Method for air quality measurement
ft	Foot
FT	Fourier transform spectroscopy (also FS)
FVC	Forced vital capacity
g	Gram
G6P	Glucose-6-phosphate
GC	Guanylate cyclase
GL	Gas chromatography
GC-MS	Gas chromatograph in combination with mass spectrometry
GM	General Motors Corporation
GMP	Guanosine 5′-phosphate; guanosine monophosphate
GSH	A tripeptide, glutathione (reduced form)
GSSG	The disulfide (oxidized) form of GSH
$H^{\bullet}$	Hydrogen (free radical)
^{3}H	Tritium; a radioactive form of hydrogen
ha	Hectare
HbO_2	Oxyhemoglobin
HNO_2	Nitrous acid (also HONO)
HNO_3	Nitric acid (also $HONO_2$)
$HO^{\bullet}$	Hydroxyl free radical (also OH)

$HO_2^{\bullet}$	Hydroperoxyl free radical
HO_2NO_2	Pernitrous acid
HO_2NO_2	Pernitric acid (also $HOONO_2$)
hr	Hour
HR	Heart rate
hv	Planck's constant (h) times the frequency of radiated energy (v) = Quanta of energy (E)
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
H_2SO_4	Sulfuric acid
IARC	International Agency for Research on Cancer
Ig	Immunoglobulins
IgA	Immunoglobulin A fraction
IgG	Immunoglobulin G fraction
IgG_1	Immunoglobulin G_1 fraction
IgG_2	Immunoglobulin G_2 fraction
IgM	Immunoglobulin M fraction
in	Inch
IR	Infrared
k	Rate constant or dissociation constants
kg	Kilograms
km	Kilometer
l	Liter (also ℓ)
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
LT50	The time required for 50% of the test animals to die when given a lethal dose
LDH	Lactic acid (lactate) dehydrogenase
LPS	Bacterial lipopolysaccharide
m	Meter
M	Molar

M	Third body (in a reaction)
MAK	Maximum permissible concentration (in Germany)
max	Maximum
MFR	Maximal flow rate
$\mu g/m^3$	Micrograms per cubic meter
mg/m^3	Milligrams per cubic meter
Mg	Magnesium
ml	Milliliter
mM	Millimoles
MMD	Mass median diameter
MMFR	Mid-maximal flow rate
mo	Month
MPC	Maximum permissible concentration (in the U.S.S.R.)
MT	Metric Ton
N	Nitrogen
N	Normal
^{13}N	A radioactive form of nitrogen
N-6-MI	N-nitrosoheptamethyleneimine
NA	Not applicable
NAAQS	National Ambient Air Quality Standard
NaCl	Sodium chloride; common table salt
NAD+	Nicotinamide-adenine dinucleotide (+ indicates oxidized form)
NADB	National Air Data Bank
NADH	Nicotinamide-adenine dinucleotide (reduced form)
NADPH	Nicotinamide-adenine dinucleotide phosphate (reduced form)
NaOH	Sodium hydroxide
NAS	National Academy of Sciences
NASN	National Air Surveillance Network
NDIR	Nondispersive infrared
NEDA	N-(1-Naphthyl)-ethylenediamine dihydrochloride
NEDS	National Emissions Data System

NEIC	National Enforcement Investigations Center
ng	Nanogram
NH_4	Ammonium ion or radial
nm	Nanometer
NO	Nitric oxide
NOHb	Nitrosylhemoglobin
NO_x	Nitrogen oxides
N_2O	Nitrous oxide
NO_2	Nitrogen dioxide
N_2O_3	Dinitrogen trioxide
N_2O_4	Dinitrogen tetroxide
NSF	National Science Foundation
O	Atomic oxygen
$O(^1D)$	Excited atomic oxygen
O_3	Ozone
OH	Hydroxyl group
$O(^3P)$	Ground state atomic oxygen
32_P	A radioactive form of phosphorus
$PaCO_2$	Alveolar partial pressure of carbon dioxide
$PaCO_2$	Arterial partial pressure of carbon dioxide
PAH	p-Ammiohippuric acid
PAN	Peroxyacetyl nitrate
PaO_2	Arterial partial pressure of oxygen
PAO_2	Alveolar partial pressure of oxygen
pH	Log of the reciprocal of the hydrogen ion concentration
PHA	Phytohemagglutinin
PO_2	Partial oxygen pressure
ppb	Parts per billion
pphm	Parts per hundred million
ppm	Parts per million
ppt	Parts per trillion

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
RM	Reference method for air quality measurement
RNA	Ribonucleic acid
RV	Residual volume
SAI	Science Applications, Inc.
SD	Standard deviation
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic-pyruvic transaminase
SH-	Sulfhydryl group
SMSA	Standard Metropolitan Statistical Area
SN	Suspended nitrates
SO_2	Sulfur dioxide
SPF	Specific pathogen free
SR_{aw}	Specific airway resistance
SRM	Standard reference material
SS	Suspended sulfates
STP	Standard temperature and pressure
TEA	Triethanolamine
Tg	Terragram; 10^6 metric tons or 10^{12} grams
TGS-ANSA	A 24-hour method for the detection of analysis of NO_2 in ambient air
TLC	Total lung capacity
TPTT	20% transport time
TSP	Total suspended particulate
USEPA	U.S. Environmental Protection Agency
UV	Ultraviolet radiation
VC	Vital capacity

VE	Ventilatory volume
VEE	Venezuelan equine encephalomyelitis (virus)
$\dot{V}$max	Maximum expiratory flow rate
V_T	Total volume
V/V	Volume per volume
WBC	White blood cells
wk	Week
yr	Year
Zn	Zinc
μg	Microgram
μl	Microliter
μm	Micrometer
>	Greater than
<	Less than
~	Approximately

GLOSSARY

$AaDO_2$: 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 mm Hg (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: CH_3CHO; 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 which 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 which produce bright shades in a wide color range. Low cost and ease of application are features which 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) which catalyzes the disassociation of phosphate (PO_4) 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 CO_2.

Acrolein: CH_2=CHCHO; a volatile, flammable, oily liquid giving off irritant vapor. Strong irritant of skin and mucuous membranes. Also called acrylic aldehyde, 2-propenal.

Acrylics (plastics): Plastics which are made from acrylic acid and are light in weight, have great breakage resistance, and a lack of odor and taste. Not resistant to scratching, burns, hot water, alcohol or cleaning fluids. Examples include Lucite and Plexiglass. Acrylics are thermoplastics and are softened by heat and hardened into definite shapes by cooling.

Acrylic fiber: The generic name of man-made fibers derived from acrylic resins (minimum of 85% acrylonitrite 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.

Advection: 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 which are dispersed or suspended in a gas.

Agglutination: The process by which suspended bacteria, cells or similar particles adhere and form into clamps.

Airborne pathogen: A disease-causing microorganism which travels in the air or on particles in the air.

Air pollutant: A substance present in the ambient atmosphere, resulting from the activity of man 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-ketoglutarate. 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 $-\overset{O}{\overset{\|}{C}}-H$.

Aldolase: An enzyme (EC 4.1.2.7) involved in metabolism of fructose which catalyzes the formation of two 3-carbon intermediates in the major pathway of carbohydrate metabolism.

Algal bloom: Sudden spurt in growth of algae which can affect water quality adversely.

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, which 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 (PAO_2): 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 (O_2/CO_2) 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 (NH_3) 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, $C_2H_4N_4$, used in areas other than croplands, that also possesses some antithyroid activity; also called amitrole.

Ammonification: Decomposition with production of ammonia or ammonium compounds, esp. by the action of bacteria on nitrogenous organic matter.

Ammonium: Anion (NH_4^+) or radical (NH_4) 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.

Anaerobic: Living, active or occurring in the absence of free oxygen.

Anaerobic bacteria: A type of microscopic organism which 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 which may be caused by depletion of oxygen delivery to the heart muscle; usually caused by coronary disease.

Angstrom (Å): A unit (10^{-8} cm) 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) which 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, $C_{14}H_8O_2$ derived from anthracene and used in the manufacture of dyes.

Anthropogenic: Of, relating to or influenced by man. An anthropogenic source of pollution is one caused by man's actions.

Antibody: Any body or substance evoked by the stimulus of an antigen and which reacts specifically with 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 antibody.

Antistatic agent: A chemical compound applied to fabrics to reduce or eliminate accumulation of static electricity.

Arachidonic acid: Long-chain fatty-acid which 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 (PaO_2): 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 non-arterial (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, 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 which 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 ($C_{17}H_{23}NO_3$) 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 macrophage, from the same organism; also, something natually and normally occurring in some part of the body.

Autotrophic: A term applied to those microorganisms which 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 which 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: CA^{2+}, Mg^{2+}, K^{+}, or Na^{+}.

Base saturation: The degree to which soil cation exchange capacity is occupied by base cations. This expressed as a percent, use charge-equivalents.

Benzenethiol: A compound of benzene and a hydrosulfide group.

Beta (β)-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 oz.) 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 which are the only N_2-fixing organisms which photosynthesize as do higher plants.

Brightener: A compound such as a dye, which 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 florescent 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 mm 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 non-neoplastic 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 which 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, C_4H_9OH, also known as butyl alcohol.

Butylated hydroxytoluene (BHT): A crystalline phenolic antioxidant.

Butylated hydroxyanisol (BHA): An antioxidant.

^{14}C labeling: Use of a radioactive form of carbon as a tracer, often in metabolic studies.

^{14}C-proline: An amino acid which has been labeled with radioactive carbon.

Calcareous: 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 degree.

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 ($C_6H_{15}ClN_2O_2$), 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 which 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 which 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, morepinephrine, or dopamine.

Cathepsins: Enzymes which 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 electostatic 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 which is contained in all vegetable fibers and in certain man-made 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 SO_4 as a catalyst.

Cellulose rayon: A regenerated cellulose which is chemically the same as cellulose except for physical differences in molecular weight and crystallinity.

Cellulose triacetate: A cellulose fiber which 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 freezing point of water is 0 and 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 grape-shaped gland.

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 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 $C_2H_{45}OH$; 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 which possesses no unshared, nonbonding valence electrons.

Chromosome: One of the bodies (46 in man) 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 CO_2. It couples the glycolytic breakdown of sugar in the cytoplasm with those reactions producing ATP 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 TLC (i.e., CC/TLC%).

Closing volume (CV): The volume exhaled after the expired gas concentration is inflected from an alveolar plateau during a controlled breathing maneuver. (Most commonly obtained during a single-breath nitrogen washout test.) Since 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 (i.e., CV/VC%).

Codon: A sequence of three nucleotides which 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_{st}): 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 in L/cm H_2O or mL/cm H_2O. Since 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 which 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.

Coproporphyrin: 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 ($C_9H_6O_2$) 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 NO_2).

Crevice corrosion: Localized corrosion occurring within crevices on metal surfaces exposed to corrosives.

Critical Load: A quantitative estimate 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 which 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 N_2. 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 which has been taken into another substance or held on its surface; the opposite of absorption or adsorption.

Desquamation: The sheading 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 percent Cu, 45 percent Al, 5 percent Zn.

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 $ArN_2^+Cl^-$, where Ar refers to an aromatic group.

Diazotizer: A chemical which, when reacted with amines (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_LO_2, D_LCO_2, D_LCO): Amount of gas (O_2, CO, CO_2) commonly expressed as milliliters of gas (STPD) 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.

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 O_2 from HbO_2. 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.

DL_{CO}: 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 hyphosis: Abnormal curvative of the spine; hunch-back.

Dose: The quantity of a substance to betaken 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}): the ratio of the tidal volume to the change in intrapleural pressure between the points of zero flow at the extremes of tidal volume in L/cm H_2O or mL/cm H_2O. Since at the points of zero airflow at the extremes of tidal volume, volume acceleration is usually other than zero, and since, particularly in abnormal states, flow may still be taking place within lungs between regions which 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 cm H_2O/L or cm H_2O/mL.

Elastomer: A synthetic rubber product which 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 non-metallic electric conductor in which-current is carried by the movement of ions; also a substance which displays these qualities when dissolved in water or another solvent.

Electronegativity: Measure of affinity for negative charges or electrons.

Electron microscopy: A technique which 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 or 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 which 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 which identifies it by function.

Eosinophils: Leukocytes (white blood cells) which 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 which 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.

Euthrophication: 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 NO_2, 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 aperatures often closed by a membrane.

FEV_t/FVC: A ratio of timed (t = 0.5, 1, 2, 3 s) forced expiratory volume (FEV_t) to forced vital capacity (FVC). The ratio is often expressed in percent 100 x FEV_t/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 which reacts chemically with the cellulose in fibers under alkaline conditions; the dye contains two chlorine atoms which 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 μm 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., 8-22 m) 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:

$FEF_{75\%}$ = Instantaneous forced exhaled flow after 75% of the forced vital capacity has been exhaled.

$FEF_{200\text{-}1200}$ = Mean forced expiratory flow between 200 mL and 1200 mL of the forced vital capacity (formerly called the maximum expiratory flow rate [MEFR]).

$FEF_{25\text{-}75\%}$ = Mean forced expiratory flow during the middle half of the forced vital capacity (formerly called the maximum mid-expiratory flow rate [MMFR]).

FEF_{max} = The maximal forced expiratory flow achieved during an forced vital capacity.

Forced expiratory volume (FEV): Denotes the volume of gas which 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 sec, symbolized $FEV_{0.5}$, $FEV_{0.75}$, $FEV_{1.0}$. These values are often expressed as a percent of the forced vital capacity; for example $(FEV_{1.0}/FVC) \times 100$.

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 determined by analysis of the characteristics of their retention in the column, since 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, q fractions, differing with respect to associated lipids and carbohydrates and in their content of antibodies (immunoglobulins).

Glomular 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 which is a principal source of energy for man and other organisms.

Glucose-6-phosphate dehydrogenase: An enzyme (EC 1.1.1.49) catalyzing the dehydrogenation of glucose-6-phosphate to 6-phospnogluconolactone.

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) which 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) which 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 mucin and when this is discharged as mucus, a goblet-shaped shell remains.

Golgi 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.

H-Thymidine: Thymine deoxyribonucleoside: One of the four major nucleosides in DNA. ^{3}H-thymidine 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 NADPH 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 measure gravimetrically 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 stimulant 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 man, 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.

Hypolimnia: 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 Ig's and all Is's probably function as antibodies.

Immunoglobulin A (IgA): A type of antibody which comprises approximately 10 to 15% of the total amount of antibodies present in normal serum.

Immunoglobulin G (IgG): A type of antibody which comprises approximately 80% of the total amount of antibodies present in normal serum. Subfractions of IgG are fractions G_1, and G_2.

Immunoglobulin M (IgM): A type of antibody which 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 man.

Inflorescence: The arrangement and development of flowers on an axis; also, a flower cluster or a single flower.

Influenza A_2/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 7×10^{-7} and 10^{-3} m (7,000 and 10,000,000 Å).

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 which 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 which 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 which 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 NO_2 currently unacceptable for air pollution work.

Klebsiella pneumoniae: A species of rod-shaped bacteria found in soil, water, and in the intestinal tract or man 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 which catalyzes the oxidation of lactate to pyruvate via transfer of H to NAD. Isomeric forms of LDH 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 which 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 which 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-pathlength 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 km); the amount of infrared 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 uses.

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 lymphocyte 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 phyocytosis. 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 CO_2, 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 ($\dot{V}_{max\ x}$): 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:

$\dot{V}_{max75\%}$ = Instantaneous forced expiratory flow when the lung is at 75% of its total lung capacity.

$\dot{V}_{max3.0}$ = Instantaneous forced expiratory flow when the lung volume is 3.0 L

Maximal expiratory flow rate (MEFR): Obsolete terminology. See $FEF_{200-1200}$ under Forced expiratory flow.

Maximal mid-expiratory flow rate (MMFR or MMEF): Synonymous with $FEF_{25-75\%}$.

Maximal ventilation (max $\dot{V}_E$): The volume of air breathed in 1 min during repetitive maximal respiratory effort. Synonymous with maximum ventilatory minute volume.

Maximal voluntary ventilation (MVV): The volume (L/min, BTPS) 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 which is exceeded in magnitude by one-half the entries in the collection.

MEFR: See $FEF_{200-1200}$ 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 which 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^{2+}) has been oxidized to Fe^{3+}. It contains oxygen in firm union with ferric (Fe^{3+}) iron and is not capable of exchanging oxygen in normal respiratory processes.

Methimazole: An anti-thyroid 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 anion: An anion associated with strong, or mineral acids such as sulfuric, nitric, or hydrochloric. These anions include NO_3^-, SO_4^{2-}, and Cl^1.

Minute ventilation ($\dot{V}_E$): 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 which 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 which 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 acrylonitrite units.

Moeity: 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 which 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 which 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).

MSA: Metropolitan statistical area.

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 membrane secreting mucus which 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 2,000-4,000 Angstroms.

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, where it normally accounts 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^-).

Nitrification: The principal natural source of nitrate in which ammonium (NH_4^+) ions are oxidized to nitrites by specialized microorganisms. Other organisms oxidize nitrites to nitrates.

Nitrifiers: Soil microorganisms that convert NH_4^+ or organic N to NO_3^-, a process referred to as nitrification. Organisms that convert NH_4^+ to NO_3^- are referred to as autotrophic nitrifiers, and organisms that convert organic N to NO_3^- are referred to as heterotrophic nitrifiers.

Nitrite: A salt or ester of nitrous acid ($NO_2^=$).

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 oxide: A compound composed of only nitrogen and oxygen. Components of photochemical smog.

Nitrogen saturation: A condition in which ecosystems are unable to accumulate anymore nitrogen.

Nitrogen washout: The multiple breath curve obtained by plotting the fractional concentration of nitrogen in expired alveolar gas vs. 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=0).

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) which hydrolyzes nucleoside 5′-phosphates into phosphoric acid (H_3PO_4) 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.

Occlusion: A point 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 which 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.

Oribitals: 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 acrylonitrite;.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 which has the ability to remove electrons from another chemical species, thereby oxidizing it; also, a substance containing oxygen which 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 NH_2 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 km above the earth's surface characterized by high ozone content produced by ultraviolet radiation.

Ozone scavenging: Removal of O_3 from ambient air or plumes by reaction with NO, producing NO_2 and O_2.

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 (PM_x): Matter in the form of small airborne liquid or solid particles, 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 one newton per square meter.

Pathogen: Any virus, microorganism, or other substance causing disease.

Pathophysiological: 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 will fall 90% of the observations.

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 which 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 NO_x 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/L 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; 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, $C_7H_8N_2S$, that is bitter or tasteless depending on a single dominant gene in the tester.

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 (E.C. sub-subclass 3.1.3).

Phosphocreatine kinase: An enzyme (EC 2.7.3.2) catalyzing the formation of creatine and ATP, 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, NO_2, PAN 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 which 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 releasing 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.

Pituary: A stalk-like gland near the base of the brain which is attached to the hypothalmus. 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 to make 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 cu. mm. 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 an lining the walls of the chest cavity.

Plume: Emission from a flue or chimney, usually distributed stream-like 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 forest.

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 ($-NH_2$) and carboxyl (-COOH) groups. Condensation reactions between the groups form amides ($-CONH_2$). 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 man-made or 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 multi-lobed nucleus.

Polymorphonuclear leukocytes: Cells which 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 which 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 which 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, $C_5H_9NO_2$, 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 gm 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.

Lung volume measurements = Tidal volume, inspiratory reserve volume, expiratory reserve volume, residual volume (four basic independent volumes).

Capacities = Combinations of basic volumes.

Total lung capacity (TLC) = Tidal volume + inspiratory reserve volume + expiratory reserve volume + residual volume; the volume of gas in the lungs at the time of maximal inspiration or the sum of all volume compartments. 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 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 (RV) = Total lung capacity ratio (RV/TLC) = 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 which 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 which represent the beginning of ventricular contraction.

Quasistatic compliance: Time dependent component of elasticity; compliance is the reciprocal of elasticity.

Rainout: 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 λ, scattered in any direction making an angle with the incident direction, is directly proportional to $1 + \cos^2$ and inversely proportional to λ^4.

Reactive dyes: Dyes which 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 NO_2, 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. RV = TLC - VC

Resin: Any of various solid or semi-solid 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 H_2O/L per sec. 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 which are instrumental in the synthesis of proteins in a cell. Aggregates are called polysomes.

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 which 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 arsenite: Na_3AsO_3, used with sodium hydroxide in the absorbing solution of a 24-hour integrated manual method for NO_2.

Sodium dithionite: A strong reducing agent (a supplier of electrons).

Sodium metabisulfite: $Na_2S_2O_5$, used in absorbing solutions of NO_2 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} = airway conductance (R_{aw}) x thoracic gas volume (TGV); liter (L) x centimeter of water per liter per second (cm H_2O/L/S).

Spectrometer: An instrument used to measure radiation spectra or to determine wavelengths of the various radiations.

Spectrophotometry: A technique in which visible, UV, 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.

Sphygomenometer: An apparatus, consisting of a cuff and a pressure gauge, which is used to measure blood pressure.

Spirometer: A mechanical device, including bellows or other sealed, moving parts, which 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 in 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 mm mercury.

Staphylococcus aureus: A spherically-shaped, infectious species of bacteria found especially on nasal mucous membrane and skin.

Static lung compliance (C_{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 NO_2 with azo dyes in air monitoring methods.

Stoma: A minute opening or pore (plural is stomata).

STPD conditions: Standard temperature and pressure, dry. These are the conditions of a volume of gas at 0°C, at 760 torr, without water vapor. A STPD volume of a given gas contains a known number of moles of that gas.

Stratosphere: That region of the atmosphere extending from 11 km above the surface of the earth to 50 km. At 50 km 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, staphlococcal, and streptococcal infections.

Sulfamethazine: An antibacterial agent of the sulfonamide group, active against homolytic streptococci, staphytococci, pneumococci and meningococci.

Sulfanilimide: A crystalline sulfonamide ($C_6H_8N_2O_2S$), the amide of sulfanilic acid and parent compound of most sulfa drugs.

Sulfhydryl group: A chemical radical consisting of sulfur and hydrogen which confers reducing potential to the chemical compound to which it is attached (-SH).

Sulfur dioxide (SO_2): 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 which 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.

Terragram (Tg): One million metric tons, 10^{12} 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 ($C_{10}H_{14}N_2O_5$) that is composed of thymine and deoxyribose; occurs as a structural part of DNA.

Tidal volume (V_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: a-d-Tocopherol is one form of Vitamin E prepared synthetically. The a 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 mm 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_L): Resistance measured by relating flow-dependent transpulmonary pressure to airflow at the mouth. Represents the total (frictional) resistance of the lung tissue (R_{ti}) and the airways (R_{aw}). $R_L = R_{aw} + R_{ti}$.

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 larnyx (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 a-amino acid to the carbonyl carbon atom of an a-keto acid.

Transmissivity (UV): The percent of ultraviolet radiation passing through a medium.

Transmittance: The fraction of the radiant energy entering an absorbing layer which reaches the layer's further boundary.

Transpiration: The process of the loss of water vapor from plants.

Triethanolamine: An amine, $(HOCH_2CH_2)_3N$, used in the absorbing solution of one analytical method for NO_2.

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 4×10^{-7} and 5×10^{-9} m (4,000 to 50Å).

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 a sum of squared deviations about a mean divided by the sample size.

Vat dyes: Dyes which have a high degree of resistance to fading by light, NO_x 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 man, 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 by the ventilatory frequency. Conditions are usually indicated as modifiers; for example:

$\dot{V}_E$ = Expired volume per minute (L/min, BTPS),

$\dot{V}_I$ = Inspired volume per minute (L/min, BTPS).

Ventilation is often referred to as "total ventilation" to distinguish it from "alveolar ventilation" (see Ventilation, alveolar).

Ventilation, alveolar ($\dot{V}_A$): 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 reinspired 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. This truly complete expiration volume times the ventilatory frequency constitutes the alveolar ventilation.

Ventilation, dead-space ($\dot{V}_D$): Ventilation per minute of the physiologic dead space (volume of gas not involved in gas exchange with the blood), BTPS, defined by the following equation:

$$\dot{V}_D = \dot{V}_E(PaCO_2 - P_ECO_2)/(PaCO_2 - P_ICO_2)$$

Ventilation/perfusion ratio ($\dot{V}_A/Q$): 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 4,000-8,000 Å.

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, which 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, which is capable of cleaving a fatty acid from a neutral fat; a lipolytic enzyme.

X-ray fluorescence spectrometry: A nondestructive technique which 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.

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