# III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

(a) Properties of Oxides of Nitrogen

Since nitrogen dioxide is frequently produced from the oxidation of nitric oxide, some consideration of the relationships of these gases is indicated. Selected chemical and physical properties of nitric oxide and nitrogen dioxide are given in Table XIII-1. [1]

Nitrogen dioxide (NO2) is one of several oxides of nitrogen. It is a reddish brown or dark orange gas with a formula weight of 46.01. Its dimer, nitrogen tetroxide (N204), is colorless. At temperatures between -9.3 C and 135 C, nitrogen dioxide and nitrogen tetroxide coexist as a mixture of gases. Below -9.3 C, a colorless solid consisting of nitrogen tetroxide is formed, while above 135 C, the gas consists mostly of nitrogen dioxide. In evaluations of occupational exposures to the mixtures of these two compounds, however, the results are customarily expressed in terms of nitrogen dioxide.

The conversion of molecular nitrogen into nitrogenous compounds is known as fixation of nitrogen. In the upper atmosphere this is brought about through photochemical processes in which nitrogen and oxygen atoms from dissociated molecules combine to form nitric oxide. At ground level, the combination of nitrogen and oxygen takes place thermally in flames, explosions, and in electric discharges. According to Jacobs, [2] the formation of nitric oxide by these reactions is given by the following equation:

$$N2 + O2 + heat = 2NO$$

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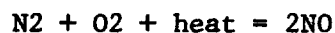
Ultraviolet energy or sufficiently high temperatures to accomplish this in the occupational environment are encountered in electric or gas welding, in the combustion of fuels, eg, in furnaces or internal combustion engines, and in the detonation of explosives. [3,4,5,6,7] Nitric oxide is also produced when nitrogen dioxide is dissolved in warm water. The net reaction is:

$$3NO_2 + H_2O = 2HNO_3 + NO \quad [2]$$
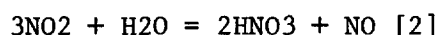
Nitrogen dioxide is formed by the spontaneous oxidation of nitric oxide in air at ordinary temperatures, the equation for this reaction is given by:

$$2NO + O_2 = 2NO_2 \quad [2]$$
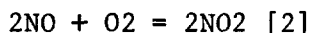
Both nitric oxide and nitrogen dioxide are formed when nitric acid reacts with reducing agents. For dilute nitric acid, the resultant mixture contains 'predominantly NO, while for concentrated nitric acid more NO_2 is produced. [2]

Nitric oxide and nitrogen dioxide rarely exist independently in the occupational environment. The rate at which nitric oxide in air is oxidized to nitrogen dioxide is expressed as:

$$d(NO_2)/dt = K(O_2)(NO)^2$$

where (NO_2) and (O_2) represent the concentrations of nitrogen dioxide and oxygen, $(NO)^2$ represents the square of the nitric oxide concentration, t is time, and K is a constant for any temperature ($K = 14.8 \times 10^9$ at 20C). [8,9]

Since the rate of oxidation is dependent upon the square of the nitric oxide concentration, oxidation is much more rapid at high concen-

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trations.   The significance of this was demonstrated by Elkins [9] who, in

1946, calculated that with a nitric oxide concentration of 200 ppm,

nitrogen dioxide would be formed at a rate of about 11 ppm/minute. If the

nitric oxide concentration were 100 ppm, the oxidation rate would drop to

2.8 ppm/minute, while with 25 ppm of nitric oxide, it would take over 5

minutes for 1 ppm of nitrogen dioxide to be formed. For this reason, both

nitric oxide and nitrogen dioxide should be sampled simultaneously.

Additional data on the theoretical oxidation rate of nitric oxide in air

are presented in Table XIII-2.

The presence of other factors, such as moisture, metal fumes, or

ultraviolet radiation, may either accelerate or slow down the actual rate

of oxidation of nitric oxide in air. [10] As a result, it is not

practicable to estimate the concentration of one of the nitrogen oxides

solely on the basis of a measurement of the other.

Mixtures of the oxides of nitrogen are also produced in other ways,

such as from the combustion of nitrogen-containing materials or from

reactions of nitric acid with metals or organic matter. At the time of

release, these mixtures may contain substantial percentages of nitrogen

dioxide. Wade et al [5] used various sampling techniques and analyses to

estimate the mixtures of nitrogen oxides produced by a number of work

operations. The results of these analyses are listed in Table III-1. It

should be noted that in operations where fixation of nitrogen from the air

occurred at high temperatures, the initial product was largely nitric

oxide. Somewhat more nitrogen dioxide is to be expected where nitrogen-

containing compounds are decomposed. Where nitric acid is a reactant in

acid dipping, nitrogen dioxide will be the predominant oxide released.

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TABLE III-1

APPROXIMATE DISTRIBUTION OF NITROGEN OXIDES
GENERATED FROM VARIOUS OPERATIONS

Source	NO2 %	NO %
Carbon arc	9	91
Oxyacetylene torch	8	92
Cellulose nitrate combustion	19	81
Diesel exhaust	35	65
Dynamite blast	52	48
Acid dipping	78	22

From Wade et al [5]

(b)   Sources of Exposure

Although nitric oxide is the oxide of nitrogen initially produced in the welding arc or flame, [4,5] its concentration in such operations is only rarely reported. In many field studies [11,12,13] and controlled laboratory experiments, [12,14,15,16] concentrations of nitric oxide have been reported as "nitrous gases" or "nitrogen oxides", whereas other field investigations [17,18] and controlled studies [19,20,21,22] have expressed concentrations of nitric oxide as "nitrogen dioxide." Differences in describing environmental levels, coupled with the variety of sampling and analytical methods used to assess the environments, virtually preclude direct comparison and interpretation between these studies.

In a simulation of cutting torch operations causing toxicity, Norwood et al [23] reported separate figures for concentrations of nitric oxide and nitrogen dioxide in 2 of 10 samples. This study showed nitrogen dioxide to be 13% of the total oxides found 15 minutes after cutting began. The

nitrogen dioxide increased to 33% after an additional 25 minutes. Detailed results are given in Table XIII-3. The measured rate of oxidation to nitrogen dioxide appears to be consistent with the theoretical oxidation rate of nitric oxide in air presented in Table XIII-2.

Theoretical predictions based on the kinetic rate of oxidation of nitric oxide to nitrogen dioxide would not be expected to hold in the case of electric arc welding because of the presence of such additional factors as ultraviolet radiation, ozone, iron fume, and moisture. For example, nitrogen dioxide is dissociated by ultraviolet radiation producing nitric oxide plus oxygen atoms. The oxygen atoms, in turn, react with nitrogen dioxide at a very rapid rate producing nitric oxide and oxygen molecules. [24] Thus, according to Silverman and Husain, [10] ultraviolet rays emitted in arc welding would counteract the oxidation of nitric oxide to nitrogen dioxide. On the other hand, ozone produced in the arc could oxidize nitric oxide to nitrogen dioxide or to even higher oxides, such as nitrogen pentoxide. They also pointed out that iron and moisture present could have a converse influence, reducing nitrogen dioxide to nitric oxide, and possibly to ammonia or nitrogen. In addition, freshly formed iron fumes may react with nitrogen oxides to produce particulate nitrites and nitrates, with a consequent reduction in the amount of gaseous oxides present. [10]

In their own welding experiments, conducted in a test chamber, Silverman and Husain [10] found that approximately equal concentrations of nitric oxide and nitrogen dioxide totalling approximately 30 ppm showed no appreciable changes in the ratio of the gases after a period of two to three hours. Here again, at such concentrations, the figures in Table

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XIII-2 indicate that very little measurable change would have been expected.

In general, it has been found that hot metals decompose nitric oxide forming the metal oxide and nitrogen. [10] This may explain the higher rate of formation of oxides of nitrogen when an oxyacetylene flame does not touch metal. [19,23] As shown in Table XIII-4, Norwood et al [23] found that as much as 250 ppm of nitrogen oxides were formed where the flame was not in contact with metal, and only 47 and 71 ppm in tests where stainless steel was being melted. Similarly, Steel and Sanderson [19] reported that the concentrations of nitrogen dioxide produced by flame alone were several times greater than the concentrations observed when metals were being flame-cut (see Table XIII-5).

The changeover from coke oven gas to natural gas in The Netherlands was followed by complaints of toxic effects in glassblowing shops. Van Mourik [25] studied the formation of nitrogen oxides in a glassblowing burner using various combinations of air and oxygen with natural gas, coke, oven gas, methane, and hydrogen. The change to natural gas caused a change in flame characteristics which led to an increased use of oxygen with a consequent increase in flame temperature. The rate of nitric oxide production is rapidly increased if the amount of oxygen in the mixture is increased, [23] as is illustrated in Table XIII-3. Furthermore, van Mourik [25] has pointed out that regardless of the nitrogen content of the gas, approximately the same amount of nitric oxide is formed, provided the flame temperature is constant. Another example [26] of occupational hazard concerns exposure to exhaust gases from ice resurfacing machines in ice arenas. Such exposures made the machine operators, as well as arena

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patrons, ill on a number of occasions. Investigations of 45 ice arenas in Minnesota showed nitrogen oxides concentrations (reported as nitrogen dioxide) as high as 40 ppm. However, concentrations of carbon monoxide were elevated, suggesting that nitrogen oxides were not the only cause of adverse effects. Since the form of nitrogen emitted from internal combustion engines exists largely as nitric oxide at the time of discharge, workers employed in automobile parking or repair garages may have occupational exposures.

The production of nitric oxide by the catalytic oxidation of ammonia is the first step in the manufacture of nitric acid. [27] Information on occupational exposures to nitric oxide from such operations has not been found. Similarly, no published information on nitric oxide exposures in other industrial operations has been found, other than those presented in Tables III-1, XIII-3, and XIII-4.

As reported by Kennedy, [28] underground blasting operations with both nitro-explosives and gunpowder produce levels of the oxides of nitrogen up to 88 ppm after normal firing of a commonly used blasting explosive, and up to 167 ppm after "misfires" (incomplete detonation). Conventional powder shots may cause momentary concentrations of up to 56 ppm and concentrations as high as 150 ppm after multiple firings.

Commins et al [29] noted concentrations of nitric oxide and nitrogen dioxide reaching several hundred ppm were produced in a freshly filled agricultural silo during various stages of the decomposition process. The maximum concentrations of nitric oxide and nitrogen dioxide were found on the fifth day after loading the silo. At that time, a distinct layer of brown gas was observed at the center of the silo. In general, con-

centrations decreased with increased height above the surface of the silage. Data are presented in Table XIII-6. Measurements were also made before and after operation of the silo filling blower. Use of the blower did reduce the levels of nitric oxide and nitrogen dioxide; however, high concentrations of these gases still remained near the surface of the silage.

It is difficult to estimate how many people are exposed to oxides of nitrogen since hazardous occupations also involve all workers located in the vicinity of operations or processes having conditions conducive to the generations of nitrogen oxidation, ie, furnaces, boilers, welding, internal combustion engines, and nitrogen oxides produced from numerous chemical processes. The difficulty in delineating the incidence of exposure has been shown by Storlazzi [30] in a study of welding and burning operations in US naval shipyards. Although only 6,000 workers were directly engaged in such activities, approximately 60,000 workers were indirectly exposed to the byproducts of these operations. NIOSH estimates that 1,500,000 workers are potentially exposed to the oxides of nitrogen. In view of the factors mentioned, this figure is low.

An estimated [3] 200,000 tons of nitrogen oxides are produced annually from industrial processses and ten million tons result from fuel combustion. These are rough estimates at best and do not distinguish between the various nitrogen oxides. From the many cases found in the medical literature, examples of which are given under Historical Reports, it is known that serious, even lethal, concentrations of nitrogen oxides may be encountered. Similar serious consequences have been reported from

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chemical processes. [31-36]

Historical Reports

One of the earliest accounts of what must have been primarily exposure to nitrogen dioxide is that of Desgranges [31] in 1804. Two carboys of concentrated nitric acid broke in a storeroom, reacted with a quantity of wood, and produced a sensation described as suffocating. The merchant entered the room for two periods of approximately five minutes each in close succession. He experienced an immediate sensation of suffocation and his hair turned yellowish red. He recovered but four hours later he became increasingly dyspneic and had painful sensation of constriction in the epigastric region. Thirteen hours after exposure, he appeared much better, but three hours later he suddenly became cyanotic and later delirious. Approximately 27 hours after exposure, he died in severe respiratory distress.

There were several subsequent reports of persons acutely exposed to the vapor of nitric acid reacting with wood or other organic material, eg, Zadek, 1916, [32] or with metals, eg, Fraenkel, 1902, [37] Wood, 1912, [38] Hortsch, 1942, [33] Darke and Warrack, 1958, [34] in many cases with fatal results. In all these cases, the onset of serious illness was characteristically delayed for several hours, after which severe pulmonary edema developed.

In 1913, Lehmann and Hasegawa [39] reported on toxic effects in cats and rabbits exposed to nitrous gases (probably nitric oxide, nitrogen dioxide, and nitric acid fumes) at concentrations between 41 and 2,039 ppm. The nitrous gases were produced by one of two methods. In the first

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method, gases produced by the reaction of nitric acid and copper were collected in a gasometer over water, transferred in a tenfold dilution with hydrogen into a paraffin oil gasometer, and then mixed with a stream of fresh air. In the second method, a small amount of air was aspirated by smoking nitric acid and then mixed with a current of fresh air. Airborne concentrations, expressed as concentrations of nitrous acid, were determined by oxidation of samples by hydrogen peroxide or by chemical reduction using potassium iodide. Cats exposed at concentrations of 41, 64, and 57 ppm for 3, 6, and 7 hours, respectively, did not show any signs during exposure or macroscopic lung changes after the animals were killed. Cats exposed at 117 ppm and above for periods from approximately 1 to 8 hours showed definite changes in respiration, and most animals died during exposure. At necropsy, pulmonary edema and methemoglobinemia were observed. Rabbits exposed at identical concentrations did not show the same signs as the cats.

In this study, [39] Hasegawa subjected himself on three separate occasions to nitrous gases at average concentrations between 62 and 158 ppm. After a 1-hour exposure at a concentration of 62 ppm, he noted laryngeal irritation and an increase in respiration rate. There were no ill effects following this exposure. In another experiment, the concentration was increased to 158 ppm. Hasegawa reported that he had to leave the room after 10 minutes of exposure. His symptoms included a feeling of suffocation, considerable coughing, mucous secretion in the nose, and tearing, all of which subsided 7 hours after exposure.

Later, accounts began to appear of severe exposures to "nitrous fumes" arising from the explosion [35] or the combustion [36] of nitro-

explosives, resulting in severe, sometimes fatal, pulmonary edema.

Severe "nitrous fume poisoning" was reported in arc welders by Adler-Herzmark [40] in 1929, and later in oxyacetylene-torch cutters by Norwood et al. [23]

The additional entity of bronchiolitis fibrosa obliterans, seen by Fraenkel [37] in 1902 in a brassfounder exposed to fumes from the pickling of castings in a mixture of nitric and sulfuric acids, has continued to be reported as a late sequel of poisoning by nitrogen oxides often following recovery in acute pulmonary edema from one [34] to three [35] or even six [41] weeks.

Peterson et al [42] in 1949 reported that toxic agents generated under certain circumstances within agricultural silos contained a high proportion of nitrogen dioxide. Exposures to these agents have resulted in "silage gas poisoning" [43] or "silo-filler's disease", [44] the symptoms of which were characteristic of nitrogen oxides poisoning including, in some cases, the sequelae bronchiolitis fibrosa obliterans. [44]

Effects on Humans

Tables XIII-7 and XIII-8 summarize clinical, epidemiologic, and experimental effects noted in humans exposed to nitrogen dioxide and nitric oxide. Evaluative or qualifying information on each study is included under the section entitled remarks.

In 1916, Zadek [32] described a group of cases of mixed nitrogen oxides poisoning with clinical and pathologic findings which were interpreted by von Oettingen [45] as effects of nitric oxide. Several carboys of crude nitric acid exploded following contact with burning wood

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shavings and excelsior in a factory fire. Of the 20 firemen exposed to what was described as dense greenish fumes, 11 were hospitalized the following day with severe headache, gastrointestinal and respiratory symptoms which appeared as early as 3 1/2 hours after exposure. Of the three most severe cases, one had nitrous acid detectable in his urine, two had detectable nitrite in the sputum, and all had spectroscopically demonstrable methemoglobinemia. One of these men died in coma about 2 days after exposure. An autopsy revealed severe lung injury in addition to methemoglobinemia. The other two severe cases, with both clinical and radiologic evidence of pneumonitis, recovered after 4-6 days. The remaining 17 men who were exposed made a fairly rapid recovery from their milder respiratory and gastrointestinal symptoms.

In 1912, Wood [38] described a case of fatal pneumonia of delayed onset following the inhalation of gas from nitric acid acting upon cadmium-silver alloy. This paper also reviewed 26 other cases from the foreign literature. However, as is often the case in the early literature, "nitric oxide" was clearly used as a nonspecific term. The author's own case, as described above, corresponds closely to the now well-known features of mixed oxides of nitrogen poisoning.

In 1930, Flury [46] presented a theoretical review of the literature on poisoning by "nitrous gases" (defined as nitric oxide, nitrogen dioxide, nitrogen tetroxide, nitrous and nitric acids, in varying proportions). This paper laid the foundation for distinguishing toxicity of nitric oxide from the higher oxides of nitrogen. Flury observed that both human and animal poisoning by "nitrous gases" fell into several distinct clinical categories including: (1) irritant-gas type, (2) reversible type, (3)

shock type, and (4) combined type. The irritant-gas type was characterized by an initial local irritation which, after a symptom-free latent period of several hours, progressed to breathlessness and cyanosis. Death occurred from pulmonary edema after 1-2 days. Immediate onset of pulmonary edema and death within a few minutes to a few hours after exposure was observed less frequently. Cases in which the illness was reversible showed breathlessness, cyanosis, vomiting, giddiness, stupefaction, delirium, fainting, unconsciousness, and severe methemoglobinemia. If the subject was promptly removed from the exposure environment, pulmonary edema did not ensue and recovery was complete. The shock type of illness showed almost instantaneous asphyxia, convulsions, and respiratory arrest. According to Flury, [46] death resulted from interruption of the pulmonary circulation. The combined type displayed symptoms characteristic of both types 1 and 2. Flury described experiments with white mice in which the distinctive effects of nitric oxide and nitrogen dioxide could be demonstrated individually. These experiments were repeated and elaborated on by Pflesser [47,48] some years later and are described under Animal Toxicity.

Nitrogen dioxide gas is an irritant to the mucous membranes and its inhalation may cause coughing, sometimes severe, which may be accompanied by mild or transient headache. [41,49] Mild dyspnea may also be present during exposure. [23] After less severe exposure, the symptoms of irritation usually subside completely for several hours. [34,49] In some cases, symptoms may persist in a mild form for several hours [23] or even days, [41] after which recovery may occur but, more commonly, if exposure to nitrogen dioxide has been severe enough, acute pulmonary edema ensues.

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Signs and symptoms include dyspnea, cough, cyanosis, and upon auscultation, moist rales are heard at the base of the lungs. [23,49] Acute pulmonary edema is usually preceded by an interval of several hours during which few, if any, symptoms appear. Many fatalities occur because of the suddenness and severity of effects and the characteristic delay in onset of up to 12 hours, by which time the exposed subject may be at home and remote from prompt medical attention. [32,33,36,40]

In some cases, severe and increasing dyspnea with fever and cyanosis ensues, usually occurring after an interval of several days to 6 weeks following recovery from the initial, though delayed, acute pulmonary edema. [34,41,44,50,51] This condition has, on occasion, developed long after the exposure, without clinical evidence of pulmonary edema. [35,52,53] This has been called bronchiolitis fibrosa obliterans on the basis of microscopic findings at necropsy. [34,53] These effects have been reported following exposure to mixed oxides of nitrogen generated from: (1) the reaction of nitric acid with various metals, [34,37,49] (2) the explosion of a vessel containing red fuming nitric acid, [53] (3) an oxyacetylene burner, [52] (4) "nitrogen dioxide gas leaking in a chemical plant," [41] and (5) gases generated under certain circumstances in agricultural silos. [44,50,51] Silo gases have been reported to contain nitrogen dioxide (35-1920 ppm), nitric oxide (30-630 ppm), and carbon dioxide (25-60% v/v). [29]

Whether or not sequelae of chronic bronchiolitis fibrosa obliterans are diagnosed or found depends upon the severity of the lesion, the sensitivity of the means of detecting residual effects, and upon individual variations. Tse and Bockman [41] in 1970 reported four cases of bronchiolitis in firemen who were exposed to nitrogen dioxide originating

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from a leak in a chemical plant. Three of the men recovered completely after six to seven weeks, ie, they had no symptoms, clinical signs, or pulmonary dysfunction, except that one had a moderately decreased diffusing capacity at six weeks after exposure. The fourth man continued to complain of dyspnea following exertion, 18 months after exposure. Serial lung function studies on this man over an 18-month period showed a progressive decrease in vital capacity with an increase in residual volume. Both the maximal breathing capacity and the lung compliance were decreased. In addition, studies revealed a decrease in arterial oxygen partial pressure. These results indicated the presence of uneven ventilation with both obstructive and restrictive impairments.

In 1957, Becklake et al [54] reported on followup studies of seven selected patients who had recovered from an episode of acute pulmonary edema following exposure to oxides of nitrogen in various mine-blasting accidents. The study periods were up to 64 months after the exposures. One patient complained of mild dyspnea, and four complained of definite breathlessness on exertion. Five cases showed a decrease in maximal breathing capacity and an increase in the "non-elastic work of breathing." The other two subjects claimed to have recovered completely from the accident. One of these two had completely normal lung function, while the other had high nonelastic resistance but normal maximal breathing capacity. It was suggested [54] that the six subjects who demonstrated residual abnormalities had some degree of bronchial and bronchiolar narrowing due to the fibrotic changes of bronchiolitis obliterans.

In 1971, Ramirez and Dowell [50] reported a followup of a case of "silo-filler's disease." Seven years after the incident, the subject's

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chest X-ray showed diffuse reticular and fine nodular markings. Except for mild hypoventilation and hypoxemia, his pulmonary function remained normal. Lung compliance and airway resistance remained normal throughout the course of followup and no impairment of maximal voluntary ventilation or expiratory flow rates were noted.

In 1973, Scott and Hunt [51] reported on four episodes of "silo filler's disease" in three farmers who were observed at 10, 28, 75, and 327 days following exposure. Serial pulmonary function tests showed acute obstructive, restrictive, and diffusion defects which cleared almost completely within the observation periods. Summarizing their review of cases of nitrogen oxides poisoning sequelae, the authors [51] concluded that chronic pulmonary insufficiency occurs following silo gas exposures in patients with preexisting "small airway disease" (predominantly chronic bronchitis and emphysema). They further stated that "animal exposure studies and lung biopsies in humans lead to speculation that the chronic disease produced might be centrilobular emphysema."

Whether or not exposure to nitrogen dioxide causes methemoglobinemia in man is still a matter of controversy. A comprehensive review article on the chemistry and pharmacology of methemoglobinemia was published by Bodansky [55] in 1951. In most cases in which methemoglobinemia was inferred or reported directly, [32,56,57] the exposures have been to mixed oxides of nitrogen with circumstantial evidence that nitric oxide was present.

The only direct information found on the effect of nitric oxide in man is furnished by a report on a contamination of anesthetic nitrous oxide by nitric oxide. The extent of contamination was greater than 1.5%. [58]

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The contaminated gas was administered to 2 female surgical patients in the course of routine general anesthesia. The first patient received a 75% concentration of the nitrous oxide in oxygen. After about 3 minutes of inhalation, cyanosis developed and rapidly became more severe, despite an increase of the oxygen to 50%. After a further 20 minutes, the nitrous oxide was discontinued. Electrocardiography showed depression of the ST segment in all leads, a sign of myocardial hypoxia. Chest X-ray screening showed several ill-defined opacities in the lung fields. Blood was obtained by femoral artery puncture and was brown in color, suggestive of methemoglobinemia. Later the presence of methemoglobin in the blood was confirmed in the laboratory. Methylene blue (10 ml of a 1% solution) was given intravenously and methemoglobin was not detected in 2 subsequent blood samples, taken 4 1/2 and 8 hours later. The patient died of cardiac arrest approximately 18 1/2 hours after the commencement of exposure to the nitric oxide. At autopsy, severe pulmonary edema was confirmed.

Before the contamination of the nitrous oxide was realized a second patient was induced with the same gas mixture. The patient became cyanotic within a short time. After only a few minutes the administration of nitrous oxide was discontinued and 100% oxygen was administered from a different cylinder. She showed some signs of respiratory distress but she later recovered fully.

Commins et al [29] in 1971 reported that the toxic gas mixture periodically present above fermenting silage and responsible for "silo fillers disease" may contain a high proportion of nitric oxide, in addition to nitrogen dioxide and carbon dioxide (see Table XIII-6). The findings described, including respiratory irritation, delayed pulmonary edema, and

bronchiolitis fibrosa obliterans were, in the opinion of many authors, [43,44,50,51,59,60,61] largely attributable to the action of the high concentration of nitrogen dioxide.

An experimental study on human subjects on the retention of nitrogen dioxide was reported in 1970 by Wagner. [62] Seven subjects were exposed to nitric oxide at several different concentrations (5, 1, 0.5 and 0.33 ppm) under conditions of normal and of maximal oral respiration. By comparative analyses of inhaled and exhaled gases, the percentage absorption of the nitric oxide was measured. The retention was uniformly high (from 85 to 93%), seemingly independent of concentration within the range tested, and somewhat higher with maximal as opposed to normal respiration. A parallel series of studies was conducted with nitrogen dioxide. Absorption rates were similar to those reported for nitric oxide.

Despite the fact that nitrogen dioxide is an irritant gas, there are few primary references in the literature as to its effects upon the eyes or mucosae other than that on the lower respiratory tract. In 1970, Morley and Silk [63] reported conjunctivitis and pharyngitis occurring in five men welding zinc-plated steel in a confined space. These effects had subsided 18 hours later when the men were reexamined. Representative atmospheric measurements showed total oxides of nitrogen, expressed as nitrogen dioxide, in the 4-20 ppm range with an average of 7.4 ppm (19 measurements). Rodin and Boyenko [64] in 1970 reported a prevalence of 64% of "chronic catarrhal processes in the upper respiratory tract" in 334 arc welders who had up to 10 years of work history. In the workers who had more than 10 years service in welding, 22% had subatrophic rhino-pharyngitis.

Hortsch [33] reported a case of right hemiplegia in a 62 year-old worker following exposure to oxides of nitrogen evolved from pickling metal objects in a 50-50 mixture of nitric and sulfuric acids. The victim also developed pneumonitis within 24 hours and died approximately 3 1/2 days after exposure. Autopsy revealed respiratory lesions and widespread hemorrhages in the brain. It is difficult to attribute the cerebrovascular damage directly to the effects of nitrogen dioxide exposure since the patient had severe hypoxemia, secondary to the pneumonitis, at the time.

An experimental self-exposure was reported by Lehmann and Hasegawa [39] in 1913. Hasegawa inhaled 62 ppm (calculated as nitric acid) for 1 hour and reported only slight irritation of the larynx and an objectionable odor, but no other overt ill effects. He inhaled 75-100 ppm for 1 hour, followed immediately by 25-75 ppm for another hour, and reported irritation with cough and an increase in pulse and respiratory rates. He was able to tolerate a concentration of 158 ppm for only 10 minutes because of coughing, irritation of the nose and throat, lacrimation, headache, nausea, and vomiting. However, he rested well after this exposure and there were no delayed aftereffects noted.

The human odor threshold for nitrogen dioxide was investigated by Henschler et al. [65] Those described as olfactorily sensitive could smell as little as 0.1 ppm of nitrogen dioxide, more than half the subjects could detect 0.2 ppm, and all recognized 0.4 ppm. However, even at 4.0 ppm not all subjects recognized the gas as nitrogen dioxide. Olfactory fatigue was observed to develop very rapidly, so that the gas was smelled for only 10 minutes at 4.0 ppm and for five minutes at 0.4 ppm. In 1970, Rumsey and Cesta [66] reported that the mean odor threshold for nitrogen dioxide in 10

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volunteers was 0.5 ppm or less.

In 1967, Abe [67] reported on experimental exposures of five healthy adult men to nitrogen dioxide at 4-5 ppm for 10 minutes. Concentration of the nitrogen dioxide was measured by simultaneous use of the Saltzman method and Kitagawa-type detection tubes. Measurements of "effective lung compliance," "inspiratory maximum viscous resistance," and "expiratory maximum viscous resistance" were made prior to the gas inhalation, immediately after exposure, and at intervals of 10, 20, and 30 minutes after inhalation had ceased. Values for effective compliance obtained 30 minutes after the cessation of exposure showed a tendency to decrease by 40% as compared with controls. Expiratory and inspiratory maximum viscous resistance were unchanged immediately after completion of exposure but gradually increased from 10 minutes after exposure and reached a maximum at 30 minutes.

In 1971, von Nieding et al [68] described the effects of low concentrations of nitrogen dioxide on the respiratory gas exchange and airway resistance in patients with chronic bronchitis. Eighty-eight chronic bronchitis patients, aged 34-72 years, breathed a nitrogen dioxide-air mixture containing from 0.5 to 5.0 ppm (as measured by the Saltzman method) either for 15 minutes or for a total of 30 breaths. Inhalation of nitrogen dioxide concentrations between 1.5 and 5.0 ppm increased airway resistance significantly. Lower concentrations had no significant effect. While the end-expiratory alveolar oxygen tension remained nearly constant during exposure at 4 and 5 ppm nitrogen dioxide, a significant decrease of the arterial oxygen tension and a corresponding increase of the end-expiratory arterial pressure difference for oxygen occurred. After

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inhalation of 2 ppm of nitrogen dioxide, there was no decrease in the arterial oxygen tension.

In 1973, von Nieding et al [69] reported an extension of their earlier studies. In 16 healthy male volunteers, carbon monoxide diffusing capacity was measured by the single-breath method, before and after inhalation of 5 ppm nitrogen dioxide for 15 minutes. A statistically significant (p < 0.01) decrease in the diffusing capacity for carbon monoxide by an average of 3.8 ml/0.1 min/0.1 torr (from 20.6 to 16.8) was observed. In 14 patients with chronic bronchitis, the arterial oxygen partial pressure was significantly depressed after 15 minutes of exposure to nitrogen dioxide at a concentration of 5 ppm. A corresponding increase in alveoloarterial oxygen pressure gradients was observed. Continued exposure for 60 minutes indicated no significant disturbances of respiratory gas exchange beyond that noted after 15 minutes of exposure. An increase in relative airway resistance was observed in 70 chronic bronchitic patients after inhalation (30 breaths) of nitrogen dioxide above 15 ppm. Relative airway resistance remained unchanged below this concentration.

Epidemiologic Studies

In 1937, Vigdortschik et al [70] reported a study of "the symptomatology of chronic poisoning with oxides of nitrogen" in 127 printing shop and sulfuric acid plant workers reportedly exposed at levels generally below 2.8 ppm. The clinical signs and symptoms reported and attributed to "oxides of nitrogen" included: dental erosion and gingivitis; emphysema and compensated pulmonary tuberculosis; cardiovascular hypotonia and

bradycardia; polycythemia rubra, granulocytosis, and basophilia; decreased osmotic fragility of red blood cells and accelerated agglutination of the blood cells; and reduced catalase index, reduced alkali reserve, reduced blood sugar and "lability of the blood sugar curve." The presence of dental erosion in many of these workers suggests that the workers were also exposed to mists of sulfuric or nitric acid. Furthermore, the authors did not describe the sampling methods, sampling locations, or analytical methods employed. Because of these deficiencies, the relevance of this study is questionable.

In 1972, Kosmider et al [71] published a study of 70 men, aged 26-48, exposed in a chemical plant for 6-8 hours daily for 4-6 years to what was described as only oxides of nitrogen. The authors reported concentrations of oxides of nitrogen between 0.4 and 2.7 ppm as nitrogen dioxide. There was no information on analytical method, sampling methods, location of sampling, frequency of sampling, the possible presence of other contaminants, or other information allowing inference or judgment as to how, or to what extent, the environment was characterized. A control group was selected consisting of 80 men of similar ages who were not exposed to oxides of nitrogen. All workers smoking more than ten cigarettes daily were excluded from both groups. The men exposed to nitrogen dioxide complained of sporadic cough with mucopurulent expectoration and dyspnea on exertion. Fine bubbling rales and "whistling" sounds were heard in some men, primarily over the lower lungs. There were no chest X-ray abnormalities. Spirometry showed slight, statistically insignificant reductions in vital capacity and maximum respiratory volume. There was an insignificant decrease in the group's mean blood pH. Carbon dioxide

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partial pressure and total carbonic acid in the blood were increased. Total serum proteins were significantly below that of the controls. There was a reduction in albumin and gamma-globulin, an increase in the alpha-1-, alpha-2-, and beta-globulins, and a statistically significant increase in the urinary hydroxyproline and acid mucopolysaccharide excretions. The authors [71] concluded from these clinical findings as well as from their animal data (see Animal Toxicity) that long-term exposure at such levels of oxides of nitrogen lead to emphysema in man. The urinary excretions mentioned above were suggested by the authors to be decomposition products of collagen (more likely of collagen and of other elements of connective tissue). They further implied that such tissue destruction is inherent in the pathogenesis of emphysema. Some of the workers were reported to have had chronic bronchitis, but a comparison between control and experimental spirometric changes on a group mean basis was statistically insignificant, a finding which is incompatible with chronic obstructive pulmonary disease. Interesting as this study is, it cannot be given full credence in view of the absence of details on the characterization of the environment, absence of spirometric changes, lack of evidence of a controlled diet in relation to urinary excretion of amino acids and glycoproteins, and omission of supporting evidence on the relation between these urinary excretions and the development of emphysema.

In 1973, Kosmider and Misiewicz [72] reported a rise in the mean total lipid level in the serum of the 70 exposed workers studied in the previous report compared with the 80 control subjects. They found a rise in the mean levels of the beta- and gamma-lipoproteins and a fall in the alpha-lipoproteins and in total serum cholesterol (both free and

esterified) in the exposed workers. The significance of these serum lipid changes in relation to exposure to oxides of nitrogen is not apparent.

In 1972, Kennedy [28] reported a study of the prevalence of emphysema in coal miners exposed sporadically to oxides of nitrogen after underground blasting operations. Some evidence of emphysema was found in 84 of the 100 miners studied. He attributed the emphysema to the nitrogen oxides exposures, but no satisfactory control group was studied to support this conclusion. Comparisons were made with other smaller-scale studies of gold miners who had been exposed only once to "nitrous fumes". The gold miners had a lower incidence of emphysema; however, the use of the gold miners as an adequate control must be questioned. The results of this study are further complicated by the association of coal worker pneumoconiosis with emphysema. [73]

In 1975, French [74] summarized results of a 4-year epidemiologic study concerned with the effects of exposure to nitrogen dioxide in communities located near TNT production plants in Chattanooga, Tennessee. [75,76,77] In 1968-69, the communities were dividied into high (0.083-0.219 ppm), medium (0.063 ppm), and low (0.031 ppm) exposure categories. Over the course of the 4-year study, ambient levels were reduced to 0.031, 0.027, and 0.024 ppm for the high-, medium-, and low-exposure communities, respectively. Results indicated that "lower respiratory" morbidity rates and the incidence of "acute respiratory disease" were significantly higher in the high- and intermediate-exposure communities compared with the low-exposure community, particularly in children below 12 years of age. Significant differences in these findings were also noted between high- and intermediate-exposure communities. There were no significant differences

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in the three communities in the prevalence of chronic respiratory symptoms such as chronic bronchitis. It is difficult to attribute the differences in acute respiratory illnesses only to the differences in exposure to nitrogen dioxide since the concentrations of suspended nitrates and total suspended particulates were also increased in the high-exposure as compared with the medium- and low-exposure communities. Suspended sulfates did not differ between communities; however, concentrations of other possible contaminants such as sulfur dioxide were not reported.

In 1955, Vigliani and Zurlo [78] made a brief comment on oxides of nitrogen in a review article on their own research into maximum acceptable concentrations of industrial poisons in the workplace. They stated that workers in 4 catalytic nitric acid plants, exposed for several years to nitrogen dioxide at concentrations averaging from 30 to 35 ppm, had no complaints or signs or symptoms of toxicity. Vigliani and Zurlo expressed the view that the reduction in 1954 by the American Conference of Governmental Industrial Hygienists of their recommended Threshold Limit Value for oxides of nitrogen from 25 ppm to 5 ppm (for nitrogen dioxide alone) was unwarranted. In their opinion, 15 ppm for "nitrous gases," calculated as 50% nitrogen dioxide and 50% nitrogen tetroxide, was acceptable, as long as no ozone was present. Data supporting this conclusion were not included.

Epidemiologic studies of the effects of nitric oxide per se have not been reported. Nitric oxide is probably involved in most occupational exposures to the mixed oxides of nitrogen or "nitrous fumes". Most of these studies [56,57,63] are difficult to evaluate in terms of a dose-response relationship because the environmental data have rarely been

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expressed in terms which permit specification of the nitric oxide concentration. In these studies, either the analytical methods did not differentiate between the different nitrogen oxides or the data were expressed entirely in terms of one oxide, [56,57,63] usually nitrogen dioxide. [56,63]

The presence of methemoglobinemia has commonly been held as indicative of nitric oxide poisoning rather than of nitrogen dioxide poisoning. [45,79,80] Such an assumption is suspect in the light of recent animal experiments. [81,82] One epidemiologic study on nitrogen fertilizer plant workers allegedly exposed to carbon monoxide, ammonia, and mixed oxides of nitrogen may be cited. [57] One hundred and seventy workers and 54 controls (mechanical maintenance workers in a woolen mill) were studied on 2 occasions, separated by two years. No environmental data were given but it was implied that the level of oxides of nitrogen (expressed in terms of nitrogen pentoxide) was above the maximum permissible concentration (5 mg/cu m or about 3 ppm in the USSR). [83] The workers had relatively high levels of carboxy- and methemoglobin in their blood as evidence of the effects of these gases. The main finding of this study was that workers under these exposure conditions developed pyridoxine (Vitamin B6) deficiency, but the mechanism for this and the individual roles of the gases were not discussed. [63]

McCord et al [56] found methemoglobin levels in the blood of four arc-welders of 2.3, 2.3, 2.5, and 2.6%, respectively, measured spectrophotometrically. They were exposed to oxides of nitrogen in the 2.0-10.3 ppm range, expressed as nitrogen dioxide. The duration of exposure prior to blood sampling was not given. The normal methemoglobin

level in man is about 1% of total hemoglobin. [84]   Even assuming the elevated methemoglobin levels to be due to the nitric oxide exposure of the order of 10 ppm, it seems unlikely that an increase in methemoglobin content of the blood of 1 or 2% would be injurious to health, even on long-term exposure.   At what point higher blood levels would pose an occupational hazard is uncertain.   Persons who are heterozygous for the trait of hereditary methemoglobin reductase (diaphorase) deficiency have this low order of methemoglobinemia throughout life and are entirely asymptomatic. [84]   Moreover, the analytical methods generally available for blood methemoglobin measurements in 1941 were rather inaccurate and a difference of only 1-2% from the physiologic normal would be well within experimental error. [85]

Animal Toxicity

Tables XIII-9 and XIII-10 summarize animal data on the inhalation toxicity of nitric oxide and nitrogen dioxide.   It is evident from these tables that exposure to the oxides of nitrogen results in diverse responses across species.   An attempt has been made to note these contrasting effects and differences between species in terms of toxic concentrations both in the tables and in the text below.

    (a)   Nitric Oxide

The first description of animal studies on the toxicity of nitric oxide, as distinct from nitrogen dioxide or mixed oxides of nitrogen ("nitrous fumes"), appears to be that given by Flury [46] in 1930. Describing work performed in Germany during World War I, he outlined an apparatus for the exposure of white mice to a stream of air in which pure

nitric oxide was continuously added. Mice exposed to the gas mixture close to the point of mixing manifested strikingly different toxic effects than those exposed in the same apparatus, but in a more distal section. In the first instance, where the content of unoxidized nitric oxide in the mixture was still relatively high, cyanosis occurred after a few minutes, the red eyegrounds (probably conjunctival or red reflux) became gray-blue, and then breathlessness appeared with paralysis and convulsions. If the mice were promptly removed and exposed to fresh air, they recovered rapidly and completely without apparent sequelae. Mice exposed in the distal part of the apparatus where, according to the author, nitrogen dioxide was predominant showed immediate signs of irritation but no cyanosis and no paralysis. Death resulted from pulmonary edema.

The following year, in their book on toxic gases, Flury and Zernik [86] gave some quantitative data on exposure of mice to nitric oxide, probably from the same experiments. Mice exposed at a concentration of 5,000 ppm died after 6-8 minutes. With inhalation at a concentration of 2,500 ppm, the animals were observed to be on their sides after 6-7 minutes of exposure and died after 12 minutes. Removal of the mice after 4-6 minutes of exposure resulted in full recovery within 24 hours.

These experiments were repeated and described in much fuller technical detail by Pflesser in 1935. [48] The clinical description of the toxic effects and mode of death of white mice exposed predominantly to nitric oxide were identical in every detail to those of Flury [46] and Flury and Zernik. [86] Pflesser [47] noted that at autopsy there was no evidence of lung injury or of pulmonary edema and that spectroscopy of the

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blood showed typical methemoglobin lines. He gave the following quantitative data:

> at 3,500 ppm, animals died in 4-5 minutes;
> at 350 ppm, all the animals died;
> at 320 ppm, half the animals died;
> at 310 ppm, all the animals survived an
> exposure of 8 hours.

By way of contrast, the effects of nitrogen dioxide exposure were quantitatively expressed as follows:

> 1,500 ppm and more was lethal to all animals;
> 1,200 ppm and less was survived by all animals.

These figures indicate a remarkably steep dose-response curve, both for nitric oxide and for nitrogen dioxide. From Pflesser's experiments, it became apparent that nitric oxide was approximately four times more toxic than nitrogen dioxide.

The question of comparative toxicities of these 2 gases is, however, complex. Experiments with albino mice and guinea pigs performed by Paribok and Grokholskaya [87] in 1962 indicated that at concentrations above 1 mg/liter (833 ppm) a 1-hour exposure to nitric oxide was more toxic than the same exposure to nitrogen dioxide. On the other hand, with 8-hour exposures at lower concentrations, nitrogen dioxide produced a higher toxic effect than nitric oxide. For nitric oxide, if the concentration is not high enough to be rapidly lethal, the animal apparently makes a complete recovery. [46,47,86] But levels of nitrogen dioxide that are not rapidly fatal may cause more persistent ill effects, in some cases resulting in death from pulmonary edema after a delay of several days. [46] The same authors exposed albino mice at various concentrations of nitric oxide and measured the level of methemoglobinemia. [87] When mice were exposed to

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nitric oxide at 2,100 ppm, 80% methemoglobin was produced in less than 30 minutes, 1,325 ppm nitric oxide produced 80% methemoglobin in about 40 minutes; and it required 6 hours of exposure to nitric oxide at 322 ppm to produce 60% methemoglobin.

The same authors exposed guinea pigs to nitric oxide at 175 ppm for 120-150 minutes and found no effect of this exposure on the rate of recovery of resting respiratory rhythm after treadmill exercise, compared with previous control measurements made on the same animals. [87]

In 1936, Zakusov [88] made a somewhat crude attempt to differentiate the effects of "nitrous gases" derived from heating nitric acid (predominantly NO2) from those associated with the action of copper metal on nitric acid (predominantly NO). Both gases were considered to be contaminated with nitric and nitrous acids. Experiments were performed on cats. The effects reported were similar to those of Flury and Zernik [86] and of Pflesser. [48] Zakusov [88] also observed that the gas derived from heating nitric acid produced more severe emphysema (as seen post mortem) following brief lethal exposures. The author based his diagnosis of emphysema on changes in lung weights, more likely indicative of pulmonary edema. He further concluded that nitric and nitrous acids may have contributed significantly to the findings.

Greenbaum and his colleagues, in an attempt to reproduce the conditions of an anesthetic accident, experimentally exposed dogs at lethal concentrations of nitric oxide and of nitrogen dioxide. [81] Either nitric oxide or nitrogen dioxide at a concentration of 5,000 ppm produced a rapid fall in arterial oxygen tension, a rise in methemoglobin concentration and a rise in arterial carbon dioxide tension, despite artificial respiration.

If exposure was continued for more than 24 minutes, all experimental animals died reportedly with overt pulmonary edema at intervals varying from 7 to 120 minutes after exposure. Nitric oxide at 20,000 ppm caused death in 15 to 50 minutes. It is interesting to note that in these animal experiments, methemoglobinemia was reported to be produced by nitrogen dioxide about as readily as by nitric oxide.

(b)   Nitrogen Dioxide

There are many studies concerned with animal exposures to nitrogen dioxide or mixed oxides of nitrogen. In order to summarize as much of this information as possible, the data are grouped by concentration range, by type of effect, and by chronology. Greater emphasis is placed upon those concentrations and effects which appear to have some potential bearing upon the development of a standard for human occupational exposure.

(1)   50 ppm and over:

In an experiment designed to determine lethal concentrations in male rats for short-term exposures to nitrogen dioxide, Gray et al [89] found that the 2-minute LC50 (the minimal concentration killing 1/2 of the animals within 2 minutes) was 1,445 ppm. The 5-minute LC50 was 833 ppm, and for 15, 30, 60, and 240 minutes the corresponding LC50s were 420, 174, 168 and 88 ppm, respectively. The cause of death at all these concentrations was stated to be pulmonary edema, although no autopsy findings were reported.

In 1962, Carson et al [90] reported results of experiments on rats, rabbits, and dogs designed to determine the LC50 for short-term exposures (5-60 minutes) as well as concentrations which, for the same exposure times, did not result in macro- or microscopic changes in the

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lungs, liver, kidneys, spleen, heart, eyes, and gastrointestinal tract. The LC50 values in rats were 416, 201, 162, and 115 ppm for 5, 15, 30, and 60 minutes of exposure, respectively. The rabbit LC50 for a 15-minute exposure was 315 ppm, considerably higher than that observed in the rat, ie, 200 ppm. Differences in the LC50 values for rats reported by Carson et al [90] and by Gray et al [89] were attributed by the former to differences in the size and age of experimental animals. However, the data of Carson et al [90] corroborated that of Gray et al [89] in showing that the product of concentration (C) and time (t) did not equal a constant (k), ie, Ct≠k. Both studies indicated that the relationship between concentration and time was best represented by an exponential function. The thresholds for nitrogen dioxide toxicity were approximately 25% of the rat LC50 values. [90] At these concentrations, dogs showed no gross or microscopic changes which were different from controls. Rats showed no gross pathologic lesions; however, microscopic studies indicated that some animals had pulmonary edema.

In 1968, Kleinerman and Cowdrey [91] exposed 48 hamsters for 21-23 hours daily to nitrogen dioxide at 50 ppm for 1-10 weeks. Over one-third of the animals died within 2 or 3 days (7 on the first day, 6 on the second day). Other animals were killed at intervals. Microscopic examination immediately after cessation of exposure showed extensive epithelial hyperplasia and hypertrophy in the region of the terminal and respiratory bronchioles and alveolar ducts. Following 10 weeks of exposure, there were extensive focal collections of inflammatory cells and hyperplastic and hypertrophied epithelial cells in the same regions. While the size of the alveolar spaces appeared enlarged in exposed animals

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compared with controls, there was no evidence of destruction of alveolar septal tissue. In animals killed 4 weeks after cessation of exposure, remarkable regression of inflammatory and epithelial hyperplastic changes were observed. Only a minimal degree of epithelial hypertrophy persisted in the respiratory bronchioles and alveolar ducts. There was no evidence of pulmonary edema, and acute inflammatory cells had virtually disappeared. These results indicated that the pulmonary lesions induced in the hamster under these exposure conditions were reversible. According to the authors, the results bring into question the belief that nitrogen dioxide, per se, causes true emphysema, ie, that due to tissue destruction.

(2)   5 to 50 ppm:

In 1952, Gray et al reported [92] experiments in which rats were exposed to the vapors of red fuming nitric acid, the airborne nitrogen dioxide content of which ranged from 9.3 to 14.3 ppm. The exposure schedule was 4 hours/day, 5 days/week. The total duration of exposure ranged from 10 to 24 days for different groups of animals. Rats examined shortly after the end of exposure showed severe rhinitis and tracheitis, with less severe pneumonitis. In many of the animals killed 8 or more weeks after the termination of exposure, the inflammatory process had subsided, but there were localized areas of emphysema in all lobes of the lung.

In 1965, Wagner et al [93] reported on the effects of exposure at 5 ppm on dogs and mice and of rabbits, guinea pigs, rats, and hamsters exposed at 5 and 25 ppm. The animals were exposed an average of 6 hours/day, 5 days/week, for periods ranging from 14 to 18 months. Dogs exposed at 5 ppm daily for 1 year showed only mild dilation of peripheral

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air spaces. At 18 months, there was additional edema and congestion, accompanied by some thickening of alveolar septa. The respiratory tissues of the rabbits were essentially normal even after 18 months of daily exposures, at either 5 or 25 ppm. Hamsters exposed at 25 ppm daily for 3 and 6 months showed minimal evidence of changes in the bronchiolar epithelium. After 12 months, there was a questionably higher incidence of mild interstitial pneumonia. Exposure of two of the strains of mice used at 5 ppm daily for 10 and 14 months produced no observable microscopic changes or other abnormalities that could be attributed to the nitrogen dioxide exposure.

Diggle and Gage [94] exposed rats to nitrogen dioxide for single 4-hour periods at concentrations of 10, 22, 36, and 45 ppm. All animals had normal trachea and lungs 4-8 days after exposure. Kleinerman and Wright [95] subjected rats, guinea pigs, and rabbits to 2-hour exposures at 15-25 ppm for either 1 day or 5 days. Animals were killed at intervals of 1, 2, 4, 7, 14, and 21 days, and the lungs were studied macro- and microscopically. Twenty-four hours after a single 2-hour exposure, the animals' lungs showed a mild degree of pulmonary edema which was confined to the respiratory bronchioles. Macrophage infiltration and epithelial regeneration were noted by the fourth day and by the end of 2 weeks epithelial repair was almost complete. The degree of morphologic change and repair was roughly proportional to the concentration of exposure. Edema and inflammation were less severe in multiple exposures (5 days) than in single exposures. Tissue repair was almost complete 7 days after exposure. In general, peribronchial and perivascular inflammation was more severe in the rat and the guinea pig than in the rabbit. In 1962, the same

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authors [96] reported results of experimental exposures of rats, rabbits, and guinea pigs at 20-25 ppm, 2 hours daily, 3-4 days a week, from 3 weeks to 18 months. The animals were killed 14 days after the completion of exposure. In 50% of the guinea pigs exposed for 15-18 months, changes judged to be equivalent to human microbullous emphysema were seen. Such changes were not, however, observed in rats or rabbits.

In 1964, Freeman and Haydon [97] presented data on rats exposed to nitrogen dioxide at 12.5, 25, 50, and 100 ppm continuously, ie, 24 hours/day, 7 days/week. At 100 ppm, the animals "began to die within 24 hours." At 50 ppm, 6 died within 48-68 days and 2 survived 76 days. At 25 ppm, all survived but failed to gain weight normally. Nine rats were exposed at 12.5 ppm. One died after 213 days, and autopsy showed pulmonary changes similar to those found in animals exposed at 25 ppm. In the surviving animals, those killed at about 40 days revealed moderate hypertrophy and hyperplasia of the bronchial and bronchiolar epithelium. The alveolar ducts and alveoli in exposed animals were more variable in size and many were much larger than those in controls. The animals exposed at 25 ppm who died between 146 and 157 days of exposure had "strikingly voluminous lungs." Haydon et al [98] exposed rabbits continuously at 8-12 ppm for 3-4 months. Various changes including emphysema-like dilatations of the peripheral alveoli were noted. In 1968, Freeman et al [99] reported results of the effects of continuous exposures at 18 ppm in rats. By the fifth day of exposure, hypertrophy of the terminal bronchial epithelium was seen, but no typical emphysema.

Riddick et al [100] continuously exposed mongrel dogs at 25 ppm for 6 months. One dog showed macroscopic bullous emphysema but all

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showed bullous alveolar enlargement microscopically. Lewis et al [101] continuously exposed beagles at 26 ppm for 191 days. One dog showed bullous emphysema, and others showed "striking increase in firmness of the lungs with scattered small bullae." Emphysema was also noted microscopically.

The effect of continuous exposure at 15 ppm in rats was studied by Freeman et al. [102] Exposures were over the natural life time of the experimental animals. The animals were killed and found to have voluminous "dry" lungs (probably meaning nonedemetous) with large functional residual capacity. Microscopically, there was epithelial hypertrophy, "emphysema-like disease", and loss of cilia.

The significance of animal studies to man with respect to the production of emphysema or emphysema-like changes must be interpreted with caution. Tyler et al [103] have reviewed the comparative micro- and macroscopic anatomy of the terminal airways and air spaces and their blood supply in humans and in many of the common experimental animal species. On the basis of morphologic relationships, the authors concluded that the rat was an inadequate model for the study of human emphysema. According to the authors, the horse represented the animal model closest to man in terms of pertinent anatomical considerations.

Kilburn and Dowell [104] exposed mongrel dogs and rabbits to nitrogen dioxide at concentrations ranging from 5 to 16 ppm for 1 hour. Lungs of both species showed minimal microscopic changes consisting of perivenular edema without alveolar edema. Electron microscopy showed marked changes in the capillary endothelium and lesser changes in the alveolar epithelium. The endothelium formed blebs that encroached and

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filled the lumens. Endothelial cell organelles, especially altered mitochondria, were found loose in capillary lumena. Platelets and polymorphonuclear leukocytes filled the lumena of involved capillaries adjoining blebs. Occasionally, platelets and leukocytes were found in edematous basement membranes. The authors concluded that brief exposure to nitrogen dioxide at low (nonlethal) concentrations has its major effects on capillary endothelium. Dowell et al [105] exposed beagles for 1 hour at concentrations of from 3 to 16 ppm. Electron microscopy revealed widespread bleb formation, loss of pinocytic vesicles, and mitochondrial swelling of endothelial cells. Exposure at only 3 ppm resulted in bleb formation in alveolar endothelium without biochemical or physiological changes.

Guinea pigs were continuously exposed at a concentration of 10 ppm for 6 weeks in a study conducted by Yuen and Sherwin. [106] An increased ratio of type 2 pneumocytes to other cells, resulting in thickening of the alveolar blood gas barrier, was observed. In 1973, Parkinson and Stephens [107] reported the results of an electron microscopic investigation of the lungs of rats exposed continously at 15 ± 2 ppm for 1, 2, and 7 days. Within 24 hours, loss of cilia was seen. The bronchiolar epithelium became less collumnar, brush cells increased in number, microvilli became smaller, and there was an increase in macrophages.

Sherwin et al [108] continuously exposed guinea pigs to nitrogen dioxide at 15 ppm for 3 months. (Because of an editorial error, confirmed by verbal communication with the senior author in September 1973, the term nitric oxide was erroneously substituted throughout this paper for

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the correct term nitrogen dioxide.) Increased proliferation of alveolar cells with an increase in the alveolar cell/alveolar space ratio was demonstrated by a cytochemical technique which measured the lactic acid dehydrogenase activity of proliferating cells.

In 1968, Sherwin et al [109] reported increased macrophage congregation in the lungs and more macrophages per epithelial cell in guinea pigs following continuous exposure at 10 ppm for 7 weeks. Gardner et al [110] exposed rabbits for 3 hours at 8, 10, or 40 ppm. At 8 ppm, there was a significant increase in intra-alveolar heterophiles. At 10 ppm, fewer macrophages contained phagocytized bacteria. At 40 ppm, there was peak infiltration of heterophiles between 6 and 9 hours after exposure. The authors speculated that nitrogen dioxide exposure might destroy opsinogenic factors and surfactant, or in some way affect the motility of macrophages. Evans et al [111] continously exposed rats at 15-17 ppm for 48 hours. The results showed that there was a large increase in the number of dividing macrophages and an increase in the total number.

Buckley and Balchum [112] exposed guinea pigs continuously at 15 ppm for 10 weeks, and at 40 ppm for 1/2 hour every 2 hours for a total of 4 1/2 hours. Oxygen consumption of tissue homogenates (lung, liver, spleen, and kidney) was studied in vitro. Lung tissue oxygen consumption was minimally changed. Liver oxygen consumption was markedly increased following the acute exposure regimen. Lactic acid dehydrogenase (LDH) and aldolase activity were increased. Buckley and Balchum [113] exposed guinea pigs at 15 ppm, 23 hours/day, for 26, 33, or 40 days. A relative decrease in fast-moving (aerobic) isozyme and an increase in slow-moving (anaerobic) isozyme were detected in the lung only. In 1969, Buckley and Loosli [114]

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reported on mice exposed continuously at 40 ppm for 6-8 weeks. An intense increase in LDH activity at the sites of nitrogen dioxide lung lesions was detected. The increase in oxygen consumption and in LDH activity suggested the stimulation of cellular activity.

Sherwin and Richters [115] exposed mice either continuously at 4-7 ppm for 14 days, or at 30 ppm for 24 hours. In all animals, leakage of tritiated serum into pulmonary lavage fluid was demonstrated, suggesting altered lung capillary permeability.

In 1965, Balchum et al [116] reported on guinea pigs exposed at 5 ppm for 4 hours/day for 5 days/week and at 15 ppm for 7 1/2 hours/day, 5 days/week, for 1 year. Only minor microscopic changes occurred but normal lung tissue serum antibodies appeared within 160 hours. The antibody titers increased thereafter with continued exposure. This is suggestive of an autoimmunization process triggered by the tissue destruction caused by nitrogen dioxide exposure.

Henry et al [117] in 1970 observed that squirrel monkeys exposed continuously at 5 ppm for 2 months and at 10 ppm for 1 month developed increased susceptibility to Klebsiella pneumoniae infection and to an influenza virus challenge given 24 hours before exposure. The authors [118] also reported that hamsters given single exposures at 15 ppm for 2 hours, followed by a 1-hour exposure to 3% volumn/volume (v/v) cigarette smoke showed decreased resistance to Klebsiella pneumoniae infection by enhanced mortality and decreased survival time. Valand et al [119] in 1970 observed that rabbits given a single 3-hour exposure at 25 ppm showed inhibition of production of interferon by their alveolar macrophages challenged by rabbit pox virus. Williams et al [120] conducted

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experiments in which rabbits were given single 3-hour exposures at 25 ppm and then challenged with an influenza virus. More virus attached to nitrogen dioxide-treated macrophages than to controls. The inability of the nitrogen dioxide-treated macrophages to produce interferon was, therefore, not due to any defect in absorption, penetration, or uncoating of the virus. Acton and Myrvik [121] exposed rabbits to nitrogen dioxide for 3 hours at 5, 15, 25, and 50 ppm. Their virus-induced resistance and phagocytic activity were suppressed by 15 ppm. Fifty ppm stimulated oxygen uptake and hexose phosphate shunt activity of macrophages.

In 1970, Matsumura [122] reported investigations on the effect of nitrogen dioxide exposures upon antigen sensitization of the airways of guinea pigs. The animals were exposed at 20, 40, or 70 ppm for 30 minutes. Thirty to 50 minutes later, they were exposed to antigen aerosols for 45 minutes (egg albumin, serum, serum albumin). Exposure to nitrogen dioxide at 70 ppm enhanced sensitization via the airways; 40 ppm or less did not. In a later study, Matsumura et al [123] exposed guinea pigs to nitrogen dioxide for 30 minutes at 50 ppm, followed 30-50 minutes later by an exposure to acetylcholine aerosol. The latter exposure caused more severe respiratory distress in the nitrogen dioxide-exposed animals than in controls.

In 1964, Boren [124] investigated the possibility of synergism between nitrogen dioxide and carbon particles. One group of mice was exposed to nitrogen dioxide for 30 minutes/day, 5 days/week, for 4 1/2 months at a concentration of 25 ppm. A second group was exposed 6 hours/day, 5 days/week for 3 months to carbon with absorbed nitrogen dioxide, the latter having an airborne concentration of 25-30 ppm. The

combined exposure gave rise to focal and destructive lung lesions, apparently tantamount to emphysema although alveolar fenestrations were rare. No such findings were observed in animals exposed only to nitrogen dioxide at concentrations of 25 ppm. There was no evidence of fibrosis. In further experiments, Boren [125] observed that the sequence of carbon particulate and irritant gas exposures influenced the outcome. Inhalation of carbon alone (at 18,000-21,000 particles/ml) produced a macrophage response. Subsequent exposure to nitrogen dioxide at 25 and 75 ppm caused lung destruction. Inhalation of nitrogen dioxide followed by particulate carbon gave a lesser macrophage response and less lung destruction.

(3)   1.0 to 5.0 ppm:

In 1954, Gray et al [126] reported on exposing rats, mice, and guinea pigs 4 hours daily, 5 days/week for 6 months to vapors of red fuming nitric acid with an average airborne nitrogen dioxide concentration of 4 ppm. Not only did the animals exhibit no pathologic effects but they had a lesser incidence of pneumonia, from which the authors suggested a therapeutic effect of nitrogen dioxide.

House [127] continuously exposed rats, mice, and monkeys at an average concentration of 4.5 ppm. The mortality was low but all species were seriously depressed and weak with poor appetite and reduced weight gain. There were no other outstanding clinical or pathologic findings. This report has been contrasted with that of MacEwen and Geckler [128] in 1968 who exposed the same species at 5.0 ppm continuously for 90 days and found no significant mortality or any remarkable changes in blood chemistry or growth. Freeman et al [129] exposed rats at 2 ppm ($\pm$ 1 ppm) continuously for their natural lifetime. The rats showed persistent

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tachypnea and all animals died of nonpulmonary diseases (predominantly of nephrosclerosis). Airflow resistance and dynamic compliance were not affected. Some microscopic changes were seen in the terminal and respiratory bronchiolar epithelium, ie, loss of exfoliative activity, reduced blebbing of cytoplasm into the airways, reduction or loss of cilia, and the appearance of rod-shaped intracytoplasmic crystalloid inclusions.

In 1972, Stephens et al [130] reported results of a study in which rats were exposed continuously at 2 ppm for periods ranging from a few hours to 21 days. They showed loss of cilia, hypertrophy, and focal hyperplasia in the epithelium of the terminal bronchioles. Animals allowed to survive returned to normal after 21 days of continuous exposure. Evans et al [131] exposed rats continuously at 2 ppm for up to 360 days. An increase in type 2 pneumocytes, ie, cuboidal rather than flattened and ultra-thin, was observed in peripheral alveoli after two days of exposure. However, the number of cells declined to control levels by the fifth day. There was no increase in cells in other alveoli or in the bronchioles. Sherwin et al [132] exposed guinea pigs at 2 ppm continuously for 1, 2, and 3 weeks. Replacement of type 1 pneumocytes (flattened and ultra-thin cells normally lining the alveoli) by type 2 pneumocytes in the alveoli was observed by a cytochemical technique based on LDH activity. A significant increase in the average area of each alveolar wall was also observed.

Chen et al [133] studied morphologic changes in the trachea and lungs of 15 mice exposed continuously for one month to nitrogen dioxide at between 1.0 and 1.5 ppm. The experimental design did not provide for the evaluation of control subjects. Animals were killed immediately following exposure and at intervals of 1 and 3 months following exposure.

Microscopic examinations revealed desquamative bronchitis in a number of animals in each sample group. Infiltration of lymphocytes was not observed in animals killed during or immediately following exposure. However, these effects were observed in 3 of 5 mice killed 3 months after exposure. The authors suggested that acute pathologic changes resulting from the exposures used in this study may lead to chronic autoimmune diseases such as chronic tracheitis or bronchitis in mice. It is difficult to assess the significance of these findings because of the inadequate controls employed.

In 1973, Arner and Rhodes [134] reported results of exposing rats at 2.9 ± 0.71 ppm for 24 hours/day, 5 days/week, for 9 months. A 12.7% mean increase in lung weights and 13% mean decrease of lung compliance were found at autopsy. There was also significant reduction in the surface-active properties of the lung-wash fluid.

Purvis and Ehrlich [135] exposed mice at 2.5 and 3.5 ppm for two hours followed by a challenge of Klebsiella pneumoniae. At the lower exposure level, there was no increased susceptibility to infection but at the higher level there was a significant increase in susceptibility for up to 27 hours after exposure. In 1970, Ehrlich et al [136] reported on single exposures of mice at 1.5, 2.5, and 3.5 ppm for two hours followed by respiratory challenge by Klebsiella pneumoniae. At the two lower levels of exposure, there was no significant increase in mortality. The threshold for this effect therefore appeared to lie in the region of 3 ppm nitrogen dioxide. Fenters et al [137] continuously exposed squirrel monkeys at 5 ppm for 169 days followed by a challenge by four intratracheal injections of a mouse-adapted influenza virus. Hemagglutination-inhibition antibody was not affected by nitrogen dioxide exposures. Initial production of

serum neutralizing antibody was influenced by gas exposure, but not after the 133rd day of exposure. In 1972, Ehrlich and Miller [138] reported results of in vitro exposures of Venezuelan equine encephalitis (VEE) virus to nitrogen dioxide at concentrations of 1.5, 5.0, and 10.0 ppm. At 5.0 ppm, the aerosol recovery and survival of VEE virus was significantly lower than in controls. Bacillus subtilis spores were exposed to nitrogen dioxide at 10 ppm but were unaffected. In 1973, Goldstein et al [139] reported on single exposures of mice at 1.9-14.8 ppm for four hours, and at 1, 2.3, and 6.6 ppm for 17 hours. The mice had been previously infected with radio phosphorus-labeled Staphylococcus aureus. With exposures above 7 ppm nitrogen dioxide, there was a progressive decrease in pulmonary bactericidal activity. Exposure at more than 2.3 ppm nitrogen dioxide prior to staphylococcal challenge also caused decreased bactericidal activity.

Coffin et al [140] studied the effects of intermittent and continuous exposures to nitrogen dioxide on mortality in mice challenged with Streptococcus pyogenes (Group C). Experimental animals were exposed on the basis of equivalent concentration-time products (Ct); for example, an exposure for 2 hours at 3.5 ppm would equal 7 ppm-hours. Results indicated that concentration was of considerably greater importance than time in determining the rate of mortality, ie, equal Ct's at different exposure times were not equally hazardous. Single exposures at 2.3 ppm for 3 hours and 1 ppm for 7 hours caused a slight but statistically insignificant increase in mortality from that of controls. The observation that mortality in mice exposed to nitrogen dioxide and then challenged with Streptococcus pyogenes does not follow a strict Ct=k relationship is

similar to that of earlier investigators [90,141] who came to similar conclusions based upon other criteria of effect. Cumulative effects of continuous exposure at 0.5, 1.5, and 3.5 ppm and intermittent (7 hours/day) exposure were also assessed by mortality figures. Exposure at 3.5 ppm resulted in a statistically higher mortality percentage than exposure at 0.5 ppm. In plotting the percent mortality as a function of exposure time, the linear regressions for exposures at 0.5 and 3.5 ppm were statistically significant at the 0.01 level, ie, from zero slope. The slope for the exposure at 1.5 ppm was significant only at the 0.25 probability level. Cumulative effects of exposure on mortality were also observed for intermittent exposures to nitrogen dioxide at 3.5 ppm. However, mortality as a function of total time of intermittent exposure was lower than that observed for continuous exposure. The authors' regression lines were from plots of mortality (linear) versus the logarithm of exposure time. Visual inspection of some of their curves suggests that a sigmoid relationship is a more likely regression than their linear regression. Thus, a plot of probit or logit mortality versus the logarithm of exposure time might have been more revealing.

Shalamberidze and Tsereteli [142] studied changes in the reproductive endocrine system of female albino rats exposed at 1.3 ppm, 12 hours daily, for 3 months. There was prolongation of the estrus cycle associated with an increased interestral period, a lengthening of the estral cycle, and a decrease in the monthly number of cycles. These changes became more pronounced with prolonged exposure. The capacity for pregnancy was not affected but the litter size and the fetal weights were decreased. This suggestion of alteration in endocrine or reproductive

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function needs to be confirmed in other species and its relevance to humans then evaluated. If such influences pertain to humans, then special restrictions governing the exposure of female workers to nitrogen oxides would be indicated.

(4)   1.0 ppm and below:

In 1965, Wagner et al [93] reported on a series of long-term, intermittent exposures of 5 animal species to nitrogen dioxide at 3 different concentrations. Dogs, rabbits, guinea pigs, rats, and hamsters were exposed to nitrogen dioxide at 1 ppm for an average of 6 hours/day, 5 days/week for up to 18 months. Comparisons between experimental and control groups indicated no significant differences in percent body weight gain. Animals exposed daily for 6 months showed no microscopic reaction attributable to the inhaled gas. After 1 year, dogs showed a general pattern of moderately dilated alveolar ducts and sacs which contained some edema fluid and an occasional macrophage. Necropsy of dogs at 18 months showed, in addition to the above findings, an occasional area of mild to moderately thickened alveolar septa with chronic inflammatory cells.

Daily exposure of guinea pigs to nitrogen dioxide at 1 ppm for 3 months revealed an essentially normal lung parenchyma, a mild hyperplasia of bronchial epithelium, and a prominence of lymph follicles. At 3 and 6 months, the reaction in rabbits exposed daily at 1 ppm was that of an increased incidence of congestion with no visible lesions or other tissue alterations. The findings in the other 2 species exposed to nitrogen dioxide at 1 ppm were not described separately, but the authors implied that they were essentially negative.

In 1965, Haydon et al [143] reported on exposing rats

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continuously at from 0.8 to 4 ppm for 16 weeks. No macroscopic emphysema was detected at autopsy, and only minimal microscopic changes were seen. In 1966, Freeman et al [144] exposed rats at 0.8 ppm continuously for their natural lifetime. The exposed rats showed a sustained tachypnea of about 20% above that of controls, but their growth and behavior were otherwise normal. Upon necropsy, only minimal morphologic changes were noted in bronchiolar epithelial cells. According to the authors, these changes were not accompanied by gross or microscopic obstructive diseases. Steadman et al [145] studied the effects on monkeys, dogs, rabbits, guinea pigs, and rats exposed at 0.5 ppm continuously for 90 days. The only effect reported was a possible slight weight loss. In 1969, Blair et al [146] studied mice exposed at 0.5 ppm for 6, 18, and 24 hours daily for 3-12 months. The expansion of lung alveoli was seen in all mice killed after 3-12 months of exposure, and the number of expanded alveoli increased with the duration of exposure. The general picture was interpreted as one of early bronchiolar inflammation with reduction of distal airway size and a concomitant expansion of alveoli. The overall lesions appeared to be consistent with the development of early focal emphysema. Kosmider et al [71] exposed guinea pigs at 1 ppm 8 hours/day for 180 days. They reported foci of emphysema and atelectasis, some bronchitis, bronchopneumonia, and extravasation of blood in the lungs. During the course of the exposures, the urinary excretion of hydroxyproline and acid mucopolysaccharides (possible degradation products of connective tissue) was increased. Total serum proteins, immunoglobulins, and weight gain were all diminished.

In 1974, Aranyi and Port [147] published the results of a study on the effects of continuous and intermittent exposure to nitrogen

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dioxide on the respiratory defense mechanisms of mice. Separate groups of animals were exposed for 3 1/2 and 7 months either continuously at 2 ppm or intermittently (5 days/week) at 0.5 ppm with daily 1-hour peaks of 2 ppm (0.5/2 ppm). Other animals were exposed for 1, 3, and 6 months at 0.5 ppm presented continuously or at 0.1 ppm with daily 3-hour peaks of 1 ppm (0.1/1 ppm) presented intermittently (5 days/week). Experimental groups were matched by appropriate controls. Effects studied included phagocytic function, cell surface morphology, and oxygen consumption of the alveolar macrophages as well as terminal airway and alveolar morphology. Results indicated that cell counts, macrophage viabilities at isolation, and oxygen consumption of macrophages were unaffected by the experimental exposures to nitrogen dioxide. The in vitro phagocytic activity of macrophages in animals exposed for 3 1/2 or 7 months at 0.5 ppm nitrogen dioxide with 2-ppm peaks was significantly reduced compared with controls. However, exposure to nitrogen dioxide at 2 ppm nitrogen dioxide for the same time periods did not cause a change from control values. Examination of macrophages by means of scanning electron microscopy indicated significant morphologic changes in macrophages in those mice exposed at 0.5/2 ppm for 7 months. No changes in macrophage morphology were found in the other exposure groups. The lungs of animals exposed for 7 months at 2 ppm or 0.5/2.0 ppm as well as those of animals exposed for 6 months at 0.1/1 ppm showed changes in alveolar and terminal airway structures described as emphysematous.

In 1969, Vaughan et al [148] examined beagles exposed to nitrogen dioxide at 0.5-1.0 ppm plus nitric oxide at 0.2 ppm, 16 hours/day for 18 months. No differences in single-breath carbon monoxide diffusing

capacity, dynamic pulmonary compliance, or total pulmonary resistance were found in these animals in comparison with unexposed controls.

Bloch et al [149] studied effects on the cardiovascular system in beagle dogs exposed to automobile exhaust or to the components of automobile exhaust including two experimental exposures to combined nitric oxide and nitrogen dioxide, ie, low nitric oxide (0.204 ppm) with high nitrogen dioxide (0.52-1.04ppm) and high nitric oxide (1.53-2.04 ppm) with low nitrogen dioxide (0.208 ppm). Ninety-six dogs were exposed to filtered air or to 1 of 7 air pollutants for 16 hours/day over 4 1/2 years. Exposure procedures as well as methods used in the characterization of the exposure environment were not described. Two of 19 dogs in the control group showed abnormal static electrocardiograms (ECG's). One of these animals had what was described as a congenital mitral insufficiency. In the 11 dogs exposed to low nitric oxide-high nitrogen dioxide, 4 showed signs of abnormal ECG's or vectorcardiograms (VCG's). However, these findings may be questioned since complete measurements were not made on all animals, and corroborative evidence of cardiovascular abnormalities in these dogs was frequently absent, eg, one dog diagnosed as having pulmonic stenosis had normal ECG's in 5 out of 6 samples as well as marginally normal VCG's. Of the 11 dogs exposed to high nitric oxide-low nitrogen dioxide, none of the animals showed consistently abnormal static or postexercise abnormalities. The authors suggested that exposure to air pollutants could lead to the development of ECG and VCG abnormalities. However, statistical tests to confirm this inference were not performed. The ommission of procedural information coupled with the observation of congenital cardiovascular defects in control animals and incomplete or

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inconsistent data in some dogs diagnosed as having abnormal cardiovascular function raises questions concerning the validity and significance of these findings.

In 1959, Ripperton and Johnston [150] reported on the exposure of rats at 0.15 and 0.5 ppm continuously for 2, 4, 5, and 6 weeks. No significant differences between experimental and control animals were detected in lung or liver tissues at necropsy. Blood catalase levels were higher in the exposed animals at five weeks exposure, but higher in the controls at six weeks. Excretion of glutamic acid and aspartic acid was higher in the exposed rats. Buell et al [151] observed spectrophotometric and microscopic changes of collagen and elastin in the lung tissue of rabbits exposed to 1 ppm for 1 hour. Animals killed immediately following exposure showed significant peak shifts in the protein absorbance spectrum as compared with controls. Preliminary microscopic evidence indicated some uncoiling of the 3-stranded twisted collagen fibers in exposed animals. Spectrophotometric peaks in the proteins of experimental animals killed 24-48 hours after exposure returned to the position of peaks exhibited by controls. The authors hypothesized that the shifts of absorbance spectra in exposed animals represented denaturation of collagen and elastin resulting from hydrolysis by nitrogen dioxide and consequent rupture of hydrogen bonds. Thomas et al [152] also exposed rats for 1 hour at 1 ppm, and for 4 hours at 0.5 ppm. The first exposure caused loss of cytoplasmic granules, disorientation, rupture, and reduction in the number of mast cells. The second exposure schedule led to degranulation of mast cells, predominantly around the mediastinal lung surface. These changes were reversible. Thomas et al [153] studied the effects of exposing rats at 1

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ppm, 4 hours/day for 6 days. At necropsy, lung lipids, extracted by solvents, showed absorption spectra characteristic of diene conjugation, typical for oxidized polynoic fatty acids. Alpha-tocopherol (Vitamin E) pretreatment was only partially effective in preventing the lipid oxidation. In 1973, Sherwin and Carlson [154] reported that guinea pigs exposed at 0.4 ppm continuously for 1 week showed higher protein levels in the lavage fluid of the lung, indicative of protein leakage into alveoli.

Ehrlich [155] exposed mice at 0.5 ppm continuously for 3 months. The mice were challenged by airborne Klebsiella pneumoniae and an increase in mortality was observed as compared with controls. Mice exposed at 3.5 ppm for only 2 hours showed no statistically significant increase in mortality whether the nitrogen dioxide exposure preceded or followed a challenge by airborne Klebsiella. In 1968, Ehrlich and Henry [156] reported on exposures of mice at 0.5 ppm continuously for 3 months or for 6-18 hours daily for one year. The first schedule led to increased susceptibility to airborne Klebsiella with enhanced mortality. The second schedule led to increased mortality of mice and reduced capacity to clear viable bacteria from the lungs. Fenters et al [157] exposed squirrel monkeys at 1.0 ppm continuously for 493 days. The monkeys were then challenged five times with a monkey-adapted influenza virus. Only the exposed animals produced serum neutralization antibody within 21 days. Microscopic examination showed slight emphysema with thickened bronchial and bronchiolar epithelium.

Ehrlich et al [158] exposed Swiss albino mice continuously at a concentration of 2 ppm or intermittently (5 days/week) at 0.5 ppm with daily 1-hour peaks of 2 ppm (0.5/2 ppm). Animals comprising the control

group were exposed to filtered air. After 12 weeks of exposure, the mice were vaccinated with A2/Taiwan influenza virus vaccine. Exposures were continued for an additional 28 weeks, and animals were killed at intervals of 2, 4, 8, 12, 16, 20, 24, and 28 weeks. Measurements were made of hemagglutination-inhibition (HI), serum neutralization antibody formation (SN), and serum immunoglobulin levels. HI antibody titers declined at a similar rate for all animals over the course of exposure. SN titers were significantly decreased for mice exposed at 0.5/2 ppm of nitrogen dioxide as compared with controls. A similar difference was not reported between controls and animals continuously exposed to nitrogen dioxide at 2 ppm. Significant depressions of SN titers were also observed in animals exposed to filtered air for 12 weeks prior to vaccination and exposed at 2 or 0.5/2 ppm of nitrogen dioxide following vaccination. SN antibody titers measured beyond the 4th postvaccination week were not significantly different between the various groups. Serum immunoglobulin concentrations measured after 12 weeks of exposure indicated a significant decrease in immunoglobulin IgA and a significant increase in IgG1 levels in mice exposed to nitrogen dioxide. Concentrations of serum IgM and IgG2 were also higher in the nitrogen dioxide exposed samples; however, statistical significance could only be established for the 0.5/2 ppm group. Measurements of immunoglobulin levels (corrected for age), made on the 28th week following vaccination, indicated no significant differences between groups in terms of serum IgA concentration except for the group maintained in filtered air for 12 weeks before vaccination and exposed at 0.5/2 ppm after vaccination. These mice showed a significant increase in IgA. Concentrations of serum IgM, IgG1, and IgG2 were significantly elevated in

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all mice exposed to nitrogen dioxide prior to or following vaccination as well as in mice continuosly exposed to nitrogen dioxide. An exception occurred in the group exposed continuously at 2 ppm nitrogen dioxide. Levels of IgG2 in this group were not significantly different from controls. The results of this study are difficult to interpret. First, it is difficult to assess the significance of the depression of SN antibody titers in animals exposed to nitrogen dioxide occurring at 2 weeks following vaccination in view of the similarities in titers between controls and NO2 exposed animals occurring beyond the 2nd week. Secondly, a number of the comparisons indicated that the 0.5/2 ppm exposures had a greater effect than the 2-ppm exposures on levels of immunoglobulins. This suggests that the degree of variability of the concentration or the peak may be important in determining immunoglobulin reactions in animals exposed to nitrogen dioxide. Finally, as the authors [158] recognized, although elevated levels of serum immunoglobulin have been identified with several chronic lung diseases, there are no data available reflecting cause-and-effect.

(c)   Carcinogenesis and Mutagenesis

In 1965, Wagner et al [93] suggested that nitrogen dioxide might be tumor-promoting to the extent of causing an acceleration of the rate of appearance of lung adenomas in a spontaneous pulmonary tumor-susceptible strain of mice. Forty-nine mice were exposed at a concentration of 5 ppm, 6 hours/day, 5 days/week, for 12-16 months. After one year of exposure, there was a greater incidence of tumors in the exposed mice than in the control group (see Table XIII-11). After 14 months of exposure, the incidence of tumors was essentially the same for controls and exposed

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animals. Experimentally exposed animals killed after 16 months of exposure showed the same number of pulmonary tumors as controls. Comments were generated as a result of these findings, criticizing the small sample size. [159] Although the incidence of tumors was greater in the exposed mice than in the controls following 12 months of exposure, subsequent statistical analysis shows this difference was not significant. In view of this statistical insignificance as well as the evident similarity in the incidence of tumors between controls and exposed animals after 14 and 16 months of exposure, the supposition that nitrogen dioxide has a tumor-promoting effect must be questioned. Further studies, with larger numbers of animals, should be performed before making inferences concerning the tumor-promoting capacity of nitrogen dioxide.

In a brief note with few supporting data, Henschler and Ross [160] reported that mice intermittently exposed to nitrogen dioxide at 40 ppm over a period of 18 months had no evidence of malignant tumors on serial section of lungs. They also found an increasing incidence of lung adenomas with a decreasing frequency of exposure, thus a decreasing dose. They added that there was not an increasing incidence of lung cancer in nitrogen dioxide-exposed workers, but gave no data in support of this conclusion. In 1968, Ross and Henschler [161] presented data on continuous exposures of hamsters to nitrogen dioxide at 40 ppm plus nitric oxide at 20 ppm for 16 months. Their results revealed no carcinogenic action of nitrogen oxides in the lung or in other organs. They reported the presence of adenomatous changes, but did not state whether there was an increase in test animals or provide data to allow a comparison with controls.

In 1973, Kuschner and Laskin [162] presented a summary progress

report on their current studies in pulmonary carcinogenesis. One hundred rats and 96 hamsters were exposed to nitrogen dioxide at approximately 25 ppm, 6 hours/day, 5 days/week, for up to 646 days. Carcinogenic findings were similar between exposed and control animals except for the appearance of an adenocarcinoma in one exposed rat. Kuschner and Laskin [163] also investigated the effects of combined nitrogen dioxide-benzpyrene (BP) exposure in rats. One group of 36 rats was exposed to fresh air while a second group of 36 rats was exposed to nitrogen dioxide at 25 ppm for 7 hours/day, 5 days/week. Thirty animals from each sample were exposed for 1 hour/day to a mixture of BP aerosol and nitrogen dioxide at concentrations of 10 mg/cu m and 18 mg/cu m (10 ppm), respectively. Preliminary results indicated the presence of squamous cell carcinoma in the lungs of one animal exposed to 25 ppm of nitrogen dioxide with repeated 1-hour exposures to combined nitrogen dioxide-benzpyrene. Similar qualitative changes in lung tissue have been observed in rats exposed to a combination of sulfur dioxide and benzo(a)pyrene. [164] However, complete results on nitrogen dioxide-benzpyrene exposures are not yet available.

Evidence of mutagenesis by nitrogen dioxide or its derivatives in animal species has not been found in the literature. However, nitrous acid, which is one of the products of the reaction of nitrogen dioxide with water at normal temperatures, [2] has been shown to have a potent mutagenic effect on lower forms of life such as the tobacco mosaic virus [165] and Escherichia coli. [166]

Correlation of Exposure and Effect

(a)   Nitric Oxide

The   effects of nitric oxide upon humans must be based upon inference from a limited number of animal experiments.   It was   interpreted   by von Oettingen [45]   that nitric oxide, per se, has no irritant properties, and that its principal direct action is to convert hemoglobin to methemoglobin. Again in the opinion of von Oettingen, [45] it is likely that the effects observed in nitric oxide poisoning are solely due to the hypoxemia which is secondary to the methemoglobinemia.

No   quantitative exposure-effect relationships can be made for nitric oxide due to the absence of any measured environmental data on human exposures.   The animal studies of Flury and Zernik [86] and Pflesser [48] show a dose-response relationship. Flury and Zernik exposed white mice to nitric oxide at 2,500 and 5,000 ppm. [86] It should be noted that the gas contained small amounts of nitrogen dioxide. At the higher concentration, the animals collapsed after 4-6.5 minutes and died after 6-8 minutes. At the lower concentration, collapse and death occurred after 6-7 minutes and 12 minutes, respectively.   Animals removed to fresh air after 4 - 6 minutes' exposure recovered completely in a few hours to one day.   In 1935, Pflesser [47] repeated these experiments in mice and observed marked cyanosis due to spectroscopically confirmed methemoglobinemia at   320-350 ppm.   There was no macro- or microscopic postmortem evidence of pulmonary irritation, either clinically or microsopically, at necropsy.

Gray [80] made a direct comparison between Pflesser's report that 8 hours' exposure to nitric oxide at 310 ppm was nonfatal to mice and his own findings that the LC50 for rats after 4 hours' exposure to NO2 was about

1/4 of that level. Such a comparison is questionable considering the different animal species used and the different modes of nitrogen dioxide production employed in the two studies.

(b)   Nitrogen Dioxide

The acute and usually delayed effects of higher concentrations of nitrogen dioxide on man are well established. After the initial response of irritant cough, which is often associated with mild headache, [41,49] and sometimes immediate but mild dyspnea, [23] there is a characteristic remission of symptoms for up to 12 hours before the onset of acute and potentially lethal pulmonary edema. [23,32,33,34,36,40,41,49] If the patient recovers from this first phase of pulmonary edema, there may be no further symptoms arising from the exposure incident. However, under certain circumstances which are probably related to the severity (total dose) of the exposure, an apparent relapse may occur. Relapses have been recorded for intervals ranging from a few days to several weeks following exposure. This takes the form of a second attack of acute dyspnea, cyanosis, cough, and fever which is usually more protracted than the first attack and may also be fatal. This symptom complex is thought to be due to an underlying pathologic lesion of the lung called bronchiolitis fibrosa obliterans. [34,41,44,50,51] The evidence suggests that, in most cases, if the patient survives this serious stage, total recovery takes place. [41,50,51]

The critical concentration of nitrogen dioxide needed to produce either acute pulmonary edema or bronchiolitis fibrosa obliterans is not known. There are considerable environmental data on sporadic human exposures to oxides of nitrogen which typically give rise to both acute

pulmonary edema and to bronchiolitis fibrosa obliterans. These conditions have not been reported at relatively steady low levels of exposure associated with those few industrial processes in which oxides of nitrogen are steadily generated and get into the workplace atmosphere. All reported cases have arisen as a result of sudden or intermittent emission of oxides of nitrogen from an accidental event such as an explosion or combustion of nitroexplosives, [44] the accidental escape or spilling of concentrated nitric acid, [34,37,53] the intermittent process of arc or gas welding, especially in a confined space, [52] or the imprudent entry into an agricultural silo which was not ventilated. [44,50,51] There are some environmental data available on the oxides of nitrogen and concomitant carbon dioxide in one farm silo filled with timothy grass, [29] but these were not related to any human exposure incident and merely indicate the potential for very high concentrations of nitrogen dioxide (up to 1920 ppm), nitric oxide (up to 630 ppm), and carbon dioxide (up to 60%).

Subacute and chronic responses to low levels of exposure to nitrogen dioxide are not well-established or defined in the human. For example, research has not definitively established that methemoglobinemia occurs in man through exposure to nitrogen dioxide, although there are theoretical considerations suggesting that it might occur. [167] In 1941, McCord et al [56] reported levels of methemoglobinemia very slightly above normal in 4 out of 14 welders exposed to "oxides of nitrogen" ranging from 2 to 10.3 ppm expressed in terms of nitrogen dioxide. The methemoglobin levels were 2.3% of the total hemoglobin in two welders, and 2.5 and 2.6% in the other two welders, barely above the physiological average normal of 1%. [84] In view of the analytical difficulties in estimating methemoglobin at that

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period, [85] McCord et al's report [56] should be treated with some caution. In contrast, Morley and Silk [63] published in 1970 a study of shipyard exposures in arc and gas welders and cutters. Using a spectrophotometric method, they observed no abnormal level of methemoglobin in 22 men following a work-time exposure to oxides of nitrogen, reported as nitrogen dioxide, at 1-15 ppm.

From theoretical considerations, nitrogen dioxide would be expected to have an irritant effect both upon general mucosal surfaces and on the lower respiratory tract. However, the direct human evidence of such general mucosal surface effects is weak. A 1970 Russian study [64] reported a prevalence of 64% of chronic catarrhal processes in the upper respiratory tract in arc welders with up to 10 years' work history. In 22% of the welders with more than 10 years' service in welding, what was translated as subatrophic rhinopharyngitis was observed. However, these reported findings were not validated by comparison with any control group and there were no environmental data presented. In addition to oxides of nitrogen, arc welders are probably exposed to a variety of dusts and fumes from coated welding rods. Morley and Silk [63] reported acute or subacute conjunctivitis and pharyngitis in 5 gas welders who worked in a confined space on welding zinc-plated steel. All complaints had subsided 18 hours later.

There has been much speculation [71,97,98,99,102,143] whether chronic low-level exposure to nitrogen dioxide, among other irritant gas air-pollutants, may be a contributory cause of chronic obstructive pulmonary disease in man. Kennedy [28] in 1972 published a study of the prevalence of emphysema in 100 British coal miners repeatedly exposed to oxides of

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nitrogen during underground blasting. Using pulmonary residual volume of more than 150% of normal as his diagnostic criterion, the author classified 84 of the miners as "probably suffering from an emphysema-like condition." Of these 84, 5 died during the period of the study and 4 of them had evidence of advanced generalized emphysema at autopsy. No definitive environmental data were given, but typical momentary concentrations of oxides of nitrogen following shot-firing were measured as high as 88 ppm. Misfires resulted in concentrations of up to 167 ppm. Graham and Runnicles, cited by Kennedy, [28] measured the concentration of nitrogen oxides in coal mines using black powder for shot-firing. Twenty-four-hour average concentrations at the coal face ranged from 0.8 to 4.5 ppm (nitric oxide + nitrogen dioxide, expressed as nitrogen dioxide), with 3-6.4 ppm in "hard headings." However, the high incidence of clinically diagnosed "probable emphysema-like condition" in coal miners could be attributed to the risk of coal workers's pneumoconiosis, which is known to be associated with focal emphysema. [73]

Kosmider et al [71] in 1972 reported an epidemiologic study on 70 chemical plant workers exposed 6-8 hours daily, for 4-6 years, to nitrogen oxides concentrations ranging from 0.4 to 2.7 ppm. There was evidence of chronic bronchitis in an unstated number of these workers. No abnormality was seen in the chest roentgenograms, and there were no significant changes in spirometric measurements or blood gases compared with controls. However, the total proteins in the blood serum were significantly lower than in controls. The exposed workers showed a reduction in albumin and gammaglobulins and an increase in the alpha-1, alpha-2, and betaglobulins. There were also significant increases in the urinary hydroxyproline and

acid mucopolysaccharides in the exposed men. The authors concluded that these abnormalities were indicative of protein synthesis inhibition and decomposition of collagen, consistent with the emphysema process.

Vigdortschik et al [70] published a study in 1937 on human exposures in sulfuric acid plants and etching operations in printing shops. The sample included 127 workers who had been exposed for 3-5 years to oxides of nitrogen at levels "mostly below 2.8 ppm." These exposures were thought to result in a number of symptoms and signs including dental erosion and gingivitis; emphysema and compensated pulmonary tuberculosis; hypotonia and bradycardia; and polycythemia rubra, granulocytosis, basophilia, and decreased osmotic fragility of erythrocytes. The evidence indicated a higher prevalence of the above conditions in the exposed workers than in carefully selected industrial worker controls, comparable in all respects except for nitrogen oxides exposure. However, the actual prevalence figures were not given. The statement that workers in sulfuric acid plants were exposed to nitrogen oxides and to no other injurious gases is questionable, especially in view of the finding of dental erosion, a well-known effect of sulfuric acid mist exposure. [168]

A small number of human experimental studies of the acute effects of low levels of nitrogen dioxide have been described. In 1967, Abe [67] reported a 40% decrease in the effective lung compliance of 5 healthy adult men 30 minutes after cessation of nitrogen dioxide exposure at 4-5 ppm for 10 minutes. Other indices of pulmonary mechanics were also altered. In 1971, von Nieding and Krekeler [68] reported increased airway resistance in 88 chronic bronchitis patients who had respired nitrogen dioxide-air mixtures containing as little as 1.5 ppm nitrogen dioxide, for either 15

minutes or for a total of 30 breaths. In 1973, von Nieding et al [69] found a statistically significant decrease in the carbon monoxide diffusing capacity of 16 healthy male volunteers following the inhalation of 5 ppm of nitrogen dioxide for 15 minutes. Patients with chronic bronchitis showed a significant increase in alveoloarterial pressure following inhalation of nitrogen dioxide for 15 minutes at 5 ppm. Increasing exposure time to 60 minutes did not alter alveoloarterial pressure from that observed during the first 15 minutes. It is not known whether any, or all, of these effects of acute low-level exposures in man are completely reversible following repeated intermittent exposures over a period of years.

Although the human studies have been helpful in delineating potentially harmful concentrations, a more scientifically rigorous approach to defining cause-and-effect relationships have been afforded by animal studies.

Much work has been done in attempts to produce emphysema in animals similar to that seen in humans by adjusting various schedules of exposure to nitrogen dioxide. Emphysematous conditions were produced in guinea pigs by Kleinerman and Wright, [96] in rabbits by Haydon et al, [98] in mongrel dogs by Riddick et al, [100] in beagles by Lewis et al, [101] and in rats by Freeman et al. [102] In all the foregoing experiments, the concentrations of nitrogen dioxide used were in the 5-50 ppm range. Few workers have claimed success in producing experimental emphysema with substantially lower concentrations of nitrogen dioxide. Blair et al [146] in 1969 reported "early focal emphysema" in mice exposed to 0.5 ppm for up to 1 year, while Kosmider et al [71] reported focal emphysema and other inflammatory lung changes in guinea pigs exposed to 1 ppm for up to 180 days.

Most researchers employing nitrogen dioxide concentrations below 5 ppm have observed subtle microscopic changes, such as reduction or loss of cilia, [99,130] hypertrophy and focal hyperplasia in the epithelium of the terminal bronchioles, [130] and a replacement of Type 1 (ultra-thin) by Type 2 (cuboidal) pneumocytes in alveoli, rather than emphysema. [131]

A problem in evaluating much of the animal studies is the imponderable relationship between the effects of continuous versus intermittent exposure. It might be assumed that for the same total dose of toxicant respired, intermittent exposure would be less harmful than continuous exposure at a correspondingly lower level. This assumption is made on the grounds that during the remissions of exposure the tissues have an opportunity to recover or repair themselves. The situation is further complicated in the case of nitrogen dioxide because it is the major constituent of the oxides of nitrogen which are, albeit at a low level, an air pollutant in most urban and heavily industrialized areas. Therefore, the typical industrial worker's exposure to nitrogen dioxide during the 14- to 16-hour daily respite from his industrial occupation will not be zero, but some fraction of his occupational exposure level. However, it is conceivable that for certain effects concentration of the toxicant might be the overwhelming determinant. Thus, for the same total respired dose, intermittent exposure might be more harmful than continuous exposure.

In view of the dose-response complexities of exposure to nitrogen dioxide, more weight should be given to those animal studies which have come as close as possible to an intermittent exposure schedule, those which parallel the typical occupational exposure schedule. One study, by Wagner et al [93] in 1965, reported the effects of exposing dogs and mice to

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nitrogen dioxide at 1 and 5 ppm, and rabbits, guinea pigs, rats, and hamsters at 1, 5, or 25 ppm. All exposures were 6 hours/day, 5 days/week, for periods ranging from 10 to 18 months. Weight gain and blood studies including serum alkaline phosphatase activities were only minimally affected in dogs. Similarly, only minimal changes in the respiratory tissues of all animals were observed. The most frequently encountered pathologic alterations, a patchy or diffuse interstitial pneumonia and a type of chronic bronchiolitis, bronchitis, or bronchiectasis (with or without peribronchitis), were seen with equal frequency in the control animals. Moreover, there was no real difference between animals exposed at the three levels of 1, 5, and 25 ppm. These negative results contrast sharply with those of Freeman and Haydon [97] on rats, Haydon et al [98] on rabbits, Riddick et al [100] and Freeman et al [102] on rats. In all these studies, exposures were continuous to nitrogen dioxide at levels at or below 25 ppm. Reported pathologic findings included moderate hypertrophy and hyperplasia of bronchial and bronchiolar epithelium in rats, [97] emphysema-like dilatations of the peripheral alveoli in rabbits [98], bullous alveolar enlargement in dogs, [100] and loss of cilia, epithelial hypertrophy, and "emphysema-like disease" in rats. [102]

Exposure at concentrations of nitrogen dioxide of 0.5 ppm may also increase susceptibility of animals to subsequent infection by Klebsiella pneumoniae or Streptococcus pyogenes. [140,155,156] This phenomenon has also been observed at somewhat higher concentrations of nitrogen dioxide (1-5 ppm) [135,136] and at concentrations in the 5-to-50 ppm range. [117,118] At these higher concentrations, decreased resistance to subsequent challenge by certain viruses [117] and inhibition of interferon

production have been reported. [119,120] Although this effect has potential human implications, no such phenomenon has been clearly described in man. The secondary bacterial invasion and ensuing bronchopneumonia of severe cases of pulmonary edema or bronchiolitis obliterans following high exposures to nitrogen dioxide are probably due to the gross tissue damage and loss of integrity of epithelium rather than to interference with immunologic or phagocytic mechanisms.

A variety of other effects of nitrogen dioxide exposures including changes in pulmonary physiology, biochemical changes in the lung, pulmonary surfactant, enzymatic changes in remote organs, changes in macrophage populations and behavior, immunologic changes, etc, have been described in this chapter but have not been reviewed again here as they do not appear to have a meaningful bearing on the development of an occupational environmental standard.

The question of carcinogenicity or mutagenicity of the oxides of nitrogen in man will require evidence before any conclusions can be made. Wagner et al [93] exposed a strain of mice susceptible to spontaneous pulmonary tumors at moderate concentrations of nitrogen dioxide and reported that such exposures accelerated the rate of tumor development. This evidence is quite tenuous since statistical comparisons between exposed and control animals showed that the differences were not significant and the final incidence of tumors was unaffected. Other long-term exposure studies of mice [160] and of hamsters [161] reported the production of adenomatous changes, but they failed to find any evidence of malignant lesions. The possibility exists that nitrogen dioxide may act as a cocarcinogen or carcinogenic "promoter"; however, data are insufficient

to support this contention at this time. Nitrous acid has been shown to be a potent mutagen for viruses [165] and for bacteria [166]; however, evidence that nitrogen dioxide and nitric oxide are mutagenic in humans has not been found.