V. DEVELOPMENT OF STANDARD

Basis for Previous Standards

On the basis of results of studies conducted with cats and rabbits, Lehmann and Hasegawa [39] stated that a concentration of oxides of nitrogen equivalent to 0.1 mg/liter of nitrous and nitric acids, calculated as nitric acid, could be withstood by humans for several hours. This is equivalent to 39 ppm of nitric acid at 25 C and 760 mmHg pressure. According to Schrenk, [214] this figure of 39 ppm was suggested for a number of years as a Maximum Allowable Concentration (MAC) for the oxides of nitrogen by several different authors and agencies in the US.

In 1937, a MAC of 10 ppm as nitrogen dioxide was suggested as an guide to manufacturers in the state of Massachusetts. [214] In a then comprehensive listing of MAC's, published in 1945, Massachusetts was the only state to list a MAC for nitrogen dioxide of 10 ppm. [215] For the states of California, Connecticut, and Oregon, and for the US Public Health Service and the American Standards Association, the MAC was 25 ppm for "nitrogen oxides." For the states of New York and Utah, MAC's of 10 and 10-40 ppm "nitrogen oxides" were recommended, respectively. As documentation for the 25 ppm MAC's, Cook [215] cited the animal experiments of La Towsky et al [216] who concluded that 3-hour exposures to oxides of nitrogen at 30 ppm produced no immediate or delayed harmful effects in guinea pigs.

In 1946, the American Conference of Governmental Industrial Hygienists (ACGIH) [217] recommended 25 ppm as the MAC for "nitrogen oxides (other than nitrous oxide)." In 1948, the ACGIH numerical recommendation

remained unchanged, but the term Threshold Limit Value (TLV) was substituted for MAC.

Gray et al [92] reported results of exposure of rats to the vapors of red fuming nitric acid, the nitrogen dioxide content of which ranged from 9 to 14 ppm. They found evidence of severe pulmonary congestion, bronchiolitis, and pneumonitis following exposures for 4 hours/day, 5 days/week, for a total exposure of 40 - 96 hours. On this basis, they [92] commented that the TLV of 25 ppm recommended at that time was too high. In 1954, Gray et al [126] published the results of a study on rats exposed on the same schedule for 6 months. The concentration of vapors from red nitric acid was 4 ppm. No toxic effects were observed. As a result, they recommended that the MAC for the oxides of nitrogen be set at 5 ppm. In 1954, the ACGIH [218] reduced their recommended TLV to 5 ppm, and, at the same time, changed the designation from "nitrogen oxides (other than nitrous oxide)" to "nitrogen dioxide." According to the ACGIH 1971 Documentation of the Threshold Limit Values, [219] the reduction to 5 ppm was based upon the results of the work of Gray et al in 1952 and 1954. [92,126]

During the period from 1948 to the mid-1950's, the 8-hour TWA concept of the TLV had evolved. In 1964, the ACGIH established 5 ppm as their recommended ceiling value for nitrogen dioxide, instead of an 8-hour TWA. [141] The basis for this more stringent recommendation was the report of Wagner et al [93] published later in 1965, which suggested a possible lung tumor accelerating capacity of nitrogen dioxide for spontaneous lung tumor-susceptible mice. [219] It was believed that by imposing this ceiling of 5 ppm, thereby effectively reducing the TWA exposure to less than 2.5 ppm,

the risk of accelerating lung tumor development in man would be minimized. [219]

The American National Standards Institute (ANSI) recommended a 5-ppm ceiling as acceptable for repeated daily exposures to nitrogen dioxide. [220] A concentration of 15 ppm was defined as the maximum "peak" for an 8-hour workday. The total exposure period at this "peak" was not to exceed 5 minutes and it was assumed that such excursions above the ceiling would be infrequent (not daily).

The present federal standard for nitrogen dioxide is 5 ppm as an 8-hour TWA. [29 CFR 1910.1000, published in the Federal Register 39:23542, June 27, 1974] This was apparently based upon the ACGIH TLV, except that the C designation (for Ceiling) was erroneously omitted.

Guidelines for nitric oxide, as distinct from nitrogen dioxide or "nitrous fumes", have been recommended only by the United States and the German Democratic Republic. In 1966, the American Conference of Governmental Industrial Hygienists [221] introduced a TLV of 25 ppm for nitric oxide. The recommendation by the German Democratic Republic in 1969 was 20 mg/cu m (16 ppm) as a maximum allowable concentration. [222]

Until 1954, nitric oxide levels were included in recommendations for nitrogen oxides. When the ACGIH changed the designation from nitrogen oxides to nitrogen dioxide, a limit for nitric oxide was not included. Therefore, nitric oxide technically remained without control recommendations for 12 years until 1966 when ACGIH recommended a limit of 25 ppm. [221] Nitric oxide was deemed to be about one-fifth as toxic as nitrogen dioxide based upon the animal experiments reported by Pflesser [48] in 1935 and in the review by Gray in 1959. [80] Pflesser's report [48] has been

misquoted to the effect that nitric oxide was found to be 4 or 5 times less toxic than nitrogen dioxide. [80,223] Von Oettingen [45] cited the paper correctly: "...the acute toxicity of nitrogen oxide is about four times greater than that of nitrogen dioxide but the latter is more insidious in its action..." The misinterpretation of Pflesser's paper was influential in the development of the ACGIH recommendation of 25 ppm for nitric oxide [221,223] which, in turn, is reflected in the current federal standard of 25 ppm as an 8-hour TWA (29 CFR 1910.1000) published in the Federal Register 39:23542, June 27, 1974.

Basis for the Recommended Environmental Standard

Inhalation of nitrogen dioxide in man at levels of the order of 50-100 ppm causes irritant cough, mild or transient headache, and breathlessness. [23,41,49] If the concentration of nitrogen dioxide is high enough, acute pulmonary edema [23,32,33,34,36,40,41,44,49] may develop after a characteristic delay of up to 12 hours. Just how high this concentration must be in man is not known because of the lack of environmental data from observed acute episodes. In some cases, after apparent recovery from the initial delayed pulmonary edema, without further exposure and after an interval of a few days up to 6 weeks, a second, more protracted lung condition called bronchiolitis fibrosa obliterans may develop. [34,41,44,50,51] Less commonly, bronchiolitis fibrosa obliterans may occur after an interval of several days to 6 weeks following exposure but without any initial clinical episode of acute pulmonary edema. [35,52,53] The critical concentrations and exposure times of nitrogen dioxide for these effects are not known, but there is circumstantial

evidence in most of the cited reports that the concentrations were very high, of the order of several hundred ppm. Moreover, it seems likely that most of the exposures have not been to nitrogen dioxide alone, but to mixtures of oxides of nitrogen of unknown proportions, principally nitric oxide and nitrogen dioxide.

There is some evidence that attacks of acute pulmonary edema [54] or of bronchiolitis obliterans, [51] due to severe exposures to nitrogen oxides, may be followed by chronic pulmonary impairment or insufficiency, possibly associated with peribronchiolar fibrosis [54] or centrilobular emphysema. [51]

It is not known whether prolonged low-level or repeated sporadic exposures to nitrogen oxides in the absence of attacks of acute pulmonary edema or bronchiolitis obliterans leads to emphysema in man. Kennedy [28] in 1972 attributed the extremely high prevalence (84%) of spirometric evidence of emphysema in 100 British coal miners to this cause. However, the study did not provide an adequate control group for differentiating emphysematous changes due to exposure to nitrogen oxides from other possible causes, such as coal workers' pneumoconiosis. Simple pneumoconioses, including coal workers' pneumoconiosis, are known to be associated with focal emphysema. [73]

In 1937, Vigdortschik et al [70] reported an epidemiologic study of 127 workers exposed to "oxides of nitrogen" and to "no other injurious gases" at levels "mostly below 2.8 ppm," in sulfuric acid plants and in print-etching shops. An increased prevalence of emphysema was reported in these workers, as compared with a control group matched in all respects but unexposed to "toxic substances." In addition, many other blood,

biochemical, and urinary abnormalities were reported, but the actual prevalence of the abnormalities was not given for either the exposed workers or the control group. Moreover, the assertion that the workers were exposed to nitrogen oxides alone and to "no other injurious gases" is questionable, especially with respect to workers in sulfuric acid plants. The presence of dental erosion was reported in the exposed workers, and dental erosion is known to be associated with exposure to sulfuric acid mist. [224,225,226]

In 1972, Kosmider et al [71] reported an epidemiologic study of 70 men exposed in a chemical plant to "oxides of nitrogen" 6-8 hours daily for 4-6 years. The concentrations of oxides of nitrogen expressed in terms of nitrogen dioxide fluctuated between 0.4 and 2.7 ppm. A control group of 80 male industrial workers of similar age but not occupationally exposed to oxides of nitrogen, was employed for comparison. All men smoking more than 10 cigarettes/day were excluded from both groups for the purposes of analysis. Spirometry showed slight, statistically insignificant reductions in vital capacity and maximum respiratory volume. There was a statistically insignificant degree of respiratory acidosis and metabolic alkalosis observed in the exposed men, as a group, in comparison with the controls. There was a statistically significant increase in the excretion of hydroxyproline and acid mucopolysaccharides in the urine of the exposed men, possibly indicative of connective tissue destruction. Based on the above evidence, the authors concluded that oxides of nitrogen, at the levels cited, probably cause emphysema in humans. They also found clinical evidence of chronic bronchitis (sporadic cough with mucopurulent expectoration, breathlessness on exertion, and fine moist rales in the lower lungs

on auscultation) in an unstated number of the exposed workers.

The results of these two epidemiologic studies [70,71] suggest an environmental limit for nitrogen dioxide substantially below 3 ppm, assuming that nitrogen dioxide alone is at least as toxic as "oxides of nitrogen" of unknown proportional composition at such levels. However, both studies have considerable weaknesses. The conclusion, in the first study by Vigdortschik et al, [70] that sulfuric acid plant workers were exposed to "oxides of nitrogen" and to "no other injurious gas" must be questioned, especially in view of the presence of dental erosion in some of the workers. The lack of information on how the environment was characterized, the lack of data on incidence of effects in the exposed vs unexposed groups, the doubtful significance of many of the findings, and the statistical insignificance of spirometric and blood gas changes raise doubts about the conclusions of Kosmider et al [71] in the second study.

In 1975, French [74] presented results of a retrospective study concerned with the effects of community exposure to nitrogen dioxide on acute and chronic respiratory illnesses in 3 communities located near Chattanooga, Tennessee. The data included revisions of environmental sampling measurements which were in error in the initial Chattanooga studies [75,76,77] made in 1968-69. The incidence of "acute respiratory disease" and "lower respiratory" morbidity rates was significantly higher in high-exposure (mean level of 0.083-0.219 ppm with a peak of 0.66 ppm) than in intermediate-exposure (mean of 0.06 ppm) communities as well as between intermediate- and low-exposure (mean of 0.031 ppm) communities. However, there were no significant differences among the 3 communities in the prevalence of "chronic respiratory" symptoms, such as chronic

bronchitis. Furthermore, the increase in "acute" diseases could be explained by the differences in suspended particulates observed in the three communities. It is important to note that the exposures in these communities were nearly continuous (approximately 24 hours/day) and, therefore, do not represent the type of exposure encountered in the occupational setting.

The short-term human experiments of Abe [67] reported in 1967 throw some light on acute effects of low concentrations of nitrogen dioxide. Five healthy male adults were exposed to nitrogen dioxide at 4-5 ppm for 10 minutes. Measurements of effective lung compliance, inspiratory maximum viscous resistance, and expiratory maximum viscous resistance were made prior to the gas inhalation, immediately after, 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% of the baseline. Expiratory and inspiratory maximum viscous resistance were unchanged immediately after completion of exposure but gradually increasing from 10 minutes after exposure, reaching a maximum at 30 minutes. Abe's results document a definite and undesirable effect at the exposure level which is the current federal standard. Moreover, the subjects were young healthy adult males, probably more fit than the average industrial worker in a population with an age range of 18-65 years.

In 1971, von Nieding and Krekeler [68] investigated the effects of low concentrations of nitrogen dioxide on the respiratory gas exchange and the airway resistance of patients with chronic bronchitis. Eighty-eight chronic bronchitis patients breathed nitrogen dioxide-air mixtures

containing 0.5-5.0 ppm for a few breaths up to 15 minutes. After inhalation of nitrogen dioxide concentrations down to 1.5 ppm the airway resistance increased significantly. Lower concentrations had no significant effect. While the end-expiratory alveolar oxygen tension remained nearly constant during inhalation of nitrogen dioxide at 4 and 5 ppm, a significant decrease of the arterial oxygen tension and, accordingly, an increase of the end-expiratory arterial tension difference for oxygen occurred. After inhalation of nitrogen dioxide at 2 ppm, there was no decrease in the arterial oxygen tension. These results indicate that exposures to nitrogen dioxide concentrations as low as 1.5 ppm may aggravate already existing respiratory impairment of sufferers from chronic bronchitis.

In 1973, von Nieding et al [69] reported further studies, including some on healthy male volunteers. The carbon monoxide diffusing capacity was measured by a single-breath method in 16 healthy male subjects before and after inhalation of nitrogen dioxide at 5 ppm for 15 minutes. A statistically significant (p less than 0.01) decrease in the diffusing capacity for carbon monoxide from 20.6 to 16.8 ml/0.1 minute/0.1 torr was observed. It is not known whether this decreased diffusing capacity would be progressive on continuation of exposure or whether it would be partially or totally reversible. Further experiments on chronic bronchitic patients indicated a significant increase in the alveolar arterial pressure following exposure for 15 minutes at 5 ppm. Increasing the exposure to 60 minutes did not change the pressure gradients from those observed after 15 minutes of inhalation.

Although a number of animal studies have been conducted in recent

years to determine the effect of exposure to nitrogen dioxide at low levels, below 5 ppm, most of the studies have employed continuous or almost continuous exposure schedules, rather than intermittent exposures parallel to the occupational exposure situation. The extrapolation of results of continuous exposure animal studies to the human occupational exposure situation has many pitfalls. As discussed earlier (see Animal Toxicity), an extrapolation from data on continuous exposure to the intermittent exposure characteristic of the occupational setting cannot be correctly performed on the assumption that effective concentration times time (Ct) is constant. In other words, the concentration producing a given effect on continuous exposure would be much lower than the concentration producing that effect by a factor greater than that predicted by differences in exposure times, and this has been verified in experimental animal exposures to soluble organic phosphate [227] as well as to nitrogen dioxide. [90,140,141]

In many of the recent animal studies, newer techniques, such as electron microscopy, biochemical and physicochemical techniques applied to lung lavage fluid, cytochemical techniques to detect cellular activity and proliferation, and bacterial and viral challenges to detect impaired resistance to infection have been introduced. Of these, some specific studies appear pertinent to man on the basis of probable similarity of pathogenesis.

In 1969, Blair et al [146] noted changes considered to be consistent with "early focal emphysema" in mice exposed at 0.5 ppm for 18 or 24 hours daily for 3-12 months. Control mice showed moderate pneumonitis but no evidence of bronchiolar obstruction or emphysema. Stephens et al [130] in

1972 reported loss of cilia, and hypertrophy and focal hyperplasia in the epithelium of the terminal bronchioles of rats exposed at 2 ppm continuously for up to 21 days. Sherwin et al [132] in 1972 reported significant increases in the average areas of alveolar walls (a change suggestive of early emphysema) in guinea pigs exposed continuously at 2 ppm for 1, 2, and 3 weeks. They also reported the replacement of Type 1 pneumocytes by Type 2 pneumocytes in the alveoli. Such a cell-type change implies thickening of the alveolar blood-gas barrier with subsequent impedance of respiratory gas exchange. In 1974, Aranyi and Port [147] demonstrated that mice exposed continuously at 2.0 ppm for 3 1/2 and 7 months or at 0.5 ppm for 1, 3, and 6 months, as well as those exposed intermittently (5 days/week) at 0.5 ppm with daily 1-hour peaks of 2 ppm (0.5/2.0 ppm) or at 0.1 ppm (0.1/1.0 ppm) with daily 3-hr peaks of 1 ppm over the same time intervals, showed no changes in blood cell counts, macrophage viability in vitro, or oxygen consumption of macrophages when compared with controls. Animals exposed at 0.5/2.0 ppm showed significant decreases of in vitro phagocytic activity of macrophages and significant morphological changes in macrophages as compared with other experimental groups and controls. The lungs of animals exposed for 7 months at 2 ppm or 0.5/2 ppm as well as those of animals exposed for 6 months to 0.1/1 ppm were said to have shown emphysematous changes in alveolar and terminal airway structures.

In contrast to the foregoing results involving continuous exposures, Wagner et al [93] in 1965 reported on intermittent exposures of 5 species of animals at 3 different levels of nitrogen dioxide for long periods. Dogs and mice were exposed at 1 and 5 ppm and rabbits, guinea pigs, rats,

and hamsters at 1, 5, and 25 ppm, all on a 6-hours/day, 5-days/week schedule, for periods ranging from 10 to 18 months. Few or no differences were noted in weight gain, blood counts, and alkaline phosphatase in dogs, or in the pathologic alterations observed in the lungs of the exposed animals of all the species studied compared with the unexposed but similarly confined control animals. The effects noted in the exposed animals may have been related to inhalation of nitrogen dioxide. However, the unexplained high incidence of similar pathologic changes in control animals may have obscured these findings. A similar problem was noted in the experiments with the tumor-susceptible mice. Mice exposed at 5 ppm for 12 months showed a higher, but statistically insignificant, incidence of tumors as compared with controls. However, the high incidence of intercurrent lesions in controls may have obfuscated experimentally induced lesions in exposed animals.

Measurement of autoimmune responses and susceptibility to challenge by pathogenic bacteria and viruses have also been used to assess the effects of exposure to nitrogen dioxide in animals. Purvis and Ehrlich [135] found a significant increase in susceptibility to infection by Klebsiella pneumoniae in mice exposed at 3.5 ppm for 2 hours. In 1970, Ehrlich et al [136] reported that mice exposed at 3.5 ppm for 2 hours and challenged with Klebsiella pneumoniae showed a significant increase in mortality relative to controls; whereas, exposures at 1.5 and 2.5 ppm, for the same period, had no effect on mortality rate. Goldstein et al [139] exposed mice at 1.9-14.8 ppm for 4 hours or 1, 2.3, and 6.6 ppm for 17 hours following infection by radiophosphorus-labeled Staphylococcus aureus. A decrease in the pulmonary bactericidal activity in the mice was observed

in the case of exposures above 7 ppm. Exposure to nitrogen dioxide at more than 2.3 ppm prior to staphylococcal challenge also caused decreased bactericidal activity.

Coffin et al [140] studied the time-dose relationship between intermittent and continuous exposures to nitrogen dioxide and mortality in mice challenged with Streptococcus pyogenes (Group C). Results of single equivalent Ct (concentration x time) exposures indicated that concentration was more important in determining the rate of mortality than time. Exposures at 2.3 ppm for 3 hours and 1 ppm for 7 hours did not appear to increase mortality above that observed in control animals. Continuous exposure at concentrations of 0.5 and at 3.5 ppm, as well as intermittent exposure (7 hours/day) at 3.5 ppm, resulted in a significant increase in mortality. Insufficient data were collected on continuous exposures at 1.5 ppm to determine the significance of the relationship between mortality and total time of exposure, or the significance of differences in mortality between this group and animals exposed at 0.5 or 3.5 ppm. Intermittent exposure at 3.5 ppm resulted in a lower mortality rate than continuous exposure at the same concentration.

Studies by Ehrlich [155] and Ehrlich and Henry [156] indicated increased susceptibility or mortality in mice challenged with airborne Klebsiella pneumoniae and exposed at 0.5 ppm continuously for 3 months or intermittently (6-18 hours/day) for 1 year. More recently, Ehrlich et al [158] vaccinated Swiss albino mice with A2/Taiwan influenza virus vaccine following nearly continuous exposure to nitrogen dioxide at 2 ppm, to nitrogen dioxide at 0.5 ppm with daily 1-hour peaks of 2 ppm (0.5/2.0 ppm), or to filtered air. Exposures were continued for 28 weeks following

vaccination, and animals were killed at intervals of 2, 4, 8, 12, 16, 20, 24, and 28 weeks. None of these exposures had a significant effect on HI antibody titers. Serum neutralizing (SN) titers were significantly below controls two weeks after vaccination in animals exposed at 0.5/2.0 ppm, but not in those exposed continuously at 2.0 ppm. In general, exposure at 0.5/2 ppm had as much of an effect, if not a greater effect, on serum IgA, IgM, IgG1, and IgG2 levels as exposure at 2.0 ppm. Whether or not there is a direct relationship between the levels of immunoglobulins and chronic pulmonary disease is still a matter of speculation. [158]

No epidemiologic studies have been found which would indicate a carcinogenic or other mutagenic effect from human exposure to nitrogen dioxide. Results from animal exposures are inconclusive. Adenomatous changes have been noted in the lungs of mice intermittently exposed at 40 ppm for 18 months [160] and hamsters continuously exposed at 40 ppm nitrogen dioxide and 20 ppm nitric oxide for 16 months. [161] But, carcinomas were not observed in the experimental animals. Other studies [93,124,163] suggest a tumor provoking or a cocarcinogenic effect of exposure to nitrogen dioxide. However, the findings of these studies are either limited in scope or of such questionable significance that it is not possible to attribute such actions to inhalation of nitrogen dioxide from the presently available evidence. Reports of human mutagenesis resulting from exposure to nitrogen dioxide have not been found in the literature.

Epidemiologic and animal studies which clearly delineate a safe level for human exposure to nitrogen dioxide are not yet available. Existing epidemiologic studies contain, for the most part, errors, omissions, and inconsistencies and are, therefore, unreliable for establishing a safe

exposure level. Difficulties in interpreting the animal data reviewed below may be attributed to a number of variables, such as species-specific responses to exposure, criteria of effect, and type (continuous versus intermittent) and duration of exposure.

Tyler et al [103] noted a number of important anatomic differences between species with respect to the vasculature of the terminal airways and spaces. These anatomic differences, coupled with differences in metabolism and other physiologic characteristics (eg, respiration rate) of various animal species, may differentially affect the deposition of inhaled nitrogen dioxide in lung tissue, and thereby give rise to species-specific pulmonary responses to exposure. Such species specificity has been indicated either by the time of onset of microscopic pathologic changes or by the toxicologic manifestations of different animal species exposed to nitrogen dioxide under the same experimental conditions. [90,93] Therefore, extreme caution must be taken in attempting to directly extrapolate experimental animal data to the responses of humans exposed to nitrogen dioxide. Considerably more effort must be expended to determine appropriate animal models of human pulmonary function so that present and future experimental animal data may be used in defining safe limits of occupational exposure.

Several criteria have been used to evaluate the toxic effects of exposure in animals. These criteria include abnormal changes in respiration, cellular morphology of the pulmonary system, weight, reproduction, and immunoglobulin levels. Susceptibility to viruses and bacteria, as well as hematologic and biochemical changes, have also been used to assess nitrogen dioxide inhalation toxicity. Of these criteria,

changes in immunoglobulin levels and susceptibility to viral and bacteria infection are least relevant to defining safe exposure levels in humans. Although elevated levels of serum immunoglobulins have been correlated with chronic lung disease, cause-and-effect relationships have not been documented. [158] Furthermore, evidence of an increased incidence of bacterial and viral infections in workers exposed to nitrogen dioxide has not been found. Indeed, a well-designed and controlled field study is needed to confirm such effects in humans.

With respect to the remaining criteria, effects noted are highly dependent upon the animal species, exposure schedule, and total time of exposure. Thus, rats continuously exposed at 0.8 ppm over their natural lifetime showed respiration rates 20% above controls. [144] However, beagle dogs exposed to nitrogen dioxide at 0.5-1.0 ppm plus nitric oxide at 0.2 ppm oxide for 16 hours/day over 18 months did not differ significantly from controls in single-breath carbon monoxide diffusing capacity, dynamic pulmonary compliance, and total pulmonary resistance. [148] Steadman et al [145] found that monkeys, dogs, rabbits, guinea pigs, and rats continuously exposed for 90 days at 0.5 ppm had a slight weight loss. In contrast, Wagner et al [93] reported no significant difference in body weight gain between control animals and animals exposed intermittently 6 hours/day, 5 days/week for up to 18 months. In terms of reproduction, Shalamberidze and Tsereteli [142] observed a prolongation of the estrus cycle and a reduction in fetal weights in female albino rats exposed at 1.3 ppm 12 hours/day for 3 months. Such findings were not observed in rats exposed at 0.07 ppm under the same experimental conditions. Apparently, the threshold for reproductive alterations in albino rats lies somewhere between 0.07 and

1.3 ppm; however, the specific concentration at which these changes begin to occur is unknown.

Minimum concentrations of exposure to nitrogen dioxide leading to macro- and microscopic changes associated with chronic obstructive disease of the lungs have not been determined. Early bronchiolar inflammation, expansion of lung alveoli, and alveolar lesions reportedly consistent with focal emphysema have been observed in mice exposed at 0.5 ppm for 6, 18, or 24 hours/day for 3-12 months, [146] and at 0.5 ppm with daily 1-hour peaks at 2 ppm for 5 days/week for 3 1/2 to 7 months. [147] Similar changes have been observed in the lung tissue of guinea pigs exposed at 1 ppm, 8 hours/day for 180 days. Conversely, only insignificant macro- and microscopic changes in lung tissue have been observed in dogs, rabbits, guinea pigs, rats, and hamsters exposed at 1 ppm, 6 hours/day for up to 18 months, [93] and in rats exposed continuously for 16 weeks at concentrations of 0.8-4.0 ppm. [143]

Although it is difficult to extrapolate animal data to occupational exposure and to human response to nitrogen dioxide inhalation, a number of important principles have emerged from animal research. First, it is apparent that a specific concentration of nitrogen dioxide on intermittent exposure is considerably less toxic than on continuous exposure. [93,140] Second, the toxic hazard associated with nitrogen dioxide during continuous exposure is primarily determined by the peak and not by the average concentration of exposure. The latter is supported by data which indicate equivalent or nearly equivalent effect on the severity of experimental respiratory infections from continuous exposure at 2.0 ppm and from continuous exposure at 0.5 ppm with 1-hour peaks at 2.0 ppm, [147,158] as

well as by evidence indicating that for equal Ct's, brief high level exposures are more hazardous than longer exposures at low concentrations. [90,140,141]

The current federal standard for nitrogen dioxide of 5 ppm was adopted from the ACGIH recommended Threshold Limit Value, except that the C designation (for ceiling) was erroneously omitted. According to the current documentation, [219] a 5-ppm ceiling should "insure against immediate injury or adverse physiological effects from prolonged daily exposure." However, evidence obtained since the time of this documentation suggests that humans with normal respiratory function may be acutely affected by exposure at or below this level. Furthermore, the conditions of workers with chronic respiratory diseases, such as chronic bronchitis, may be aggravated by exposure to nitrogen dioxide at a concentration of approximately one-third of the current federal standard. [68,69] Although much of the animal data are inconsistent and, thereby, inconclusive at this time, some studies have indicated chronic effects on respiration, [129,144] cellular morphology of the pulmonary system, [71,99,129,132-134, 146,147,154,157] reproduction, [142] immune responses, [116,117,155, 156,158] and weight gain [127] in animals exposed to nitrogen dioxide at and below the current federal standard. In view of these results, it is concluded that the federal standard of 5 ppm should be reduced.

A reduction in effective lung compliance with a corresponding increase in inspiratory and expiratory maximum viscous resistance as well as significant decreases in arterial oxygen tension and single-breath carbon monoxide diffusing capacity have been noted in normal adult males exposed to nitrogen dioxide for 10-15 minutes at 4-5 ppm. [67-69] The

threshold for the aforementioned changes in normal human respiratory mechanics is unknown, but it is obviously below 4 ppm.

Data indicating respiratory effects and toxicologic changes of the pulmonary system in animals exposed to nitrogen dioxide for either brief or long durations appear to be inconsistent or inconclusive below 2 ppm. However, studies [68,69] conducted on persons with chronic respiratory disease (bronchitis) indicate that 15-minute exposure to nitrogen dioxide at concentrations above 1.5 ppm but not at or below this level results in a decrease in arterial oxygen partial pressure, and in increases in alveolo-arterial pressure gradients and airway resistance, all of which may aggravate existing respiratory problems. Similar changes in arterial oxygen partial pressure, alveolo-arterial pressure gradients, and airway resistance have been observed in healthy subjects exposed at 4-5 ppm. Although the specific concentration at which these changes begin to occur in normal human subjects is unknown, it is likely to be at about the same or perhaps a slightly higher concentration than the one inducing pulmonary changes in humans with existing chronic bronchitis. Therefore, the environmental limit should be reduced to a ceiling value of 1 ppm to prevent acute irritant effects in the lungs of workers exposed to nitrogen dioxide. In addition, the prevention of repeated acute episodes of irritancy should lessen the risk of developing chronic obstructive lung disease.

Concerning nitric oxide, there is no direct quantitative basis for an environmental limit. No environmental data are available on exposures to nitric oxide alone. Furthermore, it is improbable that exposures to nitric oxide alone, as opposed to a mixture of nitric oxide and nitrogen dioxide,

would occur in an occupational situation. Even under experimental circumstances, it would be difficult to achieve human exposures to nitric oxide in the absence of higher oxides of nitrogen, particularly nitrogen dioxide, at concentrations above about 100 ppm because of the rapid rate of oxidation to nitrogen dioxide. [167] At concentrations below 100 ppm, it might be feasible to achieve experimental exposures to nitric oxide virtually free of nitrogen dioxide because of the much slower rate of oxidation, [10] but such experiments addressed to toxicity have not been reported. One experiment on seven human volunteers exposed at 5, 1, 0.5, and 0.33 ppm by inhalation through the mouth has been reported. [62] However, this study presented only the proportion of the inhaled gas which was absorbed, and no subjective or objective effects or durations of exposure were mentioned. The proportionate absorption of the inhaled gas containing nitric oxide or nitrogen dioxide was found to be virtually the same.

In 1941, McCord et al [56] reported a study of 4 arc-welders who were exposed, for an unstated period of time, to "nitrous gas" expressed as nitrogen dioxide at levels ranging from 2.0 to 10.3 ppm. Independent experiments have demonstrated [5] that, in the presence of an electric arc, the initial proportion of nitric oxide present in the "nitrous gas" may be above 90%. At nitric oxide concentrations around 10 ppm, the rate of oxidation is very slow (by calculation, it would take 2-3 hours for a 25% conversion to nitrogen dioxide at 20 C). [167] Therefore, it is inferred that the four welders observed by McCord et al [56] were exposed predominantly to nitric oxide rather than to nitrogen dioxide. The welders all had methemoglobin present in their blood to the extent of 2.3, 2.3,

2.5, and 2.6%, respectively. A more recent British study by Morley and Silk [63] in 1970 reported on a larger number (31) of welders and oxyacetylene flame-cutters. The workers were exposed to oxides of nitrogen, measured as nitrogen dioxide, at levels ranging up to 115 ppm. In no case was an increase of methemoglobin detected by a spectrophotometric methed.

The British anesthetic gas accidents reported in 1967 [58] would initially seem to present 2 cases of nitric oxide poisoning, inasmuch as this gas contaminated a cylinder of anesthetic nitrous oxide. As long as the nitric oxide remained mixed with nitrous oxide alone, it would remain as nitric oxide. [167] However, the nitric oxide-contaminated nitrous oxide was mixed first with 25% then 50% oxygen in the anesthetic apparatus. As the initial concentration of nitric oxide in the cylinder was stated to be "in excess of 1.5%," it would theoretically have been oxidized to nitrogen dioxide in less than 8 seconds. [167] Therefore, the exposure of the two patients might have been effectively to a mixture of nitric oxide and nitrogen dioxide, both gases being present in highly toxic concentrations and together probably accounting for the effects observed. Both patients became deeply cyanotic within 3 minutes or less of inhaling the gas mixture. The presence of an unspecified amount of methemoglobin was reported in the first patient's blood shortly after the exposure, but it was absent after 4 hours. The patient showed signs of great respiratory distress and died in cardiac arrest 18 1/2 hours after the commencement of the anesthetic. At autopsy, pulmonary edema was confirmed. When the second patient became severely cyanotic from the same anesthetic mixture, it was surmised that something was wrong with the anesthetic, its

administration was discontinued, and 100% oxygen was given. The patient showed signs of some respiratory distress but made a complete recovery. It must be stressed that the levels of exposure to nitric oxide and nitrogen dioxide suggested in these two cases are speculated approximations only. The actual gas mixture which was administered to the two patients was never analyzed; however, contamination of the nitrous oxide with nitric oxide in excess of 1.5% was determined from analyses performed by the manufacturer on other cylinders from the same production batch. [58] In addition, if fractional distillation had occurred, [167] the first gas released from the cylinder would probably have been primarily nitric oxide; thus, the first patient might have been exposed to nitric oxide in excess of 1.5%.

From these two patient exposures to nitric oxide, probably mixed to a varying degree with nitrogen dioxide, it is apparent that high concentrations (in the thousands of ppm) rapidly cause cyanosis, methemoglobinemia, and possibly death. [58] Severe lung irritation and pulmonary edema may be attributed to possible nitrogen dioxide exposure. Observations and data at lower levels of exposure to nitric oxide alone are unclear concerning a minimal level for toxic effects in humans.

Reported animal studies also provide extremely limited information on nitric oxide. In the 1930's, Pflesser [47,48] stated that the lethal concentration for 100% (LC100) of an unspecified number of white mice was about 350 ppm, the LC50 was 320 ppm, and that at 310 ppm all the animals survived an 8-hour exposure--a rather steep dose-response curve. [47,48] A subsequent study reported in 1962 by Paribok and Grokholskaya, [87] employing essentially the same experimental technique in mice as used by Pflesser, found that it took 6 hours of exposure to nitric oxide at 322 ppm

to produce a methemoglobinemia of 60%. Guinea pigs were also exposed for an unstated period to nitric oxide at 175 ppm. Little effect was observed on the rate of recovery to resting respiratory rhythm after treadmill exercise. The manner in which the mortality data on mice were presented prevent comparison with Pflesser's LC50 findings.

Von Oettingen's statement [45] that no cases of nitrogen oxide (nitric oxide) poisoning in humans had been reported in the literature is essentially true, considering the intimate association which exists between nitric oxide and nitrogen dioxide. Human [58] and animal [48,87,88] exposures to nitric oxide, whether relatively pure or as mixed with other nitrogen oxides, indicated it to be nonirritant to the respiratory tract and that it produced methemoglobinemia and rapid cyanosis at high concentrations (approximately 1,000 ppm and higher). At these levels, nitric oxide was more toxic than nitrogen dioxide. Nitrogen dioxide, on the other hand, produced pulmonary irritation followed by edema at lower concentrations. Nitric oxide, on the basis of lethality observed in animals following 2 to 8-hour exposures, had been considered to be less toxic than nitrogen dioxide [80] by a factor of 4-5 times.

At lower and sublethal concentrations (eg, 175 ppm) and 8-hour exposures, nitric oxide was found to be less toxic than nitrogen dioxide as indicated by changes in oxygen consumption and postexercise respiratory recovery. [87]

At the present time, there are no definitive data in the scientific literature concerned with chronic effects in humans or animals exposed to nitric oxide at low concentrations. It is known [46,48,58,86] that exposures at high concentrations of nitric oxide result in

methemoglobinemia and cyanosis in both humans and experimental animals, so an environmental limit is obviously needed. In the absence of data showing toxic effects for humans and animals exposed at and below 25 ppm, it is believed that the current federal standard of 25 ppm should be continued as a TWA for up to 10 hours/day and 40 hours/week to protect workers from exposure to nitric oxide. Future research should be conducted to determine concentrations of nitric oxide that result in impairment of respiratory or other biologic functions so that a soundly based standard may be promulgated.

It is recognized that many workers are exposed to the oxides of nitrogen at ambient air concentrations or at concentrations considerably below the recommended occupational limits. Under these conditions, it should not be necessary to comply with many of the provisions of this recommended standard. However, concern for worker health requires that protective measures be instituted below the enforceable limits to ensure that exposures do not exceed the standard. For this reason, "occupational exposure to the oxides of nitrogen" has been defined as exposure above half the environmental limits, thereby delineating those work situations which do not require the installation of unnecessary controls and the expenditure of health resources for provisions such as environmental and medical monitoring, and associated recordkeeping.