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13. ABSTRACT (Maximum 200 words)

Nitrogen dioxide exposure occurs in civilian and military occupations. This presentation provides an overview of nitrogen dioxide related epidemiology; available research models and issues of particular interest to both the cilivian and military sectors; clinical presentations, prophylaxis and treatment; and pathophysiology and mechanisms of injury. Throughout the presentation civilian and military issues are contrasted when pertinent. The most significant difference between the civilian and military research requirements is the need for information on chronic (with and without intermittent peaks) for the former, and information on acute high level nitrogen dioxide research for the latter. Another military requirement is predicting not only injury but incapacitation. This requirement can be compared to the need of clinicians to measure impairment for patients seeking disability. Both communities are faced with the same challenges of selecting appropriate models, understanding dosimetry and its many variables clarifying the fate of inhaled nitrogen dioxide, developing specific markers of injury, and elucidating the mechanisms of nitrogen dioxide injury for the development of prophylactic and therapeutic agents. Civilian and military cooperation and collaboration is required to optimize further NO2 research.

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Overview of nitrogen dioxide effects on the lung with emphasis on military relevance

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Abstract

Nitrogen dioxide exposure occurs in many civilian occupations as well as during military combat. Little interaction has occurred between the two communities in regards to the exchange of information about NO_2 research. This presentation provides an overview of NO_2 related epidemiology; available research models and issues of particular interest to both the civilian and military sectors; clinical presentations, prophylaxis and treatment; and pathophysiology and mechanisms of injury. Throughout the presentation civilian and military issues are contrasted when pertinent. The most significant difference between the civilian and military research requirements is the need for information on chronic (with and without intermittent peaks) for the former, and information on acute high-level NO_2 research for the latter. Another military requirement is predicting not only injury but incapacitation. This requirement can be compared to the need of clinicians to measure impairment for patients seeking disability. Both communities are faced with the same challenges of selecting appropriate models, understanding dosimetry and its many variables, clarifying the fate of inhaled NO_2, developing specific markers of injury, and elucidating the mechanisms of NO_2 injury for the development of prophylactic and therapeutic agents. Further research is required in these areas and it is hoped that this symposium will be the first attempt to join civilian and military resources and expertise for future research cooperation and collaboration.

Key words: Nitrogen dioxide; Occupational; Military; Clinical; Pathophysiology; Inhalation; Pulmonary; Toxicity; Overview

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1. Introduction

Adverse effects of exposure to ambient or occupational levels of nitrogen dioxide (NO_2) is of civilian and military importance. Humans may be exposed to low levels of NO_2 on a chronic or intermittent basis as seen in environmental pollution exposures. Alternatively, exposure to NO_2 may also be a single, acute high-dose event as seen in occupational accidents or in military or combat scenarios. The need to understand the mechanisms of NO_2 toxicity in order to develop preventative and treatment modalities is of importance to both communities. Additionally, similar research challenges face both civilian and military scientists. Knowledge of mutually relevant research issues would maximize research efforts and resources for the further elucidation of NO_2 toxicity. The models to study NO_2 toxicity and the mechanisms defined may serve to examine other gas toxicities which are applicable to both civilian and military occupations. Finally, both civilian and military communities have the need to develop methods to predict pulmonary injury and incapacitation (impairment) and to establish criteria for human exposure.

The development of methods to predict pulmonary injury and incapacitation of soldiers in combat scenarios is of utmost importance to the military

Table 1
Civilian and military human exposure limits and combat levels for NO_2

Exposure limits	NO_2 (ppm)
American Conference of Governmental Industrial Hygienist, 8 h Time weighted average (TWA)[a]	3
American Conference of Governmental Industrial Hygienist, Short-term exposure limits (STEL)[b]	5
National Institute of Occupational Safety and Health (IDLH)[c]	50
Military emergency exposures limits based on 1985 National Research Council recommendations and Army Medical Department review[d]	100
Peak levels observed in combat simulations	100

[a]TWA is defined as the time-weighted average concentration for a normal 8 h workday and a 40 h workweek, to which all workers may be repeatedly exposed without adverse effects.
[b]STEL is defined as the concentration to which workers can be exposed continuously for a 15 min period without suffering acute or chronic adverse effects.
[c]IDLH is defined as immediately dangerous to life and health; it is the maximal level from which one could escape within 30 min without any escape-impairing symptoms or any irreversible health effects.
[d]100 ppm/5 min or 50 ppm for 15 min.

Table 2
1985 National Research Council Commission of Life Sciences Summary of NO_2 effects for humans

Effects	Concentration (ppm)	Time (min)
Immediate incapacitation with respiratory and eye injury followed by death	1000	15
Immediate respiratory and eye irritation with progressive respiratory injury and death	100	60
Immediate respiratory and eye irritation with possible subacute and chronic pulmonary lesions	50	60
Immediate respiratory irritation with chest pain	25	60
Acute reversible respiratory function effects	5	60
Equivocal respiratory function effects and impaired dark adaption of vision	1	60

for the operational integrity of a fighting unit as well as for the triage and treatment of the injured soldier. The US Army has used a variety of sources to develop its criteria for the exposure of soldiers in training and combat situations. These sources include the civilian literature (Table 1) and the National Research Council (NRC) Commission of Life Sciences summary of NO_2 effects and recommendations for exposure criteria for oxides of nitrogen (D.L. Davis, pers. commun.), (Table 2 and 3). Sole reliance on civilian literature and recommendations was inadequate for the US Army due to the differences between civilian and military needs, particularly during combat. These differences include: (1) higher levels of toxic gases in combat situations than those accepted by civilian standards, (2) higher risks encountered during combat by the military versus the civilian sector, and (3) the impact that incapacitation (physical and mental degradation in performing military

Table 3
1985 National Research Council Commission of Life Sciences Recommendations for maximal emergency NO_2 exposures for humans

Concentration (ppm)	Time (min)
10	30
25	10
50	5

Table 4
1985 US Army summary of NO_2-induced human incapacitation[a]

Concentration	Incapacitation
300–500 ppm/few min	Immediate incapacitation or fatal injury
150–200 ppm/30 min	No immediate incapacitation, but subsequent fatal injury
50–100 ppm/20–30 min	No immediate incapacitation, but non-fatal permanent injury
1–50 ppm/1–30 min	No immediate incapacitation, non-fatal reversible injury

[a]Based on the review and conclusions of the National Academy Science, the Army Medical Bioengineering Research Development Laboratory, Walter Reed Army Institute of Research and the Army Environmental Hygiene Agency

tasks) has on the outcome of a battle. For these reasons the Army Medical Department at the Walter Reed Army Institute of Research and other governmental agencies reviewed the NRC recommendations and proposed tentative military exposure criteria (Table 4 and 5). These criteria were based on the NRC recommendations in addition to other *military-unique* considerations such as the use by soldiers of breathing masks connected to ventilation systems within armored vehicles. Concurrent with the establishment of these initial criteria, an in-house toxic gas research program was established at Walter Reed Army Institute of Research as well as at Los Alamos National Laboratory. The purpose of this research was specifically to determine the threshold levels of NO_2 injury which cause gross and histologic pathology and affect performance in small and large animal models. Data from the small animal experiments conducted at Los Alamos National Laboratory guided the large animal studies performed at Walter Reed Army Institute of

Table 5
1985 US Army acceptable human criteria for within-armored vehicle levels of NO_2 during live fire testing

Concentration (ppm)	Time (min)
50	15
100	5

Table 6
1989 US Army human incapacitation criteria for levels of NO_2 during live fire testing[a]

Concentration	Incapacitation
< 125 ppm/min prior to masking (30 s)	Negligible risk for injury or incapacitation
125–250 ppm/min	Some risk of impairment for exercising soldier
250–750 ppm/min	Varying degrees of incapacitation
750 ppm/min	100% incapacitation

[a]Assuming a tripling of ventilation of soldiers during combat.

Research. The current criteria used by the Army Live Fire Testing Program are shown in Table 6. These criteria will be updated as new information emerges from in-house and extramural research.

The purpose of this presentation is to provide an overview of NO_2-related epidemiology; available research models and issues of particular interest to this audience; clinical presentations, prophylaxis and treatment; and pathophysiology and mechanisms of injury. Throughout this presentation, civilian and military issues regarding NO_2 will be contrasted when pertinent. More detailed discussions on NO_2 chemistry, dosimetry, effects in small and large animal models and in humans, as well as antioxidant treatment, will be presented in the following papers.

2. Epidemiology

There is a paucity of data concerning the true worldwide prevalence or incidence of NO_2 exposure, the development of NO_2 related disease, or its contribution to morbidity and mortality. The multiple gases and particulates which comprise outdoor and indoor pollution and the spontaneous conversion of NO_2 to other oxides make epidemiological studies designed to investigate the injurious effects of NO_2 difficult. The US National Ambient Air Quality Standard for NO_2 is 0.053 ppm (annual arithmetic mean). Nonetheless, NO_2 is present in abnormally high concentrations (0.05–0.2 ppm) with superimposed spikes (peaks) as high as 0.5 ppm in densely populated urban areas with poor air quality (South Coast Air Quality Management District, 1981; Morrow, 1984; Chang et al., 1986). The major source of NO_2 in these areas is the combustion of fossil fuels, particularly in automobiles.

Another source of NO_2 pollution that has gained recognition is the use of gas-fired appliances in the home which may produce NO_2 levels greater than 4 ppm (Samet et al., 1987; Leaderer et al., 1984). Studies which exposed humans to ambient levels of 0.5–7.5 ppm for 10 min to 2 h have demonstrated increases in airway resistance, decreases in lung compliance and diffusing capacity, but only at the higher concentrations (>2.5 ppm) (Von Neiding et al., 1970; Beil and Ulmer, 1976; Abe, 1976). Airway hyperresponsiveness may be elicited in normal humans after exposure to 2.0 ppm × 1 h (Mohsenin, 1987a) and in asthmatic patients after exposure to as low as 0.5 ppm × 1 h (Mohsenin, 1987b). In cigarette smokers levels of 50–250 ppm of NO_2 have been reported (Norman and Keith, 1965; Chrzanowski et al., 1980; Stavert et al., 1986). Cigarette smokers serve as a very interesting population to study because of protective or maladaptive mechanisms which may develop in this population chronically exposed to relatively high levels of NO_2.

In contrast to the civilian community, low level exposure to NO_2 is not considered a health hazard risk to the military. Low levels of NO_2 have been measured in outdoor environments simulating soldiers firing small and large weapons. In the outdoors, gases are rapidly dissipated and diluted by ambient air. However, studies measuring ambient levels of NO_2 after the firing of weapons within enclosures have not been conducted by the US Army. Furthermore, examinations using more sensitive morphological and biochemical methodologies after exposure to sub-lethal levels of NO_2 have not been performed in humans by the US Army. However, the US Army has investigated the pulmonary effects of various levels of NO_2 (100–1500 ppm × 15–30 min), the effect of NO_2 (100 ppm × 15 min) on post-exposure exercise performance and the contribution of exercise to NO_2-induced pulmonary injury in a rodent model (Stavert et al., 1987a,b; Stavert and Lehnert, 1988). Similar exercise studies have not been performed in humans by the US Army, although, in civilian studies, humans have been exposed to NO_2 in concentrations of 0.50 ppm–4 ppm for 20 min–2 h followed by exercise (Folinsbee et al., 1978; Kerr et al., 1979).

More retrospective and anecdotal information is available on the accidental or occupational exposure of humans to high levels (>200 ppm) of NO_2 in the occupations of silo filling, manufacture of nitroglycerin and other explosives, fire-fighting, welding, and in the aerospace industry (Lowry and Schuman, 1956; Horvath et al., 1978; Hatton et al., 1977). More unusual accounts of NO_2 exposure have been reported such as the report of an exposure of 116 spectators, cheer leaders and hockey players at an ice hockey arena. Ice resurfacing equipment was found to be the source of the pollution (Hedberg et al., 1989). In this accidental exposure, nitrogen dioxide levels were not measured during the two games, during which spectators and

players complained of cough, dyspnea, chest pain, headache, hemoptysis and weakness. Though nitrogen dioxide was measured at a level of 4 ppm 2 days after the exposure, levels during the accidental exposure were likely much higher since the arena ventilation system had been operable for 2 days at the time of this NO_2 level measurement.

In the military, accidental and combat exposures to high levels of NO_2 may occur in fires within aircraft, ships, submarines, and armored vehicles or by the penetration of munitions into any of these structures. In 1984, Congress mandated the establishment of the Joint Live Fire Test Program to test the combat vulnerability of US weapon systems in a realistic manner and to gain insight into design changes necessary to improve crew survivability on the battle field (Department of Defense Authorization Act, 1986). This program heralded a new emphasis on crew vulnerability and survivability in addition to weapon system vulnerability and lethality. In the testing of one armored vehicle, oxides of nitrogen were found to be the major non-fragment insult. In live fire tests, peak levels over 2000 ppm, decreasing to greater than 500 ppm at 1 min and 20 ppm at 5 min were reported for an armored vehicle penetrated by a high temperature shaped charge. The source of the NO_2 in this scenario was the combustion of stowed munitions and the fixation of atmospheric nitrogen by the high temperature shaped charge jet penetrating the vehicle. As a result of these findings, vehicle design modifications to include the compartmentalization of munitions, an automatic fire-extinguishing system, an automatic ventilation system and the crew safety requirement to use personnel face masks have helped to minimize the health effects of fires and their toxic products.

3. Experimental models and research issues

The pulmonary toxicity of NO_2 has been described in animals and humans, in isolated perfused lung models and in cultured cells (Morrow, 1984; Lowry and Schuman, 1956; Lee, 1980; The National Research Council, 1977; Horvath, 1980; Postlethwait et al., 1990; Patel and Block, 1986). When comparing studies, it is important to note the animal species used in the study and the contributions of animal size, minute ventilation and respiratory tract anatomical differences; and exposure duration and pattern. In this presentation NO_2 levels will be divided into 'acute high-dose', 'acute moderate dose', 'acute low-dose', 'subchronic' and 'chronic'. These classifications are arbitrary; however, they take into account the animal species, the average weight and respiratory rate of the animal species, and the gas concentration in ppm and duration of exposure. There is controversy concerning whether NO_2 follows Haber's Law — dose concentration multiplied by the exposure duration is equal to a constant ($C \times T = K$) — throughout the dose-

response curve. However, a standard accepted format which would minimally provide gas concentration (including peak concentrations), duration of exposure, respiratory rate and pattern, and the weight of the experimental animal would further aid in comparing studies. The differences in cumulative dose and dose rate-dependency may be explained by the high reactivity of NO_2, the toxic endpoint measured, or the respiratory pattern of the animal (Stavert and Lehnert, 1988; Postlethwait et al., 1990; Gelzleichter et al., 1992a). In studies involving chronic and/or intermittent exposure to NO_2, the importance of such reporting would be tempered by other factors such as the influence of the natural protective and adaptive processes occurring over time. Animals used in NO_2 models include mice, rats, hamsters, rabbits, dogs, sheep, monkeys and humans (The National Research Council, 1977; Januszkiewicz et al., 1992; Mohsenin and Gee, 1987). Knowledge of the physiological and anatomical nuances of each species is critical in the interpretation of research results. The isolated perfused lung (IPL) model has served as a very useful research tool in following the fate of inhaled NO_2 (Postlethwait and Birdani, 1989). The most commonly employed source of lung for the IPL model has been the rat. In the use of an IPL model, the determination of lung circulation (bronchial-pulmonary anastomosis versus non-bronchial pulmonary anastomosis) has been shown to be important as injury markers measured in perfusate effluent may be modified by the contribution of the bronchial circulation (Postlethwait et al., 1990).

Age-related differences in the susceptibility to NO_2 have been noted in newborn animals in contrast to adolescent or adult animals, with both newborn and adolescent lungs demonstrating less susceptibility (Chang et al., 1986). These differences in susceptibility may be accounted for by a higher fat content in nursing rats than in weaned rats and the higher percentage of the more resistant Type II alveolar epithelial cells in juvenile rats than in adult rats. Exposure of cells in culture to NO_2 have been used to investigate cytokine release (Devalia et al., 1993). The use of cell culture models may be useful in further elucidating some of the mechanisms of NO_2 injury.

Numerous research issues are of particular current interest to both the civilian and military and require further resolution. Effects of NO_2 alone versus NO_2 concomittant with combinations of other reaction products (i.e., NO, NO_3, N_2O_3, N_2O_4, N_2O_5) are difficult, but important to decipher. Additionally, in many occupational and combat situations, gases occur in combination with other gases or particulates such as sulfuric acid (H_2SO_4), hydrogen chloride (HCl), ozone (O_3), or a multitude of hydrocarbons. This is the case when an armored vehicle is hit by an incoming round. The inside environment of a burning armored vehicle may include NO_2, NO, CO, CO_2, halon, HFl, HCl, HBr, NH_3, SO_2, acrolein, and carbon, metal and spall lin-

ing particulates. The additive or synergistic effects of these combinations must be determined in the assessment of injury (Sagai and Ichnose, 1991; Schlesinger, 1987; Gelzleichter et al., 1992b; Mustafa et al., 1984). The differences in injury caused by varying NO_2 concentrations and patterns of NO_2 exposure constitute other research concerns which have implications for various occupations and environmental scenarios. For instance, differences exist in the injury produced by low-level chronic and acute high-level NO_2 exposures. In low level chronic exposure injury may result in emphysema-like picture or fibrosis in animals. Conversely, after acute high-level exposure, the injury is characterized by edema, destruction of bronchiolar epithelium, loss of cilia, flattened epithelial cells, Type I alveolar epithelial cell destruction and Type II alveolar epithelial cell proliferation and shape change, inflammatory cell infiltration, and disruption of epithelial tight junctions (Lafuma et al., 1987 Gordon et al., 1986a,b; Blank et al., 1988; Evans et al., 1993). The diverse patterns of exposure studied include single, intermittent, continuous, and continuous with intermittent boluses (Guidotti, 1980; Frederick et al., 1987; Gelzleichter et al., 1992b).

Most studies in the civilian literature have investigated the effects of relatively low level NO_2 exposure whereas the US Army studies have concentrated on acute high-level NO_2 exposure (Januszkiewicz et al., 1992). Some studies have investigated the differences in distribution of histological damage as well as biochemical alterations (Kubota et al., 1987; Cavanagh and Morris, 1987). Correlation between histological and biochemical alterations may not necessarily be present. For example, the bronchioles, alveolar ducts and alveoli are known to be more susceptible to NO_2 injury, at least when examined histologically. However, in one study where rats were exposed to NO_2 (40 ppm × 1 h) via a whole-body inhalation chamber, lipid peroxidation, as measured by thiobarbituric acid-reactive material, was significant only in the trachea, rather than in the lung parenchyma (Cavanagh and Morris, 1987). This disparity could be explained by the reduction in antioxidant capacity of the trachea.

4. Clinical presentations, prophylaxis and treatment

Human exposure to low-level NO_2 (0.6–5 ppm) may cause no symptoms or signs though subclinical physiologic, and/or biochemical changes may occur (Mohsenin, 1988; Frampton et al., 1989; Horvath, 1980; Abe, 1976). Morphometric evaluation of samples of human lung exposed to these low levels might demonstrate changes seen in other animal models. High-level NO_2 (>200 ppm) causes a biphasic clinical response characterized by acute laryngospasm and bronchospasm followed by the development of pulmonary edema in 8–24 h (Table 7). In 1–4 weeks, the development of Bron-

Table 7
Clinical Presentations of NO_2 exposure

Laryngospasm[a]
Reflex respiratory arrest[a]
Airway Hyperresponsiveness: in susceptible individuals[b]
Bronchospasm[a]
Pulmonary edema[c]
Bronchiolitis obliterans[d]
Alveolar Proteinosis[e]
Chronic obstructive pulmonary disease
Death[f]

[a]May be an acute event.
[b]Observed after NO_2 exposure of 0.5 ppm.
[c]Observed at 8–24 h.
[d]Observed at 1–4 weeks.
[e]Observed at 3 months (1 case report).
[f]May be an acute or delayed event.

chiolitis obliterans can occur with concomitant exacerbation of earlier symptoms (Table 7). Chronic obstructive pulmonary disease with emphysema or fibrosis is controversial and may be long-term sequelae of NO_2 exposure in animals (Stavert et al., 1986; Blank et al., 1988). Development of obstructive and/or restrictive pulmonary function tests and radiographic hyperinflation in humans suggest the development of chronic obstructive pulmonary disease, fibrosis and emphysema (Horvath, 1978) (Table 7). Airway hyperreactivity in the susceptible human has been described after a 1-h exposure to as little as 2 ppm (Mohsenin, 1988). A recent report described the development of alveolar proteinosis following NO_2 exposure (Dawkins et al., 1991). This is not surprising as the mechanism in alveolar proteinosis is speculated to be a defect in Type II alveolar cells or of surfactant. Type II alveolar cells produce surfactant, Clara cells produce surfactant apoproteins and alveolar macrophages are involved in the recycling of old surfactant. All of these cells are affected by exposure to NO_2. Symptoms of high-level exposure to NO_2 include cough, dyspnea, hemoptysis and chest pain, vomiting, headache, vertigo, weakness, loss of consciousness and death (Horvath et al., 1978; Hedberg et al., 1989). Signs of high-level exposure include tachypnea, tachycardia, cyanosis, rales, rhonchi, wheezes, radiographic bilateral pulmonary infiltrates or increased bronchial markings, abnormal pulmonary function tests (Horvath et al., 1978; Hedberg et al., 1989).

The primary treatment of NO_2 toxicity in humans is supportive therapy directed at hypoxemia, ventilatory failure, infection and any other complications. Steroids, *N*-acetylcysteine and lipoic acid have been used with varying degrees of efficacy (Horvath et al., 1978; Hedberg et al., 1989). Prophylaxis is a superior alternative for the military as soldiers going into combat could

possibly benefit from easily delivered aerosolized and/or dietary antioxidants. Vitamin C, Vitamin E, glutathione, lipoic acid or taurine or a combination thereof have been used in humans or animals as prophylactic agents for NO_2-induced pulmonary effects (Mohsenin, 1987; Gordon et al., 1986a; Shoaf et al., 1989; Elsayed, 1982).

5. Pathophysiology

Various animal studies, both early and more current, have described pathophysiologic changes that include increased airway resistance, decreased tidal volume, increased respiratory rate, hypoxemia, mild methemoglobinemia and decreased lung compliance in response to "acute high-dose" NO_2 exposure (The National Research Council, 1977; Januszkiewicz et al., 1992; Januszkiewicz and Mayorga, 1993). A biphasic response consisting of immediate and delayed reactions has been observed in a sheep model after exposure to 500 ppm × 20 min in our laboratory (Januszkiewicz and Mayorga, 1993). In this sheep study an immediate effect is manifested by an increased respiratory rate which compensated for a reduction in tidal volume. The magnitude of tidal volume reduction has been noted to vary depending on animal species (The National Research Council, 1977; Januszkiewicz and Mayorga, 1993). The delayed response in the sheep study was observed at 6 h and continued to increase up to 24 h (Januszkiewicz and Mayorga, 1993). This delayed response is represented by an increase in airway resistance, decreased lung compliance and hypoxemia.

NO_2 exposure has produced varying results regarding the association of NO_2 with decreased viral and bacterial defense mechanisms (Ehrlich, 1966; McGrath and Oyerides, 1985). In one murine study using exposures of 4–30 ppm NO_2 for 4 h, suppression of both macrophage and polymorphonuclear neutrophilic phagocytic response was reported, though the level of NO_2 producing this effect was dependent on the bacteria used in the experiments (Jakab, 1987). However, another study which exposed mice to 5 ppm NO_2 for 7 days showed no significant changes in macrophage function, antibody production, cell-mediated immunity or susceptibility to viral infections. A significant difference was noted only in the reduction in the ratio of the number of plaque-forming cells to sheep erythrocytes (Lefkowitz et al., 1986). The different results in these two studies may be accounted for by the differences in NO_2 concentrations. Human epidemiologic studies likewise have demonstrated conflicting results regarding the association of NO_2 and the immune system. A higher incidence of respiratory infections has been reported in association with indoor and outdoor levels of NO_2 (Pearlman et al., 1971; Shy et al., 1970a,b; Love et al., 1982; Melia et al., 1977; Speizer et al., 1981). Other studies have not corroborated this association (Florey et al., 1979; Keller et al., 1979; Melia et al., 1982). In hamsters exposed to higher

"acute moderate dose" NO_2 (7–30 ppm × 24 h) ciliary loss, inflammatory cell infiltration, hyperplasia of goblet cells and Type II pneumocytes, mucinous and cellular debris within bronchioles was found (Gordon et al., 1986a; Case et al., 1983). When "acute moderate-dose" NO_2 exposure, sufficient to cause more pronounced histological changes, the more classical NO_2 lesion was noted as characterized by bronchiolar epithelial destruction, Type I pneumocyte destruction, Type II pneumocyte hyperplasia and hypertrophy, Type I regeneration, proteinaceous edema and fibrin in alveoli, interstitial edema, and mononuclear and polymorphonuclear leukocyte infiltration (Stavert and Lehnert, 1990; Man et al., 1990).

'Acute low-dose' NO_2 exposure in a dog study demonstrates that the injury measured may be dependent on the sensitivity of injury indices. In lightly anesthetized beagle dogs exposed to 37 ppm NO_2 for 4 h, gross and histological findings were negative, however, subtle electron microscopic changes were observed (Guidotti, 1980). These changes included variable endothelial width and frequency of vesicle formation, and an increase in endothelial redundancy (Guidotti, 1980). Furthermore, morphometric changes in NO_2-exposed lungs were characterized in the interstitium by a greater surface-to-volume ratio compatible with an alteration in shape and in the capillary endothelium by a greater volume ratio, surface-to-volume ratio and number of vesicles consistent with increased thickness, shape change and greater number of picocytic vesicles (Guidotti, 1980).

In a rat model, 'subchronic' exposure to NO_2 (0.5–2 ppm × 23 h × 6 weeks) produced fenestration, blebbing and disruption of plasma membrane of Type I pneumocytes and elongation and thinning of Type II pneumocytes with lamellar bodies (Chang et al., 1986). Morphometric changes included an increase in the volume of the alveolar-capillary barrier (volume of Type II epithelial cells, fibroblasts, interstitial matrix and alveolar macrophages), an increase in the surface area and surface density of alveolar basement membrane, an increase in the surface area of Type I and II epithelial cells, and an increase in the density of the interstitium (Chang et al., 1986). In hamsters, 'chronic' exposure to 30 ppm × 5 or 9 months caused a decrease in the number of plasma membrane adjacent points of contact in the alveolar and bronchiolar tight junctions. Bronchioles showed disruption of tight junctions and reduction in fibrils and fibril fragmentation (Gordon et al., 1986b).

6. Mechanisms of NO_2 injury

Though the exact mechanism of NO_2 toxicity is unknown, a direct effect through lipid peroxidation of cellular membranes and the oxidation of reducing equivalents and proteins has been hypothesized (Cavanagh and Morris, 1987; Menzel). Whole lung or lung segments have been analyzed for

evidence of lipid or protein oxidation, oxidative enzymes or antioxidant compounds and enzymes. These include thiobarbituric acid-reactive substances, non-protein sulfhydryls, NADP-dependent cytochrome P-450 reductase, glutathione peroxidase, glutathione reductase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione-*S*-transferase (Ospital et al., 1981; Crapo et al., 1978; Chow et al., 1974; Sagai and Inchinose, 1987). Variable results have been reported dependent on the NO_2 exposure dose, duration and animal species examined. An argument against direct NO_2 lipid peroxidation is the observance of transformation of the majority of radiolabelled inhaled NO_2-nitrogen to nitrite in rat IPL perfusate (Postlethwait and Bidani, 1989). Only about 30% of radiolabelled NO_2-N could be accounted for in soluble and weakly insoluble elements, but not in lipid elements (Postlethwait and Bidani, 1989). Though non-specific markers of cell death and epithelial and capillary leakage have been reported (Man et al., 1990; Johnson et al., 1990), some studies have examined bronchial alveolar lavage fluid for cell composition and proteins such as α-protease inhibitor (Mohsenin and Gee, 1987; Januszkiewicz and Mayorga, 1993; Man et al., 1990; Patel et al., 1990) looking for more specific markers and mechanisms of injury. In a study in which sheep were exposed to 500 ppm of NO_2 for 20 min, bronchoalveolar lavage fluid (BALF) analysis demonstrated a decrease in alveolar macrophages, an increase in epithelial cells and an increase in protein and albumin (Januszkiewicz and Mayorga, 1993). These changes were most marked at 6 h post-exposure. There was no significant difference in total cell count or the percentage of polymorphonuclear leukocytes throughout the sampling points (1/2, 6, 24 h). A similar observation was reported at 1 h post exposure in another study in which dogs were exposed to 200 ppm $NO_2 \times 1$ h (Man et al., 1990). Because of the occurrence of emphysema in both NO_2 toxicity and in a the human genetic deficiency of α_1 protease inhibitor (α_1-PI), oxidation of proteins such as α_1-PI, has been examined. In one human study (α_1-PI) activity was found to be elevated in the BALF from human subjects exposed to low-level NO_2 and intermittent exercise (Mohsenin and Gee, 1987). This finding was not corroborated in another study where subjects were subjected to similar experimental conditions (Patel et al., 1990). The contrasting results in these studies may have been due to the differences in NO_2 concentrations and/or in the variability in α_1-PI measurements. More recently, there have been reports of the role of NO_2 or its reaction products in cell signalling by the modulation of ligand binding on the pulmonary endothelial cell membrane through a PLA_1 mechanism, by production of diacylglycerol and activation of protein kinase C (Patel et al., 1991; Patel et al., 1992). Secondary effects mediated by the initiation and propagation of other injurious pathways are very plausible. A recent study showed elevation of granulo-

cyte/macrophage colony stimulating factor, tumor necrosis factor and interleukin 8 in the culture media of human bronchial cells exposed to 400 ppb NO_2(80).

7. Conclusion

This is the first symposium solely dedicated to the exchange of information concerning mutually relevant NO_2 research issues among representatives of the civilian and military communities. Differences in the research needs of the civilian and the military sectors have been described with the most significant dissimilarity consisting of the military's need for research in the area of acute, high-level NO_2 exposure in contrast to the civilian need for chronic (with and without peaks), intermittent low-level NO_2 research. However, the research challenges of understanding NO_2 dosimetry related variables, the fate of inhaled NO_2, and the primary and secondary mechanisms of NO_2 injury; and of developing preventative and therapeutic modalities, are the same for civilian and military investigators. Though there has been substantial progress in the elucidation of NO_2 toxicity, further research is required in these areas. Continued use of simpler models such as in vitro systems, cell culture and isolated perfused lung injury systems will be very useful in further examination of mechanisms of NO_2-induced injury. However, results from the use of simpler systems will require validation in whole animal systems where mechanisms may be complex and totally different. The following presentations in this symposium will expand on the areas discussed in this overview. It is hoped that this presentation has served to provoke interest in the many NO_2 toxicological issues of concern to both the civilian and military sectors and will lead to a continued exchange in knowledge and expertise in inhalation toxicology.

The views of the author do not reflect the position of the Department of the Army or the Department of Defense.

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