Proposed 1: 8/2003

ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)
FOR
NITRIC OXIDE
(CAS Reg. No. 10102-43-9)

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. Three levels— AEGL-1, AEGL-2 and AEGL-3— are developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that can produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation, or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SUMMARY

Nitric oxide (NO) is an endogenous molecule that mediates the biological action of endothelium-derived relaxing factor. The chemical is an important regulator of the functions of the cardiovascular, immune, and nervous systems. The toxicity of NO is associated with both methemoglobin formation and oxidation to nitrogen dioxide. Inhaled NO has been used therapeutically to treat adult respiratory distress syndrome, persistent pulmonary hypertension of the newborn, pulmonary hypertension in congenital heart disease and diaphragmatic hernia, pulmonary hypertension following thoracic organ transplantation, idiopathic pulmonary hypertension, and chronic obstructive pulmonary disease. Nitric oxide is also a component of air pollution and is generally measured as part of the total oxides of nitrogen ($NO + NO_2$) present.

Data were insufficient for derivation of AEGL values. Generally, therapeutic levels of 20-80 ppm for 24 hours or 100 ppm for 20 minutes have not resulted in adverse effects among treated patients. Methemoglobin levels increased to 9.4-9.6% in lung transplantation or pulmonary hypertension patients after treatment with 80 ppm NO for up to 108 hours (Adatia et al. 1994, Wessel et al. 1994). In all cases, a reduction in NO concentration resulted in a reduction of methemoglobin levels. Methemoglobinemia >7% occurred in 13/37 newborns treated with 80 ppm NO for persistent pulmonary hypertension. The average time to peak level in all patients was 19.6 hours and the highest level of methemoglobin was 11.9% at 8 hours in one patient (Davidson et al. 1998).

Most of the experimental animal studies available focused on the therapeutic use of NO in an animal model of human disease. Lethality studies in dogs, rats, and mice lacked complete concentration-response information, were confounded by possible NO_2 contamination, or were secondary citations in which the original source could not be obtained. From these studies, however, it appears that in the absence of lung injury, the mechanism of toxicity of NO is methemoglobin formation.

NO is unstable in air and undergoes spontaneous oxidation to NO_2 making experimental effects difficult to separate and studies difficult to perform (U.S. EPA 1993). Studies on the conversion of NO to NO_2 in medicinal applications have found the conversion to be significant in an atmospheric concentration of O_2 (20.9%) at room temperatures. While on the laboratory scale, closed system experiments clearly indicate the potential for the production of NO_2, the chemical kinetics of NO conversion during a large-scale atmospheric release and dispersion are not well documented. As a result, the conversion of NO to NO_2 during the atmospheric release is of concern to emergency planners, but the NAC is unable to provide any significant guidance, other than to indicate that a significant fraction of the NO will be converted to NO_2 and present an atmospheric hazard that is generally more toxic than just NO.

Therefore, AEGL values for NO are not recommended. Because conversion to NO_2 is expected to occur in the atmosphere, and because NO_2 is more toxic than NO, the AEGL values

for NO_2 are recommended for use with emergency planning for NO. The NAC recognizes, however, that short-term exposures below 80 ppm NO should not constitute a health hazard.

Summary of AEGL Values for Nitric Oxide[a]

Classification	10-Minute	30-Minute	1-Hour	4-Hour	8-Hour	Endpoint (Reference)
AEGL–1[b] (Non-disabling)	NR	NR	NR	NR	NR	
AEGL–2[b] (Disabling)	NR	NR	NR	NR	NR	
AEGL–3[b] (Lethal)	NR	NR	NR	NR	NR	

NR, not recommended

[a]AEGL values for nitrogen dioxide should be used for emergency planning.

[b]Short-term exposures to below 80 ppm NO should not constitute a health hazard.

References

Adatia, I., C. Lillehei, J.H. Arnold, J.E. Thompson, R. Palazzo, J.C. Fackler, and D.L. Wessel. 1994. Inhaled nitric oxide in the treatment of postoperative graft dysfunction after lung transplantation. Ann. Thorac. Surg. 57:1311-1318.

Davidson, D., E.S. Barefield, J. Kattwinkel, G. Dudell, M. Damask, R. Straube, J. Rhines, C.-T. Chang, and the I-NO/PPHN study group. 1998. Inhaled nitric oxide for the early treatment of persistent pulmonary hypertension of the term newborn: a randomized, double-masked, placebo-controlled, dose-response, multicenter study. Pediatrics 101:325-334.

U.S. EPA (U.S. Environmental Protection Agency). 1993. Air Quality Criteria for Oxides of Nitrogen, Vol. I-III. Office of Research and Development, U.S. EPA, Research Triangle Park, NC.

Wessel, D.L., I. Adatia, J.E. Thompson, and P.R. Hickey. 1994. Delivery and monitoring of inhaled nitric oxide in patients with pulmonary hypertension. Crit. Care Med. 22:930-938.

1. INTRODUCTION

Nitric oxide (NO) is an endogenous molecule that mediates the biological action of endothelium-derived relaxing factor. The chemical is an important regulator of the functions of the cardiovascular, immune, and nervous systems (Moncada et al. 1991; Rossaint et al. 1996). One of the most important functions of NO is relaxation of the vascular smooth muscles. Because of this action, inhaled NO has been used to treat adult respiratory distress syndrome, persistent pulmonary hypertension of the newborn, pulmonary hypertension in congenital heart disease and diaphragmatic hernia, pulmonary hypertension following thoracic organ transplantation, idiopathic pulmonary hypertension, and chronic obstructive pulmonary disease (Troncy et al. 1997a).

The major mechanism of toxicity of NO is the binding of hemoglobin (U.S. EPA 1993b). Inhaled NO is absorbed into the bloodstream and binds to hemoglobin forming nitrosylhemoglobin which is rapidly oxidized to methemoglobin (Maeda et al. 1987; Sharrock et al. 1984; U.S. EPA 1993b). The affinity of NO for hemoglobin is about 1500 times greater than that of carbon monoxide (Gibson and Roughton 1957) and the binding and formation of methemoglobin is NO concentration- and time-dependent (Maeda et al. 1987; Ripple et al. 1989; Sharrock et al. 1984). Potentiating possible toxicity, NO reacts quantitatively with oxygen in air to form nitrogen dioxide which causes pulmonary edema: $2NO + O_2 \rightarrow 2NO_2$. Nitrogen dioxide then reacts with water to form nitric acid (NIOSH 1976). For this reason, careful monitoring of NO_2 concentrations has been suggested when NO is used therapeutically at concentrations $\geq$80 ppm especially when coadministered with oxygen (Miller et al. 1994; Foubert et al. 1992).

Unintended exposure during the therapeutic use of NO may also occur. During inhalation therapy, environmental levels of NO rose to a maximum of 0.462 ppm in a nonventilated room without exhaust, but with normal air conditioning, the levels were 0.075 ppm (Markhorst et al. 1996). Hospital compressed air tanks were found to be contaminated with 5-8 ppm NO on days that corresponded to welding activity near the intake port; otherwise, tank levels were similar to ambient levels (Pinsky et al. 1997).

Nitric oxide is also a component of air pollution and is generally measured as part of the total oxides of nitrogen ($NO + NO_2$) present. Anthropogenic sources of nitrogen oxides include automobiles, electric utilities, industrial boilers, gas stoves, space heaters, kerosene heaters, wood stoves, and tobacco products (U.S. EPA 1993b). Cigarette smoke contains 400-1000 ppm NO (Fullerton and McIntyre 1996). In the atmosphere, NO reacts with ozone to produce NO_2 and oxygen (U.S. EPA 1993b).

Selected physicochemical properties of nitric oxide are listed in Table 1.

TABLE 1. PHYSICOCHEMICAL DATA FOR NITRIC OXIDE

Parameter	Value	Reference
Common name	nitric oxide	
Synonyms	nitrogen monoxide	Budavari et al., 1996
CAS registry no.	10102-43-9	
Chemical formula	NO	Budavari et al., 1996
Molecular weight	30.01	Budavari et al., 1996
Physical state	colorless gas	Budavari et al., 1996
Vapor pressure	26,000 torr at 20°C	ACGIH, 1991
Vapor density (air = 1)	1.04	Budavari et al., 1996
Melting/boiling point	-163.6°C/-151.7°C	Budavari et al., 1996
Solubility in water	4.6 mL/100 mL (20°C) 0.006 g/100 g (24°C)	Budavari et al., 1996 U.S. EPA, 1993a
Conversion factors in air	1 ppm = 1.25 mg/m^3 1 mg/m^3 = 0.8 ppm	NIOSH, 1976
Reactivity	combines with O_2 to form NO_2	Budavari et al., 1996

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

Following induction of anesthesia with nitrous oxide and oxygen, a woman became cyanotic within 2 minutes. Treatment with methylene blue reversed the methemoglobinemia, but she developed severe pulmonary edema several hours later and died of cardiac arrest. A second patient also became cyanotic after initiation of anesthesia and the nitrous oxide was discontinued immediately. Several hours later the second patient developed some respiratory distress but recovered completely after oxygen and steroid therapy. It was determined that the nitrous oxide cylinder had been contaminated with NO (Clutton-Brock 1967). The possible exposure concentration was not determined nor was the contribution of the formation of nitrogen dioxide addressed by the study author. Greenbaum et al. (1967) made several assumptions about retention volume, time to cyanosis, and ventilation rate and estimated that the contamination must have been 1% (10,000 ppm) NO or higher.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

Methemoglobin levels rose to 9.4% in one lung transplantation patient after treatment with 80 ppm NO for 8 hours. A reduction in concentration to 40 ppm over 4 hours resulted in a decrease to 6.6%, and a further reduction to 20 ppm for the next 12 hours reduced methemoglobin levels to 0.9% (Adatia et al. 1994). A Japanese newborn developed a methemoglobin level of 40% after 26 hours of exposure to 80 ppm; the level reduced to 3.9% within 20 minutes of infusion with methylene blue and gradual reduction of the NO concentration over one hour then discontinuation. No methemoglobin levels were reported prior to the 26-hour timepoint. The infant survived with no indications of hypoxic brain damage at 4 months of age (Nakajima et al. 1997).

The therapeutic use of NO has been studied extensively in patients with acute respiratory distress syndrome (ARDS). Manktelow et al. (1997) reviewed data collected over 5 years from ARDS patients treated with NO inhalation therapy. In general, patients received 20 ppm NO for 48 hours with a reduction to 10 ppm for the next 8 days. No patient had an adverse response to NO and 58% of all patients had clinically significant responses to NO measured as increases in the inspiratory fraction of oxygen and decreases in pulmonary vascular resistance. Another review (Troncy et al. 1997b) found that the optimal concentration of NO was between 0.5 and 40 ppm for producing the greatest improvement in hypoxia score among ARDS patients. These results were confirmed in a more recent study in which ARDS patients were treated with 1-40 ppm for 30 minutes. Concentration-dependent decreases in pulmonary capillary pressure and post-capillary resistance were observed with a maximum effect at 20 ppm (Benzing et al. 1998). Other studies confirm improvements in oxygenation and/or pulmonary artery pressure in ARDS patients treated with 40 ppm for 20 minutes (Doering et al. 1997), 0.1-2 ppm for 15-20 minutes (Puybasset et al. 1994), 100 ppm for 20 minutes (Wenz et al. 1997), and 0.1-100 ppm for 15 minutes (Gerlach et al. 1993). Mortality was not affected by NO inhalation in any of these studies. A large increase in cardiac output was reported for one patient with ARDS and acute right heart failure treated with 20 ppm NO for 3 days; methemoglobin levels were $\leq$1.7% (Benzing et al. 1997).

Newborns and children diagnosed with hypoxemic respiratory failure (Abman et al. 1994; Day et al. 1997; Goldman et al. 1997) or persistent pulmonary hypertension (Goldman et al. 1995; Ichida et al. 1997; Kinsella et al. 1997; Nakagawa et al. 1997; Wessel et al. 1997) showed decreased pulmonary artery pressure and/or improved oxygen saturation when treated with 10 ppm for up to 24 hours, 20 ppm for up to 4 hours, 60 ppm for 10 minutes, or 80 ppm for up to 12 hours. Two studies reported longer term therapies in which hypoxemic newborns were treated with 10 ppm NO for a duration of 6-331 hours (Biban et al. 1998) and newborns with persistent pulmonary hypertension were treated with 80 ppm for a mean duration of 65.1 hours (Davidson et al. 1998). The large variation in duration of exposure is explained by the fact that

in most of these trials treatment was continued until success or failure criteria were met as defined by the study protocol.

NO inhalation has also been used therapeutically to treat patients with lung or heart disease and following surgery. Decreased pulmonary artery pressure occurred in adult patients with chronic obstructive pulmonary disease treated with 40 ppm for 20 minutes (Roger et al. 1997) and with pulmonary fibrosis treated with 2 ppm for 10 minutes (Yoshida et al. 1997). Pulmonary vascular resistance was also significantly reduced in preterm infants treated with 20 ppm for 2 hours followed by 5 ppm for 70 hours (Subhedar and Shaw 1997), in patients with heart failure treated with up to 80 ppm for 5 minutes (Semigran et al. 1994), in patients following left ventricular assist device implantation treated with 25-40 ppm for up to 48 hours (Wagner et al. 1997), and in patients following lung transplantation treated with 80 ppm for 15 minutes with a decrease to 10 ppm for up to 69 hours (Adatia et al. 1994). Patients with congestive heart failure had increased oxygen uptake and decreased pulmonary hypertension when administered 20 ppm NO during light exercise (no duration given) (Matsumoto et al. 1997) and attenuation of excessive increases in tidal volume, which contribute to exercise-induced hyperventilation, when exposed to 30 ppm for about 20 minutes (Bocchi et al. 1997). Decreased pulmonary artery pressure, increased cardiac output, and/or increased oxygen arterial saturation occurred in infants treated with 20 ppm NO for 4-250 hours (Journois et al. 1994) or 50 ppm for a mean of 41 hours (methemoglobin, 1.4%) (Schulze-Neick et al. 1997) following surgery for congenital heart defects.

NO inhalation of 20 ppm had no effect on PaO_2 during one-lung ventilation in patients undergoing thoracoscopic procedures, although when combined with i.v. almitrine, it limited the decrease of PaO_2 (Moutafis et al. 1997).

2.2.2. Epidemiologic Studies

As a component of air pollution, NO levels have been studied in association with various diseases, however, other pollutants such as NO_2 and O_3 were also involved. In Helsinki, Finland, emergency room admissions due to ischemic cardiac diseases were significantly correlated with nitric oxide and ozone levels. NO levels ranged from 7-467 $\mu g/m^3$ (5.6-373.6 ppb) during the three year study (Pönkä and Virtanen 1996). In Copenhagen, Denmark, NO and NO_X (NO + NO_2) were significantly associated with the number of emergency medical contacts for children who had respiratory illnesses. The yearly mean concentration of NO was 229 $\mu g/m^3$ (183.2 ppb) and higher NO concentrations correlated with higher NO_X concentrations which were linked to traffic pollution (Keiding et al. 1995). In contrast, no relationship was found between exposure to oxides of nitrogen and respiratory symptoms or decline in FEV_1 among British coal miners exposed to peak NO concentrations in the range of 4-100 ppm (Robertson et al. 1984).

2.2.3. Experimental Studies

Seven male and five female healthy volunteers were exposed to 40 ppm NO through a tight facial mask for 2 hours (Luhr et al. 1998). Concentrations of nitrogen dioxide were closely monitored and did not exceed 2.3 ppm. No changes in blood pressures, heart rates, or peripheral oxygen saturation were noted during exposure. Mean methemoglobin concentration increased from 0.63% to 1.13% during inhalation of NO.

NO was administered by inhalation at 80 ppm for 10 minutes to four groups of volunteers: healthy adults, adults with hyperreactive airways during provocation with methacholine, patients with bronchial asthma, and patients with chronic obstructive pulmonary disease. Bronchodilatory effects were measured as changes in specific airway conductance. No unusual smell, taste, or discomfort was noted and no individual reacted with bronchoconstriction during NO exposure. NO did not affect airway conductance in healthy adults or in patients with pulmonary disease. However, NO inhalation modulated the methacholine-induced bronchoconstriction toward bronchodilation in individuals with hyperreactive airways and increased airway conductance in patients with asthma (Högman et al. 1993a).

Ten healthy volunteers, 8 patients with pulmonary hypertension, and 10 cardiac patients were exposed to 40 ppm NO for 5 minutes (Pepke-Zaba et al. 1991). No clinical signs of toxicity were reported by any individual. Pulmonary vascular resistance was significantly reduced in patients with pulmonary hypertension and in cardiac patients; this was not measured in the healthy volunteers. Systemic vascular resistance was not affected in any patient or volunteer. Methemoglobin levels in the volunteer group rose from 0.33% with air to 0.42% after NO.

Eight healthy adult male volunteers were exposed to 1 ppm NO for 2 hours while performing intermittent light exercise consisting of pedaling a stationary bicycle for 15 minutes of every half hour (Kagawa 1982). Pulmonary function tests were performed after 1 and 2 hours of exposure and after 1 hour of postexposure recovery. No clinical symptoms in any volunteer were associated with exposure. A small but significant ($p \leq 0.05$) decrease in airway conductance was observed in 4/8 individuals during NO exposure that resolved in all but 2 subjects 1 hour postexposure; no significant difference in the group mean occurred. As a group, a significant reduction in the percentage increase of maximal expiratory flow at 50% of forced vital capacity while breathing a He-O_2 mixture was noted at the end of the exposure period. However, since this reduction was not accompanied by a reduction in forced vital capacity or an increase in the alveolar plateau slope, the author questioned its biological relevance. In a similar study, respiratory resistance was significantly increased (10-12%) in healthy adults and smokers exposed to $\geq$20 ppm for 15 minutes (von Nieding et al. 1973).

In another report, specific airway conductance was significantly ($p \leq 0.05$) increased in healthy men exposed to 80 ppm NO for 4 minutes following methacholine-induced bronchoconstriction (Sanna et al. 1994). The bronchodilator action of NO described in the second report is consistent with experiments in rabbits and guinea pigs summarized below.

Pulmonary vasoconstriction was induced in one healthy male volunteer by inhalation of a hypoxic gas mixture (Dupuy et al. 1995). NO was then administered at 10, 20, and 80 ppm for 15-minute intervals. NO induced a dose-dependent, rapid, consistent, and reversible decrease in pulmonary artery pressure, but no distress, discomfort, or pain were noted from exposure. In a similar experiment, healthy volunteers breathed a 12% O_2 atmosphere to induce hypoxic pulmonary vasoconstriction. Addition of 40 ppm NO to the inspired gas resulted in a decrease of pulmonary artery pressure to baseline levels within 10 minutes (Frostell et al. 1993).

In several studies inhaled NO has been shown to affect bleeding times or platelet aggregation although adverse clinical effects have not been demonstrated. The bleeding-time ratio increased to 1.33 in six healthy volunteers exposed to 30 ppm for 15 minutes, but returned to near normal 60 minutes after exposure (Högman et al. 1993b). Platelet aggregation was inhibited after 4 hours in mechanically ventilated neonates treated with 2-80 ppm NO for hypoxic respiratory failure (Cheung et al. 1998). Cardiopulmonary bypass surgery in children with congenital heart defects resulted in a decrease in platelet numbers by 50%; with the therapeutic use of 20 ppm NO after surgery (duration not specified), platelet numbers decreased by 70%. However, no prolonged bleeding after withdrawal of indwelling catheters or drainage tubes was detected in those patients treated with NO (Breuer et al. 1998).

NO had no effect on left ventricular function in normal healthy adults exposed to 20 ppm for 10 minutes and no increase in methemoglobin levels was found (Hayward et al. 1997).

2.3. Developmental/Reproductive Toxicity

No information was found regarding the developmental or reproductive toxicity of nitric oxide in humans.

2.4. Genotoxicity

No increase in chromosome aberrations was found in human peripheral blood lymphocytes following a 2-hour exposure to 40 ppm NO (Luhr et al. 1998). No other information was found regarding the genotoxicity of nitric oxide in humans.

2.5. Carcinogenicity

No information was found regarding the carcinogenicity of nitric oxide in humans.

2.6. Summary

NO has been used extensively in adults and children to lower pulmonary vascular resistance caused by acute respiratory distress syndrome, hypoxemic respiratory failure, persistent pulmonary hypertension, other heart or lung disease, and organ transplantation. The

toxicity of NO is associated with methemoglobin formation and oxidation to nitrogen dioxide. Contamination of anesthesia gases has resulted in one fatality, but exposure concentrations were not measured. Therapeutic levels of 20-80 ppm for 24 hours or 100 ppm for 20 minutes have not resulted in adverse effects among treated patients. However, an infant given 80 ppm for 26 hours developed clinically significant levels of methemoglobin which were rapidly lowered with infusion of methylene blue and reduction of the NO concentration. Effects of NO on the airways are somewhat variable. It appears that NO may have either no effect or cause bronchoconstriction in normal subjects, but result in bronchodilation in individuals with chemically-induced bronchoconstriction or asthma.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Dogs

Greenbaum et al. (1967) exposed dogs to 0.5% NO (5000 ppm) for 25 minutes or to 2% (20,000 ppm) for 7-50 minutes. All dogs died either at the end of exposure or within 16 minutes after exposure. Death was associated with a reduction in arterial oxygen content caused by methemoglobinemia, low arterial Po_2 due to pulmonary edema, and/or acidemia. Concurrent studies were conducted in which dogs were exposed to nitrogen dioxide. No distinction was seen between the effects of either gas and it is probable that the pulmonary effects observed for nitric oxide were actually from the formation of nitrogen dioxide within the system prior to inhalation by the dogs. This is supported by the authors' observation that considerable oxidation to nitrogen dioxide occurred as indicated by the contents of the reservoir bag of the inhalation system which were visibly brown. Further, methemoglobin levels increased as a function of time and concentration of exposure to NO. Administration of methylene blue did not return arterial oxygen content to safe levels in all dogs and the dogs died with methemoglobin levels of 3-5% which could not have contributed to mortality. The authors also stated that the cause of pulmonary edema was the action of nitrogen dioxide on the alveolar lining fluid forming nitric and nitrous acids which, in turn, cause denaturing of proteins, rupture of lysosomes, and the development of chemical pneumonitis.

3.1.2. Rats

To assess acute lung injury caused by inhalation of NO, rats were exposed to 500-1500 ppm for 5-30 minutes (Stavert and Lehnert 1990). By the end of the exposure to 1000 ppm for 30 minutes, the animals were cyanotic and 11/20 died within half an hour of termination of exposure. Deaths were attributed to methemoglobin formation although levels were not measured in this study. Exposures of up to 1500 ppm for 15 minutes or 1000 ppm for 30

minutes produced no increases in lung weight and did not result in any histopathological changes in the lungs.

Groups of 5 rats/sex were exposed for 6 hours by nose-only inhalation to 0, 80, 200, 300, 400, or 500 ppm NO (Waters et al. 1998). Concentrations of ≥300 ppm were lethal and methemoglobin levels were significantly elevated at concentrations of ≥200 ppm. No histopathological changes in animals exposed to 200 ppm were observed with light microscopy, but interstitial edema attributed to NO_2 contamination (2.6 ppm) was seen with the electron microscope. Further details of the results or experimental procedures were not available in the abstract.

3.1.3. Mice

In a series of experiments, mice were exposed to "predominantly" NO (Pflesser 1935). Exposure to 350 and 3500 ppm resulted in death of all animals while complete survival occurred following exposure to 310 ppm for up to 8 hours. An 8-hour LC_{50} was given as 320 ppm. Death appeared to be due to methemoglobin formation and at necropsy no evidence of lung injury or pulmonary edema was observed.

3.2. Nonlethal Toxicity

3.2.1. Dogs

Anesthetized beagle dogs (3-4 per group) were exposed to 0, 80, 160, 320, or 640 ppm NO for 6 hours (Mihalko et al. 1998, Wilhelm et al. 1998). One animal in the 640 ppm group died. Decreased arterial oxygen concentrations were measured following exposure to 320 and 640 ppm and increased minute volumes and decreased systemic arterial pressures were observed at 640 ppm. Methemoglobin levels were 3, 6.6, 24, and 78%, respectively. Further details of the results and experimental procedures were not available in the abstracts.

The pulmonary vasodilating effects of NO have been demonstrated in several canine models of lung injury including hypoxia (Channick et al. 1994, Romand et al. 1994), oleic acid-induced injury (Romand et al. 1994, Putensen et al. 1994ab, Zwissler et al. 1995), pulmonary microembolism (Zwissler et al. 1995), cardiac transplant (Chen et al. 1997), and pulmonary shunt (Hopkins et al. 1997). Following lung injury, dogs were given NO at concentrations ranging from 40 to 80 ppm for up to 40 minutes. In all studies, NO significantly decreased pulmonary vascular resistance, decreased pulmonary artery pressure, and/or improved ventilation-perfusion mismatch. Where measured, methemoglobin levels did not exceed 1.1% (Channick et al. 1994, Putensen et al. 1994a, Romand et al. 1994).

3.2.2. Rabbits

NO inhalation has been shown to attenuate the effects of experimentally-induced lung injury in the rabbit. Rabbits were given 20 ppm NO for 6 hours with or without prior endotoxin-induced lung injury. In control animals, NO had no effect on pulmonary artery pressure, mean arterial pressure, heart rate, central venous pressure, or oxygenation. Pulmonary hypertension and deterioration of oxygenation by endotoxin were less pronounced in rabbits receiving NO, but the inflammatory response was not reduced. After 6 hours, methemoglobin levels did not exceed 1.5% (Nishina et al. 1997). In another study of endotoxin-induced lung injury, increased survival occurred in rabbits treated with 10 ppm for 90 minutes (7/7 vs 5/9 controls), but improvement of pulmonary gas exchange was not demonstrated (Uchida et al. 1996).

The influence of inhaled NO on airway responsiveness to acetylcholine (ACH) in normal and hyperresponsive rabbits was investigated (Mensing et al. 1997). Following ACH provocation, animals were treated with 150 or 300 ppm for 5-10 minutes. No effects of NO inhalation were seen at ACH concentrations of ≤2%, however NO significantly reduced airway resistance caused by 4% and 8% ACH. Animals were then made hyperresponsive to ACH by exposure to ammonium persulfate. NO inhalation at 300 ppm significantly decreased the response to ACH to almost the same level as before ammonium persulfate. Similar results were obtained with methacholine-induced bronchoconstriction (Högman et al. 1993c). Rabbits were exposed to increasing concentrations of nebulized methacholine with or without inhalation of 80 ppm NO and airway resistance was measured after 5 minutes. During NO inhalation, there was no significant increase in methacholine-induced airway resistance.

In rabbits exposed to 30 or 300 ppm for 15 minutes bleeding times increased 46% and 72%, respectively, but there were no changes in hematocrit, whole blood or plasma viscosity, erythrocyte aggregation tendency, or erythrocyte deformability (Högman et al. 1993b, 1994).

3.2.3 Pigs

Inhalation of 20, 40, or 80 ppm NO for 5 minutes by healthy pigs resulted in slight, but significant ($p = 0.04$), reductions in pulmonary artery pressure (Goldstein et al. 1997). The effects of inhaled NO have also been studied in porcine models of adult and neonatal pulmonary hypertension. Dose-related decreases in pulmonary artery pressure and input resistance and increases in vascular efficiency have been observed in adult pigs administered 10-80 ppm for up to 20 minutes following vasoconstriction induced by hypoxia (Hillman et al. 1997), thromboxane administration (Goldstein et al. 1997), and oleic acid administration (Shah et al. 1994). No effects on cardiac output, systemic arterial pressure, or left ventricular contractility were observed in any study. Inhalation of 40 ppm for 30 minutes by pigs with oleic acid induced lung injury resulted in sustained improvements in pulmonary artery pressure, oxygen partial pressure, and intrapulmonary shunt fraction which deteriorated to control levels following termination of NO inhalation (Shah et al. 1994). NO inhalation did not cause histopathologic changes in the lungs and methemoglobin levels were 1.7% following exposure to 80 ppm (Shah et al. 1994).

The effects of inhaled NO were studied in a porcine model of neonatal pulmonary hypertension (Nelin et al. 1994). Pigs, approximately 13 days old, were administered room air, 25 ppm NO, hypoxia, or hypoxia plus 25 ppm NO for 15 minutes each. NO inhalation significantly reduced pulmonary artery pressure both alone and following hypoxia with no changes in dynamic lung compliance, pulmonary resistance, hemoglobin, hematocrit, or methemoglobin. At the end of the experiment, 1000 ppm NO was administered to one animal for 15 minutes which resulted in methemoglobin levels of 20%.

3.2.4. Sheep

Frostell et al. (1991) examined the effects of inhalation of 5-80 ppm NO on the normal and acutely constricted pulmonary circulation in awake lambs. Dose-response data were collected for a 6-minute inhalation and toxicity data were collected after 1 and 3 hours. Pulmonary constriction was induced by either infusion of the endoperoxide analoge of thromboxane, U46619, or by hypoxia. In normal lambs, 80 ppm for 6 minutes did not affect either pulmonary artery pressure or pulmonary vascular resistance. However, in lambs with constricted pulmonary circulation, a dose-related increase in vasodilation occurred with significantly reduced pulmonary artery pressure at 5 ppm and an almost complete vasodilator response at 40 and 80 ppm. Systemic vasodilation did not occur. Inhalation of 80 ppm for 1 and 3 hours did not increase extravascular lung water or methemoglobin levels, or modify lung histology compared with control lambs.

Decreased pulmonary artery pressure has also been demonstrated in several other ovine models of experimental pulmonary hypertension. The therapeutic effects of NO described by Frostell et al. (1991) were confirmed in another study (DeMarco et al. 1996) in which 80 ppm for 3 hours completely reversed U46619-induced pulmonary hypertension without affecting systemic circulation. In this study, maximum methemoglobin levels of 4.7% occurred in the last half hour. A similar dose-dependent reduction in pulmonary artery pressure was shown over a range of concentrations of 4-512 ppm NO with maximum effect at 64 ppm within 5-10 minutes. Inhalation of 512 ppm for 20 minutes resulted in methemoglobin levels of 11% (Dyar et al. 1993). In newborn lambs with persistent pulmonary hypertension, significantly increased survival occurred in lambs treated with 80 ppm for 23 hours; no evidence of lung injury from NO inhalation was observed. Arterial oxygen tension in the NO treated lambs was significantly greater (63 vs 14 mm Hg) within 15 minutes and continued to increase over time. At the end of the study, methemoglobin levels were 3% (Zayek et al. 1993).

Decreased pulmonary artery pressure and increased arterial oxygenation occurred in sheep treated with 20 ppm for 48 hours following lung injury from smoke inhalation, but airway inflammation was not reduced (Ogura et al. 1994). In premature lambs with hyaline membrane disease, exposure to 20 ppm NO for 5 hours did not significantly change oxidative stress parameters or induce lung inflammation (Storme et al. 1998).

3.2.5. Guinea Pigs

Pulmonary resistance was significantly decreased in guinea pigs exposed to 300 ppm for 6 minutes. In the same study, 5-300 ppm for 10 minutes resulted in a dose-related, rapid, consistent, and reversible reduction of pulmonary resistance and an increase in lung compliance following methacholine-induced bronchoconstriction (Dupuy et al. 1992).

3.2.6. Rats

The effects of NO on discrimination learning and brain activity were studied in rats (Groll-Knapp et al. 1988). Rats were exposed to 10 or 50 ppm for 180 minutes and the test atmospheres maintained during behavioral testing and EEG examination. The high concentration significantly reduced the number of correct trials and the total number of lever presses in the operant conditioning chamber. Both concentrations resulted in increased amplitudes and prolonged peak latencies of the auditory evoked potentials assessed on EEG. Maximum methemoglobin levels were 3.98%. The authors suggested that the effects could be due, in part, to diminished oxygen carrying capacity related to methemoglobin formation.

The effects of inhaled NO on hyperoxic lung injury in rats were investigated (Garat et al. 1997). Animals were exposed to 10 or 100 ppm NO while breathing 21% or 100% oxygen for 40 hours. No toxic effects on any lung parameter were observed at either NO concentration under normoxic conditions. In hyperoxic conditions, inhalation of 10 ppm prevented increases in thiobarbituric acid reactive substances and wet to dry lung weight ratios, had no effect on the alveolar barrier impermeability to protein, and improved alveolar liquid clearance. These effects did not occur at 100 ppm NO with hyperoxia and the lack of protection may have been due to the formation of nitrogen dioxide in the exposure chambers.

Rats were exposed to an atmosphere of oxides of nitrogen that was produced by mixing nitrogen dioxide and nitric oxide (Brown et al. 1983). The ratio of each chemical was not specified or measured in the exposure chambers. Exposures were to 518 ppm for 5 minutes or to 1435 ppm for 1 minute. No clinical signs of toxicity were observed during exposure to either concentration but “stertorous respirations” appeared within 24 hours. Histologically, initial lung damage showed thickening and blebbing of the alveolar epithelium followed by a latent period of about 6 hours after which development of edema of the interstitium and alveolar septum was observed. The early changes were attributed to a direct oxidant effect. Both clinical signs and histiological findings were more severe following exposure to 518 ppm for 5 minutes.

3.3. Developmental/Reproductive Toxicity

No information was found regarding the developmental or reproductive toxicity of exogenously administered nitric oxide in animals. Growth retardation and hind limb reduction

were found in the offspring of rats given 0.3 and 1.0 mg/mL of N^G-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, in the drinking water on gestation days 13-19 (Shepard 1995).

3.4. Genotoxicity

Three-week old male Sprague-Dawley rats were exposed by inhalation to 9, 19, or 27 ppm NO for 3 hours, maintained overnight before sacrifice, and lung cells were isolated. At the high dose, there was a significant increase in mutations to ouabain resistance in lung cells. Chromosome aberrations were not observed following exposure to NO but were induced by exposure to NO_2 (Isomura et al. 1984).

A dose-related increase in the number of revertants of *Salmonella typhimurium* (TA1535) occurred when culture dishes were exposed to atmospheres containing 0-20 ppm nitric oxide for 30 minutes. Oxygen was required and mutation was inhibited by antioxidants. Cytotoxicity was seen at 50 ppm nitric oxide (Arroyo et al. 1992).

3.5. Carcinogenicity

No information was found regarding the carcinogenicity of nitric oxide in animals.

3.6. Summary

Most of the experimental animal studies available focused on the therapeutic use of NO in an animal model of human disease. Lethality studies in dogs, rats, and mice lacked complete concentration-response information, were confounded by possible NO_2 contamination, or were secondary citations in which the original source could not be obtained. From these studies, however, it appears that in the absence of lung injury, the mechanism of toxicity of NO is methemoglobin formation.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Approximately 85-92% of NO is absorbed into the body by humans breathing normally during inhalation exposure to 0.33-5.0 ppm (0.4-6.1 mg/m^3) (Yoshida and Kasama 1987). In contrast, about 35% of the total amount of NO delivered is taken up by the lungs in patients with acute lung injury given 5-40 ppm NO as ongoing therapy (Westfelt et al. 1997). Once absorbed, inhaled NO reacts with hemoglobin to form nitrosylhemoglobin from which nitrite and nitrate are generated. Most of the nitrates are excreted in the urine with a small portion secreted into the oral cavity through the salivary glands and transformed to nitrite. Nitrate in the intestine is reduced to ammonia through nitrite, reabsorbed into the body, and converted to urea (Yoshida and Kasama 1987). Pigs given 10-80 ppm sequentially for 10-minute periods followed by 40

ppm for 30 minutes, showed a concentration-related increase in plasma nitrites and nitrates with a combined concentration of 67 μmol/L at the end of exposure compared to a baseline of 30 μmol/L (Shah et al. 1994). A high ^{15}N content was found in serum and urine of rats after inhalation of 138-880 ppm ^{15}NO, and within 24 hours, about 40% of the inhaled ^{15}N was excreted into the urine. Small amounts of ^{15}N were found in lung, trachea, liver, kidney, and muscle (Yoshida et al. 1980).

Nitrate (10.4 μmol/L) has been detected in the broncho-alveolar lavage fluid of healthy children from the metabolism of endogenous NO in the lower respiratory tract (Grasemann et al. 1997).

4.2. Mechanism of Toxicity

From the available studies, it appears that the major mechanism of toxic action of NO is the binding of hemoglobin (U.S. EPA 1993b). Inhaled NO is absorbed into the bloodstream and binds to hemoglobin forming nitrosylhemoglobin which is rapidly oxidized to methemoglobin (Maeda et al. 1987, Sharrock et al. 1984, U.S. EPA 1993b). The affinity of NO for hemoglobin is about 1500 times greater than that of carbon monoxide (Gibson and Roughton 1957) and the binding and formation of methemoglobin is NO concentration- and time-dependent (Maeda et al. 1987, Ripple et al. 1989, Sharrock et al. 1984). Experiments with rats (Maeda et al. 1987) and rabbits (Sharrock et al. 1984) show that NO binding to hemoglobin is rapidly reversible with a half-life of 15-20 minutes when the animals are placed in clean air.

The signs and symptoms of methemoglobinemia in humans are summarized in Table 2. As can be seen, clinical signs do not appear until methemoglobin levels are 15-20% and toxicity is not evident until levels of about 30%.

TABLE 2. SIGNS AND SYMPTOMS IN HUMANS ASSOCIATED WITH METHEMOGLOBIN CONCENTRATIONS

Methemoglobin Concentration (%)	Signs and Symptoms
1.1	Normal level
1-15	None
15-20	Clinical cyanosis (chocolate brown blood); no hypoxic symptoms
30	Fatigue; recovery without treatment
20-45	Anxiety, exertional dyspnea, weakness, fatigue, dizziness, lethargy, headache, syncope, tachycardia
45-55	Decreased level of consciousness
55-70, ~60	Hypoxic symptoms: semistupor, lethargy, seizures, coma, bradycardia, cardiac arrhythmias
>70	Heart failure from hypoxia; high incidence of mortality
>85	Lethal

Sources: Kiese 1974, Seger 1992

In most of the human and animal experimental studies and the human case reports described previously, methemoglobin levels were <5% even after exposure to as much as 50 ppm NO for 41 hours (human infant) or 80 ppm for 23 hours (lamb). Methemoglobin levels rose to 9.4% in one lung transplantation patient after treatment with 80 ppm NO for 8 hours. A reduction in concentration to 40 ppm over 4 hours resulted in a decrease to 6.6%, and a further reduction to 20 ppm for the next 12 hours reduced methemoglobin levels to 0.9% (Adatia et al. 1994). In one patient with pulmonary hypertension, methemoglobin levels rose to 9.6% after 108 hours of treatment with 80 ppm and in another patient levels were 14% after 18 hours (Wessel et al. 1994). An American Indian patient with pulmonary hypertension treated with 80 ppm for 6 hours developed levels of 9.4% which decreased rapidly with a reduction in NO to 40 ppm (Wessel et al. 1994). Methemoglobinemia >7% occurred in 13/37 newborns treated with 80 ppm NO for persistent pulmonary hypertension. The average time to peak level in all patients

was 19.6 hours and the highest level of methemoglobin was 11.9% at 8 hours in one patient (Davidson et al. 1998).

Despite the relatively low levels of methemoglobin measured in most studies, clinically significant levels have been reported. A newborn (Japanese) developed a methemoglobin level of 40% after 26 hours of exposure to 80 ppm; the level reduced to 3.9% within 20 minutes of infusion with methylene blue and reduction of the NO concentration (Nakajima et al. 1997). Sheep administered 512 ppm for 20 minutes (Dyar et al. 1993) and pigs given 1000 ppm for 15 minutes (Nelin et al. 1994) developed levels of 11% and 20%, respectively. Cyanosis appeared in dogs within 3-8 minutes of exposure to 0.5% or 2% NO (5000 ppm or 20,000 ppm) and methemoglobin levels were 5-25%. However, levels reached 100% in one dog that died after exposure to 2% (20,000 ppm) for 50 minutes (Greenbaum et al. 1967). A single 6-hour exposure of dogs to 80, 160, 320, or 640 ppm resulted in methemoglobin levels of 3, 6.6, 24, and 78%, respectively (Wilhelm et al. 1998). Rats exposed to 1000 ppm NO for 30 minutes appeared cyanotic and 11/20 died due to methemoglobin formation but levels were not measured (Stavert and Lehnert 1990).

While the main toxicological effect of inhaled NO is the induction of methemoglobin, that of NO_2 is the formation of pulmonary edema. Methemoglobin levels did not increase in rats exposed to 40 ppm NO_2 despite a slight elevation (0.2%) in nitrosylhemoglobin levels (Oda et al. 1980). Rats exposed to 1000 ppm NO for 30 minutes appeared cyanotic and 11/20 died due to methemoglobin formation but no changes in lung weight or histopathology were observed. In the same study, increased lung weight occurred following exposure to 50 ppm NO_2 for 30 minutes and histopathological changes were observed after exposure to 25 ppm for 30 minutes (Stavert and Lehnert 1990). Other studies have failed to show any effect of NO on the respiratory tract of humans (Manktelow et al. 1997, Högman et al. 1993a, Pepke-Zaba et al. 1991, Kagawa 1982), mice (Pflesser 1935), pigs (Nelin et al. 1994), or lambs (Frostell et al. 1991). A concentration of 10 ppm, but not 100 ppm, offered protection against hyperoxic lung injury in rats and it is likely that the higher concentration of NO resulted in significant NO_2 formation (Garat et al. 1997). NIOSH (1976), summarized the effects of NO_2 in humans as initial irritation with mild dyspnea during exposure followed by delayed onset of pulmonary edema after several hours of apparent recovery. A similar toxic response, including interstitial fibrosis, has been shown in five species of animals following acute inhalation exposure to NO_2 (Hine et al. 1970) and in rats exposed to mixed oxides of nitrogen (Brown et al. 1983). These results indicate that NO_2 has a direct toxic action on the respiratory tract, but NO does not.

The relative toxicities of NO and NO_2 are complex. NIOSH (1976) summarized experiments by Paribok and Grokholskaya (1962) in mice and guinea pigs. At concentrations >833 ppm for 1 hour, NO was more toxic than NO_2, however, at lower concentrations, NO_2 was more toxic. It appears that for NO, if the concentration is not high enough to be lethal due to methemoglobin formation, the animal recovers completely. On the other hand, concentrations of

NO_2 that are not rapidly lethal may cause more persistent effects and in some cases cause death from pulmonary edema after a delay of several days (NIOSH 1976).

4.3. Oxides of Nitrogen

NO is unstable in air and undergoes spontaneous oxidation to NO_2 making experimental effects difficult to separate and studies difficult to perform (U.S. EPA 1993b). Studies on the conversion of NO to NO_2 in medicinal applications have found the conversion to be significant in an atmospheric concentration of O_2 (20.9%) at room temperature. The delivery of 100 ppm NO in 21% O_2 through a pediatric tube (d=0.009m l=0.9m) at a flow rate of 2 L/min is calculated to produce 1.13 ppm NO_2 (Lindberg and Rydgren 1998). For 80 ppm NO, a concentration commonly used therapeutically, 5 ppm NO_2 is calculated to form by 3 minutes in air (Foubert et al. 1992). NO reacts quantitatively with oxygen in air to form NO_2 which then reacts with water to form nitric acid (NIOSH 1976). For this reason, careful monitoring of NO_2 concentrations has been suggested when NO is used therapeutically at concentrations $\geq$80 ppm especially when coadministered with oxygen (Miller et al. 1994, Foubert et al. 1992). Figure 1 summarizes the reactions of the oxides of nitrogen.

$$2NO + O_2 \rightarrow 2NO_2 \text{ (minor at ambient temp)}$$

$$NO + O_3 \rightarrow NO_2 + O_2$$

$$NO + HO_2\cdot \rightarrow NO_2 + HO\cdot$$

$$NO + RO_2\cdot \rightarrow NO_2 + RO\cdot$$

$$NO_2 + HO\cdot \rightarrow HNO_3$$

$$2NO_2 \rightarrow N_2O_4$$

Figure 1: Environmental reactions of the oxides of nitrogen

This family of reaction paths is temperature dependent, but in general favors NO_2 production. While on the laboratory scale, closed system experiments clearly indicate the potential for the production of NO_2, the chemical kinetics of NO conversion during a large-scale atmospheric release and dispersion are not well documented. The estimation of the concentration isopleths following an accidental release would require the use of a finite element model along with several assumptions as to the chemical rate constants. As a result, the conversion of NO to NO_2 during the atmospheric release is of concern to emergency planners, but the NAC is unable to provide any significant guidance, other than to indicate that a significant fraction of the NO will be converted to NO_2 and present an atmospheric hazard that is generally more toxic than just NO.

4.4. Other Relevant Information

No information was available to allow comparison of NO toxicity between species or between individuals. Concentrations used in animal models of human diseases are similar to those used therapeutically in humans with no adverse effects. Because the major toxic action of NO is binding to hemoglobin resulting in methemoglobinemia, little inter- or intra-species variation is expected. In addition, NO is administered for extended periods of time to critically ill patients with only slight increases in methemoglobin concentrations.

5. DATA ANALYSIS FOR AEGL-1

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

5.1. Summary of Human Data Relevant to AEGL-1

Human data relevant to derivation of AEGL-1 are limited to case reports of either slightly increased methemoglobin levels or no adverse effects during NO administration to therapeutically lower pulmonary artery pressure. Methemoglobin levels rose to 9.4% in one lung transplantation patient after treatment with 80 ppm NO for 8 hours. A reduction in concentration to 40 ppm over 4 hours resulted in a decrease to 6.6%, and a further reduction to 20 ppm for the next 12 hours reduced methemoglobin levels to 0.9% (Adatia et al. 1994). In one patient with pulmonary hypertension methemoglobin levels rose to 9.6% after 108 hours of treatment with 80 ppm and in another patient levels were 14% after 18 hours (Wessel et al. 1994). An American Indian patient with pulmonary hypertension treated with 80 ppm for 6 hours developed methemoglobin levels of 9.4% which decreased rapidly with a reduction in NO to 40 ppm (Wessel et al. 1994). The average time to peak methemoglobin levels in newborns

treated with 80 ppm was 19.6 hours and the highest level of methemoglobin was 11.9% at 8 hours in one patient (Davidson et al. 1998).

No adverse toxicity was reported for newborns and children diagnosed with hypoxemic respiratory failure (Abman et al. 1994, Day et al. 1997, Goldman et al. 1997) or persistent pulmonary hypertension (Goldman et al. 1995, Ichida et al. 1997, Kinsella et al. 1997, Nakagawa et al. 1997, Wessel et al. 1997) when treated with 10-80 ppm for 10 minutes to 24 hours. Methemoglobin levels were not reported in these cases.

5.2. Summary of Animal Data Relevant to AEGL-1

Animal data relevant to derivation of AEGL-1 were limited to one abstract. A single 6-hour exposure of dogs to 80, 160, 320, or 640 ppm resulted in methemoglobin levels of 3, 6.6, 24, and 78%, respectively (Wilhelm et al., 1998). Most experimental animal studies were either on lethality or as models of human disease in which the therapeutic effects of NO were assessed.

5.3. Derivation of AEGL-1

AEGL-1 values were not derived. The NAC recommends the use of the AEGL values for NO_2 for emergency planning. The proposed AEGL values for NO_2 are given in Appendix A. In several human case reports a concentration of 80 ppm for 6-108 hours resulted in methemoglobin levels of approximately 10% without adverse toxicity (Adatia et al. 1994, Wessel et al. 1994). Therefore, concentrations below 80 ppm NO should not constitute a health hazard.

6. DATA ANALYSIS FOR AEGL-2

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

6.1. Summary of Human Data Relevant to AEGL-2

Human data relevant to AEGL-2 were not found.

6.2. Summary of Animal Data Relevant to AEGL-2

Animal data relevant to AEGL-2 were not found.

6.3. Derivation of AEGL-2

AEGL-2 levels were not derived because no human or animal data were available upon which to base the calculations. The NAC recommends the use of the AEGL values for NO_2 for emergency planning. The proposed AEGL values for NO_2 are given in Appendix A.

7. DATA ANALYSIS FOR AEGL-3

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

7.1. Summary of Human Data Relevant to AEGL-3

The one report of human fatality due to NO contamination of anesthetic gases does not contain concentration or duration information. However, an infant developed a methemoglobin level of 40% after 26 hours of exposure to 80 ppm (Nakajima et al., 1997). This level is clearly life-threatening in a newborn.

7.2. Summary of Animal Data Relevant to AEGL-3

Animal data relevant to derivation of AEGL-3 were not found. Lethality studies in dogs (Greenbaum et al. 1967), rats (Stavert and Lehnert 1990), and mice (Pflesser 1935) lacked complete dose-response information, were confounded by possible NO_2 contamination, or were secondary citations in which the original source could not be obtained. An approximate 30-minute LC_{50} of 1000 ppm was reported by Stavert and Lehnert (1990) at which 11/20 rats died. These studies were valuable, however, in determining the mechanism of toxicity of NO, i.e., methemoglobin formation in the absence of lung injury. Sheep developed methemoglobin levels of only 11% after exposure to 512 ppm for 20 minutes (Dyar et al. 1993) and pigs developed levels of 20% after exposure to 1000 ppm for 15 minutes (Nelin et al. 1994), although these levels are not life-threatening.

7.3. Derivation of AEGL-3

Data are insufficient for derivation of AEGL-3 values. A methemoglobin level of 40% in an infant is clearly life-threatening, but this level did not occur until after 26 hours of continuous exposure to 80 ppm. The concentration of 80 ppm has been safely used in many clinical applications for durations less than 26 hours, although higher concentrations for shorter durations would be expected to result in critical levels of methemoglobin formation. Lethality studies in animals lacked sufficient concentration-response information to calculate either an LC_{01} value or the concentration of NO which results in formation of critical methemoglobin levels.

Importantly, in the event of an accidental release of NO, attention should be given to the formation of NO_2 in the atmosphere. Careful monitoring of the concentrations of both NO and NO_2 is warranted. Therefore, AEGL-3 values are not proposed because of conflicting data and lack of sound toxicity information. The NAC recommends the use of the AEGL values for NO_2 for emergency planning. The proposed AEGL values for NO_2 are given in Appendix A.

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity Endpoints

AEGL values for NO are not recommended. Because conversion to NO_2 is expected to occur in the atmosphere, and because NO_2 is more toxic than NO, the AEGL values for NO_2 are recommended for use with emergency planning for NO (Appendix A). The NAC recognizes, however, that short-term exposures below 80 ppm NO should not constitute a health hazard.

8.2. Comparison with Other Standards and Guidelines

Standards and guidance levels for workplace and community exposures are listed in Table 3. An occupational TWA of 25 ppm has been adopted by several groups (ACGIH 2003, NIOSH 2003, OSHA 1999). International standards are also 25 ppm for a workday (Ministry of Social Affairs and Employment 2000, Swedish National Board of Occupational Safety and Health 1996). In addition, Sweden has adopted 50 ppm as a short-term exposure limit and the immediate danger to life and health (IDLH) of 100 ppm (NIOSH 1996) is based on human and animal data for oxides of nitrogen due to lack of useful data on nitric oxide.

TABLE 3. Extant Standards and Guidelines for Nitric Oxide

Guideline	Exposure Duration				
	10 minute	30 minute	1 hour	4 hour	8 hour
AEGL-1	NR	NR	NR	NR	NR
AEGL-2	NR	NR	NR	NR	NR
AEGL-3	NR	NR	NR	NR	NR
IDLH (NIOSH)[a]		100 ppm			
REL-TWA (NIOSH)[b]					25 ppm
PEL-TWA (OSHA)[c]					25 ppm
TLV-TWA (ACGIH)[d]					25 ppm
MAC (The Netherlands)[e]					25 ppm
OEL-LLV (Sweden)[f]					25 ppm
OEL-CLV (Sweden)[g]	50 ppm				

NR: not recommended

[a]**IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH 1996) represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects.

[b]**NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average)** (NIOSH 2003) is defined analogous to the ACGIH-TLV-TWA.

[c]**OSHA PEL-TWA (Occupational Health and Safety Administration, Permissible Exposure Limits - Ceiling)** (OSHA 1999) is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[d]**ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** (ACGIH 2003) is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

[e]**MAC (Maximaal Aanvaaarde Concentratie [Maximal Accepted Concentration])** (SDU Uitgevers [under the auspices of the Ministry of Social Affairs and Employment], The Hague, The Netherlands 2000) is defined analogous to the ACGIH-TLV-TWA.

[f]**OEL-LLV (Occupational Exposure Limits - Level Limit Value)** (Swedish National Board of Occupational Safety and Health, 1996) is an occupational exposure limit value for exposure during one working day.

[g]**OEL-CLV (Occupational Exposure Limits - Ceiling Limit Value)** (Swedish National Board of Occupational Safety and Health, 1996) is an occupational exposure limit value for exposure during a reference period of fifteen minutes.

8.3. Data Adequacy and Research Needs

Data were not available for derivation of AEGL values. The available studies did not contain full concentration-response information, and many did not account for conversion of NO to NO_2. NO_2 is more toxic than NO and the rate of conversion in the event of an accident is unknown.

9. REFERENCES

Abman, S.H., J.L. Griebel, D.K. Parker, J.M. Schmidt, D. Swanton, and J.P. Kinsella. 1994. Acute effects of inhaled nitric oxide in children with severe hypoxemic respiratory failure. J. Pediatr. 124:881-888.

Adatia, I., C. Lillehei, J.H. Arnold, J.E. Thompson, R. Palazzo, J.C. Fackler, and D.L. Wessel. 1994. Inhaled nitric oxide in the treatment of postoperative graft dysfunction after lung transplantation. Ann. Thorac. Surg. 57:1311-1318.

Arroyo, P.L., V. Hatch-Pigott, H.F. Mower, and R.V. Cooney. 1992. Mutagenicity of nitric oxide and its inhibition by antioxidants. Mut. Res. 281:193-202.

ACGIH (American Conference of Governmental Industrial Hygienists, Inc.). 1991. Nitric Oxide. In: Documentation of the Threshold Limit Values and Biological Exposure Indicies, 6th ed., ACGIH, Cincinnati, OH, pp. 1090-1092.

ACGIH (American Conference of Governmental Industrial Hygienists, Inc.). 2003. TLVs® and BEIs® Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indicies, ACGIH, Cincinnati, OH, p. 43.

Benzing, A., G. Mols, U. Beyer, and K. Geiger. 1997. Large increase in cardiac output in a patient with ARDS and acute right heart failure during inhalation of nitric oxide. Acta Anaesthesiol. Scand. 41:643-646.

Benzing, A., G. Mols, J. Guttmann, H. Kaltofen, and K. Geiger. 1998. Effect of different doses of inhaled nitric oxide on pulmonary capillary pressure and on longitudinal distribution of pulmonary vascular resistance in ARDS. Br. J. Anaesth. 80:440-446.

Biban, P., D. Trevisanuto, A. Pettenazzo, P. Ferrarese, E. Baraldi, and F. Zacchello. 1998. Inhaled nitric oxide in hypoxaemic newborns who are candidates for extracorporeal life support. Eur. Respir. J. 11:371-376.

Bocchi, E.A., J.O. Auler, G.V. Guimarâes, M.J. Carmona, M. Wajngarten, G. Bellotti, and F. Pileggi. 1997. Nitric oxide inhalation reduces pulmonary tidal volume during exercise in severe chronic heart failure. Am. Heart J. 134:737-744.

Breuer, J., G. Leube, P. Mayer, S. Gebhardt, L. Sieverding, L. Häberle, M. Heinemann, and J. Apitz. 1998. Effects of cardiopulmonary bypass and inhaled nitric oxide on platelets in children with congenital heart defects. Eur. J. Pediatr. 157:194-201.

Brown, R.F.R., W.E. Clifford, T.C. Marrs, and R.A. Cox. 1983. The histopathology of rat lung following short term exposures to mixed oxides of nitrogen (NO_X). Br. J. Exp. Path. 64:579-593.

Budavari, S., M.J. O'Neil, A. Smith, P.E. Heckelman, and J.F. Kinneary (Eds.). 1996. The Merck Index, 11th ed. Rahway, NJ:Merck and Co., Inc., p. 1041.

Channick, R.N., J.W. Newhart, F.W. Johnson, and K.M. Moser. 1994. Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction in dogs. A practical nitric oxide delivery and monitoring system. Chest 105:1842-1847.

Chen, E.P., H.B. Bittner, R.D. Davis, and P. Van Trigt. 1997. Effects of nitric oxide after cardiac transplantation in the setting of recipient pulmonary hypertension. Ann. Thorac. Surg. 63:1546-1555.

Cheung, P.Y., E. Salas, P.C. Etches, E. Phillipos, R. Schulz, M.W. Radomski. 1998. Inhaled nitric oxide and inhibition of platelet aggregation in critically ill neonates. Lancet 351:1181-1182.

Clutton-Brock, J. 1967. Two cases of poisoning by contamination of nitrous oxide with higher oxides of nitrogen during anaesthesia. Br. J. Anaesth. 39:338-392

Davidson, D., E.S. Barefield, J. Kattwinkel, G. Dudell, M. Damask, R. Straube, J. Rhines, C.-T. Chang, and the I-NO/PPHN study group. 1998. Inhaled nitric oxide for the early treatment of persistent pulmonary hypertension of the term newborn: a randomized, double-masked, placebo-controlled, dose-response, multicenter study. Pediatrics 101:325-334.

Day, R.W., E.M. Allen, and M.K. Witte. 1997. A randomized, controlled study of the 1-hour and 24-hour effects of inhaled nitric oxide therapy in children with acute hypoxemic respiratory failure. Chest 112:1324-1331.

DeMarco, V., J.W. Skimming, T.M. Ellis, and S. Cassin. 1996. Nitric oxide inhalation: Effects on the ovine neonatal pulmonary and systemic circulations. Reprod. Fertil. Dev. 8:431-438.

Doering, E.B., C.W. Hanson, D.J. Reily, C. Marshall, and B.E. Marshall. 1997. Improvement in oxygenation by phenylephrine and nitric oxide in patients with adult respiratory distress syndrome. Anesthesiology 87:18-25.

Dupuy, P.M., S.A. Shore, J.M. Drazen, C. Frostell, W.A. Hill, and W.M. Zapol. 1992. Bronchodilator action of inhaled nitric oxide in guinea pigs. J. Clin. Invest. 90:421-428.

Dupuy, P.M., J.-P. Lançon, M. Françoise, and C.G. Frostell. 1995. Inhaled cigarette smoke selectively reverses human hypoxic vasoconstriction. Intensive Care Med. 21:941-944.

Dyar, O., J.D. Young, L. Xiong, S. Howell, and E. Johns. 1993. Dose-Response relationship for inhaled nitric oxide in experimental pulmonary hypertension in sheep. Br. J. Anaesth. 71:702-708.

Foubert, L., B. Fleming, R. Latimer, M. Jonas, A. Oduro, C. Borland, and T. Higenbottam. 1992. Safety guidelines for use of nitric oxide. Lancet 339:1615-1616.

Frostell, C., M.D. Fratacci, J.C. Wain, R. Jones, and W.M. Zapol. 1991. Inhaled nitric oxide: A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. Circulation 83:2038-2047.

Frostell, C.G., H. Blomquist, G. Hedenstierna, J. Lundberg, and W.M. Zapol. 1993. Inhaled nitric oxide selectively reverses human hypoxic pulmonary vasoconstriction without causing systemic vasodilation. Anesthesiology 78:427-435.

Fullerton, D.A. and R.C. McIntyre. 1996. Inhaled nitric oxide: therapeutic applications in cardiothoracic surgery. Ann. Thorac. Surg. 61:1865-1864.

Garat, C., C. Jayr, S. Eddahibi, M. Laffon, M. Meignan, and S. Adnot. 1997. Effects of inhaled nitric oxide or inhibition of endogenous nitric oxide formation on hyperoxic lung injury. Am. J. Respir. Crit. Care Med. 155:1957-1964.

Gerlach, H., R. Rossaint, D. Pappert, and K.J. Falke. 1993. Time-course and dose-response of nitric oxide inhalation for systemic oxygenation and pulmonary hypertension in patients with adult respiratory distress syndrome. Eur. J. Clin. Invest. 23:449-502.

Gibson, Q.H. and F.J.W. Roughton. 1957. The kinetics and equilibria of the reactions of nitric oxide with sheep haemoglobin. J. Physiol. 136:507-526.

Goldman, A.P., R.C. Tasker, S. Hosiasson, T. Henrichsen, and D.J. Macrae. 1997. Early response to inhaled nitric oxide and its relationship to outcome in children with severe hypoxemic respiratory failure. Chest 112:752-758.

Goldman, A.P., P.G. Rees, and D.J. Macrae. 1995. Is it time to consider domiciliary nitric oxide? Lancet 345:199-200.

Goldstein, D.J., D.A. Dean, A. Smerling, M.C. Oz, D. Burkoff, and M.L. Dickstein. 1997. Inhaled nitric oxide is not a negative inotropic agent in a porcine model of pulmonary hypertension. J. Thorac. Cardiovasc. Surg. 114:461-466.

Grasemann, H., I. Ioannidis, H. de Groot, and F. Ratjen. 1997. Metabolites of nitric oxide in the lower respiratory tract of children. Eur. J. Pediatr. 156:575-578.

Greenbaum, R., J. Bay, M.D. Hargreaves, M.L. Kain, G.R. Kelman, J.F. Nunn, C. Prys-Roberts, and K. Siebold. 1967. Effects of higher oxides of nitrogen on the anaesthetized dog. Brit. J. Anaesth. 39:393-404.

Groll-Knapp, E., M. Haider, K. Kienzl, A. Handler, and M. Trimmel. 1988. Changes in discrimination learning and brain activity (ERP's) due to combined exposure to NO and CO in rats. Toxicology 49:441-447.

Hayward, C.S., W.V. Kalnins, P. Rogers, M.P. Feneley, P.S. MacDonald, and R.P. Kelly. 1997. Effect of inhaled nitric oxide on normal human left ventricular function. J. Am. Coll. Cardiol. 30:49-56.

Hillman, N.D., I.M. Cheifetz, D.M. Craig, P.K. Smith, R.M. Ungerleider, and J.N. Meliones. 1997. Inhaled nitric oxide, right ventricular efficiency, and pulmonary vascular mechanics: Selective vasodilation of small pulmonary vessels during hypoxic pulmonary vasoconstriction. J. Thoracic. Cardiovasc. Surg. 113:1006-1013.

Hine, C.H., F.H. Meyers, and R.W. Wright. 1970. Pulmonary changes in animals exposed to nitrogen dioxide, effects of acute exposures. Toxicol. Appl. Pharmacol. 16:201-213.

Högman, M., C.G. Frostell, H. Hedenström, and G. Hedenstierna. 1993a. Inhalation of nitric oxide modulates adult human bronchial tone. Am. Rev. Respir. Dis. 148:1474-1478.

Högman, M., C. Frostell, H. Arnberg, and G. Hedenstierna. 1993b. Bleeding time prolongation and NO inhalation. Lancet 341:1664-1665.

Högman, M., C. Frostell, H. Arnberg, and G. Hedenstierna. 1993c. Inhalation of nitric oxide modulates methacholine-induced bronchoconstriction in the rabbit. Eur. Respir. J. 6:177-180.

Högman, M., C. Frostell, H. Arnberg, B. Sandhagen, and G. Hedenstierna. 1994. Prolonged bleeding time during nitric oxide inhalation in the rabbit. Acta Physiol. Scand. 151:125-129.

Hopkins, S.R., E.C. Johnson, R.S. Richardson, H. Wagner, M. De Rosa, and P.D. Wagner. 1997. Effects of inhaled nitric oxide on gas exchange in lungs with shunt or poorly ventilated areas. Am. J. Respir. Crit. Care Med. 156:484-491.

Ichida, F., K. Uses, S. Tsubata I. Hashimoto, Y. Hamamichi, K. Fukahara, A. Murakami, and T. Miyawaki. 1997. Additive effect of beraprost on pulmonary vasodilation by inhaled nitric oxide in children with pulmonary hypertension. Am. J. Cardiol. 80:662-664.

Isomura, K., M. Chikahira, K. Teranishi, and K. Hamada. 1984. Induction of mutations and chromosome aberrations in lung cells following in vivo exposure of rats to nitrogen oxides. Mut. Res. 136:119-125.

Journois, D., P. Pouard, P. Mauriat, T. Malhère, P. Vouhé, and D. Safran. 1994. Inhaled nitric oxide as a therapy for pulmonary hypertension after operations for congenital heart defects. J. Thorac. Cardiovasc. Surg. 107:1129-1135.

Kagawa, J. 1982. Respiratory effects of 2-hr exposure to 1 ppm nitric oxide in normal subjects. Environ. Res. 27:485-490.

Keiding, L., A.K. Rindel, and D. Kronborg. 1995. Respiratory illnesses in children and air pollution in Copenhagen. Arch. Environ. Health 50:200-206.

Kiese, M. 1974. Methemoglobinemia: A comprehensive treatise. CRC Press, Cleveland, Ohio.

Kinsella, J.P., W.E. Truog, W.F. Walsh, R.N. Goldberg, E. Bancalari, D.E. Mayock, G.J. Redding, R.A. deLemos, S. Sardesai, D.C. McCurin, S.G. Moreland, G.R. Cutter, and S.H. Abman. 1997. Randomized, multicenter trial of inhaled nitric oxide and high-frequency oscillatory ventilation in severe, persistent pulmonary hypertension of the newborn. J. Pediatr. 131:55-62.

Lindberg, L. and G. Rydgren. 1998. Production of nitrogen dioxide in a delivery system for inhlation of nitric oxide: a new equation for calculation. Br. J. Anaesth. 80:213-217.

Luhr, O.R., C.G. Frostell, R. Heywood, S. Riley, and P.-A. Lönnqvist. 1998. Induction of chromosome aberrations in peripheral blood lymphocytes after short time inhalation of nitric oxide. Mut. Research 414:107-115.

Maeda, N., K. Imaizumi, K. Kon, and T. Shiga. 1987. A kinetic study on functional impairment of nitric oxide-exposed rat erythrocytes. Environ. Health Persp. 73:171-177.

Manktelow, C., L.M. Bigatello, D. Hess, and W.E. Hurford. 1997. Physiologic determinants of the response to inhaled nitric oxide in patients with acute respiratory distress syndrome. Anesthesiology 87:297-307.

Markhorst, D.G., T. Leenhoven, J.W. Uiterwijk, J. Meulenbelt, and A.J. van Vught. 1996. Occupational exposure during nitric oxide inhalational therapy in a pediatric intensive care setting. Intensive Care Med. 22:954-958.

Matsumoto, A., S. Momomura, Y. Hirata, T. Aoyagi, S. Sugiura, and M. Omata. 1997. Inhaled nitric oxide and exercise capacity in congestive heart failure. Lancet 349:999-1000.

Mensing, T., W. Marek, and X. Baur. 1997. Modulation of airway responsiveness to acetylcholine by nitric oxide in a rabbit model. Lung 175:367-377.

Mihalko, P.J., C.R. Hassler, R.R. Moutvic, T. Vinci, R.L. Hamlin, and S.J. Waters. 1998. Effects of inhaled nitric oxide on cardiovascular and pulmonary function in the dog. Toxicologist 42:250.

Miller, O.I., D.S. Celermajer, J.E. Deanfield, and D.J. Macrae. 1994. Guidelines for the safe administration of inhaled nitric oxide. Arch. Dis. Child. 70:F47-F49.

Moncada, S., R.M.J. Palmer, and E.A. Higgs. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol. Rev. 43:109-142.

Moutafis, M., N. Liu, N. Dalibon, G. Kuhlman, L. Ducros, M.-H. Castelain, and M. Fischler. 1997. The effects of inhaled nitric oxide and its combination with intravenous alitrine on Pao_2 during one-lung ventilation in patients undergoing thoracoscopic procedures. Anesth. Analg. 85:1130-1135.

Nakagawa, T.A., A. Morris, R.J. Gomez, S.J. Johnston, P.T. Sharkey, and A.L. Zaritsky. 1997. Dose response to inhaled nitric oxide in pediatric patients with pulmonary hypertension and acute respiratory distress syndrome. J. Pediatr. 131:62-69.

Nakajima, W., A. Ishida, H. Arai, and G. Takada. 1997. Methaemoglobinaemia after inhalation of nitric oxide in infant with pulmonary hypertension. Lancet 350:1002-1003.

National MAC List. 2000. The Hague, SDU Uitgevers (under the auspices of the Ministry of Social Affairs and Employment) The Netherlands.

Nelin, L.D., J. Moshin, C.J. Thomas, P. Sasidharan, and C.A. Dawson. 1994. The effect of inhaled nitric oxide on the pulmonary circulation of the neonatal pig. Pediatr. Res. 35:20-24.

NIOSH (National Institute for Occupational Safety and Health). 1976. NIOSH criteria for a recommended standard.... occupational exposure to oxides of nitrogen (nitrogen dioxide and nitric oxide). U.S. Department of Health, Education, and Welfare, Washington, D.C., HEW publication No. (NIOSH) 76-149, 195pp.

NIOSH (National Institute for Occupational Safety and Health). 1996. Documentation for Immediately Dangerous to Life or Health Concentrations (IDLHs). NIOSH, Cincinnati, OH. Retrieved on-line 8/8/2003.

NIOSH (National Institute for Occupational Safety and Health). 2003. NIOSH Pocket Guide to Chemical Hazards. NIOSH, Cincinnati, OH. p. 224.

Nishina, K., K. Mikawa, Y. Takao, and H. Obara. 1997. Inhaled nitric oxide does not prevent endotoxin-induced lung injury in rabbits. Acta Anaesthesiol. Scand. 41:399-407.

Oda, H., H. Nogami, and T. Nakajima. 1980. Reaction of hemoglobin with nitric oxide and nitrogen dioxide in mice. J. Toxicol. Environ. Health 6:673-678.

Ogura, H., W.G. Cioffi, B.S. Jordan, C.V. Okerberg, A.A. Johnson, A.D. Mason, and B.A. Pruitt. 1994. The effect of inhaled nitric oxide on smoke inhalation injury in an ovine model. J. Trauma 37:294-302.

OSHA (Occupational Safety and Health Administration). 1999. Table Z-1. Limits for Air Contaminants. 29 CFR (§1910.1000).

Paribok, V.P. and N.V. Grokholskaya. 1962. Comparative study of the toxicity of nitric oxide and nitrogen dioxide. Farmakol. Toksik. 25:741-746. (Russian) Cited in NIOSH, 1976.

Pepke-Zaba, J., T.W. Higenbottam, A.T. Dinh-Xuan, D. Stone, and J. Wallwork. 1991. Inhaled nitric oxide as a cause of selective pulmonary vasodilation in pulmonary hypertension. Lancet 338:1173-1174.

Pflesser, G. 1935. The significance of nitric oxide in poisoning by nitrous gases. Naunyn-Schmiedeberg Arch. Exp. Pathol. Pharmakol. 179:545-547. (German) Cited in NIOSH, 1976.

Pinsky, M.R., F. Genc, K.H. Lee, and E. Delgado. 1997. Contamination of hospital compressed air with nitric oxide. Unwitting replacement therapy. Chest 111:1759-1763.

Pönkä, A. and M. Virtanen. 1996. Low-level air pollution and hospital admissions for cardiac and cerebrovascular diseases in Helsinki. Am. J. Public Health 86:1273-1280.

Putensen, C., J. Räsänen, J.B. Downs, and F.A. Lopez. 1994a. Effect of endogenous and inhaled nitric oxide on the ventilation-perfusion relationships in oleic-acid lung injury. Am. J. Respir. Crit. Care Med. 150:330-336.

Putensen, C., J. Räsänen, F.A. Lopez, and J.B. Downs. 1994b. Continuous positive airway pressure modulates effect of inhaled nitric oxide on the ventilation-perfusion distributions in canine lung injury. Chest 106:1563-1569.

Puybasset, L., J.J. Rouby, E. Mourgeon, T.E. Stewart, P. Cluzel, M. Arthaud, P. Poète, L. Bodin, A.M Korinek, and P. Viars. 1994. Inhaled nitric oxide in acute respiratory failure: dose-response curves. Intensive Care Med. 20:319-327.

Ripple, G., T. Mundie, D.M. Stavert, and B.E. Lehnert. 1989. Kinetics of methemoglobin formation and elimination as a function of inhaled nitric oxide concentration and minute ventilation. Toxicologist 9:754.

Robertson, A., J. Dodgson, P. Collings, and A. Seaton. 1984. Exposure to oxides of nitrogen: respiratory symptoms and lung function in British coal miners. Br. J. Ind. Med. 41:214-219.

Roger, N., J.A. Barberà, J. Roca, I. Rovira, F.P. Gómez, and R. Rodriguez-Roisin. 1997. Nitric oxide inhalation during exercise in chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 156:800-806.

Romand, J.-A., M.R. Pinsky, L. Firestone, H.A. Zar, and J.R. Lancaster. 1994. Effect of inhaled nitric oxide on pulmonary hemodynamics after acute lung injury in dogs. J. Appl. Physiol. 76:1356-1362.

Rossaint, R., T. Busch, and K. Falke. 1996. Nitric oxide inhalation therapy in acute respiratory distress syndrome: intended effects and possible side effects. Meth. Enzymol. 269:442-453.

Sanna, A., A. Kurtansky, C. Veriter, and D. Stânescu. 1994. Bronchodilator effect of inhaled nitric oxide in healthy men. Am. J. Respir. Crit. Care Med. 150:1702-1704.

Schulze-Neick, I., M. Bültmann, H. Verner, A. Gamillscheg, M. Vogel, F. Berger, R. Rossaint, R. Hetzer, and P.E. Lange. 1997. Right ventricular function in patients treated with inhaled nitric oxide after cardiac surgery for congenital heart disease in newborns and children. Am. J. Cardiol. 80:360-363.

Seger, D.L. 1992. Methemoglobin-forming chemicals. In: J.B. Dullivan and G.R. Krieger, Eds., Hazardous Materials Toxicology: Clinical Principals of Environmental Health. Williams & Wilkins Co., Baltimore, pp. 800-806.

Semigran, M.J., B.A. Cockrill, R. Kacmarek, B.T. Thompson, W.M. Zapol, G.W. Dec, and M.A. Fifer. 1994. J. Am. Coll. Cardiol. 24:982-988.

Shah, N.S., D.K. Nakayama, T.D. Jacob, I. Nishio, T. Imai, T.R. Billiar, R. Exler, S.A. Yousem, E.K. Motoyama, and A.B. Peitzman. 1994. Efficacy of inhaled nitric oxide in a porcine model of adult respiratory distress syndrome. Arch. Surg. 129:158-164.

Sharrock, P., P. Levy, and M. Massol. 1984. Blood analysis of rabbits exposed to nitrogen monoxide. Chemosphere 13:959-964.

Shepard, T.H. (Ed.) 1995. Catalog of Teratogenic Agents, 8th ed. Baltimore: The Johns Hopkins University Press, p. 304.

Stavert, D.M. and B.E. Lehnert. 1990. Nitric oxide and nitrogen dioxide as inducers of acute pulmonary injury when inhaled at relatively high concentrations for brief periods. Inhal. Toxicol. 2:53-67.

Storme, L., F. Zerimech, Y. Riou, A. Martin-Ponthieu, L. Devisme, C. Slomianny, S. Klosowski, E. Dewailly, F. Cneude, M. Zandecki, B. Dupuis, and P. Lequien. 1998. Inhaled nitric oxide neither alters oxidative stress parameters

nor induces lung inflammation in premature lambs with moderate hyaline membrane disease. Biol. Neonate 73:172-181.

Subhedar, N.V. and N.J. Shaw. 1997. Changes in oxygenation and pulmonary haemodynamics in preterm infants treated with inhaled nitric oxide. Arch. Dis. Child. 77:F191-F197.

Swedish National Board of Occupational Safety and Health. 1996. Occupational Exposure Limit Values, Adopted 28th August 1996. p. 56.

Troncy, E., M. Francœur, and G. Blaise. 1997a. Inhaled nitric oxide: clinical applications, indications, and toxicology. Can. J. Anaesth. 44:973-988.

Troncy, E., J.-P. Collet, J.-G. Guimond, L. Blair, M. Charbonneau, and G. Blaise. 1997b. Should we treat acute respiratory distress syndrome with inhaled nitric oxide? Lancet 350:111-112.

Uchida, T., K. Ichikawa, K. Yokoyama, C. Mitaka, H. Toyooka, and K. Amaha. 1996. Inhaled nitric oxide improved the outcome of severe right ventricular failure caused by lipopolysaccharide administration. Intensive Care Med. 22:1203-1206.

U.S. EPA (U.S. Environmental Protection Agency). 1993a. Monograph for Nitric Oxide CAS No. 10102-43-9. Office of Pollution, Prevention and Toxics, U.S. EPA, Washington, D.C. 20pp.

U.S. EPA (U.S. Environmental Protection Agency). 1993b. Air Quality Criteria for Oxides of Nitrogen, Vol. I-III. Office of Research and Development, U.S. EPA, Research Triangle Park, NC.

von Nieding, G., H.M. Wagner, and H. Krekeler. 1973. Investigation of the acute effects of nitrogen monoxide on lung function in man. In: Proceedings of the third international clean air congress; October; Duesseldorf, Federal Republic of Germany. Verein Deutscher Ingenieure, pp. A14-A16. Cited in U.S. EPA, 1993a.

Wagner, F., M. Dandel, G. Günther, M. Loebe, I. Schulze-Neick, U. Laucke, R. Kuhly, Y. Weng, and R. Hetzer. 1997. Nitric oxide inhalation in the treatment of right ventricular dysfunction following left ventricular assist device implantation. Circulation 96:291-296.

Waters, S.J., P.J. Mihalko, C.R. Hassler, A.W. Singer, and P.C. Mann. 1998. Acute and 4-week toxicity evaluation of inhaled nitric oxide in rats. Toxicologist 42:253.

Wenz, M., R. Steinua, H. Gerlach, M. Lange, and G. Kaczmarczyk. 1997. Inhaled nitric oxide does not change transpulmonary angiotensin II formation in patients with acute respiratory distress syndrome. Chest 112:478-483.

Wessel, D.L., I. Adatia, J.E. Thompson, and P.R. Hickey. 1994. Delivery and monitoring of inhaled nitric oxide in patients with pulmonary hypertension. Crit. Care Med. 22:930-938.

Wessel, D.L., I. Adatia, L.J. Van Marter, J.E. Thompson, J.W. Kane, A.R. Stark, and S. Kourembanas. 1997. Improved oxygenation in a randomized trial of inhaled nitric oxide for persistent pulmonary hypertension of the newborn. Pediatrics 100:888.

Westfelt, U.N., S. Lundin, and O. Stenquist. 1997. Uptake of inhaled nitric oxide in acute lung injury. Acta Anaesthesiol. Scand. 41:818-823.

Wilhelm, J.A., P. Veng-Pedersen, P.J. Mihalko, and S.J. Waters. 1998. Pharmacokinetic modeling of methemoglobin concentration-time data in dogs receiving inhaled nitric oxide. Toxicologist 42:213.

Yoshida, M., O. Taguchi, E.C. Gabazza, H. Yasui, T. Kobayashi, H. Kobayashi, K. Maruyama, and Y. Adachi. 1997. The effect of low-dose inhalation of nitric oxide in patients with pulmonary fibrosis. Eur. Respir. J. 10:2051-2054.

Yoshida, K. and K. Kasama. 1987. Biotransformation of nitric oxide. Environ. Health Persp. 73:201-206.

Yoshida, K., K. Kasama, M. Kitabatake, M. Okuda, and M. Imai. 1980. Metabolic fate of nitric oxide. Int. Arch. Occup. Env. Health 46:71-77.

Zayek, M., L. Wild, J.D. Roberts, and F.C. Morin. 1993. Effect of nitric oxide on the survival rate and incidence of lung injury in newborn lambs with persistent pulmonary hypertension. J. Pediatr. 123:947-952.

Zwissler, B., M. Welte, O. Habler, M. Kleen, and K. Messmer. 1995. Effects of inhaled prostacyclin as compared with inhaled nitric oxide in a canine model of pulmonary microembolism and oleic acid edema. J. Cardiothorac. Vasc. Anesth. 9:634-640.