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Acute Exposure Guideline Levels for Selected Airborne Chemicals

VOLUME 11

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Preface

Extremely hazardous substances (EHSs)[2] can be released accidentally as a result of chemical spills, industrial explosions, fires, or accidents involving railroad cars and trucks transporting EHSs. Workers and residents in communities surrounding industrial facilities where EHSs are manufactured, used, or stored and in communities along the nation's railways and highways are potentially at risk of being exposed to airborne EHSs during accidental releases or intentional releases by terrorists. Pursuant to the Superfund Amendments and Reauthorization Act of 1986, the U.S. Environmental Protection Agency (EPA) has identified approximately 400 EHSs on the basis of acute lethality data in rodents.

As part of its efforts to develop acute exposure guideline levels for EHSs, EPA and the Agency for Toxic Substances and Disease Registry (ATSDR) in 1991 requested that the National Research Council (NRC) develop guidelines for establishing such levels. In response to that request, the NRC published *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* in 1993. Subsequently, *Standard Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Substances* was published in 2001, providing updated procedures, methodologies, and other guidelines used by the National Advisory Committee (NAC) on Acute Exposure Guideline Levels for Hazardous Substances and the Committee on Acute Exposure Guideline Levels (AEGLs) in developing the AEGL values.

Using the 1993 and 2001 NRC guidelines reports, the NAC—consisting of members from EPA, the Department of Defense (DOD), the Department of Energy (DOE), the Department of Transportation (DOT), other federal and state governments, the chemical industry, academia, and other organizations from the private sector—has developed AEGLs for more than 270 EHSs.

In 1998, EPA and DOD requested that the NRC independently review the AEGLs developed by NAC. In response to that request, the NRC organized within its Committee on Toxicology (COT) the Committee on Acute Exposure Guideline Levels, which prepared this report. This report is the eleventh volume in that series. AEGL documents for bis-chloromethyl ether, chloromethyl

[2]As defined pursuant to the Superfund Amendments and Reauthorization Act of 1986.

methyl ether, chlorosilanes, nitrogen oxides, and vinyl chloride are each published as an appendix in this report. The committee concludes that the AEGLs developed in these appendixes are scientifically valid conclusions based on the data reviewed by NAC and are consistent with the NRC guideline reports. AEGL reports for additional chemicals will be presented in subsequent volumes.

The committee's review of the AEGL documents involved both oral and written presentations to the committee by the authors of the documents. The committee examined the draft documents and provided comments and recommendations for how they could be improved in a series of interim reports. The authors revised the draft AEGL documents based on the advice in the interim reports and presented them for reexamination by the committee as many times as necessary until the committee was satisfied that the AEGLs were scientifically justified and consistent with the 1993 and 2001 NRC guideline reports. After these determinations have been made for an AEGL document, it is published as an appendix in a volume such as this one.

The five interim reports of the committee that led to this report were reviewed in draft form by individuals selected for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of the five committee interim reports, which summarize the committee's conclusions and recommendations for improving NAC's AEGL documents for bis-chloromethyl ether (interim reports 18 and 19a), chloromethyl methyl ether (interim reports 11, 18, and 19a), chlorosilanes (interim reports 18 and 19a), nitrogen oxides (interim reports 15, 18, and 19a), and vinyl chloride (interim reports 16, 18, and 19a): Deepak Bhalla (Wayne State University), Harvey Clewell (The Hamner Institutes for Health Sciences), Sidney Green, Jr. (Howard University), A. Wallace Hayes (Harvard School of Public Health), Rogene Henderson (Lovelace Respiratory Research Institute [retired]), Sam Kacew (University of Ottawa), James McDougal (Wright State University [retired]), Charles Reinhardt (DuPont Haskell Laboratory [retired]), Andrew Salmon (California Environmental Protection Agency), Joyce Tsuji (Exponent, Inc.), and Judith Zelikoff (New York University).

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of this volume before its release. The review of interim report 11 was overseen by Rakesh Dixit (MedImmune/AstraZeneca Biologics, Inc.), and interim reports 15, 16, 18, and 19a were overseen by Robert Goyer (University of Western Ontario [retired]). Appointed by the NRC, they were responsible for making certain that an independent examination of the interim reports was carried out in accordance with institutional

procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

The committee gratefully acknowledges the valuable assistance provided by the following persons: Ernest Falke and Iris A. Camacho (both from EPA) and George Rusch (Risk Assessment and Toxicology Services). The committee also acknowledges Susan Martel, the project director for her work this project. Other staff members who contributed to this effort are James J. Reisa (director of the Board on Environmental Studies and Toxicology), Radiah Rose (manager of editorial projects), Mirsada Karalic-Loncarevic (manager of the Technical Information Center), and Tamara Dawson (program associate). Finally, I would like to thank all members of the committee for their expertise and dedicated effort throughout the development of this report.

Donald E. Gardner, *Chair*
Committee on Acute Exposure
Guideline Levels

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Acute Exposure Guideline Levels for Selected Airborne Chemicals

VOLUME 11

National Research Council Committee Review of Acute Exposure Guideline Levels of Selected Airborne Chemicals

This report is the eleventh volume in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals*.

In the Bhopal disaster of 1984, approximately 2,000 residents living near a chemical plant were killed and 20,000 more suffered irreversible damage to their eyes and lungs following accidental release of methyl isocyanate. The toll was particularly high because the community had little idea what chemicals were being used at the plant, how dangerous they might be, or what steps to take in an emergency. This tragedy served to focus international attention on the need for governments to identify hazardous substances and to assist local communities in planning how to deal with emergency exposures.

In the United States, the Superfund Amendments and Reauthorization Act (SARA) of 1986 required that the U.S. Environmental Protection Agency (EPA) identify extremely hazardous substances (EHSs) and, in cooperation with the Federal Emergency Management Agency and the U.S. Department of Transportation, assist local emergency planning committees (LEPCs) by providing guidance for conducting health hazard assessments for the development of emergency response plans for sites where EHSs are produced, stored, transported, or used. SARA also required that the Agency for Toxic Substances and Disease Registry (ATSDR) determine whether chemical substances identified at hazardous waste sites or in the environment present a public health concern.

As a first step in assisting the LEPCs, EPA identified approximately 400 EHSs largely on the basis of their immediately dangerous to life and health values, developed by the National Institute for Occupational Safety or Health. Although several public and private groups, such as the Occupational Safety and Health Administration and the American Conference of Governmental Industrial

Hygienists, have established exposure limits for some substances and some exposures (e.g., workplace or ambient air quality), these limits are not easily or directly translated into emergency exposure limits for exposures at high levels but of short duration, usually less than 1 hour (h), and only once in a lifetime for the general population, which includes infants (from birth to 3 years of age), children, the elderly, and persons with diseases, such as asthma or heart disease.

The National Research Council (NRC) Committee on Toxicology (COT) has published many reports on emergency exposure guidance levels and spacecraft maximum allowable concentrations for chemicals used by the U.S. Department of Defense (DOD) and the National Aeronautics and Space Administration (NASA) (NRC 1968, 1972, 1984a,b,c,d, 1985a,b, 1986a, 1987, 1988, 1994, 1996a,b, 2000a, 2002a, 2007a, 2008a). COT has also published guidelines for developing emergency exposure guidance levels for military personnel and for astronauts (NRC 1986b, 1992, 2000b). Because of COT's experience in recommending emergency exposure levels for short-term exposures, in 1991 EPA and ATSDR requested that COT develop criteria and methods for developing emergency exposure levels for EHSs for the general population. In response to that request, the NRC assigned this project to the COT Subcommittee on Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances. The report of that subcommittee, *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* (NRC 1993), provides step-by-step guidance for setting emergency exposure levels for EHSs. Guidance is given on what data are needed, what data are available, how to evaluate the data, and how to present the results.

In November 1995, the National Advisory Committee (NAC)[1] for Acute Exposure Guideline Levels for Hazardous Substances was established to identify, review, and interpret relevant toxicologic and other scientific data and to develop acute exposure guideline levels (AEGLs) for high-priority, acutely toxic chemicals. The NRC's previous name for acute exposure levels—community emergency exposure levels (CEELs)—was replaced by the term AEGLs to reflect the broad application of these values to planning, response, and prevention in the community, the workplace, transportation, the military, and the remediation of Superfund sites.

AEGLs represent threshold exposure limits (exposure levels below which adverse health effects are not likely to occur) for the general public and are applicable to emergency exposures ranging from 10 minutes (min) to 8 h. Three levels—AEGL-1, AEGL-2, and AEGL-3—are developed for each of five expo-

[1]NAC completed its chemical reviews in October 2011. The committee was composed of members from EPA, DOD, many other federal and state agencies, industry, academia, and other organizations. From 1996 to 2011, the NAC discussed over 300 chemicals and developed AEGLs values for at least 272 of the 329 chemicals on the AEGLs priority chemicals lists. Although the work of the NAC has ended, the NAC-reviewed technical support documents are being submitted to the NRC for independent review and finalization.

sure periods (10 min, 30 min, 1 h, 4 h, and 8 h) 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 ppm [parts per million] or mg/m^3 [milligrams per cubic meter]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory 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 adverse health effects or death.

Airborne concentrations below AEGL-1 represent exposure levels that can produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic nonsensory adverse 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 idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY OF REPORT ON GUIDELINES FOR DEVELOPING AEGLS

As described in *Guidelines for Developing Community Emergency Exposure Levels for Hazardous Substances* (NRC 1993) and the NRC guidelines report *Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals* (NRC 2001a), the first step in establishing AEGLs for a chemical is to collect and review all relevant published and unpublished information. Various types of evidence are assessed in establishing AEGL values for a chemical. These include information from (1) chemical-physical characterizations, (2) structure-activity relationships, (3) in vitro toxicity studies, (4) animal toxicity studies, (5) controlled human studies, (6) observations of humans involved in chemical accidents, and (7) epidemiologic studies. Toxicity data from human studies are most applicable and are used when available in preference to data from animal studies and in vitro studies. Toxicity data from

inhalation exposures are most useful for setting AEGLs for airborne chemicals because inhalation is the most likely route of exposure and because extrapolation of data from other routes would lead to additional uncertainty in the AEGL estimate.

For most chemicals, actual human toxicity data are not available or critical information on exposure is lacking, so toxicity data from studies conducted in laboratory animals are extrapolated to estimate the potential toxicity in humans. Such extrapolation requires experienced scientific judgment. The toxicity data for animal species most representative of humans in terms of pharmacodynamic and pharmacokinetic properties are used for determining AEGLs. If data are not available on the species that best represents humans, data from the most sensitive animal species are used. Uncertainty factors are commonly used when animal data are used to estimate risk levels for humans. The magnitude of uncertainty factors depends on the quality of the animal data used to determine the no-observed-adverse-effect level (NOAEL) and the mode of action of the substance in question. When available, pharmacokinetic data on tissue doses are considered for interspecies extrapolation.

For substances that affect several organ systems or have multiple effects, all end points (including reproductive [in both genders], developmental, neurotoxic, respiratory, and other organ-related effects) are evaluated, the most important or most sensitive effect receiving the greatest attention. For carcinogenic chemicals, excess carcinogenic risk is estimated, and the AEGLs corresponding to carcinogenic risks of 1 in 10,000 (1×10^{-4}), 1 in 100,000 (1×10^{-5}), and 1 in 1,000,000 (1×10^{-6}) exposed persons are estimated.

REVIEW OF AEGL REPORTS

As NAC began developing chemical-specific AEGL reports, EPA and DOD asked the NRC to review independently the NAC reports for their scientific validity, completeness, and consistency with the NRC guideline reports (NRC 1993, 2001a). The NRC assigned this project to the COT Committee on Acute Exposure Guideline Levels. The committee has expertise in toxicology, epidemiology, occupational health, pharmacology, medicine, pharmacokinetics, industrial hygiene, and risk assessment.

The AEGL draft reports were initially prepared by ad hoc AEGL development teams consisting of a chemical manager, chemical reviewers, and a staff scientist of the NAC contractors—Oak Ridge National Laboratory and subsequently Syracuse Research Corporation. The draft documents were then reviewed by NAC and elevated from "draft" to "proposed" status. After the AEGL documents were approved by NAC, they were published in the *Federal Register* for public comment. The reports were then revised by NAC in response to the public comments, elevated from "proposed" to "interim" status, and sent to the NRC Committee on Acute Exposure Guideline Levels for final evaluation.

The NRC committee's review of the AEGL reports prepared by NAC and its contractors involves oral and written presentations to the committee by the authors of the reports. The NRC committee provides advice and recommendations for revisions to ensure scientific validity and consistency with the NRC guideline reports (NRC 1993, 2001a). The revised reports are presented at subsequent meetings until the committee is satisfied with the reviews.

Because of the enormous amount of data presented in AEGL reports, the NRC committee cannot verify all of the data used by NAC. The NRC committee relies on NAC for the accuracy and completeness of the toxicity data cited in the AEGL reports. Thus far, the committee has prepared ten reports in the series *Acute Exposure Guideline Levels for Selected Airborne Chemicals* (NRC 2001b, 2002b, 2003, 2004, 2007b, 2008b, 2009, 2010a,b, 2011). This report is the eleventh volume in that series. AEGL documents for bis-chloromethyl ether, chloromethyl methyl ether, chlorosilanes, nitrogen oxides, and vinyl chloride are each published as an appendix in this report. The committee concludes that the AEGLs developed in these appendixes are scientifically valid conclusions based on the data reviewed by NAC and are consistent with the NRC guideline reports. AEGL reports for additional chemicals will be presented in subsequent volumes.

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Appendixes

4

Nitrogen Oxides[1]

Acute Exposure Guideline Levels

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 (min) to 8 hours (h). Three levels—AEGL-1, AEGL-2, and AEGL-3—are developed for each of five exposure periods (10 and 30 min and 1, 4, and 8 h) 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, nonsensory

[1]This document was prepared by the AEGL Development Team composed of Carol Wood (Oak Ridge National Laboratory), Gary Diamond (Syracuse Research Corporation), Chemical Managers George Woodall and Loren Koller (National Advisory Committee [NAC] on Acute Exposure Guideline Levels for Hazardous Substances), and Ernest V. Falke (U.S. Environmental Protection Agency). The NAC reviewed and revised the document and AEGLs as deemed necessary. Both the document and the AEGL values were then reviewed by the National Research Council (NRC) Committee on Acute Exposure Guideline Levels. The NRC committee has concluded that the AEGLs developed in this document are scientifically valid conclusions based on the data reviewed by the NRC and are consistent with the NRC guidelines reports (NRC 1993, 2001).

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 concentrations that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, nonsensory 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 idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

SUMMARY

Nitrogen oxide compounds occur from both natural and anthropogenic sources. Nitrogen dioxide (NO_2) is the most ubiquitous of the oxides of nitrogen and has the greatest impact on human health. Nitrogen tetroxide (N_2O_4) is a component of rocket fuels. Very few inhalation toxicity data are available on N_2O_4. Nitric oxide (NO) is an endogenous molecule that mediates the biologic action of endothelium-derived relaxing factor. The toxicity of NO is associated with methemoglobin formation and oxidation to NO_2. NO is also a component of air pollution and is generally measured as part of the total oxides of nitrogen ($NO + NO_2$).

The reactions of the oxides of nitrogen consist of a family of reaction paths that is temperature dependent and generally favors NO_2 production. A significant fraction of N_2O_4 and NO will be converted to NO_2. Since NO_2 is the most ubiquitous and the most toxic of the oxides of nitrogen, AEGL values derived from NO_2 toxicity data are considered applicable to all oxides of nitrogen. NO_2 exists as an equilibrium mixture of NO_2 and N_2O_4, but the dimer is not important at ambient concentrations (EPA 1993). When N_2O_4 is released, it vaporizes and dissociates into NO_2, making it nearly impossible to generate a significant concentration of N_2O_4 at atmospheric pressure and ambient temperatures without generating a vastly higher concentration of NO_2. Almost no inhalation toxicity data are available on N_2O_4 because of this effect, and no information was found on the interactions of nitrogen trioxide (N_2O_3).

NO is unstable in air and undergoes spontaneous oxidation to NO_2 making experimental effects difficult to separate and studies difficult to perform (EPA 1993). Studies on the conversion of NO to NO_2 in medicinal applications have found the conversion to be significant at an atmospheric concentration of oxygen (20.9%) at room temperature. NO reacts 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 ≥80 ppm, especially when coadministered with oxygen (Foubert et al. 1992; Miller et al. 1994). Although closed-system experiments on a laboratory scale 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 about the chemical-rate constants. As a result, the conversion of NO to NO_2 during the atmospheric release is of concern to emergency planners. In photochemical smog, NO_2 absorbs sunlight at wavelengths between 290 and 430 nanometers (nm) and decomposes to NO and oxygen (EPA 1993).

AEGL values were based on studies of NO_2, the predominant form of the nitrogen oxides, and values are considered applicable to all nitrogen oxides. Values for N_2O_4 in units of ppm have been calculated on a molar basis. 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 National Advisory Committee recognizes, however, that short-term exposures to NO below 80 ppm should not constitute a health hazard.

NO_2 is an irritant to the mucous membranes and might cause coughing and dyspnea during exposure. After less severe exposure, symptoms might persist for several hours before subsiding (NIOSH 1976). With more severe exposure, pulmonary edema ensues with signs of chest pain, cough, dyspnea, cyanosis, and moist rales heard on auscultation (NIOSH 1976; Douglas et al. 1989). Death from NO_2 inhalation is caused by bronchospasm and pulmonary edema in association with hypoxemia and respiratory acidosis, metabolic acidosis, shift of the oxyhemoglobin dissociation curve to the left, and arterial hypotension (Douglas et al. 1989). A characteristic of NO_2 intoxication after the acute phase is a period of apparent recovery followed by late-onset bronchiolar injury that manifests as bronchiolitis fibrosa obliterans (NIOSH 1976; NRC 1977; Hamilton 1983; Douglas et al. 1989). In addition, experiments with laboratory animals indicate that exposure to NO_2 increases susceptibility to infection (Henry et al. 1969; EPA 1993) due, in part, to alterations in host pulmonary defense mechanisms (Gardner et al. 1969).

For AEGL-1, a concentration of 0.5 ppm was adopted for all time points. Although the response of asthmatics to NO_2 is variable, asthmatics were identified as a potentially susceptible population. The evidence indicates that some asthmatics exposed to NO_2 at 0.3-0.5 ppm might respond with either

subjective symptoms or slight changes in pulmonary function that are not clinically significant. In contrast, some asthmatics did not respond to NO_2 at concentrations of 0.5-4 ppm. Because of the weight of evidence, the study by Kerr et al. (1978, 1979) was considered the most appropriate for derivation of AEGL-1 values. They reported that 7/13 asthmatics experienced slight burning of the eyes, slight headache, and chest tightness or labored breathing with exercise when exposed at 0.5 ppm for 2 h; at this concentration, the odor of NO_2 was perceptible but the subjects became unaware of it after about 15 min. No changes in any pulmonary function tests were found immediately following the chamber exposure (Kerr et al. 1978, 1979). Therefore, 0.50 ppm was considered a no-adverse-effect level for the asthmatic population. Since asthmatics are potentially the most susceptible population, no uncertainty factor was applied. Time scaling was not performed because adaptation to mild sensory irritation occurs. In addition, animal responses to NO_2 exposure have demonstrated a much greater dependence on concentration than on time; therefore, extending the 2-h concentration to 8 h should not exacerbate the human response.

Supporting studies for AEGL-1 effects report findings similar to the key studies. Significant group mean reductions in forced expiratory volume (FEV_1) (-17.3% with NO_2 vs. -10.0% with air) and specific airway conductance (-13.5% with NO_2 vs. -8.5% with air) occurred in asthmatics after exercise when exposed at 0.3 ppm for 4 h and 1/6 individuals experienced chest tightness and wheezing (Bauer et al. 1985). The onset of effects was delayed when exposures were by oral-nasal inhalation as compared with oral inhalation, and might have resulted from scrubbing within the upper airway. In a similar study, asthmatics exposed at 0.3 ppm for 30 min at rest followed by 10 min of exercise had significantly greater reductions in FEV_1 (10% with NO_2 vs. 4% with air) and partial expiratory flow rates at 60% of total lung capacity, but no symptoms were reported (Bauer et al. 1986). In a preliminary study with 13 asthmatic subjects exposed at 0.3 ppm for 110 min, slight cough and dry mouth and throat and significantly greater reduction in FEV_1 occurred after exercise (11% vs. 7%); however, in a larger study, no changes in pulmonary function were measured and no symptoms were reported in 21 asthmatic subjects exposed to concentrations up to 0.6 ppm for 75 min (Roger et al. 1990).

Human data also were used as the basis for AEGL-2 values. Three healthy male volunteers experienced discomfort from exposure to NO_2 at 30 ppm for 2 h (Henschler et al. 1960). Three individuals exposed at 30 ppm for 2 h perceived an intense odor on entering the chamber, but odor perception quickly diminished and was completely absent after 25-40 min. One individual experienced a slight tickling of the nose and throat mucous membranes after 30 min, the two others after 40 min. From 70 min and longer, all subjects experienced a burning sensation and an increasingly severe cough for the next 10-20 min, but coughing decreased from 100 min. However, the burning sensation continued and moved into the lower sections of the airways and was finally felt deep in the chest. At that time, marked sputum secretion and dyspnea were noted. Toward the end of the exposure, the subjects reported the exposure conditions to be bothersome

and barely tolerable. A sensation of pressure and increased sputum secretion continued for several hours after exposure ceased (Henschler et al. 1960). The point-of-departure is considered a threshold for AEGL-2 effects because the effects experienced by the subjects would not impair ability to escape and the effects were reversible after cessation of exposure.

AEGL-3 values were based on animal data and supported by a human case report. A study of monkeys exposed to NO_2 at 10-50 ppm for 2 h (Henry et al. 1969) was used to derive the AEGL-3 values. Monkeys exposed at 50 and 35 ppm had markedly increased respiratory rates and decreased tidal volumes, but only slight effects were observed at 15 and 10 ppm. Mild histopathologic changes in the lungs were observed at 10 and 15 ppm, whereas marked changes in lung structure were found at 35 and 50 ppm. The alveoli were expanded with septal wall thinning, bronchi were inflamed with proliferation or erosion of the surface epithelium, and lymphocyte infiltration was seen with edema. In addition to the effects on the lungs, interstitial fibrosis (35 ppm) and edema (50 ppm) of cardiac tissue, glomerular tuft swelling in the kidney (35 and 50 ppm), lymphocyte infiltration in the kidney and liver (50 ppm), and congestion and centrilobular necrosis in the liver (50 ppm) were observed.

The AEGL-3 values are supported by a case study of a welder. Pulmonary edema, confirmed on x-ray and requiring medical intervention, resulted from exposure to NO_2 at approximately 90 ppm for up to 40 min (Norwood et al. 1966). If this exposure scenario is used for derivation of AEGL-3 values with an uncertainty factor of 3, the values are nearly identical to those derived using the data on monkeys. The AEGL-3 values also are below the concentrations at which lethality first occurred in five animal species: 75 ppm for 4 h in the dog and 1 h in the rabbit, 50 ppm for 1 h in the guinea pig, and 50 ppm for 24 h in the rat and mouse (Hine et al. 1970).

For AEGL-2 and AEGL-3, the 10- and 30-min, and 1-, 4-, and 8-h AEGL end points were calculated using the equation $C^n \times t = k$, with $n = 3.5$ (ten Berge et al. 1986). The value of n was calculated by ten Berge et al. (1986) using the data of Hine et al. (1970) in five species of laboratory animals. A total uncertainty factor of 3 was applied, which includes 3 for intraspecies variability and 1 for interspecies variability. Use of a greater intraspecies uncertainty factor was considered unnecessary because the mechanism of action for a direct-acting respiratory irritant is not expected to differ greatly among individuals (see Section 4.2 for detailed information regarding the mechanism of respiratory toxicity). An interspecies uncertainty factors was considered unnecessary because human data were used as the point-of-departure for AEGL-2 values, the end point in the monkey study was below the definition of AEGL-3, human data support the AEGL-3 point-of-departure and derived values, the mechanism of action does not vary between species with the target at the alveoli, and the respiratory tract of humans and monkeys is similar.

The AEGLs values for NO_2, NO, and N_2O_4 are presented in Tables 4-1 and 4-2.

TABLE 4-1 Summary of AEGL Values for Nitrogen Dioxide and Nitric Oxide

Classification	10 min	30 min	1 h	4 h	8 h	End Point[a] (Reference)
AEGL-1[b] (ondisabling)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	Slight burning of the eyes, slight headache, chest tightness or labored breathing with exercise in 7/13 asthmatics (Kerr et al. 1978, 1979)
AEGL-2 (disabling)	20 ppm (38 mg/m^3)	15 ppm (28 mg/m^3)	12 ppm (23 mg/m^3)	8.2 ppm (15 mg/m^3)	6.7 ppm (13 mg/m^3)	Burning sensation in nose and chest, cough, dyspnea, sputum production in normal volunteers (Henschler et al. 1960)
AEGL-3 (lethal)	34 ppm (64 mg/m^3)	25 ppm (47 mg/m^3)	20 ppm (38 mg/m^3)	14 ppm (26 mg/m^3)	11 ppm (21 mg/m^3)	Marked irritation, histopathologic changes in lungs, fibrosis and edema of cardiac tissue, necrosis in liver, no deaths in monkeys (Henry et al. 1969)

[a]Some effects might be delayed.
[b]The sweet odor of NO_2 may be perceptible to most individuals at this concentration; however, adaptation occurs rapidly.

TABLE 4-2 Summary of AEGL Values for Nitrogen Tetroxide

Classification	10 min	30 min	1 h	4 h	8 h	End Point[a] (Reference)
AEGL-1[b] (nondisabling)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	Slight burning of the eyes, slight headache, chest tightness or labored breathing with exercise in 7/13 asthmatics (Kerr et al. 1978, 1979)
AEGL-2 (disabling)	10 ppm (38 mg/m^3)	7.6 ppm (28 mg/m^3)	6.2 ppm (23 mg/m^3)	4.1 ppm (15 mg/m^3)	3.5 ppm (13 mg/m^3)	Burning sensation in nose and chest, cough, dyspnea, sputum production in normal volunteers (Henschler et al. 1960)
AEGL-3 (lethal)	17 ppm (64 mg/m^3)	13 ppm (47 mg/m^3)	10 ppm (38 mg/m^3)	7.0 ppm (26 mg/m^3)	5.7 ppm (21 mg/m^3)	Marked irritation, histopathologic changes in lungs, fibrosis and edema of cardiac tissue, necrosis in liver, no deaths in monkeys (Henry et al. 1969)

[a]Some effects might be delayed.
[b]The sweet odor of NO_2 may be perceptible to most individuals at this concentration; however, adaptation occurs rapidly.

1. INTRODUCTION

NO_2 is the most ubiquitous of the oxides of nitrogen and has the greatest impact on human health. NO_2, which exists as an equilibrium mixture of NO_2 and N_2O_4, is a reddish-brown gas with a sweet odor, is heavier than air, and reacts with water (EPA 1993; Mohsenin 1994). NO_2 is shipped under pressure and the equilibrium between NO_2 and N_2O_4 is altered with changes in pressure, with N_2O_4 becoming predominant at very high pressures. NO_2 is a free radical with sufficient stability to exist in relatively high concentrations in ambient air (Mohsenin 1994). NO is also a component of air pollution and is generally measured as part of the total oxides of nitrogen ($NO + NO_2$) present. NO reacts with oxygen in air to form NO_2: $2NO + O_2 \rightarrow 2NO_2$ (NIOSH 1976).

The major source of atmospheric nitrogen oxides is from the combustion of fossil fuels for heating, household appliances, power generation, and in motor vehicles. Consequently, the chemicals are a major contributor to smog and a concern for indoor air quality. Ambient concentrations in urban air pollution episodes in the United States have been measured between 0.1 and 0.8 ppm as a maximum hourly average with short-term peaks as high as 1.27 ppm. Indoor NO_2 concentrations might reach a maximum 1-h concentration of 0.25-1.0 ppm, with peak concentrations as high as 2-4 ppm where gas appliances or kerosene heaters are used (Mohsenin 1994).

N_2O_4 is a commonly used as a rocket propellant (Yue et al. 2004). Toxicity data on N_2O_4 show effects similar to those of NO_2.

NO is an endogenous molecule that mediates the biologic action of endothelium-derived relaxing factor. 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 binding of hemoglobin (EPA 1993). NO reacts with oxygen in air to form NO_2, possibly potentiating toxicity, and causing pulmonary edema. For this reason, careful monitoring of NO_2 concentrations has been suggested when NO is used therapeutically at concentrations $\geq$80 ppm, especially when administered with oxygen (Foubert et al. 1992; Miller et al. 1994).

No toxicity data or information on the uses or sources of N_2O_3 were found. Information on the chemical interactions of N_2O_3 with the other oxides of nitrogen was not available. Therefore, N_2O_3 was not considered further.

NO_2 is an irritant of the mucous membranes and might cause coughing and dyspnea during exposure. After less severe exposure, symptoms might persist for several hours before subsiding (NIOSH 1976). With more severe exposure, pulmonary edema ensues with chest pain, cough, dyspnea, cyanosis, and moist rales heard on auscultation (NIOSH 1976; Douglas et al. 1989). Death from NO_2 inhalation is caused by bronchospasm and pulmonary edema in association with hypoxemia and respiratory acidosis, metabolic acidosis, shift of

the oxyhemoglobin dissociation curve to the left, and arterial hypotension (Douglas et al. 1989). A characteristic of NO_2 intoxication after the acute phase is a period of apparent recovery followed by late-onset bronchiolar injury that manifests as bronchiolitis fibrosa obliterans (NIOSH 1976; NRC 1977; Hamilton 1983; Douglas et al. 1989).

Selected physical and chemical properties of NO_2, N_2O_4, and NO are presented in Tables 4-3, 4-4, and 4-5, respectively.

TABLE 4-3 Physical and Chemical Properties for Nitrogen Dioxide

Parameter	Value	Reference
Common name	Nitrogen dioxide	
CAS registry no.	10102-44-0	
Chemical formula	NO_2	Budavari et al. 1996
Molecular weight	46.01	Budavari et al. 1996
Physical state	Reddish-brown gas	Budavari et al. 1996
Melting point	-9.3°C	Budavari et al. 1996
Boiling point	21.15°C	Budavari et al. 1996
Vapor density (air = 1)	1.58	Budavari et al. 1996
Solubility in water	0.037 mL at 35°C	Mohsenin 1994
Vapor pressure	720 mm Hg at 20°C; 800 mm Hg at 25°C	EPA 1990; ACGIH 1991
Flammability	Does not burn	Budavari et al. 1996
Conversion factors in air	1 ppm = 1.88 mg/m^3 1 mg/m^3 = 0.53 ppm	EPA 1993
Reactivity	Decomposes in water forming nitric oxide and nitric acid	Budavari et al. 1996

TABLE 4-4 Physical and Chemical Properties for Nitrogen Tetroxide

Parameter	Value	Reference
Common name	Dinitrogen dioxide	
CAS registry no.	10544-72-6	
Chemical formula	N_2O_4	Lide 1988
Molecular weight	92.01	Lide 1988
Physical state	Colored liquid	Lide 1988
Melting point	-9.3°C	Lide 1988; Kushneva and Gorshkova 1999
Boiling point	21.5°C	Lide 1988; Kushneva and Gorshkova 1999
Vapor density (air = 1)	1.45 at 20°C	Lide 1988
Solubility in water	No data	
Vapor pressure	760 mm Hg at 21°C	Lide 1988
Conversion factors in air	1 ppm = 3.70 mg/m^3 1 mg/m^3 = 0.27 ppm	Calculated
Reactivity	Reacts violently with organic compounds; reacts with water	Lide 1988; Kushneva and Gorshkova 1999

TABLE 4-5 Physical and Chemical Properties for Nitric Oxide

Parameter	Value	Reference
Common name	Nitric oxide	
Synonyms	Nitrogen monoxide	Budavari et al. 1996
CAS Reg. 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
Melting point	-163.6°C	Budavari et al. 1996
Boiling point	-151.7°C	Budavari et al. 1996
Vapor density (air = 1)	1.04	Budavari et al., 1996
Solubility in water	4.6 mL/100 mL (20°C)	Budavari et al. 1996
Vapor pressure	26,000 mm Hg at 20°C	ACGIH 1991
Conversion factors in air	1 ppm = 1.25 mg/m^3 1 mg/m^3 = 0.8 ppm	NIOSH 1976
Reactivity	Combines with oxygen to form NO_2	Budavari et al. 1996

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

Book (1982) used allometric scaling based on minute volume and LC_{50} (lethal concentration, 50% lethality) values for NO_2 for five animal species to calculate a human 1-h LC_{50} of 174 ppm. Concentrations >200 ppm were reported to induce immediate symptoms of bronchospasm and pulmonary edema and might cause syncope, unconsciousness, and quick death (Douglas et al. 1989).

Clinical responses to "acute" inhalation of high concentrations of NO_2 based on occupational exposures are presented in Table 4-6 (NRC 1977). Durations of exposure were not specified except for the statement that workers in a nitric acid manufacturing plant in Italy were exposed to average concentrations of 30-35 ppm for an unspecified number of years with no adverse signs or symptoms.

Following induction of anesthesia with nitrous oxide and oxygen, a woman became cyanotic within 2 min. 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 NO_2 addressed in the study. Greenbaum et al. (1967) made several assumptions about retention volume, time-to-cyanosis, and ventilation rate and estimated that the contamination by NO must have been 1% (10,000 ppm) or greater.

TABLE 4-6 Effects of Acute Exposure to High Concentrations Nitrogen Dioxide

Concentration (ppm)	Effect
0.4	Approximate odor threshold
15-25	Respiratory and nasal irritation
25-75	Reversible pneumonia and bronchiolitis
150-300+	Fatal bronchiolitis and bronchopneumonia

Source: NRC 1977.

2.2. Nonlethal Toxicity

2.2.1. Case Reports

2.2.1.1. Nitrogen Dioxide

Probably the most well-known occupational manifestation of NO_2 toxicity is that of silo filler's disease. In a silo, the gas that accumulates above the silage is depleted of oxygen, is rich in carbon dioxide, and contains a mixture of nitrogen oxides, mainly NO_2, which can reach concentrations of 200-4,000 ppm within 2 days (Lowry and Schuman 1956; Douglas et al. 1989). The term silo filler's disease was first used by Lowry and Schuman in 1956 in an article that described the clinical progression of the disease: inhalation of irritant gas from a silo; immediate cough and dyspnea with a sensation of choking; apparent remission 2-3 weeks after exposure; second phase of illness accompanied by fever and progressively more severe dyspnea, cyanosis, and cough; inspiratory and expiratory rales; discrete nodular densities on the lung; and neutrophilic leukocytosis (Lowry and Schuman 1956). Douglas et al. (1989) reported on 17 patients examined at the Mayo Clinic between 1955 and 1987 after exposure to silo gas. Ocular irritation was described during exposure, acute lung injury occurred in 11 individuals, and 16 had persistent or delayed symptoms of dyspnea, cough, chest pain, and rapid breathing. One patient died and autopsy revealed diffuse alveolar damage with hyaline membranes and hemorrhagic pulmonary edema and acute edema of the airways. Bronchiolitis fibrosa obliterans developed in one patient many years later; however, prophylactic administration of corticosteroids might have prevented chronic obstructive pulmonary disease in the other patients. Similar case reports and outcomes of silo filler's disease and industrial exposure were described in earlier literature (Grayson 1956; Lowry and Schuman 1956; Milne 1969).

A welder developed shortness of breath and chest discomfort during the use of an acetylene torch for metal-cutting in a poorly ventilated water main; the worker had spent approximately 30 min welding in the confined space before being forced to vacate. Several hours later, the worker became so short of breath that he could not sleep. Chest x-ray 18 h after exposure revealed pulmonary edema, and a pulmonary function test showed 42% of the predicted value for

forced vital capacity (FVC). The individual was admitted to the hospital and treated with antibiotics and oxygen. The patient fully recovered 21 days after exposure. Simulation of the accident produced an NO_2 concentration of 90 ppm within 40 min and total oxides of nitrogen in excess of 300 ppm (Norwood et al. 1966). It was assumed that the individual was exposed to at least 90 ppm of NO_2 during the welding operation and that the outcome could have been more severe, or even fatal, without medical intervention.

An outbreak of NO_2-induced respiratory illness was reported among players and spectators at two high school hockey games (Hedberg et al. 1989). Patients presented with acute onset of cough, hemoptysis, or dyspnea during or within 48 h of attending the hockey game. No changes in lung function were measured 10 days and 2 months after exposure. NO_2 concentrations were not measured in the arena during the outbreak, but the source was traced to a malfunctioning motor in the ice resurfacer. Other cases of respiratory illness in hockey players, referees, and spectators have been associated with elevated NO_2 concentrations in the arena because of malfunctioning resurfacers or ventilation systems, combined with elevated carbon monoxide concentrations (Smith et al. 1992; Soparkar et al. 1993; Karlson-Stiber et al. 1996; Morgan 1995). Attempts to measure NO_2 concentrations in the arenas or to reconstruct the situations were described by the authors as not indicative of the actual exposure scenario that resulted in adverse effects.

Morley and Silk (1970) described a number of cases in which welders involved in ship repair and shipbuilding were exposed to nitrous fumes. Symptoms included dyspnea, cough, headache, tightness or pain in chest, nausea, and cyanosis. Most patients recovered after treatment with oxygen and antibiotics; however, one man died 43 days later from viral pneumonia. Two individuals admitted to the hospital with cyanosis, dyspnea, and pulmonary edema, were exposed to NO_2 at 30 ppm during a 40-min welding operation. However, the authors noted that seven other individuals present at the time were unaffected.

A railroad tank car ruptured at a chemical plant, releasing a cloud of NO_2 in a small community (Bauer et al. 1998). In the first 30 h after the release, the most common symptoms reported in emergency room visits were headache, burning eyes, and sore throat. Most air samples collected 3-7 h after the release showed concentrations of 0 ppm with one sample of 1.4 ppm. No attempt was made to correlate symptoms with estimated exposure.

Acute toxic reactions were described in four firemen exposed to NO_2 that originated from a leak in a chemical plant (Tse and Bockman 1970). Concentrations were not reported and exposure durations were defined as “barely a few minutes” to “about ten minutes.” Initial responses included headache, a dry hacking cough, pulmonary edema, sinusitis, and upper respiratory tract irritation; effects cleared within several days. Four to six weeks after exposure, three of the patients developed fever, chest tightness, shortness of breath, and a productive cough; these effects subsided and the patients remained asymptomatic. The fourth

patient developed chronic pulmonary insufficiency, consisting of dyspnea on exertion, despite normal chest x-ray.

Four cases of exposure to unknown concentrations of nitrous fumes were reported for individuals involved in the use of an oxyacetylene burner during a leak at a chemical plant or in shotfiring (Jones et al. 1973). Three patients presented with pulmonary edema, one of which progressed to bronchiolitis obliterans; the fourth patient presented with clinical features of bronchiolitis obliterans. All recovered completely following corticosteroid treatment.

2.2.1.2. Nitrogen Tetroxide

A large number of patients were treated for respiratory complaints following release of a cloud of N_2O_4 from a railroad tank car. The most common symptoms were headache, burning eyes, and sore throat; an abnormal lung exam and an abnormal chest x-ray were also reported for some individuals, but these findings were not further defined (Bauer et al. 1996). No pulmonary edema or deaths were attributed to the accident. However, six individuals were diagnosed with reactive airways dysfunction syndrome (RADS) 3 months after exposure (Conrad et al. 1998). Concentrations of oxides of nitrogen in the cloud were not reported.

2.2.1.3. Nitric Oxide

Methemoglobin concentrations rose to 9.4% in one lung transplantation patient after treatment with NO at 80 ppm for 8 h. A reduction in NO concentration to 40 ppm over 4 h reduced methemoglobin concentrations to 6.6%, and a further reduction of NO to 20 ppm for 12 h decreased the methemoglobin concentration to 0.9% (Adatia et al. 1994). A Japanese newborn developed a methemoglobin concentration of 40% after being exposed to NO at 80 ppm for 26 h; the concentration decreased to 3.9% within 20 min of infusion with methylene blue and gradual reduction of the NO concentration over 1 h then discontinuation. No methemoglobin concentrations were reported before the 26-h time point. 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. Manktelow et al. (1997) reviewed data collected over 5 years from patients treated with NO inhalation therapy. In general, patients received NO at 20 ppm for 48 h, 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 for producing the greatest improvement in hypoxia score among patients with acute respiratory distress syndrome ranged from 0.5 to 40 ppm. This range

was confirmed in a more recent study in which patients were treated with NO at 1-40 ppm for 30 min. 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 pulmonary artery pressure in patients with acute respiratory distress syndrome treated with NO at 40 ppm for 20 min (Doering et al. 1997), 0.1-2 ppm for 15-20 min (Puybasset et al. 1994), 100 ppm for 20 min (Wenz et al. 1997), and 0.1-100 ppm for 15 min (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 acute respiratory distress syndrome and acute right heart failure treated with NO at 20 ppm for 3 days; methemoglobin concentrations were ≤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 improved oxygen saturation when treated with NO at 10 ppm for up to 24 h, 20 ppm for up to 4 h, 60 ppm for 10 min, or 80 ppm for up to 12 h. Two studies reported longer-term therapies in which hypoxemic newborns were treated with NO at 10 ppm for 6-331 h (Biban et al. 1998) and newborns with persistent pulmonary hypertension were treated with 80 ppm for a mean duration of 65.1 h (Davidson et al. 1998). The large variation in exposure duration 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 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 NO at 40 ppm for 20 min (Roger et al. 1997) and with pulmonary fibrosis treated with 2 ppm for 10 min (Yoshida et al. 1997). Pulmonary vascular resistance also was significantly reduced in preterm infants treated with NO at 20 ppm for 2 h, followed by 5 ppm for 70 h (Subhedar and Shaw 1997), in patients with heart failure treated with up to 80 ppm for 5 min (Semigran et al. 1994), in patients implanted with a left ventricular assist device treated at 25-40 ppm for up to 48 h (Wagner et al. 1997), and in lung-transplant patients treated at 80 ppm for 15 min, with a decrease to 10 ppm for up to 69 h (Adatia et al. 1994). Patients with congestive heart failure had increased oxygen uptake and decreased pulmonary hypertension when administered NO at 20 ppm during light exercise (duration not specified) (Matsumoto et al. 1997) and attenuation of excessive increases in tidal volume, which contribute to exercise-induced hyperventilation, when exposed to NO at 30 ppm for about 20 min (Bocchi et al. 1997). Decreased pulmonary artery pressure, increased cardiac output, and increased oxygen arterial saturation occurred in infants treated with NO at 20 ppm for 4-250 h (Journois et al. 1994) or at 50 ppm for a mean of 41 h (methemoglobin, 1.4%) (Schulze-Neick et al. 1997) after surgery for congenital heart defects.

Inhalation of NO at 20 ppm had no effect on PaO_2 (arterial partial pressure of oxygen) during one-lung ventilation in patients undergoing thoracoscopic procedures. However, when combined with intravenous almitrine, it limited the decrease of PaO_2 (Moutafis et al. 1997).

2.2.2. Epidemiologic Studies

Several epidemiologic studies associating ambient NO_2 exposure with an increase in the prevalence of respiratory illness have been inconclusive. Increased odds ratios (1.2-1.7) were found for bronchitis, chronic cough, and chest illness but not for wheeze or asthma in children from six U.S. cities with annual average NO_2 concentration of 0.0065-0.0226 ppm (Dockery et al. 1989). No association was found between long-term differences in NO_2 concentrations (change of 0.0106 ppm/6-week average) and mean annual rates of respiratory episodes in children from urban and rural regions in Switzerland; however, the duration of symptoms was increased (Braun-Fahrlaender et al. 1992). An increase in the cases of croup in children was associated with total suspended particulate matter and NO_2 (Schwartz et al. 1991), and decreased lung function in children was linked to sulfur dioxide (SO_2) in combination with NO_2 (Mostardi et al. 1981). Symptoms of chronic obstructive pulmonary disease have been linked to exposure to total oxidants (>0.1 ppm), NO_2, and sulfates, but not to NO_2 alone (Detels et al. 1981; Euler et al. 1988). Combined effects of NO_2, SO_2, particulate matter, hydrogen sulfide (H_2S), and other pollutants were considered as contributing factors to a positive association between the occurrence of upper respiratory infections in children (<2 and 6 years of age) and living in polluted areas of Finland (Jaakkola et al. 1991).

In a more recent study, children from 12 communities in California were assessed for respiratory disease prevalence and pulmonary function (Peters et al. 1999a,b). Wheeze prevalence was correlated with concentrations of nitric acid and NO_2 in boys, whereas regression analysis showed that NO_2 was significantly associated with lower FVC, FEV_1, and maximal midexpiratory flow in girls. When these data were analyzed by month (Millstein et al. 2004), wheezing during the spring and summer months was not associated with either nitric acid or NO_2. However, among asthmatics, the monthly prevalence of asthma medication use was associated with monthly concentrations of ozone, nitric acid, and acetic acid (Millstein et al. 2004). Similar results were reported for eight areas of Switzerland in which an average increase in NO_2 of 10 $\mu g/m^3$ was associated with decreases in FVC (Schindler et al. 1998).

Several recent studies have attempted to describe the correlation between NO_2 concentrations and mortality or respiratory symptoms by pooling large datasets from multiple cities or countries. One of these studies used information collected from up to 12 cities in Canada. These authors found that an approximate 20 ppb increase in NO_2 was positively associated with a 2.25% increase in mortality (Burnett et al. 2004), intrauterine growth retardation (odds

ratio of 1.14-1.16) (Liu et al. 2007), a 17.72% increase in the incidence of sudden infant death syndrome (Dales et al. 2004), increased numbers of hospitalizations from cardiac disease (Cakmak et al. 2006), and greater asthma hospitalizations in children of 6-12 years of age (Lin et al. 2003). However, many of the positive findings in Canada were also positively correlated with other pollutants, such as particulate matter, ozone, and SO_2. Similarly, a significant association of NO_2 with cardiovascular and respiratory mortality was found in 30 European cities (Samoli et al. 2006) and in nine French cities (Le Tertre et al. 2002), but evidence of confounding effects of black smoke, SO_2, and ozone were also found in both studies.

Asthma and allergy prevalence in conjunction with NO_2 concentrations also have been assessed in multiple city or country studies. Positive correlations were found for asthma attacks, tightness in the chest, wheeze, and allergic rhinitis in children from eight Japanese communities (Shima et al. 2002) and in 13 areas of Italy, with the most pronounced effects in the warmer Mediterranean areas (de Marco et al. 2002). An increased incidence of morning symptoms was associated with a 6-day average increase in NO_2 (odds ratio of 1.48) in asthmatic children from eight U.S. cities (Mortimer et al. 2002). In a cross-sectional study of five countries, long-term NO_2 concentrations were correlated with sensitivity to inhaled allergens, but not to prevalence of bronchitis or asthma (Pattenden et al. 2006). No association was found between NO_2 concentrations and asthma, allergic rhinitis, or atopic dermatitis in children from six French cities (Pénard-Morand et al. 2005).

As a component of air pollution, NO concentrations have been studied in association with various diseases; however, other pollutants such as NO_2 and ozone were also involved. In Helsinki, Finland, emergency room admissions from ischemic cardiac diseases were significantly correlated with NO and ozone concentrations. NO concentrations were 7-467 $\mu g/m^3$ (5.6-373.6 ppb) during the 3-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 NO at peak concentrations of 4-100 ppm (Robertson et al. 1984).

Epidemiologic studies of indoor NO_2 also have been inconclusive. One study found no evidence of any short-term association between prevalence of respiratory symptoms in infants and median indoor and outdoor concentrations of NO_2 at 6.8 and 12.6 ppb, respectively (Farrow et al. 1997). Similarly, no

associations were found between indoor NO_2 and wheeze or asthma in children from seven Japanese communities (Shima and Adachi 2000). Other studies found a significant increase in the occurrence of sore throat, colds, and absences from school among children exposed to hourly peak concentrations of

NO_2 at ≥80 ppb from unvented gas heating in the classrooms (Pilotto et al. 1997), increased respiratory illness in children from homes using gas cooking where NO_2 concentrations in the children's bedroom were 4-169 ppb (Florey et al. 1979), and slight decreases in FVC and peak expiratory flow among adult asthmatics exposed at >0.3 ppm while cooking on a gas range (Goldstein et al. 1988). Similarly, Neas et al. (1991) found that a 15 ppb increase the mean annual concentration of NO_2 in the household was associated with an increased cumulative incidence of attacks of shortness of breath, with wheeze, chronic wheeze, chronic cough, chronic phlegm, or bronchitis in children.

As part of a review of the National Ambient Air Quality Standards (NAAQS) for NO_2, EPA (1995) conducted a meta-analysis of studies that examined the respiratory effects in children living in homes with gas stoves. Conclusions drawn from that analysis were that children (5-12 years of age) had an increased risk of about 20% for developing respiratory symptoms and disease with each increase of 0.015 ppm in estimated 2-week average NO_2 exposure (mean weekly concentrations in bedrooms 0.008-0.065 ppm) and that no evidence for increased risk was found for infants <2 years old. Several limitations of this meta-analysis have been noted, including the following: uncertainty between monitored vs. actual exposure concentration; peak and average exposures could not be distinguished by the method used; and confounding effects of other gas combustion byproducts. In context of the NAAQS review, it was noted that indoor exposures do not mimic outdoor exposures (EPA 1995). EPA (2008) performed an Integrated Health Assessment for Oxides of Nitrogen in support of the 2010 revision of the NAAQS for NO_2. The assessment concluded that recent epidemiology studies confirm previous findings that short-term NO_2 exposure is associated with respiratory symptoms and increased airway responsiveness, especially in children and asthmatics. In considering the uncertainties associated with the epidemiologic evidence, the EPA (2008) assessment noted that it is difficult to determine ''the extent to which NO_2 is independently associated with respiratory effects or if NO_2 is a marker for the effects of another traffic-related pollutant or mix of pollutants."

Several occupations result in exposure to NO_2 concentrations higher than ambient concentrations. In diesel bus garage workers, NO_2 concentrations of ≥0.3 ppm, along with respirable particulates, were associated with work-related symptoms of cough; itching, burning, or watering eyes; difficult breathing; chest tightness; and wheeze, but there were no reductions in pulmonary function (Gamble et al. 1987). In contrast, no relationship was found between respiratory symptoms or decline in FEV_1 among British coalminers and exposure to peak NO_2 concentrations of up to 14 ppm; controls were matched for age, dust exposure, smoking habits, coal rank, and type of work (Robertson et al. 1984). No differences in pulmonary function were noted among shipyard welders exposed to average concentrations of oxides of nitrogen of 0.04 ppm (Peters et al. 1973). Slight increases in prevalence of bronchitis (17.2 vs. 12.6%) and colds (37.5 vs. 30.7%) were noted in traffic officers exposed to automobile exhaust containing mean concentrations of NO_2 of 0.045-0.06 ppm (Speizer and Ferris 1973).

In conclusion, indoor air quality might be more significant than outdoor air quality in the prevalence of respiratory illness from NO_2. An early review of epidemiology studies that assessed ambient air quality (EPA 1993) yielded insufficient evidence to reach any conclusion about the long- or short-term health effects of NO_2. EPA (2008) concluded that recent epidemiology studies confirmed an association between ambient NO_2 concentrations with respiratory symptoms and airway reactivity in children and asthmatics, but cautioned that it was unclear whether NO_2 was the proximate toxicant or a marker for other air contaminants. Review of epidemiology studies that assessed indoor air quality in homes with gas stoves, found that meta-analysis yielded insufficient evidence that NO_2 had an effect on infants 2 years and younger while several considerations limited the interpretation of the positive results for children aged 5-12 years.

2.2.3. Experimental Studies

2.2.3.1. Nitrogen Dioxide

Healthy Subjects

The odor threshold for NO_2 in air has been reported as 0.4 ppm for recognition and 4.0 ppm for less than 100% identification (NIOSH 1976). In an experimental study, the odor of NO_2 was perceived by 3/9 volunteers exposed at 0.12 ppm and by 8/13 subjects at 0.22 ppm. At concentrations of ≤4 ppm, the volunteers perceived the odor for 1-10 min, but the duration of perception was not directly related to concentration. The olfactory response to NO_2 returned 1-1.5 min after cessation of exposure (Henschler et al. 1960). There appears to be a difference between perception and recognition concentrations and the volunteers perceiving the odor at the lowest concentrations were described as "olfactory sensitive."

Studies of healthy individuals exposed to NO_2 at <2 ppm have shown no effects on pulmonary function or symptoms. In several studies, healthy men and women were exposed to NO_2 at 0.6 ppm for 1-3 h with intermittent or continuous exercise. No significant effects were observed in any study on pulmonary function, cardiovascular function, metabolism, or symptoms of exposure (Folinsbee et al. 1978; Adams et al. 1987; Frampton et al. 1991; Hazucha et al. 1994). No changes in pulmonary function occurred following exposure to NO_2 at 1.5 ppm for 3 h or to a baseline of 0.05 ppm with intermittent peaks of 2 ppm; however, continuous exposure to 1.5 ppm for 3 h resulted in a slight but significantly greater decrease in FEV_1 and FVC in response to carbachol (Frampton et al. 1991). Pulmonary function was not affected in competitive athletes exposed to NO_2 at 0.18 and 0.30 ppm for 30 min during heavy exercise (Kim et al. 1991) or in healthy adults exposed at 0.3 ppm for 4 h with intermittent exercise (Smeglin et al. 1985).

Studies at higher concentrations of NO_2 indicate an apparent threshold before pulmonary function is affected. No changes in pulmonary function, airway reactivity, or indications of irritation were measured in healthy adults exposed to NO_2 at 1 ppm for 2 h, 2 ppm for 3 h (Hackney et al. 1978), 2 ppm for 4 h (Devlin et al. 1992), 3 ppm for 2 h (Goings et al. 1989), or 2.3 ppm for 5 h (Rasmussen et al. 1992). Normal subjects exposed to 2 ppm for 1 h developed an increase in airway reactivity to methacholine challenge without changes in lung volume or pulmonary function (Mohsenin 1988). No statistically significant effects on airway resistance, symptoms, heart rate, skin conductance, or self-reported emotional state were found in healthy volunteers exposed to NO_2 at 4 ppm for 1 h and 15 min with intermittent light and heavy exercise (Linn and Hackney 1983). However, a significant decrease in mean (n = 11) alveolar oxygen partial pressure by 8 mm Hg and a significant increase in mean (n = 11) airway resistance from 1.51 to 2.41 cm H_2O/(L/s) occurred in healthy volunteers exposed at 5 ppm for 2 h with 6/11 individuals responding (von Nieding et al. 1979). Similarly, a 10-min exposure to NO_2 at 4-5 ppm resulted in increased expiratory and inspiratory flow resistance in five healthy males; the effect was greatest 30 min after exposure (Abe 1967).

Henschler et al. (1960) performed several experiments on healthy, male volunteers. They reported that a 2-h exposure to NO_2 at 20 ppm did not cause any irritation when preceded by several exposures to lower concentrations during the preceding days; however, exposure at 30 ppm for 2 h caused definite discomfort. Three individuals exposed to NO_2 at 30 ppm for 2 h perceived an intense odor on entering the chamber; odor detection quickly diminished and was completely absent after 25-40 min. One individual experienced a slight tickling of the nose and throat mucous membranes after 30 min, and the others after 40 min. All subjects experienced a burning sensation after 70 min and an increasingly severe cough for the next 10-20 min, but coughing decreased after 100 min. However, the burning sensation continued and moved into the lower sections of the airways and was finally felt deep in the chest. At that time, marked sputum secretion and dyspnea were noted. Toward the end of the exposure, the subjects reported the exposure conditions to be bothersome and barely tolerable. A sensation of pressure and increased sputum secretion continued for several hours after cessation of exposure (Henschler et al. 1960).

In a similar experiment (Henschler and Lütge 1963), groups of four or eight healthy, male volunteers were exposed to NO_2 at 10 ppm for 6 h or to 20 ppm for 2 h. All subjects noted the odor on entering the chamber, but it diminished rapidly. At 20 ppm, minor scratchiness of the throat was reported after about 50 min, and 3/8 experienced slight headaches toward the end of the exposure period. Methemoglobin concentrations remained within the normal range in all subjects after exposure.

Biochemical changes in bronchoalveolar lavage fluid and blood also have been studied in healthy adults exposed to NO_2. Exposures at 2 ppm for 4 h (Devlin et al. 1992) or 6 h (Frampton et al. 1992) caused an influx of polymorphonuclear leukocytes in bronchoalveolar lavage fluid, 2.3 ppm for 5 h resulted in a decrease

in serum-glutathione-peroxidase activity (Rasmusen et al. 1992), 1 and 2 ppm for 3 h caused a decrease in red-blood-cell membrane acetylcholinesterase activity, 2 ppm for 3 h resulted in an increase in peroxidized red-blood-cell lipids and glucose-6-phosphate dehydrogenase activity (Posin et al. 1978), and 3 or 4 ppm for 3 h resulted in a decrease in α-1-protease inhibitor activity but not in enzyme concentration in bronchoalveolar lavage fluid (Mohsenin and Gee 1987). After exposure to NO_2 at 2 ppm for 4 h, neutrophilic inflammation was detected in bronchial washings but no changes in inflammatory cells were observed in endobronchial biopsy samples (Blomberg et al. 1997). Mucociliary activity was completely stopped in healthy individuals 45 min after a 20-min exposure to NO_2 at 1.5 and 3.5 ppm (Helleday et al. 1995).

Asthmatic Subjects

Studies of the effects of NO_2 on pulmonary function in asthmatics are inconclusive and conflicting. No consistent changes in pulmonary function or reported symptoms were found in exercising asthmatic adults and adolescents exposed to NO_2 at 0.12 or 0.18 ppm for 40 min (Koenig et al. 1987); 0.12 ppm for 1 h at rest (Koenig et al. 1985); 0.2 ppm for 2 h with intermittent exercise (Kleinman et al. 1983), 0.3 ppm for 30 min (Rubinstein et al. 1990), 1 h (Vagaggini et al. 1996), or 4 h with exercise (Morrow and Utell 1989); 0.5 ppm for 1 h at rest (Mohsenin 1987); up to 0.6 ppm for 75 min with intermittent exercise (Roger et al. 1990); and up to 1 ppm for 4 h (Sackner et al. 1981). No statistically significant differences between control and NO_2 exposure were found for airway resistance, symptoms, heart rate, skin conductance, or self-reported emotional state of asthmatic subjects exposed to NO_2 at 4 ppm for 75 min with intermittent exercise (Linn and Hackney 1984).

Kerr et al. (1978, 1979) studied the effects of NO_2 on pulmonary function and reported other symptoms that were not reported in many other studies. The subjects were asked note symptoms they experienced during exposure to NO_2 at 0.5 ppm for 2 h, specifically cough, sputum, irritation of mucus membranes, and chest discomfort. The odor of NO_2 was perceptible but the subjects became unaware of it after about 15 min. Seven of 13 asthmatic subjects reported symptoms with exposure, compared with only 1/10 normal subjects and 1/7 subjects with chronic bronchitis. In the group of asthmatics, two had slight burning of the eyes, one had a slight headache, three reported chest tightness, and one had labored breathing with exercise, compared with slight nasal discharge in the normal and chronic bronchitis individuals. No changes in any pulmonary function tests were found immediately after the exposure.

Significant group mean reductions in FEV_1 (-17.3% with NO_2 vs. -10.0% with air) and specific airway conductance (-13.5% with NO_2 vs. -8.5% with air) occurred in asthmatic subjects after exposure during exercise to NO_2 at 0.3 ppm for 4 h, and 1/6 individuals experienced chest tightness and wheezing (Bauer et al. 1985). The onset of effects was delayed when exposures were by oral-nasal

inhalation compared with oral inhalation; the delay might have resulted from scrubbing within the upper airway. In a similar study, 15 asthmatic subjects exposed at rest to NO_2 at 0.3 ppm for 20 min followed by 10 min of exercise had significantly greater reductions in FEV_1 (-10 vs. -4% with air) and partial expiratory flow rates at 60% of total lung capacity, but no symptoms were reported (Bauer et al. 1986). In a preliminary study with 13 asthmatic subjects exposed to NO_2 at 0.3 ppm for 110 min, slight cough, dry mouth and throat, and significantly greater reduction in FEV_1 (-11 vs. -7%) occurred after exercise; however, in a larger study, no changes in pulmonary function were measured and no symptoms were reported when 21 asthmatic subjects were exposed to NO_2 at concentrations up to 0.6 ppm for 75 min (Roger et al. 1990). The mean drop in FEV_1 for asthmatics during a 3-h to NO_2 at 1 ppm with intermittent exercise (-2.5%) was significantly greater than the drop during air exposure (-1.3%); concentrations of 6-keto-prostaglandin$_{1\alpha}$ were decreased and concentrations of thromboxane B_2 and prostaglandin D_2 were increased bronchoalveolar lavage fluid after NO_2 exposure (Jörres et al. 1995).

Studies on the effects of NO_2 on airway hyper-reactivity in asthmatic subjects also have been inconclusive. Methacholine responsiveness in asthmatics was not increased following exposure to NO_2 at 0.25 ppm for 20 min at rest, plus 10 min of exercise (Jörres and Magnussen 1991), or by exposure to 0.1 ppm for 1 h at rest (Hazucha et al. 1983). Exposure at 0.1 ppm for 1 h caused an increase in specific airway resistance in 3/20 asthmatic subjects (the other 17 individuals had little or no response) and enhanced the bronchoconstrictor effect of carbachol in 13/20 asthmatic subjects, but the remaining seven subjects were unaffected. When the study was repeated in four individuals (two responders and two nonresponders) exposed to NO_2 at 0.2 ppm, the results were variable; the two nonresponders were still unaffected, while one responder had an equal response and the other had a greater response to carbachol challenge compared with the response at 0.1 ppm (Orehek et al. 1976). Slight but significant potentiation of airway reactivity in asthmatic subjects occurred from exposure to NO_2 at 0.5 ppm for 1 h followed by methacholine challenge (Mohsenin 1987), 0.3 ppm for 40 min followed by isocapnic cold air hyperventilation (Bauer et al. 1986), 0.2 ppm for 2 h followed by methacholine challenge (Kleinman et al. 1983), and 0.25 ppm for 30 min followed by isocapnic hyperventilation (Jörres and Magnussen 1990). A significantly greater decrease in FEV_1 from challenge with house-dust-mite antigen was reported for asthmatic subjects compared with controls (-7.76 vs. -2.85%) following exposure to NO_2 at 0.4 ppm for 1 h (Tunnicliffe et al. 1994), but no significant changes were found in a similar study using a 6-h exposure (Devalia et al. 1994). Exposure of asthmatic subjects to NO_2 at 0.4 ppm for 3 h significantly decreased the amount of inhaled allergen required to decrease FEV_1 by 20%, but no changes in airway responsiveness occurred following exposure to 0.2 ppm for 6 h; these results suggest a concentration threshold rather than a duration effect (Jenkins et al. 1999).

Folinsbee (1992) conducted a meta-analysis of 20 studies that measured airway responsiveness in asthmatic subjects following exposure to NO_2. Eight

different agents were used to induce nonspecific airway responsiveness and the analysis was restricted to exposures of 0.2-0.3 ppm. The fraction of asthmatic subjects with an increase in airway responsiveness was significant ($p \leq 0.01$) following exposures at rest, but not with exercise. When only those studies that used a cholinergic agonist were analyzed, similar results were found in that a greater proportion of subjects showed an increased response when exposed during rest than during exercise.

Subjects with Chronic Lung Disease

Studies of NO_2 on pulmonary function in patients with chronic lung disease or bronchitis are conflicting. No significant differences in pulmonary function or symptom were observed in patients with chronic respiratory illness exposed at rest to NO_2 at 0.3 ppm for 4 h (Hackney et al. 1992), in patients with chronic obstructive pulmonary disease exposed at up to 2 ppm for 1 h with intermittent exercise (Linn et al. 1985), and in patients with chronic bronchitis exposed at 0.5 ppm for 2 h with exercise (Kerr et al. 1978, 1979). In contrast to these reports, forced expiratory volume of patients with chronic obstructive pulmonary disease significantly decreased from 18.8 L after exposure to air to 13.6 L after exposure to NO_2 at 0.3 ppm for 1 h (Vagaggini et al. 1996). A significant reduction in FVC that progressed during exercise (from -1.2 to -8.2%) occurred in elderly patients with chronic obstructive pulmonary disease exposed to NO_2 at 0.3 ppm for 4 h, while no effects were seen in an age- and gender-matched healthy control group (Morrow and Utell 1989; Morrow et al. 1992).

The effects of NO_2 on respiratory gas exchange were investigated in patients with chronic bronchitis. Inhalation of NO_2 at 4 and 5 ppm for 15-60 min significantly decreased the carbon-monoxide diffusing capacity and arterial pO_2 (partial pressure of oxygen), with no progressive changes over time. Exposure at 5 ppm for 15 min resulted in an average decrease in carbon monoxide diffusion capacity of 3.8 mL/min/Torr and a decrease in arterial pO_2 from an average of 76.5-71.3 Torr. A slight, but statistically significant, increase in airway resistance (approximately 20-30% above the initial value) was measured at concentrations of 1.6-5 ppm for 5 min; no effects occurred at ≤ 1.5 ppm (von Nieding et al. 1973a; von Nieding and Wagner 1979).

2.2.3.2. Nitric Oxide

Seven male and five female healthy volunteers were exposed to NO at 40 ppm through a tight facial mask for 2 h (Luhr et al. 1998). Concentrations of NO_2 were closely monitored and did not exceed 2.3 ppm. No changes in blood pressure, heart rate, 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 min to four groups of volunteers: healthy adults, adults with hyper-reactive 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 when exposed to NO. NO did not affect airway conductance in healthy adults or in patients with pulmonary disease. However, inhalation of NO attenuated the methacholine-induced bronchoconstriction in individuals with hyper-reactive airways and increased airway conductance in patients with asthma (Högman et al. 1993a).

Ten healthy volunteers, eight patients with pulmonary hypertension, and 10 cardiac patients were exposed to NO at 40 ppm for 5 min (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, but not in healthy volunteers. No effect on systemic vascular resistance was observed in any patient or volunteer. Methemoglobin concentrations in the volunteer group increased from 0.33% with air to 0.42% with NO.

Eight healthy adult male volunteers were exposed to NO at 1 ppm for 2 h while performing intermittent light exercise consisting of pedaling a stationary bicycle for 15 min of every half hour (Kagawa 1982). Pulmonary-function tests were performed after 1 and 2 h of exposure, and 1 h after exposure ceased. 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 and resolved in all but two subjects 1 h post-exposure; no significant difference in the group mean was found. As a group, a significant reduction in the percentage increase of maximal expiratory flow at 50% of FVC while breathing a helium-oxygen mixture was noted at the end of the exposure period. However, since this reduction was not accompanied by a reduction in FVC or an increase in the alveolar plateau slope, the author questioned its biologic relevance. In a similar study, respiratory resistance was significantly increased (10-12%) in healthy adults and smokers exposed to ≥20 ppm for 15 min (von Nieding et al. 1973b).

In another report, specific airway conductance was significantly ($p \leq 0.05$) increased in healthy men exposed to NO at 80 ppm for 4 min following methacholine-induced bronchoconstriction (Sanna et al. 1994). The bronchodilator action of NO described in the 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-min 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% oxygen atmosphere to induce hypoxic pulmonary vasoconstriction. Addition of NO at 40 ppm to the

inspired gas decreased pulmonary artery pressure to baseline levels within 10 min (Frostell et al. 1993).

In several inhalation studies, NO was shown to affect bleeding times or platelet aggregation, although adverse clinical effects were not demonstrated. The bleeding-time ratio increased to 1.33 in six healthy volunteers exposed at 30 ppm for 15 min, but returned to near normal 60 min after exposure (Högman et al. 1993b). Platelet aggregation was inhibited after 4 h in mechanically ventilated neonates treated with NO at 2-80 ppm 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 NO at 20 ppm 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 at 20 ppm for 10 min and no increase in methemoglobin concentrations was found (Hayward et al. 1997).

2.3. Developmental and Reproductive Toxicity

No information was found regarding the developmental or reproductive toxicity of nitrogen oxides in humans.

2.4. Genotoxicity

No information was found regarding the genotoxicity toxicity of NO_2 in humans.

No increase in chromosome aberrations was found in human peripheral blood lymphocytes after exposure to NO at 40 ppm for 2 h (Luhr et al. 1998). No other information was found regarding the genotoxicity of NO in humans.

2.5. Carcinogenicity

No information was found regarding the carcinogenicity of nitrogen oxides in humans.

2.6. Summary

In humans, exposure to NO_2 at $\geq$15 ppm causes immediate irritation with pulmonary edema followed by a latent period of apparent recovery in healthy individuals. A second phase of symptoms can occur after several hours or days, which include fever with progressively more severe dyspnea, cyanosis, and cough, and inspiratory and expiratory rales. The concentration causing death in humans is approximately $\geq$150 ppm, but no duration of exposure was given. Most case reports do not contain information on concentrations or durations of

exposure; however, welders exposed at 30 and 90 ppm for 40 min experienced varying degrees of dyspnea, cough, headache, chest tightness, nausea, and cyanosis, and hospitalization was required for pulmonary edema at the higher concentration (Norwood et al. 1966; Morley and Silk 1970). Similar symptoms and respiratory complaints were reported following release of a cloud of N_2O_4 from a railroad tank car (Bauer et al. 1996).

Epidemiologic studies on the long-term effects of elevated concentrations of NO_2 are conflicting. It is likely that increases in respiratory illnesses are from NO_2 in combination with other pollutants and that short-term peak concentrations are more detrimental than chronic, low-level exposures. Evidence suggests that children (5-12 years old) have a greater risk for developing respiratory disease from long-term exposure to higher concentrations, but infants do not.

Experimental studies with both healthy and asthmatic individuals exposed to NO_2 are inconclusive. Negative results were obtained in many studies with exposures up to 4 ppm for 1 h; however, other studies report positive effects on pulmonary function at lower concentrations. In the studies that found statistically significant differences with NO_2 exposure, the changes were within 10% of the measured value after air-only exposure and of questionable biologic significance even for asthmatic subjects. However, the available evidence also suggests that asthmatic subjects may experience an increase in airway responsiveness at 0.2-0.3 ppm.

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 NO_2. Contamination of anesthesia gases has resulted in one fatality, but exposure concentrations were not measured. Therapeutic concentrations of 20-80 ppm for 24 h or 100 ppm for 20 min have not resulted in adverse effects among treated patients. However, an infant exposed at 80 ppm for 26 h developed clinically significant concentrations 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 might have either no effect or cause bronchoconstriction in normal subjects, but might result in bronchodilation in individuals with chemically-induced bronchoconstriction or asthma.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

Acute lethality data from NO_2 were found for several species. One group of investigators (Hine et al. 1970) studied the effects of varying concentration and duration of exposure in five different species of laboratory animal; these

results are described separately by species below and are summarized in Table 4-7. In this study, deaths generally occurred within 2-8 h of exposure and the majority within 24 h. Additional data from rabbit, rat, and mouse studies were available and agree with the results of the Hine et al. (1970) study. LC_{50} values for N_2O_4 were listed for four species, but duration of exposure was not specified; effects were similar to those described following NO_2 exposure. With NO, 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.

TABLE 4-7 Summary of Nitrogen Dioxide Mortality in Five Species[a]

Concentration (ppm)	Time (h)	Rat	Mouse	Guinea Pig	Rabbit	Dog
50	1	0/17	0/5	1/6	0/4	0/1
	8	0/12	0/5	4/6	0/4	0/2
	24	3/10	5/10	—	0/4	—
75	1	3/31	1/6	1/4	1/8	0/2
	2	1/12	2/6	3/4	0/6	0/2
	4	7/12	5/6	2/4	2/8	1/3
	8	12/12	6/6	4/4	6/8	1/4
100	0.5	0/5	2/10	1/2	1/3	0/2
	2	8/8	13/14	3/4	2/4	1/3
	4	29/29	10/10	—	3/4	2/2
	8	—	10/10	—	—	—
150	0.5	2/10	—	3/4	—	—
	1	10/13	—	—	1/6	2/3
	2	10/12	—	3/3	—	—
	4	4/4	—	—	3/4	—
200	0.08	6/12	4/6	2/2	0/2	—
	0.17	8/12	6/6	—	1/2	—
	0.33	5/5	6/6	—	2/4	2/2
	0.50	4/4	—	—	—	—

[a]Deaths generally occurred within 2-8 h after exposure and the majority within 24 h.
Source: Adapted from Hine et al. 1970.

3.1.1. Dogs

Greenbaum et al. (1967) exposed mongrel dogs (n = 1/concentration) to NO_2 at 0.1% (1,000 ppm) for 136 min, 0.5% (5,000 ppm) for 5-45 min, or 2% (20,000 ppm) for 15 min. All dogs that were exposed at 0.5% for 35-45 min or 2% for 15 min died. They exhibited shallow respiration and gasping, and death was from pulmonary edema. Fluid was visible in the tracheobronchial tree at necropsy. Cyanosis from methemoglobin formation (78%) was noted in one animal exposed at 2% for 15 min. At concentrations of 0.5% and 2%, arterial pO_2 and systemic arterial pressure were reduced. The authors stated that pulmonary edema was caused by the action of NO_2 on the alveolar lining fluid, which formed nitric and nitrous acids that denatured proteins, ruptured lysosomes, and caused chemical pneumonitis.

Hine et al. (1970) studied the effects of varying concentration and duration of NO_2 exposure on mongrel dogs. Animals (n = 1-4) were exposed to NO_2 at 5-250 ppm for 30 min to 24 h. At concentrations of ≥40 ppm, signs of toxicity included lacrimation, reddening of the conjunctivae, and increased respiration, which became labored and difficult as the concentration increased. Mortality was first observed at 75 ppm for 4 h (see Table 4-7). Gasping and spasmodic respiration were observed, and pulmonary edema was found at necropsy. Histologic findings in the lungs included bronchiolitis, desquamated bronchial epithelium, infiltration by polymorphonuclear cells, and edema.

Kushneva and Gorshkova (1999) reported an LC_{50} of 260 mg/m^3 (70 ppm) for N_2O_4. The cause of death was pulmonary edema; duration of exposure was not specified and no experimental details were provided.

Greenbaum et al. (1967) exposed dogs to NO at 0.5% (5,000 ppm) for 25 min or at 2% (20,000 ppm) for 7-50 min. All dogs died either within 16 min of exposure or at the end of exposure. Death was associated with a reduction in arterial oxygen caused by methemoglobinemia, low arterial pO_2 from pulmonary edema, and acidemia. Concurrent studies described above were conducted in which dogs were exposed to NO_2. No difference in the effects of either gas was observed, and it is probable that the pulmonary effects observed for NO were from the formation of NO_2 within the test system prior to inhalation by the dogs. This assumption is supported by the authors' observation that considerable oxidation to NO_2 occurred, as indicated by the brown contents of the reservoir bag of the inhalation system. Further, methemoglobin concentrations increased as a function of time and NO concentration. Administration of methylene blue did not return arterial oxygen to safe levels in all dogs and the dogs died with methemoglobin concentrations of 3-5%. The authors stated that the cause of pulmonary edema was the action of NO_2 on the alveolar lining fluid forming nitric and nitrous acids that denatured proteins, ruptured lysosomes, and caused chemical pneumonitis.

3.1.2. Rabbits

The 15-min LC_{50} for NO_2 was 315 ppm in the rabbit (strain not specified; n = 5). Clinical signs of toxicity included severe respiratory distress, ocular irritation, 10-15% body-weight suppression for 2 days, and death; time-to-death varied from 30 min to 3 days. Gross pathology revealed darkened areas on the surface of the lungs. Histopathologic changes in the lungs of survivors 7 and 21 days after exposure included focal accumulation of intra-alveolar macrophages, some proliferation of the alveolar lining epithelium, and varying amounts of inflammatory cells (Carson et al. 1962).

Hine et al. (1970) studied the effects of varying concentration and duration of NO_2 exposure rabbits (strain not specified). Animals (n = 2-8) were exposed to NO_2 at 5-200 ppm for 30 min to 24 h. At concentrations of ≥40 ppm, signs of toxicity included lacrimation, reddening of the conjunctivae, and increased respiration, which became labored and difficult as the intoxication increased. One death was observed at 75 ppm for 1 h but none occurred after 2 h, which makes attributing the death after 1 h to NO_2 questionable (see Table 4-7). The rabbits were gasping and had spasmodic respiration at the end of the study, and pulmonary edema was observed at necropsy. Histologic findings in the lungs included bronchiolitis, desquamated bronchial epithelium, infiltration by polymorphonuclear cells, and edema.

In a similar study, rabbits (strain not specified; n = 3) were exposed to NO_2 at 125, 175, 250, 400, 600, or 800 ppm for 10 min (Meulenbelt et al. 1994). Two of three animals exposed at 800 ppm died 7-21 h after exposure. Lung weights were significantly greater and lung homogenates contained greater amounts of protein and higher concentrations of lactate dehydrogenase, glutathione peroxidase, and glutathione-dehydrogenase activity in animals exposed at ≥250 ppm. Bronchoalveolar lavage fluid from animals exposed to ≥175 ppm contained greater amounts of protein and albumin, and higher concentrations of lactate dehydrogenase and angiotensin converting enzyme activity than unexposed controls and all treated groups had increased numbers of neutrophilic leucocytes. Dose-related increases in severity of centriacinar catarrhal pneumonitis, macrophage influx, and neutrophilic leucocytes were observed on histopathologic examination of the lungs. Edema occurred at ≥250 ppm, subpleural hemorrhaging at ≥400 ppm, and desquamation of the bronchiolar epithelium was seen at ≥600 ppm.

Kushneva and Gorshkova (1999) list an LC_{50} of 320 mg/m^3 (86 ppm) for N_2O_4, with the cause of death pulmonary edema; duration of exposure was not given and no experimental details were included.

3.1.3. Guinea Pigs

Hine et al. (1970) also studied the effects of varying concentration of NO_2 and duration of exposure in the guinea pig (strain not specified). Animals (n = 2-

6) were exposed to NO_2 at 5-200 ppm for 30 min to 8 h. At concentrations of ≥40 ppm, signs of toxicity included lacrimation, reddening of the conjunctivae, and increased respiration, which became labored and difficult as the intoxication increased. Deaths first occurred at 50 ppm for 1 h (see Table 4-7). Guinea pigs exhibited gasping and spasmodic respiration, and lung edema was observed at necropsy. Histologic findings in the lungs included bronchiolitis, desquamated bronchial epithelium, infiltration by polymorphonuclear cells, and edema.

To determine the sensitivity of adult and neonate animals to NO_2, Duncan-Hartley guinea pigs (ages 5, 10, 21, 45, 55, and 60 days) were exposed continuously for 3 days to NO_2 at 2 or 10 ppm (Azoulay-Dupuis et al. 1983). A total of 17-27 animals were studied in each age group. Exposure of neonates before weaning included the dam. At 10 ppm, clinical signs of toxicity in adults over 45 days of age included difficulty in moving, reduced food and water consumption, and hyperventilation. Body weight gain was decreased until 21 days, and body weight was reduced after 45 days in all exposed animals. These effects were most pronounced in the dams. Mortality in the high-concentration group increased with age; deaths occurred in 4% of 5-day-old animals, up to 60% in 55-day-old animals died, and 67% in dams. Most of the older animals died after the first 24 h, whereas the younger animals died later in the 3-day period. At 2 ppm, lung histopathology was normal until animals were 45 days of age, when thickening of the alveolar walls, infiltration by polymorphonuclear neutrophils, and alveolar edema were observed. In dams, bronchioles were devoid of cilia in some areas. At 10 ppm, guinea pigs of all ages were affected by these changes, and were more pronounced in older animals.

3.1.4. Rats

The 5-, 15-, 30-, and 60-min LC_{50} values for NO_2 in the male rat (100-120 g; strain not specified; n = 10) are 416, 201, 162, and 115 ppm, respectively. Clinical signs of toxicity included severe respiratory distress, ocular irritation, 10-15% body weight suppression, and death; time-to-death varied from 30 min to 3 days. Gross pathology revealed darkened areas on the surface of the lungs, and purulent nodules involving the entire lungs was found in some of the survivors (Carson et al. 1962).

An older study reported LC_{50} values for NO_2 in male rats (200-300 g; strain not specified; n = 10) of 1,445 ppm for 2 min, 833 ppm for 5 min, 420 ppm for 15 min, 174 ppm for 30 min, 168 ppm for 60 min, and 88 ppm for 240 min (Gray et al. 1954). Deaths were attributed to pulmonary edema. The differences in LC_{50} values between this study and Carson et al. (1962) might be from differences in the size and age of the rats used the studies.

Meulenbelt et al. (1992a,b) investigated the effects of NO_2 concentration and duration of exposure in Wistar rats. The effect of concentration was studied by exposing 6-9 rats/group to NO_2 at 25, 75, 125, 175, or 200 ppm for 10 min. No signs of toxicity were observed at 25 ppm. Stertorous respiration was heard in

animals exposed at 175 and 200 ppm. Rats exposed at ≥75 ppm had significantly increased lung weight, and subpleural hemorrhages and pale discolorations of the lung were observed during gross examination. Histologic changes in the lungs included atypical pneumonia, edema, focal desquamation of the terminal bronchiolar epithelium, increased numbers of macrophages and neutrophilic leucocytes, and interstitial thickening of the centriacinar septa (175 and 200 ppm only), with the severity increasing at the higher concentrations. One rat died in both the 175- and 200-ppm groups after 14-20 h of exposure. Biochemical changes in bronchoalveolar lavage fluid included concentration-dependent increases in protein and albumin concentrations, angiotensin converting enzyme activity, β-glucuronidase activity, and neutrophilic leukocytes.

Duration of exposure was investigated by exposing 6 rats/group to NO_2 at 175 ppm for 10, 20, or 30 min or at 400 ppm for 5, 10, or 20 min (Meulenbelt et al. 1992a,b). Stertorous respiration was heard in animals at both concentrations for all exposure durations, and lung weight was significantly higher than that of the controls. At 175 ppm, 5/6 rats died in the 20- and 30-min groups exposed at 400 ppm, 6/6 rats died in the 10- and 20-min groups. Necropsy revealed foamy, seroanguinous fluid in the trachea, subpleural bleeding, and pale discoloration. Histologic alterations were similar to those described above. Methemoglobin concentrationss, measured after exposure at 175 ppm for 10 min, were not elevated, but plasma nitrate concentrations were significantly greater than controls.

Hine et al. (1970) also studied the effects of varying NO_2 concentration and duration of exposure in Long-Evans rats. Animals (n = 4-31) were exposed to NO_2 at 5-250 ppm at duration of 30 min to 24 h. At concentrations of ≥40 ppm, signs of toxicity included lacrimation, reddening of the conjunctivae, and increased respiration, which became labored and difficult as the intoxication increased. Mortalities were first observed at 50 ppm for 24 h (see Table 4-7). Animals exhibited gasping and spasmodic respiration, and pulmonary edema was observed at necropsy. Histologic findings in the lungs included bronchiolitis, desquamated bronchial epithelium, infiltration by polymorphonuclear cells, and edema.

Kushneva and Gorshkova (1999) reported an LC_{50} of 105 mg/m^3 (28 ppm) for N_2O_4, with the cause of death pulmonary edema; duration of exposure was not specified and no experimental details were provided.

To assess acute lung injury caused by inhalation of NO, rats were exposed at 500-1,500 ppm for 5-30 min (Stavert and Lehnert 1990). At 1,000 ppm for 30 min, the animals were cyanotic and 11/20 died within 30 min after exposure ended. Deaths were attributed to methemoglobin formation, although concentrations of methemoglobin were not measured in this study. At concentrations up to 1,500 ppm for 15 min or at 1,000 ppm for 30 min, NO produced no increases in lung weight and did not result in any histopathologic changes in the lungs.

Groups of five male and five female rats were exposed for 6 h to NO at 0, 80, 200, 300, 400, or 500 ppm by nose-only inhalation (Waters et al. 1998). Concentrations of ≥300 ppm were lethal, and methemoglobin concentrations were significantly elevated at ≥200 ppm. No histopathologic changes in animals

exposed at 200 ppm were observed with light microscopy, but interstitial edema attributed to NO_2 contamination (2.6 ppm) was seen by electron microscopy. Further details of the results and experimental procedures were not available in the abstract.

3.1.5. Mice

BALB/c mice (n = 5-7) were exposed to NO_2 at 5, 20, or 40 ppm for 12 h (Hidekazu and Fujio 1981). Body weight was markedly decreased 1 and 2 days after exposure at 20 and 40 ppm, and 3/38 (7.8%) animals exposed at 40 ppm died within 2 days of exposure.

Hine et al. (1970) studied the effects of varying concentration and duration of exposure in Swiss-Webster mice. Animals (n = 5-14) were exposed to NO_2 at 5-250 ppm for durations of 30 min to 24 h. At concentrations of ≥40 ppm, signs of toxicity included lacrimation, reddening of the conjunctivae, and increased respiration, which became labored and difficult as the intoxication increased. Mortality was first observed at 50 ppm for 24 h (see Table 4-7). Animals exhibited gasping and spasmodic respiration, and lung edema was observed at necropsy. Histologic findings in the lungs included bronchiolitis, desquamated bronchial epithelium, infiltration by polymorphonuclear cells, and edema.

Kushneva and Gorshkova (1999) reported an LC_{50} of 190 mg/m^3 (51 ppm) for N_2O_4, with the cause of death pulmonary edema. Duration of exposure was not specified and no experimental details were provided.

In a series of experiments, mice were exposed to "predominantly" NO (Pflesser 1935). All of the animals exposed at 350 and 3,500 ppm died, and all animals exposed at 310 ppm for up to 8 h survived. The 8-h LC_{50} was reported as 320 ppm. Death appeared to be from methemoglobin formation; at necropsy, no evidence of lung injury or pulmonary edema was observed.

3.2. Nonlethal Toxicity

3.2.1. Monkeys

Squirrel monkeys (n = 2-6/group) were exposed to NO_2 at 10-50 ppm for 2 h and respiratory function monitored during exposure (Henry et al. 1969). Exposure at 35 or 50 ppm resulted in a markedly increased respiratory rate and decreased tidal volume, which returned to normal 7 days post-exposure. Only slight effects on respiratory function were noted at 15 and 10 ppm. Mild histopathologic changes in the lungs were noted after exposure at 10 and 15 ppm; however, marked changes in lung structure were observed after exposure at 35 and 50 ppm. At 35 ppm, areas of the lung were collapsed and had basophilic alveolar septa, alveoli were expanded with septal wall thinning, the bronchi were moderately inflamed and had some proliferation of the surface epithelium. At 50 ppm, extreme vesicular dilatation of alveoli or total collapse

was observed, lymphocyte infiltration was seen with extensive edema, and surface erosion of the epithelium of the bronchi was found. In addition to the effects on the lungs, interstitial fibrosis (35 ppm) and edema (50 ppm) of cardiac tissue, glomerular tuft swelling in the kidney (35 and 50 ppm), lymphocyte infiltration in the kidney and liver (50 ppm), and congestion and centrilobular necrosis in the liver (50 ppm) were observed. Although no animals died following the single exposure to NO_2 at 50 ppm, one animal died after a second exposure 2 months after the first exposure, suggesting that some of the lesions were irreversible.

3.2.2. Dogs

Carson et al. (1962) conducted a series of experiments on dogs (breed not specified; n = 2) at target concentrations of NO_2 at 50% and 25% of the LC_{50} for the rat (see Section 3.1.4). The actual concentrations varied slightly, but were within 10% of the target. Dogs exposed to NO_2 at 164 ppm for 5 min, 85 ppm for 15 min, or 53 ppm for 60 min (approximately 50% of the rat LC_{50}) had some respiratory distress, a mild cough, and ocular irritation, all of which cleared within 2 days of exposure. Dogs exposed at 125 ppm for 5 min, 52 ppm for 15 min, or 39 ppm for 60 min (approximately 25% of the rat LC_{50}) showed only mild sensory effects. No gross or microscopic lesions were found in any dog.

Greenbaum et al. (1967) exposed mongrel dogs (n = 1) to NO_2 at 0.1% (1,000 ppm) for 136 min or at 0.5% (5,000 ppm) for 5-45 min. The dog exposed at 0.1% remained in good condition throughout the exposure. Exposures at 0.5% for 15 and 22 min were not lethal, but resulted in respiratory distress and gave rise to anxiety for about 2 h and then resolved without therapy. Histopathologic examination of the lungs was not performed.

No treatment-related changes in behavior or clinical signs were observed in mongrel dogs (n = 1) exposed to NO_2 at 10-40 ppm for 6 h (Henschler and Lütge 1963).

Mongrel dogs (number not specified) exposed to NO_2 at 20 ppm for up to 24 h showed minimal signs of irritation and changes in behavior. Microscopic lesions were described as questionable evidence of lung congestion and interstitial inflammation for up to 48 h post-exposure (Hine et al. 1970).

Pulmonary ultrastructural changes were examined in beagle dogs (n = 1) exposed to NO_2 at 3-16 ppm for 1 h (Dowell et al. 1971). Intra-alveolar edema occurred in most dogs exposed at $\geq$7 ppm and was associated with impaired surfactant activity and lung compliance. Ultrastructural alterations included wide-spread bleb formation, loss of pinocytic vesicles, and mitochondrial swelling of endothelial cells. Exposure at 3 ppm resulted in bleb formation in the alveolar endothelium (observed by electron microscopy) without biochemical or physiologic changes.

Anesthetized beagle dogs (3-4 per group) were exposed to NO at 0, 80, 160, 320, or 640 ppm for 6 h (Mihalko et al. 1998; Wilhelm et al. 1998). One

animal in the 640-ppm group died. Decreased arterial oxygen concentrations were measured following exposure at 320 and 640 ppm, and increased minute volumes and decreased systemic arterial pressures were observed at 640 ppm. Methemoglobin concentrations 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 (Putensen et al. 1994a,b; Romand et al. 1994; 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 min. In all studies, NO significantly decreased pulmonary vascular resistance, decreased pulmonary artery pressure, and improved ventilation-perfusion mismatch. Methemoglobin concentrations did not exceed 1.1% (Channick et al. 1994; Putensen et al. 1994a; Romand et al. 1994).

3.2.3. Rabbits

Rabbits (strain not specified; number not specified) exposed to NO_2 at 20 ppm for up to 24 h showed minimal signs of irritation and changes in behavior. Microscopic lesions were described as questionable evidence of lung congestion and interstitial inflammation for up to 48 h post-exposure (Hine et al. 1970).

Rabbits exposed to NO_2 at 10 ppm for 2 h showed accelerated alveolar particle clearance (Vollmuth et al. 1986) and altered pulmonary arachidonic acid metabolism (Schlesinger et al. 1990). Continuous exposure of rabbits to NO_2 at 3.6 ppm for 6 days did not cause morphologic changes in the lungs (Hugod 1979).

Rabbits (strain, sex, and number not specified) exposed continuously for up to 20 h to NO_2 at 7, 14, or 28 ppm had an increase in polymorphonuclear neutrophils in the lavage fluid throughout the exposure (Gardner et al. 1977).

NO has been shown to attenuate the effects of experimentally-induced lung injury in the rabbit. Rabbits were given NO at 20 ppm for 6 h 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 exposed to NO, but the inflammatory response was not reduced. Methemoglobin concentrations did not exceed 1.5% after 6 h (Nishina et al. 1997). In another study of endotoxin-induced lung injury, increased survival occurred in rabbits treated with 10 ppm for 90 min (7/7 vs. 5/9 controls), but improvement in pulmonary gas exchange was not demonstrated (Uchida et al. 1996).

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

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

3.2.4. Pigs

Inhalation of NO at 20, 40, or 80 ppm for 5 min by healthy pigs resulted in slight, but significant ($p = 0.04$), reductions in pulmonary artery pressure (Goldstein et al. 1997). The effects of NO also have 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 NO at 10-80 ppm for up to 20 min after vasoconstriction induced by hypoxia (Hillman et al. 1997), thromboxane administration (Goldstein et al. 1997), or oleic acid administration (Shah et al. 1994). No effects on cardiac output, systemic arterial pressure, or left ventricular contractility were observed in any study. Exposure to NO at 40 ppm for 30 min by pigs with 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 exposure (Shah et al. 1994). NO inhalation did not cause histopathologic changes in the lungs, and methemoglobin concentrations were 1.7% after exposure at 80 ppm (Shah et al. 1994).

The effects of NO were studied in a porcine model of neonatal pulmonary hypertension (Nelin et al. 1994). Pigs (approximately 13 days old) were administered room air, NO at 25 ppm, nitrogen in 14% oxygen (hypoxia), or NO at 25 ppm in 14% oxygen for 15 min. NO significantly reduced pulmonary artery pressure both alone and after hypoxia, with no changes in dynamic lung compliance, pulmonary resistance, hemoglobin, hematocrit, or methemoglobin.

At the end of the experiment, NO at 1,000 ppm was administered to one animal for 15 min, which resulted in a methemoglobin concentration of 20%.

3.2.5. Sheep

Lung mechanics, hemodynamics, and blood chemistry were assessed in crossbred sheep (n = 5-6) exposed by nose- or lung-only (to mimic mouth breathing) to NO_2 at 500 ppm for 15 min; in another group exposed by lung-only, bronchoalveolar lavage fluid was examined after a 20-min exposure to NO_2 at 500 ppm (Januszkiewicz and Mayorga 1994). No changes in hemodynamics or blood chemistry occurred in either group. Mean inspired minute ventilation was significantly increased, resulting in increased breathing rate and decreased mean tidal volume, in the lung-only exposure group, but not the nose-only group. Both nose- and lung-only exposure groups had significantly increased lung resistance and decreased dynamic lung compliance. Histopathologic examination of tissue from the lung-only exposed group revealed exudative fluid distributed in a patchy, lobular pattern, with mild neutrophil infiltration; little evidence of exudation was seen in the nose-only exposed group. Epithelial cell number and total protein in bronchoalveolar lavage fluid were significantly increased in the animals exposed to NO_2, while macrophage number was decreased.

Airway reactivity to aerosolized carbachol was evaluated in crossbred sheep (n = 4-10) exposed to NO_2 at 7.5 or 15 ppm for 2 h (Abraham et al. 1980). Group means for pulmonary resistance, bronchial reactivity to carbachol, and static lung compliance were similar to those from controls at both concentrations. However, after exposure to NO_2 at 7.5 ppm, 5/9 animals showed at 57% increase in pulmonary resistance after carbachol exposure. At 15 ppm, 9/10 animals responded with either bronchoconstriction or hyper-reactivity. In a concurrent experiment, sheep were exposed to NO_2 at 15 ppm for 4 h (Abraham et al. 1980). Mean pulmonary resistance was significantly increased from the pre-exposure value, but there were no changes in pulmonary hemodynamics or clinical signs of distress.

Frostell et al. (1991) examined the effects of inhalation of NO at 5-80 ppm on the normal and acutely constricted pulmonary circulation in awake lambs. Dose-response data were collected for a 6-min exposure, and toxicity data were collected after 1 and 3 h. Pulmonary constriction was induced by either infusion of the endoperoxide analog of thromboxane, U46619, or by hypoxia. In normal lambs, exposure to NO at 80 ppm for 6 min did not affect pulmonary artery pressure or 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 NO at 80 ppm for 1 and 3 h did not increase extravascular lung water or

methemoglobin concentrations, or modify lung histology compared with control lambs.

Decreased pulmonary artery pressure also has 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 exposures to NO at 80 ppm for 3 h completely reversed U46619-induced pulmonary hypertension without affecting systemic circulation. In this study, maximum methemoglobin concentrations of 4.7% were reached in the last half hour. A similar dose-dependent reduction in pulmonary artery pressure was shown at concentrations of 4-512 ppm, with maximum effect at 64 ppm within 5-10 min. Inhalation of NO at 512 ppm for 20 min resulted in methemoglobin concentrations 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 h; 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 min and continued to increase over time. At the end of the study, methemoglobin concentrations were 3% (Zayek et al. 1993).

Decreased pulmonary artery pressure and increased arterial oxygenation occurred in sheep treated with NO at 20 ppm for 48 h 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 NO at 20 ppm for 5 h did not significantly change oxidative stress parameters or induce lung inflammation (Storme et al. 1998).

3.2.6. Guinea Pigs

Guinea pigs (strain not specified; number not given) exposed to NO_2 at 20 ppm for up to 24 h showed minimal signs of irritation and changes in behavior. Microscopic lesions were described as questionable evidence of lung congestion and interstitial inflammation for up to 48 h post-exposure (Hine et al. 1970). Guinea pigs exposed at 9 and 13 ppm for 2 h or at 5.2 and 6.5 ppm for 4 h had significantly increased respiratory rate and decreased tidal volume with complete recovery after cessation of exposure (Murphy et al. 1964).

Hartley guinea pigs (n = 5-16) on an ascorbic-acid-deficient diet had increased lung lavage fluid protein following exposure to NO_2 at 4.8 ppm for 3 h and increased wet lung weight, increased nonprotein-sulfhydryl and ascorbic-acid content of the lungs, and decreased α-tocopherol content of the lungs following exposure to NO_2 at 4.5 ppm for 16 h. These changes were not seen in animals on normal guinea pig diets (Hatch et al. 1986). Similarly, vitamin-C-deficient male Hartley guinea pigs (n = 3-12) exposed to NO_2 at 1, 3, or 5 ppm for 72 h had significantly increased protein and lipid content in lavage fluid (Selegrade et al. 1981). No effects were seen at 0.4 ppm. At 5 ppm, 50% of the animals died and histopathology revealed multifocal interstitial pneumonia.

When the exposure to 5 ppm was shortened to 3 h, lavage protein was increased with a peak effect 15 h post-exposure.

Guinea pigs (strain not specified; n = 12-18) were exposed to NO_2 at 20, 40, or 70 ppm for 30 min followed by a 30-min exposure to aerosolized albumin; this regimen was repeated 5-7 times at intervals of several days (Matsumura 1970). During the first exposure at 70 ppm, labored breathing, though not severe, was observed in "some" animals, but was not seen with subsequent exposures. Immediately after the fifth exposure to antigen, one-half of the animals in the 70-ppm group showed enhanced airway sensitization (anaphylactic attacks). No effects were seen at 20 or 40 ppm.

Changes in airway responsiveness to histamine were investigated in Hartley guinea pigs (number not specified) exposed to NO_2 at 7-146 ppm for 1 h (Silbaugh et al. 1981). Pulmonary-function measurements and histamine challenge tests were performed 2 h before and at about 10 min and 2 and 19 h after exposure to NO_2. Increased sensitivity to histamine occurred at concentrations ≥40 ppm for 10-min exposures, but returned to baseline thereafter. Significant concentration-related increases in breathing frequency and decreased tidal volume were measured at 10 min (exact concentrations not specified) and remained correlated with concentration at 2 and 19 h.

Pulmonary resistance was significantly decreased in guinea pigs exposed to NO at 300 ppm for 6 min. In the same study, exposure at 5-300 ppm for 10 min 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).

3.2.7. Hamsters

Syrian golden hamsters (n = 5) were administered NO_2 at 28 ppm for 6, 24, or 48 h and histopathologic changes in the lungs were examined by light and electron microscopy (Case et al. 1982; Gordon et al. 1983). The bronchiolar epithelium showed ciliary loss and surface-membrane damage, loss of ciliated cells, and epithelial flattening at 24 and 48 h and epithelial hyperplasia, nonciliated cell hypertrophy, and loss of tight junctions between type I pneumocytes at 48 h.

3.2.8. Ferrets

Weanling domestic ferrets (n = 4-6; 6 weeks of age) were exposed to NO_2 at5, 10, 15, or 20 ppm for 4 h (Rasmussen 1992). A transient inflammatory response was evident as a significantly increased number of neutrophils in the lavage fluid for up to 48 h post-exposure at all concentrations. Morphometrically, dose-related decreased alveolar size and thickened alveolar walls indicative of exposure were observed in the lungs.

3.2.9. Rats

Only one study was found in which rats were exposed to N_2O_4; pulmonary lesions were similar to those described following NO_2 exposure. Male Wistar rats exposed to N_2O_4 at 43 ppm for 15 min had increased lung weight, lung edema, and hemorrhaging (Yue et al. 2004). The chamber atmosphere was generated by injecting liquid N_2O_4 and heating to evaporate it. Thus, it is likely that much of the dimer was converted to NO_2.

Pulmonary injury from NO_2 indicated by increases in lung weight was assessed in male Fischer 344 rats (n = 6-12) after exposure to NO_2 at 10, 25, or 50 ppm for 5, 15, or 30 min or at 100 ppm for 5 or 15 min (Stavert and Lehnert 1990). No significant changes in lung weight occurred in rats exposed at 10 ppm for 30 min or at 25-50 ppm for up to 15 min. Significant increases in lung wet weight and right cranial-lobe dry weight were found after exposure at 50 ppm for 30 min or at 100 ppm for 5 and 15 min. However, histologic evidence of lung injury was seen in animals exposed at 25 ppm for 30 min, 50 ppm for ≥5 min, and 100 ppm for 5 and 15 min. Findings included accumulation of fibrin, increased numbers of polymorphonuclear neutrophils and macrophages, extravasated erythrocytes, and type II pneumocyte hyperplasia, the severity of which increased with concentration and duration of exposure.

In an expanded study, Lehnert et al. (1994) determined that NO_2 concentration was more important than exposure duration in the severity of lung injury. Male Fischer 344 rats (n = 8-12) were exposed to NO_2 at 25, 50, 75, 100, 150, 200, or 250 ppm for 5-30 min. Lung wet weight was significantly increased after exposure at ≥150 ppm for 5 min, 100 ppm for 15 min, or 75 ppm for 30 min and further increases were observed as exposure duration increased. The pulmonary edematous response to a given concentration was not proportional to duration; however, increasing concentrations produced proportional increases in lung wet weight when similar exposure durations were compared. Histologically, fibrin and type II cell hyperplasia were observed after 5-min exposures at ≥50 ppm, and the severity increased proportionally to concentration. As further confirmation of concentration-dependent lung injury, rats were exposed to 1-min bursts of NO_2 at 500-2,000 ppm. The severity of pulmonary edema (measured by lung wet weight) was directly proportional to exposure concentration. The authors concluded that brief exposures to high concentrations of NO_2 are more injurious than longer-duration exposures to lower concentrations. Dietary taurine (an antioxidant) was not protective against the increase in lung wet weight, and exercise potentiated the severity of the pulmonary edema.

The concentration-dependent response of the lung to NO_2 was confirmed in another study in which Sprague-Dawley rats (n = 5-6) were exposed at 3.6-14.4 ppm for 6-24 h/day for 3 days (Gelzleichter et al. 1992). Increases in protein content and cell types in lavage fluid demonstrated that the magnitude of lung injury was a function of exposure concentration.

Carson et al. (1962) conducted a series of experiments of NO_2 at concentrations approximating 50, 25, and 15% of the rat LC_{50}. At the 50% LC_{50}, rats (strain not specified; n = 30) exposed to 190 ppm for 5 min, 90 ppm for 15 min, or 72 ppm for 60 min showed signs of severe respiratory distress and ocular irritation lasting about 2 days; lung-to-body weight ratios were significantly increased during the first 48 h after exposure. Pathologic examination showed darkened areas of the lungs, pulmonary edema, and an increased incidence of chronic murine pneumonia. Rats exposed at 104 ppm for 5 min, 65 ppm for 15 min, or 28 ppm for 60 min (about 25% of the LC_{50} values) showed some respiratory distress or mild signs of nasal irritation but lung-to-body-weight ratios were increased only at 104 and 65 ppm. No gross lesions were observed, but pulmonary edema was seen microscopically. No adverse clinical signs of toxicity or pathologic changes were seen in rats exposed at 15% of the LC_{50} (74 and 33 ppm for 5 and 15 min, respectively).

Histologic changes were examined in the lungs of male rats (strain and number of animals not specified) exposed to NO_2 at 17 ppm continuously (Stephens et al. 1972). After 2 h, there was some pre- and post-capillary engorgement in the alveoli. Loss of cilia and occasional alveolar type-I cell swelling were detectable by 4 h, the terminal bronchiolar epithelium had become uniform by 16 h, maximal macrophage numbers were reached by 24 h, cellular hypertrophy had begun by 48 h, and mitotic figures became more prevalent in the epithelium of the terminal bronchiole between 16 and 48 h. Type-I alveolar cells appeared to be the most sensitive to NO_2 insult.

Results similar to those described above were obtained in a morphologic study of the Wistar-rat lung (number of animals not specified) after exposure to NO_2 at 20 ppm for 20 h (Hayashi et al. 1987). Cytoplasmic blebbing occurred in a small number of type-I cells immediately after exposure. Swelling and hyperplasia of type-II cells and pinocytotic vesicles of endothelial cells in capillaries followed by interstitial edema in the alveolar walls were observed between days 5 and 15 postexposure. Twenty days after exposure, the lesions lessened and the lungs appeared normal after 35 days. Other studies have confirmed alveolar and interstitial edema, bronchiolitis, bronchiolar epithelial-cell hyperplasia, loss of cilia, necrosis of type-I cells, and type-II cell hyperplasia 1-3 days after exposure at 26 ppm for 24 h (Schnizlein et al. 1980; Hillam et al. 1983) or at 20 ppm for 24 h (Rombout et al. 1986).

Long-Evans rats (number of animals not specified) exposed to NO_2 at 20 ppm for up to 24 h showed minimal signs of irritation and changes in behavior. Microscopic lesions were described as questionable evidence of lung congestion and interstitial inflammation for up to 48 h post-exposure (Hine et al. 1970).

The effects of NO_2 on the lung neonatal and adult Sprague-Dawley rats (number of animals not specified). Animals (1-40 days old) were exposed to NO_2 at 14 ppm for 24, 48, or 72 h continuously. Before weaning (20 days old), exposure resulted in only minor injury and loss of cilia from epithelial cells lining the terminal airways. Subsequent to weaning, there was a progressive

increase in lung injury; maximum response was reached at about 35 days of age (Stephens et al. 1978).

In a similar study to determine the sensitivity of adult and neonate animals to NO_2, Wistar rats (number of animals not specified; 5-60 days of age) were continually exposed at 2 or 10 ppm for 3 days (Azoulay-Dupuis et al. 1983). Exposure of the litters before weaning included the dam. No clinical signs of toxicity or deaths were observed in animals of any age except for body weight loss in dams of the 10 ppm-group. At 2 ppm, lung histopathology was normal in all animals. At 10 ppm, fibrinous deposits were observed in the alveoli and the tracheal and bronchiolar epithelia were occasionally devoid of cilia in animals of ≥45 days of age.

Alterations in lavage fluid have been assessed in male Long Evans rats (n = 6) after exposure to NO_2 at 10, 20, 30, or 40 ppm for 4 h. Cell-free lavage fluid contained elevated lactate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, glutathione dehydrogenase, acid phosphatase, and aryl sulfatase activity levels after exposure at ≥30 ppm. Total protein and sialic acid were increased after exposure at ≥20 ppm. Protein and sialic-acid concentrations and acid-phosphatase activity were similar to those in plasma, indicating transudation into the airways (Guth and Mavis 1985). Increases in lactate dehydrogenase, malate dehydrogenase, and glutathione-dehydrogenase activity were significantly attenuated in animals on diets providing 1,000 mg/kg of α-tocopherol, suggesting that lipid peroxidation is involved in NO_2-induced lung injury (Guth and Mavis 1986). Antioxidants in the lung were depleted, lipid-peroxidation products were elevated, and total cell count in bronchoalveolar lavage fluid and alveolar macrophage count were decreased, while epithelial cell count was increased after exposure of male Sprague-Dawley rats (n = 5) at 200 ppm for 15 min (Elsayed et al. 2002). Another study found changes in fatty-acid composition of alveolar lavage phospholipids after Wistar rats (n = 6) were exposed to NO_2 at 20 ppm for 12 h (Kobayashi et al. 1984). Increases in lavageable protein, polymorphonuclear lymphocytes, and alveolar macrophages also were observed aftermale Fischer 344 rats (number not secified) were exposed to NO_2 at 100 ppm for 15 min (Lehnert et al. 1994).

Changes in minute ventilation, V_E, were measured in male Fischer 344 rats (n = 12) after exposure to NO_2 at 100, 300, or 1,000 ppm for 1-20 min (Lehnert et al. 1994). In general, reductions in V_E were greater with the higher concentrations. For example, reductions of about 7 and 15% were measured during 15- and 20-min exposures at 100 ppm, while a reduction of about 20% and 28% were measured during 1- and 2-min exposures to 1,000 ppm. Similarly, male Sprague-Dawley rats (n = 5) exposed at 200 ppm for 15 min showed a decrease in minute ventilation that was from a decline in tidal volume but not in frequency of breathing (Elsayed et al. 2002).

Changes in lung immunity after exposure to NO_2 have been described as increased specific IgE, IgA, and IgG titers after exposure at 87 ppm for 1 h (Siegel et al. 1997) or 5 ppm for 3 h (Gilmour 1995), increased number of IgG anti-sheep red blood cell antibody-forming cells in the lung-associated lymph

nodes (Schnizlein et al. 1980), and cell proliferation in the spleen and thoracic lymph nodes (Hillam et al. 1983) after exposure at 26 ppm for 24 h.

Male Porton rats (n = 4) were exposed to an atmosphere of oxides of nitrogen that was produced by mixing NO_2 and NO (Brown et al. 1983). The ratio of each chemical was not specified or measured in the exposure chambers. Exposures were at 518 ppm for 5 min or to 1,435 ppm for 1 min. No clinical signs of toxicity were observed but "stertorous respirations" appeared within 24 h. Histologically, initial lung damage showed thickening and blebbing of the alveolar epithelium, followed by a latent period of about 6 h, after which development of edema of the interstitium and alveolar septum was observed. The early changes were attributed to a direct oxidant effect. Clinical signs and histologic findings were more severe following exposure at 518 ppm for 5 min.

The effects of NO on discrimination learning and brain activity were studied in rats (Groll-Knapp et al. 1988). Rats were exposed to NO at 10 or 50 ppm for 180 min, and the test atmospheres were 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 by electroencephalography. Maximum methemoglobin concentrations were 3.98%. The authors suggested that the effects could be, in part, from diminished oxygen-carrying capacity related to methemoglobin formation.

The effects of NO on hyperoxic lung injury in rats were investigated (Garat et al. 1997). Animals were exposed to NO at 10 or 100 ppm while breathing 21% or 100% oxygen for 40 h. No toxic effects on any lung parameter were observed at either concentration under normoxic conditions. Under hyperoxic conditions, NO at 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 with hyperoxia, and the lack of protection might have been from the formation of NO_2 in the exposure chambers.

3.2.10. Mice

Swiss-Webster mice (number not specified) exposed to NO_2 at 20 ppm for up to 24 h showed minimal signs of irritation and changes in behavior. Histologically, there was questionable evidence of lung congestion and interstitial inflammation for up to 48 h post-exposure (Hine et al. 1970). The voluntary running activity of mice on an activity-wheel was 80% and 17% of pre-exposure levels at 7.7 and 20.9 ppm, respectively, during 6-h exposures (Murphy et al. 1964).

Female CD-1 mice (n = 29-60) were examined for phenobarbital-induced sleeping time after a 3-h, whole-body exposure to NO_2 at 0.125-5.0 ppm (Miller

et al. 1980). Sleeping time was significantly increased in animals exposed at ≥0.25 ppm compared with air-exposed controls. The authors stated that no effects in males were observed until after 3 days of exposure (data not included). In contrast, the effect in females decreased after the third day of exposure suggesting some tolerance might have developed.

The alveolar septum from two female NMRI mice was examined microscopically 36 h after exposure to NO_2 at 35 ppm for 6 h (Dillmann et al. 1967). Morphometric measurements found that the arithmetic mean thickness of the alveoli was approximately 1.5 times that of unexposed controls. No changes in the numbers or types of cells present were observed and no interstitial edema was found with ultrastructure examination by electron microscopy.

Male CD-1 mice (n = 5-9) were exposed to NO_2 at 50-140 ppm for 1 h and biochemical and histologic responses were assessed immediately and 48 h after exposure (Siegel et al. 1989). Immediately after exposure at 140 ppm, cell death was visible in the terminal bronchioles and there were significant increases in protease-inhibitor activity, pulmonary protein, and lung wet weight. Two days after exposure at 140 ppm, the histologic damage was exacerbated with complete obliteration of the alveolar structure, progressive edema and congestion of the lungs, hypertrophy and hyperplasia of the epithelial cells, and increased numbers of intra-alveolar macrophages and neutrophils. In addition, there were dose-related increases in β-glucuronidase, lactate-dehydrogenase, and choline-kinase activity, as well as increased protease-inhibitor activity, pulmonary protein, and lung wet weight.

To examine the effects of NO_2 on gaseous exchange in the lung, JCL:ICR mice (n = 6) were exposed at 5, 10, or 20 ppm for 24 h (Suzuki et al. 1982). Significantly increased lung wet weight and lung water content occurred at 10 and 20 ppm. The gaseous exchange and metabolic rate of oxygen and carbon dioxide were accelerated in animals exposed at 5 ppm, while gaseous exchange in the lung was inhibited in animals exposed at 10 and 20 ppm.

Continuous exposure of C56Bl/6 mice (n = 60) to NO_2 at 20 ppm for 4 days resulted in significantly decreased food consumption and body weight, but no deaths (Bouley et al. 1986).

3.3. Developmental and Reproductive Toxicity

The postnatal effects of prenatal exposure to NO_2 were investigated (Tabacova et al. 1985). Pregnant Wistar rats (n = 20) were exposed to NO_2 at 0.265, 0.053, 0.53, or 5.3 ppm for 6 h/day throughout pregnancy. Maternal effects were not reported or discussed. Pup viability and body weight of the 5.3-ppm group were significantly less ($p \leq 0.05$) than those of controls on lactation day 21. Exposure at ≥0.53 ppm resulted in developmental delays and exposure at ≥0.053 ppm caused disturbances in neuromotor development. Also at the two highest concentrations, hexobarbital sleeping time was increased in the offspring and correlated with altered biochemical parameters in the liver.

No information was found regarding the developmental or reproductive toxicity of exogenously administered NO in animals. Growth retardation and hind-limb reduction were found in the offspring of rats given N^G-nitro-L-arginine methyl ester, a NO-synthase inhibitor, at 0.3 and 1.0 mg/mL in drinking water on gestation days 13-19 (Shepard 1995).

3.4. Genotoxicity

Three-week-old male Sprague-Dawley rats were exposed by inhalation to NO_2 at 8, 15, 21, or 27 ppm for 3 h or to NO at 9, 19, or 27 ppm for 3 h. Animals were maintained overnight before sacrifice, and lung cells were isolated. At NO_2 concentrations of ≥15 ppm and NO concentrations of 27 ppm, mutations to ouabain resistance in lung cells was increased. Concentration-dependent increases in chromosome aberrations were observed with NO_2 at 8 and 27 ppm, the only concentrations analyzed for aberrations. Chromosome aberrations were not observed following exposure to NO (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 NO at 0-20 ppm for 30 min. Oxygen was required and mutation was inhibited by antioxidants. Cytotoxicity was seen with NO at 50 ppm (Arroyo et al. 1992).

3.5. Carcinogenicity

The effect of NO_2 on promotion of lung tumorigenesis induced by *N*-bis(2-hydroxypropyl)-nitrosamine (BHPN) was investigated in male Wistar rats (Ichinose et al. 1991). Animals were given a single intraperitoneal injection of BHPN at 0.5 g/kg body weight at 6 weeks of age and exposed to NO_2 at 0.04, 0.4, or 4.0 ppm for 17 months. The incidence of pulmonary tumors in rats exposed to BHPN and NO_2 at 4 ppm was 12.5% (n.s.), with adenomas found in 4/40 rats (10%) and adenocarcinomas found in 1/40 rats (2.5%). One adenoma was found in the control group (2.5%) and one in the 0.04-ppm group, but none in the 0.4-ppm group. In addition, marked bronchiolar mucosal hyperplasia was found in 17/40 rats (42.5%, $p \leq 0.001$) in the group exposed to BHPN and NO_2 at 4.0 ppm.

No information was found regarding the carcinogenicity of NO or N_2O_4 in animals.

3.6. Summary

Five- to 60-min LC_{50} values for NO_2 in the rat ranged from 416 to 115 ppm, respectively, in one study (Carson et al. 1962) and from 833 to 168 ppm in another study (Gray et al. 1954). The 15-min LC_{50} for rabbits was 315 ppm (Carson et al. 1962). In a study using varying concentration and duration of exposure, the first mortalities were observed in dogs exposed at 75 ppm for 4 h,

in rabbits at 75 ppm for 1 h, in guinea pigs at 50 ppm for 1 h, and in rats and mice at 50 ppm for 24 h (Hine et al. 1970). Histologic alterations of the lungs following death included bronchiolitis, desquamated bronchial epithelium, infiltration by polymorphonuclear cells, and edema. Enhanced susceptibility to infection was shown in monkeys after exposure to NO_2 at 50 ppm for 2 h (Henry et al. 1969) and in mice exposed at 2 or 3.5 ppm for 3 h (Ehrlich 1978).

Pulmonary edema and histologic alterations induced by exposure to NO_2 have been characterized in dogs, sheep, guinea pigs, hamsters, rats, and mice. Numerous studies in rats have confirmed alveolar and interstitial edema, bronchiolitis, bronchiolar epithelial-cell hyperplasia, loss of cilia, necrosis of type-I cells, and type-II cell hyperplasia 1-3 day after exposure to NO_2 at 26 ppm for 24 h (Schnizlein et al. 1980; Hillam et al. 1983) or at 20 ppm for 20 h (Hayashi et al. 1987) or 24 h (Rombout et al. 1986).

Neonates appeared less sensitive to NO_2 than adult animals; progressive increases in lung injury and deaths were seen in older rats and guinea pigs (Stephens et al. 1978; Azoulay-Dupuis et al. 1983).

Only one study was found in which rats were exposed to N_2O_4, and pulmonary lesions were similar to those described after NO_2 exposure. No studies of exposure to N_2O_3 were found.

For NO, most of the experimental animal studies focused on the therapeutic use of NO in animal models of human disease. Lethality studies in dogs, rats, and mice lacked complete concentration-response information, some of the studies were confounded by possible NO_2 contamination, or the study was a secondary citation 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

Total respiratory-tract absorption by humans exposed to NO_2 at 0.29-7.2 ppm for ≤30 min during quiet respiration and during exercise was 81-90% and 91-92%, respectively, in healthy adults, and 72% and 87%, respectively, in asthmatic subjects (EPA 1993). In monkeys exposed to NO_2 at 0.30-0.91 ppm for <10 min, 50-60% of the inspired gas was retained during quiet respiration and was distributed throughout the lungs (Goldstein et al. 1977). While the isolated rat lung, ventilated with NO_2 at 5 ppm for 90 min, retained 36% of the NO_2 (Postlethwait and Mustafa 1981), the majority of labeled NO_2 (exposure parameters not specified) was retained by the upper-respiratory tract of the rat (Russell et al. 1991).

Pulmonary absorption of NO_2 has been studied using in-vivo and in-vitro models. Uptake appears to be governed by the reaction between inhaled NO_2 and constituents of the pulmonary surface lining layer, which forms nitrite (Postlethwait and Bidani 1990, 1994). NO_2 uptake is saturable, with absorption

proportional to inspired dose (Saul and Archer 1983; Postlethwait and Bidani 1994) and increased as temperature increases to a maximum of NO_2 at 10.6 μg/min in an isolated lung model (Postlethwait and Bidani 1990). The predominant reaction in the lungs involves hydrogen abstraction by readily oxidizable tissue components, such as proteins and lipids, to form nitrous acid and the nitrite radical (Postlethwait and Bidani 1994), and reaction with water to form nitrous and nitric acids (Goldstein et al. 1977).

Distribution of inhaled NO_2 or its metabolites is via the blood stream (Goldstein et al. 1977). Nitrite formed in the lungs is oxidized to nitrate by interactions with red blood cells after diffusion into the vascular space (Postlethwait and Mustafa 1981). Mice exposed to NO_2 at 40 ppm had slight (0.2%) nitrosylhemoglobin but no methemoglobin (Oda et al. 1980), and an increase in both nitrite and nitrate that reached equilibrium in 10 and 30 min, respectively (Oda et al. 1981). After cessation of exposure, the half-life of nitrite was several minutes and that of nitrate about 1 h (Oda et al. 1981). Urinary excretion of nitrate has been shown to be have a linear relationship to the inhaled concentration of NO_2 (Saul and Archer 1983).

Approximately 85-92% of NO is absorbed into the body by humans breathing normally when exposed to NO at 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 NO at 5-40 ppm 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 sequentially exposed to NO at 10-80 ppm for 10-min periods, followed by 40 ppm for 30 min, showed a concentration-related increase in plasma nitrites and nitrates with a combined concentration of 67 μmol/L at the end of exposure compared with 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 ^{15}NO at 138-880 ppm, and within 24 h, 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 bronchoalveolar 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

4.2.1. Nitrogen Dioxide

NO_2 is an irritant to the mucous membranes and might cause coughing and dyspnea during exposure. After less severe exposure, symptoms might persist

for several hours before subsiding (NIOSH 1976). With more severe exposure, pulmonary edema ensues with signs of chest pain, cough, dyspnea, cyanosis, and moist rales heard on auscultation (NIOSH 1976; Douglas et al. 1989). Death from NO_2 inhalation is caused by bronchospasm and pulmonary edema in association with hypoxemia and respiratory acidosis, metabolic acidosis, shift of the oxyhemoglobin dissociation curve to the left, and arterial hypotension (Douglas et al. 1989). A characteristic of NO_2 intoxication after the acute phase is a period of apparent recovery followed by late-onset bronchiolar injury that manifests as bronchiolitis fibrosa obliterans (NIOSH 1976; NRC 1977; Hamilton 1983; Douglas et al. 1989).

Toxicity from acute exposure can be described in one of three categories: (1) immediate death after very heavy exposure, (2) delayed symptoms with development of edema within 48 h, and (3) apparent recovery from immediate effects but later chronic chest disease of varying severity (NRC 1977; Hamilton 1983). Morphologic and biochemical changes in the lungs during these phases were studied in mice exposed at 140 ppm for 1 h (Siegel et al. 1989). Immediately after exposure, cell death was noted in areas adjacent to the distal terminal bronchioles, and protease inhibitor activity, lung protein content, and lung wet weight were significantly elevated. Two days after exposure, the histologic damage was exacerbated with complete obliteration of the alveolar structure, progressive edema and congestion of the lungs, hypertrophy and hyperplasia of the epithelial cells, and increased numbers of intra-alveolar macrophages and neutrophils. In addition, there were dose-related increases in β-glucuronidase, lactate-dehydrogenase, and choline-kinase activity as well as increased protease-inhibitor activity, pulmonary protein, and lung wet weight. Pulmonary injury is characterized by loss of ciliated cells, disruption of tight capillary junctions, degeneration of type-I cells, and proliferation of type-II cells (Siegel et al. 1989; Elsayed 1994).

The predominant reaction in the lungs involves hydrogen abstraction by readily oxidizable tissue components, such as proteins and lipids, to form nitrous acid and the nitrite radical (Postlethwait and Bidani 1994; EPA 1995) and reaction with water to form nitrous and nitric acids (Greenbaum et al. 1967; Goldstein et al. 1977). This reaction can lead to one mechanism by which NO_2 causes pulmonary injury, lipid peroxidation. NO_2 is a free radical that can attack unsaturated fatty acids in the cell membrane forming carbon and oxygen centered radicals in a chain reaction (Ainslie 1993; Elsayed 1994; EPA 1995). This hypothesis is supported by studies on the effects of antioxidants on NO_2 exposure in humans and animals. Four-week supplementation with vitamins C and E before exposure at 4 ppm for 3 h resulted in a marked decrease in the amount of conjugated dienes and attenuated the decrease in elastase activity inhibitory capacity in the alveolar lining fluid of healthy volunteers (Mohsenin 1991). Guinea pigs maintained on an ascorbic-acid-deficient diet had increased lung lavage fluid protein following exposure to NO_2 at 4.8 ppm for 3 h and increased wet lung weight, increased nonprotein sulfhydryl and ascorbic acid content of the lungs, and decreased α-tocopherol content of the lungs following

exposure at 4.5 ppm for 16 h. These changes were not seen in animals maintained on normal guinea pig diets (Hatch et al. 1986). Rats exposed at 30 and 40 ppm for 4 h had elevations of lactate-dehydrogenase, MDH, and glutathione-dehydrogenase activity in lavage fluid, which were significantly attenuated in animals maintained on diets with α-tocopherol at 1,000 mg/kg (Guth and Mavis 1986). Another study found changes in fatty-acid composition of alveolar lavage phospholipids in rats exposed to NO_2 at 10 ppm for 12 h (Kobayashi et al. 1984).

4.2.2. Nitric Oxide

From the available studies, it appears that the major mechanism of toxic action of NO is the binding of hemoglobin (EPA 1993). Inhaled NO is absorbed into the bloodstream and binds to hemoglobin forming nitrosylhemoglobin, which is rapidly oxidized to methemoglobin (Sharrock et al. 1984; Maeda et al. 1987; EPA 1993). The affinity of NO for hemoglobin is about 1,500 times greater than that of carbon monoxide (Gibson and Roughton 1957) and the binding and formation of methemoglobin is dependent on NO concentration and time (Sharrock et al. 1984; Maeda et al. 1987; Ripple et al. 1989). Experiments with rats (Maeda et al. 1987) and rabbits (Sharrock et al. 1984) show that binding of NO to hemoglobin is rapidly reversible, with a half-life of 15-20 min when animals are placed in clean air.

The signs and symptoms of methemoglobinemia in humans are summarized in Table 4-8. Clinical signs do not appear until methemoglobin concentrations are 15-20% and toxicity is not evident until about 30%.

TABLE 4-8 Signs and Symptoms Associated with Methemoglobin Concentrations

Methemoglobin Concentration (%)	Signs and Symptoms in Humans
1.1	Normal concentration
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 consciousness
55-70, ~60	Hypoxic symptoms (semi-stupor, 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 earlier in this chapter, methemoglobin concentrations were <5% even after exposure to NO at as much as 50 ppm for 41 h (human infant) or at 80 ppm for 23 h (lamb). Methemoglobin concentrations rose to 9.4% in one lung transplantation patient after treatment with NO at 80 ppm for 8 h. A reduction in concentration to 40 ppm over 4 h resulted in a decrease to 6.6%, and a further reduction to 20 ppm for the 12 h reduced methemoglobin concentrations to 0.9% (Adatia et al. 1994). In one patient with pulmonary hypertension, methemoglobin concentration rose to 9.6% after 108 h of treatment with NO at 80 ppm; in another patient, the concentration was 14% after 18 h (Wessel et al. 1994). An American Indian patient with pulmonary hypertension treated with NO at 80 ppm for 6 h developed methemoglobin concentrations 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 for persistent pulmonary hypertension with NO at 80 ppm. The average time to peak concentration in all patients was 19.6 h and the highest concentration was 11.9% at 8 h in one patient (Davidson et al. 1998).

Despite the relatively low concentrations of methemoglobin measured in most studies, clinically significant concentrations have been reported. A newborn (Japanese) developed a methemoglobin concentration of 40% after 26 h of exposure at 80 ppm; the concentration was reduced to 3.9% within 20 min of infusion with methylene blue and reduction in the NO concentration (Nakajima et al. 1997). Sheep administered NO at 512 ppm for 20 min (Dyar et al. 1993) and pigs exposed at 1,000 ppm for 15 min (Nelin et al. 1994) developed methemoglobin concentrations of 11% and 20%, respectively. Cyanosis appeared in dogs within 3-8 min of exposure to NO at 0.5 or 2% (5,000 or 20,000 ppm) and methemoglobin concentrations were 5-25%. However, concentrations reached 100% in one dog that died after exposure at 2% (20,000 ppm) for 50 min (Greenbaum et al. 1967). A single 6-h exposure of dogs to NO at 80, 160, 320, or 640 ppm resulted in methemoglobin concentrations of 3, 6.6, 24, and 78%, respectively (Wilhelm et al. 1998). Rats exposed to NO at 1,000 ppm for 30 min appeared cyanotic and 11/20 died from methemoglobin formation but concentrations were not measured (Stavert and Lehnert 1990).

The main toxicologic effect of inhaled NO is the induction of methemoglobin, whereas that of NO_2 is pulmonary edema. Methemoglobin concentrations did not increase in rats exposed to NO_2 at 40 ppm despite a slight elevation (0.2%) in nitrosylhemoglobin concentrations (Oda et al. 1980). Rats exposed to NO at 1,000 ppm for 30 min appeared cyanotic and 11/20 died from methemoglobin formation, but no changes in lung weight or histopathology were observed. In the same study, increased lung weight occurred following exposure to NO_2 at 50 ppm for 30 min and histopathologic changes were observed after exposure at 25 ppm for 30 min (Stavert and Lehnert 1990). Other studies have failed to show any effect of NO on the respiratory tract of humans (Kagawa 1982; Pepke-Zaba et al. 1991; Högman et al. 1993a; Manktelow et al. 1997), mice (Pflesser 1935), pigs (Nelin et al. 1994), or lambs (Frostell et al.

1991). An NO concentration of 10 ppm, but not 100 ppm, offered protection against hyperoxic lung injury in rats, and it is probable 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 that 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 h, 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 from 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. Chemical Transformation of Nitrogen Oxides

Figure 4-1 summarizes the reactions of the oxides of nitrogen. This family of reaction pathways is temperature dependent, but in general favors NO_2 production. The National Advisory Committee was unable to provide any significant guidance, other than to indicate that a significant fraction of the N_2O_4 and NO will be converted to NO_2. Because NO_2 is the most ubiquitous and the most toxic of the oxides of nitrogen, AEGL values derived from NO_2 toxicity data were considered applicable to all oxides of nitrogen.

$$2NO + O_2 \rightarrow 2NO_2$$

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

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

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

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

$$N_2O_4 \rightarrow 2NO_2$$

FIGURE 4-1 Environmental reactions of the oxides of nitrogen.

NO_2 exists as an equilibrium mixture of NO_2 and N_2O_4 but the dimer is not important at ambient concentrations (EPA 1993). The two compounds are phase-related forms with N_2O_4 favored in the liquid phase and NO_2 favored in the gaseous phase. An equilibrium distribution is reached, which favors the lowest energy state in the phase. As a result, when N_2O_4 is released, it vaporizes and dissociates into NO_2, making it nearly impossible to generate a significant concentration of N_2O_4 at atmospheric pressure and ambient temperature without generating a vastly higher concentration of NO_2. Because of this effect, almost no inhalation toxicity data are available on N_2O_4, and a rate for the reaction was not found. No information was found on the interactions of N_2O_3.

NO is unstable in air and undergoes spontaneous oxidation to NO_2 making experimental effects difficult to separate and studies difficult to perform (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 oxygen (20.9%) at room temperature. The delivery of NO 100 ppm in 21% oxygen through a pediatric tube (d = 0.009 m, l = 0.9 m) at a flow rate of 2 L/min is calculated to produce NO_2 at 1.13 ppm (Lindberg and Rydgren 1998). For NO at 80 ppm, a concentration commonly used therapeutically, about 5 ppm of NO_2 is calculated to form after 3 min in air (Foubert et al. 1992). NO reacts 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 (Foubert et al. 1992; Miller et al. 1994).

While 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. In photochemical smog, NO_2 absorbs sunlight of wavelengths between 290 and 430 nm and decomposes to NO and O (EPA 1993).

4.4. Other Relevant Information

4.4.1. Species Variability

Several studies indicate that there is a size-dependent species sensitivity to NO_2; larger animals are apparently less sensitive than smaller animals. Dogs showed only mild signs of irritation at concentrations that caused pulmonary edema in rats (Carson et al. 1962). Dogs also survived exposures to NO_2 at 1,000 ppm for 136 min and at 5,000 ppm for up to 22 min (Greenbaum et al. 1967) and sheep survived exposure at 500 ppm for 15-20 min (Januszkiewicz and Mayorga 1994). In contrast, 15-min and 1-h LC_{50} values in the rat were 201-

420 and 115-168 ppm, respectively (Gray et al. 1954; Carson et al. 1962). On the basis of the available data, humans are not more sensitive than larger laboratory animals. For example, irritation was reported for humans exposed to NO_2 at 30 ppm for 2 h (Henschler et al. 1960), dogs exposed at 20 ppm for 24 h (Hine et al. 1970), and monkeys exposed at 35 ppm for 2 h (Henry et al. 1969).

Elsayed et al. (2002) examined species variability to NO_2 through dosimetry; the calculated total inspired dose from experimental measurements in rats and sheep was compared with the theoretical dose of an average human. Whether normalized for body weight, lung volume, or alveolar surface area, the total effective dose was greater in rats then sheep then humans. Taking physiologic and anatomical factors into consideration, rats had a much higher effective dose than the larger animals. The authors concluded that NO_2 toxicity is associated with inhaled-dose distribution per unit lung volume or lung surface rather than per unit body mass (Elsayed et al. 2002).

No information was available to allow comparison of NO toxicity between species. 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 interspecies variation is expected.

4.4.2. Susceptible Populations

For chronic, low-level exposures to NO_2, EPA (1995) has identified two populations as potentially at risk from NO_2 exposure: children (5-12 years old) and persons with pre-existing respiratory disease. Conclusions drawn from epidemiology studies were that 5-12 year-old children had an increased risk of about 20% for developing respiratory symptoms and disease with each increase of 0.015 ppm in estimated 2-week average NO_2 exposure (mean weekly concentrations in bedrooms was 0.008-0.065 ppm) and that no evidence for increased risk was found for infants <2 years old. These conclusions are supported somewhat by animal data in which adult animals were more sensitive than neonates to the effects of NO_2 (Stephens et al. 1978; Azoulay-Dupuis et al. 1983). Reduced ventilatory reserves may prevent individuals with respiratory disease from resuming normal activity following exposure to NO_2 (EPA 1995). However, it is not certain whether these populations also are at particular risk from acute exposure scenarios.

Taken together, the data summarized in Section 2.2.3.1 indicate that some asthmatic subjects exposed to NO_2 at 0.3-0.5 ppm may respond with either subjective symptoms or slight changes in pulmonary function of no clinical significance. At approximately these same concentrations of NO_2, subsequent exposure of asthmatic subjects to an agent that causes nonspecific airway responsiveness resulted in slight hyper-reactivity, but the response is not more severe than to NO_2 alone (e.g., while some asthmatic subjectss respond to

bronchial challenge and to NO_2, the response to the challenge is not additively increased from prior exposure to NO_2). In contrast, some asthmatic subjects did not respond to NO_2 with changes in pulmonary function or symptoms at concentrations of 0.5-4 ppm. The responses of healthy individuals to NO_2 also are variable, with some, but not all, having slight changes in pulmonary function at 5 ppm. All reported responses in both asthmatic and healthy subjects at the concentrations discussed were slight and of questionable biologic or clinical significance.

Conclusions regarding differences in susceptibility between healthy and asthmatic individuals are difficult to draw from the available data because of the high variability in responses among both groups. There is only one study that has measured the responses of both healthy and asthmatic individuals with the same study protocol (Linn and Hackney 1983). Dose-response patterns were not discernible at low concentrations and clear thresholds were not apparent. Some individuals reported clinical symptoms in the absence of changes in pulmonary function, while other individuals had measurable changes in pulmonary function tests but no symptoms. One proposed explanation for the variability in the responses of asthmatic subjects to inhaled NO_2 is the existence of a subgroup of "responders." From one laboratory, several asthmatic subjects were identified as equally responsive to NO_2 at 0.3 ppm in more than one study (Bauer et al. 1985, 1986). However, the investigators could find no common identifiers for these "responders," such as degree of baseline obstruction or their inherent airway reactivity to carbachol or cold air (Utell 1989). Although some individuals had a measurable response at lower concentrations, the magnitude of the reported changes was not biologically or clinically significant in either asthmatic subjects or healthy individuals.

No information was available to allow comparison of NO toxicity between individuals. Because the major toxic action of NO is binding to hemoglobin resulting in methemoglobinemia, little intraspecies variation is expected. In addition, NO is administered for extended periods of time to critically ill patients with only slight increases in methemoglobin concentrations.

4.4.3. Concentration-Response Relationship

As discussed below for AEGL-2 and -3 levels, extrapolations were made to each of the time points using the equation $C^n \times t = k$, where $n = 3.5$ (ten Berge et al. 1986). The value of n was calculated by ten Berge et al. (1986) on basis of data from Hine et al. (1970). The large value of n indicates that concentration is more important than duration for the effects of exposure to NO_2. Support for this supposition also comes from Gardner et al. (1979), who showed that short-term exposure to high concentrations resulted in greater effects (as measured by mortality in a infectivity model using mice) than exposure to lower concentrations administered over a longer duration.

4.4.4. Susceptibility to Infection

To determine the effects of NO_2 on resistance to infection, squirrel monkeys were challenged with *Klebsiella pneumoniae* within 24 h after exposure. No deaths occurred from exposure to NO_2 alone; however, 3/3 monkeys died within 72 h after exposure to NO_2 at 50 ppm 2 h, followed by challenge with *K. pneumoniae*; massive infection was present in the lungs and other organs. No death occurred in monkeys exposed at 10 ppm for 2 h and challenged with *K. pneumoniae* 3-5 days later, but bacteria were still present in lung tissue at necropsy up to 46 days after challenge indicating reduced clearance (Henry et al. 1969).

Numerous studies have reported enhanced susceptibility of mice to infectious agents following exposure to NO_2. Most of these studies have been reviewed by EPA (1993) and only a few are described here. Gardner et al. (1977) demonstrated that the concentration-time relationship was linear for 20% mortality using an infectivity model in mice challenged with *Streptococcus pyogenes*; NO_2 exposures ranged from 0.5 to 28 ppm for 6 min to 12 months. Similarly, mortality was increased in mice challenged with *S. pyogenes* in response to short-term exposure to a high concentration of NO_2 compared with a lower concentration administered over a longer duration when the concentration × time product was held constant. A single 3-h exposure to NO_2 at 2.0 or 3.5 ppm enhanced the susceptibility of three strains of mice to streptococcal pneumonia and influenza infection, as seen by excess mortality and reduced survival time (Ehrlich 1978). Mice exercised in a motorized wheel during exposure to NO_2 at 3 ppm for 3 h and challenged with *S. pyogenes* had significantly increased mortality compared with nonexercised animals (Illing et al. 1980). Pulmonary bacterial defenses against *Staphylococcus aureus* were suppressed following exposure of Swiss mice to concentrations of NO_2 at ≥4 ppm for 4 h (Jakab 1987). Significantly decreased pulmonary bactericidal activity was shown in Swiss mice infected with *S. aureus* then exposed to NO_2 at 7, 9.2, or 14.8 ppm for 4 h, or exposed at 2.3 or 6.6 ppm for 17 h prior to infection. Histologically the lungs of mice exposed at ≥9.2 ppm for 4 h showed vascular hyperemia, while those from mice exposed at ≥2.3 ppm for 17 h had minor vascular hyperemia and interstitial edema (Goldstein et al. 1973). Enhanced susceptibility to infection was observed in CD-1 mice exposed to NO_2 at 5 ppm for 6 h/day on two consecutive days prior to inoculation with murine cytomegalovirus, followed by exposure at 5 ppm for 6 h/day for 4 consecutive days; there was no histologic evidence of lung injury (Rose et al. 1989). Continuous exposure of mice to NO_2 at 20 ppm for 4 days resulted in impairment of acquired resistance (decreased ED_{50}) in C57Bl/6 mice immunized prior to challenge with *K. pneumoniae* (Bouley et al. 1986).

Alterations in host-defense mechanisms have been demonstrated in rabbits. Male and female New Zealand rabbits exposed for 3 h to varying concentrations of NO_2 had an increase in polymorphonuclear neutrophils obtained by pulmonary lavage at ≥8 ppm, with the peak infiltration 6-9 h after

the end of exposure (Gardner et al. 1969). In other experiments, these authors demonstrated that the response persisted up to 72 h post-exposure and that phagocytic activity was inhibited.

Mice also have been used extensively as a model for immune function alterations following NO_2 exposure. Decreases in splenic and thymic weights, cellularity, plaque-forming cell responses, and hemagglutinins, along with decreased body weight, were observed in C56Bl/6 mice exposed to NO_2 at 20 ppm for 48 h (Azoulay-Dupuis et al. 1985). Significant suppression of primary antibody responses (hemagglutinins and plaque-forming cells) also were seen in BALB/c mice following exposure at 20 or 40 ppm for 12 h (Hidekazu and Fujio 1981). Phytohemagglutinin and bacterial-lipopolysaccharide responses were depressed in mice exposed continuously to NO_2 at 0.5 or 0.1 ppm, with daily 3-h peaks (5 days/week) of 0.25, 0.5, or 1.0 ppm (Maigetter et al. 1978). Other effects of NO_2 on cellular and humoral immunity have been reviewed by EPA (1993), but are not relevant to derivation of AEGL values.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

The evidence indicates that some asthmatic subjects exposed to NO_2 at 0.3-0.5 ppm might respond with either subjective symptoms or slight changes in pulmonary function of no clinical significance. Some asthmatic subjects exposed at approximately same concentrations might show slight hyper-reactivity to a bronchial challenge, but the response is no more severe than the response to NO_2 alone (e.g., while some asthmatic subjects respond to a bronchial challenge and to NO_2, the response to the challenge is not additively increased from prior exposure to NO_2). In contrast, some asthmatic subjects did not respond to NO_2 at concentrations of 0.5-4 ppm. The responses of healthy individuals to NO_2 exposures also are variable, with some, but not all, responding at 5 ppm.

Kerr et al. (1978, 1979) reported that 7/13 asthmatic subjects experienced slight burning of the eyes, slight headache, chest tightness, and labored breathing with exercise when exposed to NO_2 at 0.5 ppm for 2 h; at that concentration, the odor of NO_2 was perceptible but the subjects lost awareness of it after about 15 min. No changes in any pulmonary function tests were found immediately following the chamber exposure (Kerr et al. 1978, 1979). Significant group-mean reductions in FEV_1 (-17.3 vs. -10.0%) and specific airway conductance (-13.5 vs. -8.5%) occurred in asthmatic subjects after exercise when exposed at 0.3 ppm for 4 h, and 1/6 individuals experienced chest tightness and wheezing (Bauer et al. 1985). The onset of effects was delayed when exposures were by oral-nasal inhalation compared with oral inhalation. This delay may result from scrubbing within the upper airway. In a similar study, asthmatic subjects exposed at 0.3 ppm for 30 min at rest, followed by 10 min of exercise, had significantly greater reductions in FEV_1 (10% vs. 4% with air) and partial expiratory flow rates at 60% of total lung capacity, but no

symptoms were reported (Bauer et al. 1986). In a preliminary study with 13 asthmatic subject exposed at 0.3 ppm for 110 min, slight cough, dry mouth and throat, and significantly greater reduction (11% vs. 7%) in FEV_1 occurred after exercise; however, in a larger study, no changes in pulmonary function were measured and no symptoms were reported when 21 asthmatic subjects were exposed at concentrations up to 0.6 ppm for 75 min (Roger et al. 1990). The mean drop in FEV_1 for asthmatic subjects during a 3-h exposure to NO_2 at 1 ppm (2.5%) with intermittent exercise was significantly greater than the drop during air (1.3%) exposure with intermittent exercise; in bronchoalveolar lavage fluid, concentrations of 6-keto-prostaglandin$_{1\alpha}$ were decreased and thromboxane B_2 and prostaglandin D_2 were increased after NO_2 exposure (Jörres et al. 1995).

5.2. Summary of Animal Data Relevant to AEGL-1

Animal data relevant to derivation of AEGL-1 are limited. Slight irritation was noted in squirrel monkeys exposed to NO_2 at 10 and 15 ppm for 2 h (Henry et al. 1969) and mild sensory effects occurred in dogs exposed at 125 ppm for 5 min, 52 ppm for 15 min, or 39 ppm for 60 min (Carson et al. 1962).

5.3. Derivation of AEGL-1

AEGL values were based on studies of NO_2, the predominant form of the nitrogen oxides, and the values are considered applicable to all nitrogen oxides. Values for N_2O_4 in units of ppm have been calculated on a molar basis. 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 in emergency planning for NO. However, short-term exposures below an NO concentration of 80 ppm should not constitute a health hazard.

The study by Kerr et al. (1978, 1979) was considered the most appropriate to use as the basis for AEGL-1 values. Asthmatic subjects exposed to NO_2 at 0.5 ppm 2 h showed clinical signs but no changes in pulmonary function. Since asthmatic subjects are potentially the most susceptible population, no uncertainty factor was applied. Therefore, a concentration of 0.94 mg/m^3 (NO_2 or NO at 0.50 ppm or N_2O_4 at 0.25 ppm) was adopted for all time points (see Table 4-9), because adaptation to mild sensory irritation occurs. In addition, animal responses to NO_2 have demonstrated a much greater dependence on concentration than on time; therefore, extending the 2-h concentration to 8 h should not exacerbate the human response.

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Human data relevant to AEGL-2 values are limited but consistent. Henschler et al. (1960) performed several experiments on healthy, male volunteers

and found that exposure to NO_2 at 30 ppm for 2 h caused definite discomfort. Three individuals exposed at 30 ppm for 2 h perceived an intense odor on entering the chamber, but odor detection quickly diminished and was completely absent after 25-40 min. One individual experienced a slight tickling of the nose and throat mucous membranes after 30 min, and others after 40 min. From 70 min, all subjects experienced a burning sensation and an increasingly severe cough for the next 10-20 min, but coughing decreased after 100 min. However, the burning sensation continued and moved into the lower sections of the airways and was finally felt deep in the chest. At that time, marked sputum secretion and dyspnea were noted. Toward the end of the exposure, the subjects' condition was described as bothersome and barely tolerable. A sensation of pressure and increased sputum secretion continued for several hours after cessation of exposure (Henschler et al. 1960). In a similar experiment (Henschler and Lütge 1963), groups of four or eight healthy male volunteers were exposed at 10 ppm for 6 h or at 20 ppm for 2 h. All subjects noted the odor on entering the chamber, but detection diminished rapidly. At 20 ppm, minor scratchiness of the throat was felt after about 50 min and three of eight subject experienced slight headaches near the end of the exposure period.

6.2. Summary of Animal Data Relevant to AEGL-2

Several animal studies are relevant to AEGL-2 derivation. Hine et al. (1970) noted lacrimation, reddening of the conjunctivae, and increased respiration in five species exposed to NO_2 at ≥40 ppm for varying durations. Lethality did not occur until concentrations and durations reached 75 ppm for 4 h in the dog and 1 h in the rabbit, 50 ppm for 1 h in the guinea pig, and 50 ppm for 24 h in the rat and mouse. At 20 ppm for 24 h, all species showed minimal signs of irritation and changes in behavior with histopathologic lesions described as questionable evidence of lung congestion and interstitial inflammation.

Exposure of monkeys to NO_2 at 35 ppm for 2 h resulted in irritation as measured by changes in lung function and microscopic lesions in the lung (Henry et al. 1969). The histologic lesions in the lung were characterized by Siegel et al. (1989) following exposure of mice at 140 ppm for 1 h. Carson et al. (1962) conducted a series of experiments in dogs and rats. Mild irritation and some respiratory effects, but no gross or microscopic lesions, were noted in dogs exposed to NO_2 at 53 or 39 ppm for 1 h, while rats exposed at 72 ppm for 1 h showed signs of severe respiratory distress and ocular irritation as well as gross lesions in the lung and evidence of infection.

TABLE 4-9 AEGL-1 Values for Nitrogen Dioxide, Nitric Oxide, and Nitrogen Tetroxide

Chemical	10 min	30 min	1 h	4 h	8 h
NO_2 and NO	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)
N_2O_4	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)

Developmental delays and disturbances in neuromotor development were reported for rat pups following maternal exposure to NO_2 (Tabacova et al. 1985). However, these effects were reported to have occurred at levels near ambient concentrations and are well below those of most other studies in both humans and animals.

6.3. Derivation of AEGL-2

AEGL values were based on studies of NO_2, the predominant form of the nitrogen oxides, and the values are considered applicable to all nitrogen oxides. Values for N_2O_4 in units of ppm were calculated on a molar basis. 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 in emergency planning for NO.

On the basis of both human and animal data, it appears that NO_2 at concentrations of ≥30 ppm are required before marked irritation, discomfort, and respiratory effects occur. Therefore, 30 ppm for a 2-h exposure in humans (Henschler et al. 1960) was used to derive AEGL-2 values. The point-of-departure was considered a threshold for AEGL-2 effects, because the effects noted by the subjects would not impair the ability to escape and the effects were reversible after cessation of exposure. Values were scaled for 10- and 30-min and 1-, 4-, and 8-h AEGL-2 end points using the equation $C^n \times t = k$ using $n = 3.5$ (ten Berge et al. 1986). The value of n was calculated by ten Berge et al. (1986) from data on all species tested by Hine et al. (1970). An intraspecies uncertainty factor of 3 was applied to account for sensitive subpopulations because the mechanism of action for a direct-acting irritant is not expected to differ greatly among individuals (see Section 4.2 for detailed information regarding the mechanism of respiratory toxicity). The application of additional uncertainty factors would make the values inconsistent with some of the experimental data on asthmatic subjects, such as the no-adverse-effect concentration of 4 ppm in the study by Linn and Hackney (1984). AEGL-2 values are presented in Table 4-10.

These levels are not expected to cause severe effects because coal miners were exposed to peak NO_2 concentrations of 14 ppm without adverse consequences (Robertson et al. 1984), and it can be assumed that the peak levels were not sustained longer than a few minutes. Similar AEGL-2 values are derived from a study of mice exposed NO_2 at 140 ppm for 1 h (Siegel et al. 1989), with the application of an uncertainty factor of 10, and from a study of monkeys exposed at 35 ppm for 2 h (Henry et al. 1969), with the application of an uncertainty factor of 3. If the animal data from either Hine et al. (1970) or Carson et al. (1962) are used, the AEGL-2 values are even more conservative than those derived with the use of human data.

TABLE 4-10 AEGL-2 Values for Nitrogen Dioxide, Nitric Oxide, and Nitrogen Tetroxide

Chemical	10 min	30 min	1 h	4 h	8 h
NO_2 and NO	20 ppm (38 mg/m^3)	15 ppm (28 mg/m^3)	12 ppm (23 mg/m^3)	8.2 ppm (15 mg/m^3)	6.7 ppm (13 mg/m^3)
N_2O_4	10 ppm (38 mg/m^3)	7.6 ppm (28 mg/m^3)	6.2 ppm (23 mg/m^3)	4.1 ppm (15 mg/m^3)	3.5 ppm (13 mg/m^3)

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

A welder was hospitalized with pulmonary edema after exposure to NO_2 at approximately 90 ppm for 30-40 min (Norwood et al. 1966). It is possible that without medical intervention, the exposure could have been fatal.

Concentrations of NO_2 greater than150 ppm are probably fatal to humans because of bronchospasm and pulmonary edema (NRC 1977; Douglas et al. 1989). A human 1-h LC_{50} of 174 ppm was estimated from data on five animal species (Book 1982); however, the data were not considered valid experimental data on which to base AEGL-3 values. No other human data were relevant to derivation of AEGL-3 values.

7.2. Summary of Animal Data Relevant to AEGL-3

Squirrel monkeys (n = 2-6/group) were exposed to NO_2 at 10-50 ppm for 2 h, and respiratory function was monitored during exposure (Henry et al. 1969). NO_2 alone resulted in a markedly increased respiratory rate and decreased tidal volume at concentrations of 50 or 35 ppm, but caused only slight effects at 15 and 10 ppm. Mild histopathologic changes in the lungs were noted after exposure at 10 and 15 ppm; however, marked changes in lung structure were observed at 35 and 50 ppm. At 35 ppm, areas of the lung were collapsed with basophilic alveolar septa; in other areas, the alveoli were expanded with septal-wall thinning, and the bronchi were moderately inflamed with some proliferation of the surface epithelium. At 50 ppm, extreme vesicular dilatation or total collapse of alveoli, lymphocyte infiltration with extensive edema, and surface erosion of the bronchial epithelium were observed. In addition to the effects on the lungs, interstitial fibrosis (35 ppm) and edema (50 ppm) of cardiac tissue, glomerular tuft swelling in the kidney (35 and 50 ppm), lymphocyte infiltration in the kidney and liver (50 ppm), and congestion and centrilobular necrosis in the liver (50 ppm) were observed.

Rats exposed at 72 ppm for 60 min (approximately 50% of the LD_{50}) showed signs of severe respiratory distress and ocular irritation lasting about 2

days; lung-to-body-weight ratios were significantly increased during the first 48 h after exposure (Carson et al. 1962).

Lethality from NO_2 in five animal species first occurred at 75 ppm for 4 h in the dog and 1 h in the rabbit, 50 ppm for 1 h in the guinea pig, and 50 ppm for 24 h in the rat and mouse (Hine et al. 1970). In general, the larger animals, including humans, are less susceptible to toxicity from NO_2 inhalation than rodents.

7.3. Derivation of AEGL-3

AEGL values were based on studies of NO_2, the predominant form of the nitrogen oxides, and values are considered applicable to all nitrogen oxides. Values for N_2O_4 in units of ppm have been calculated on a molar basis. 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 in emergency planning for NO.

The data from the monkey are considered the best available for derivation of AEGL-3 values. Signs of marked irritation and severe lung histopathology were observed from exposure to NO_2 at 50 ppm for 2 h. This exposure scenario was extrapolated to the 10- and 30-min and 1-, 4-, and 8-h time points using the equation $C^n \times t = k$ where $n = 3.5$ (ten Berge et al. 1986). The value of n was calculated by ten Berge et al. (1986) from the data on all species studied by Hine et al. (1970). A total uncertainty factor of 3 was applied, which includes a 3 for intraspecies variability and a 1 for interspecies variability. Use of a greater intraspecies uncertainty factor was considered unnecessary, because the mechanism of action for direct-acting respiratory irritants is not expected to differ greatly among individuals (see Section 4.2 for detailed information regarding the mechanism of respiratory toxicity). Because the end point in the monkey study is below the definition of AEGL-3, human data support the point-of-departure and derived values, and the respiratory tracts of humans and monkeys are similar, an interspecies uncertainty factor is not considered necessary. The mechanism of action of NO_2 does not vary between species with the target at the alveoli. AEGL-3 values for NO_2, NO, and N_2O_4 are presented in Table 4-11.

TABLE 4-11 AEGL-3 Values for Nitrogen Dioxide, Nitric Oxide, and Nitrogen Tetroxide

Chemical	10 min	30 min	1 h	4 h	8 h
NO_2 and NO	34 ppm (64 mg/m^3)	25 ppm (47 mg/m^3)	20 ppm (38 mg/m^3)	14 ppm (26 mg/m^3)	11 ppm (21 mg/m^3)
N_2O_4	17 ppm (64 mg/m^3)	13 ppm (47 mg/m^3)	10 ppm (38 mg/m^3)	7.0 ppm (26 mg/m^3)	5.7 ppm (21 mg/m^3)

The AEGL-3 values are supported by human data from a welder. Pulmonary edema, confirmed on x-ray, resulted from exposure to NO_2 at approximately 90 ppm for up to 40 min (Norwood et al. 1966). If this exposure scenario is used for derivation of AEGL-3 values and an uncertainty factor of 3 is applied, the 10- and 30-min and 1-, 4-, and 8-h values are 45, 33, 27, 18, and 15 ppm, respectively. Similar results are obtained from a study in rats exposed to NO_2 at 72 ppm for 1 h (Carson et al. 1962), and an uncertainty factor of 3 is applied. In addition, the AEGL-3 values are below the concentrations at which lethality first occurred in five animal species (Hine et al. 1970).

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity End Points

AEGL values for NO_2 and NO are summarized in Table 4-12, and the values for N_2O_4 are in Table 4-13. Values were derived on the basis of data on NO_2, and are considered applicable to the other oxides of nitrogen. Values for N_2O_4 in units of ppm have been calculated on a molar basis.

8.2. Comparison with Other Standards and Criteria

Standards and guidelines for workplace and community exposures to NO_2 are presented in Table 4-14. No standards or guidelines for exposure to N_2O_4 were found. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a Threshold Limit Value (TLV) of 3 ppm for workers (ACGIH 2003), and the Occupational Safety and Health Administration's Permissible Exposure Limit (PEL) is a ceiling concentration of 5 ppm (29 CFR§1910.1000[1999]). The Immediately Dangerous to Life or Health (IDLH) value of the National Institute for Occupational Safety and Health (NIOSH) is 20 ppm (NIOSH1994a), which is exactly between the 30-min AEGL-2 and AEGL-3 values. The IDLH is reportedly based on acute inhalation data in humans, but no primary references were listed in the documentation; NIOSH notes that the IDLH may be a conservative value because of the lack of relevant acute toxicity data on workers exposed at concentrations above 20 ppm. Emergency Response Planning Guidelines (ERPGs) (AIHA 2003), based on human and animal data, are similar to the 1-h AEGL values. The National Research Council 1-h Emergency Exposure Guidance Level (EEGL) is 1 ppm for workplace conditions (NRC 1985). The occupational exposure limits of ACGIH, Germany, The Netherlands, and Sweden are 2-5 ppm.

In addition to the standards in Table 4-14, air-quality standards also have been developed for NO_2. The National Ambient Air Quality Standard is 0.053 ppm (40 CFR §50.11[1997]) with Significant Harm Levels of 2 ppm for a 1-h average and 0.5 ppm for a 24-h average (40 CFR §51.151[1987]). The Level of Concern is 5 ppm (EPA 1987). California has adopted 0.25 ppm as the standard for a 1-h exposure to NO_2 to protect sensitive individuals (CalEPA 2007).

TABLE 4-12 Summary of AEGL Values for Nitrogen Dioxide and Nitric Oxide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (nondisabling)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)	0.50 ppm (0.94 mg/m^3)
AEGL-2 (disabling)	20 ppm (38 mg/m^3)	15 ppm (28 mg/m^3)	12 ppm (23 mg/m^3)	8.2 ppm (15 mg/m^3)	6.7 ppm (13 mg/m^3)
AEGL-3 (lethal)	34 ppm (64 mg/m^3)	25 ppm (47 mg/m^3)	20 ppm (38 mg/m^3)	14 ppm (26 mg/m^3)	11 ppm (21 mg/m^3)

TABLE 4-13 Summary of AEGL Values for Nitrogen Tetroxide

Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (nondisabling)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)	0.25 ppm (0.94 mg/m^3)
AEGL-2 (disabling)	10 ppm (38 mg/m^3)	7.6 ppm (28 mg/m^3)	6.2 ppm (23 mg/m^3)	4.1 ppm (15 mg/m^3)	3.5 ppm (13 mg/m^3)
AEGL-3 (lethal)	17 ppm (64 mg/m^3)	13 ppm (47 mg/m^3)	10 ppm (38 mg/m^3)	7.0 ppm (26 mg/m^3)	5.7 ppm (21 mg/m^3)

TABLE 4-14 Extant Standards and Guidelines for Nitrogen Dioxide

	Exposure Duration				
Guideline	10 min	30 min	1 h	4 h	8 h
AEGL-1	0.50 ppm	0.50 ppm	0.50 ppm	0.50 ppm	0.50 ppm
AEGL-2	20 ppm	15 ppm	12 ppm	8.2 ppm	6.7 ppm
AEGL-3	34 ppm	25 ppm	20 ppm	14 ppm	11 ppm
ERPG-1 (AIHA)[a]			1 ppm		
ERPG-2 (AIHA)			15 ppm		
ERPG-3 (AIHA)			30 ppm		
EEGL (NRC)[b]			1 ppm	0.25 ppm	0.12 ppm
IDLH (NIOSH)[c]		20 ppm			
TLV-STEL (ACGIH)[d]	5 ppm				
REL-STEL (NIOSH)[e]	1 ppm				
PEL-STEL (OSHA)[f]	1ppm				
TLV-TWA (ACGIH)[g]					3 ppm
PEL-C (OSHA)[h]					5 ppm
MAK (Germany)[i]					5 ppm
MAK Peak Exposure (Germany)[j]	5 ppm				
MAC (The Netherlands)[k]					2.0 ppm
OEL-LLV (Sweden)[l]					2 ppm
OEL-CLV (Sweden)[m]	5 ppm				

[a]ERPG (Emergency Response Planning Guidelines, American Industrial Hygiene Association) (AIHA 2003).

The ERPG-1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing other than mild, transient adverse health effects or without perceiving a clearly defined objectionable odor.
The ERPG-2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair an individual's ability to take protective action.
The ERPG-3 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing life-threatening health effects.
[b]EEGL (Emergency Exposure Guidance Levels, National Research Council) (NRC 1985) is the concentration of contaminants that can cause discomfort or other evidence of irritation or intoxication in or around the workplace, but avoids death, other severe acute effects, and long-term or chronic injury.
[c]IDLH (Immediately Dangerous to Life or Health, National Institute of Occupational Safety and Health) (NIOSH 1994a) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms, or any irreversible health effects. The IDLH for NO_2 is based on acute inhalation toxicity data in humans.
[d]TLV-STEL (Threshold Limit Value-Short-Term Exposure Limit, American Conference of Governmental Industrial Hygienists) (ACGIH 2003) is defined as a 15-minTWA exposure that should not be exceeded at any time during the workday even if the 8-h TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 min and should not occur more than 4 times per day. There should be at least 60 min between successive exposures in this range.
[e]REL-STEL (Recommended Exposure Limits-Short Term Exposure Limit, National Institute of Occupational Safety and Health) (NIOSH 2010a) is defined analogous to the ACGIH TLV-STEL.
[f]PEL-STEL (Permissible Exposure Limits-Short Term Exposure Limit, Occupational Health and Safety Administration) (NIOSH 2010 3) is defined analogous to the ACGIH TLV-STEL.
[g]TLV-TWA (Threshold Limit Value - Time-Weighted Average, American Conference of Governmental Industrial Hygienists) (ACGIH 2003) is the TWA concentration for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.
[h]PEL-C (Permissible Exposure Limits-Ceiling, Occupational Health and Safety Administration) (29CFR§1910.1000[1999]) is a value that must not be exceeded during any part of the workday.
[i]MAK (Maximale Argeitsplatzkonzentration [Maximum Workplace Concentration]) (Deutsche Forschungsgemeinschaft [German Research Association](DFG 2002) is defined analogous to the ACGIH TLV-TWA.
[j]MAK Spitzenbegrenzung (Peak Limit [Category I, 1]) (German Research Association (DFG 2002) constitutes the average concentration to which workers can be exposed for up to 15 min, with no more than 1 excursion per work shift and a minimum of 1 h between excursions.
[k]MAC (Maximaal Aanvaaarde Concentratie [Maximal Accepted Concentration]) (Dutch Expert Committee for Occupational Standards, The Netherlands (MSZW 2004 is defined analogous to the ACGIH TLV-TWA.

[l]OEL-LLV (Occupational Exposure Limits - Level Limit Value) (Swedish Work Environment Authority 2005) is an occupational exposure limit value for exposure during 1 working day.
[m]OEL-CLV (Occupational Exposure Limits - Ceiling Limit Value) (Swedish Work Environment Authority 2005) is an occupational exposure limit for exposure during a reference period of 15 min.

Standards and guidance levels for workplace and community exposures to NO are presented in Table 4-15. An occupational time-weighted average of 25 ppm has been adopted by several groups (29CFR§1910.1000[1999]; ACGIH 2003; NIOSH 2010b). International standards also are 25 ppm for a workday (MSZW 2004; Swedish Work Environmental Authority 2005). In addition, Sweden has adopted 50 ppm as a short-term exposure limit, and the IDLH of 100 ppm (NIOSH 1994b) is based on human and animal studies of oxides of nitrogen because of the lack of useful data on NO.

8.3. Data Adequacy and Research Needs

Data on the effects of NO_2 on asthmatic subjects and individuals with respiratory disease were inconsistent and inconclusive. Additional studies that correlate severity of disease with individual responses would be helpful.

TABLE 4-15 Extant Standards and Guidelines for Nitric Oxide

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

[a]IDLH (Immediately Dangerous to Life or Health, National Institute of Occupational Safety and Health) (NIOSH 1994b) represents the maximum concentration from which one could escape within 30 min without any escape-impairing symptoms, or any irreversible health effects.
[b]TLV-TWA (Threshold Limit Value - Time Weighted Average, American Conference of Governmental Industrial Hygienists) (ACGIH 2003) is the TWA concentration for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.
[c]PEL-TWA (Permissible Exposure Limits – Ceiling, Occupational Health and Safety Administration) (29 CFR§1910.1000 [1999]) is defined analogous to the ACGIH TLV-TWA, but is for exposures of no more than 10 h/day, 40 h/week.

[d]REL-TWA (Recommended Exposure Limits - time weighted average, National Institute of Occupational Safety and Health) (NIOSH 2010b) is defined analogous to the ACGIH TLV-TWA.
[e]MAC (Maximaal Aanvaaarde Concentratie [Maximal Accepted Concentration]) (Dutch Expert Committee for Occupational Standards, The Netherlands (MSZW 2004) is defined analogous to the ACGIH TLV-TWA.
[f]OEL-LLV (Occupational Exposure Limits - Level Limit Value) (Swedish Swedish Work Environment Authority 2005) is an occupational exposure limit value for exposure during 1 working day.
[g]OEL-CLV (Occupational Exposure Limits - Ceiling Limit Value) (Swedish Swedish Work Environment Authority 2005) is an occupational exposure limit value for exposure during a reference period of 15 min.

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APPENDIX A

DERIVATION OF AEGL VALUES FOR NITROGEN OXIDES

Derivation of AEGL-1 Values

Key Studies:	Kerr, H.D., T.J. Kulle, M.L. McIlhany, and P. Swidersky. 1978. Effects of Nitrogen Dioxide on Pulmonary Function in Human Subjects: An Environmental Chamber Study. EPA/600/1-78/025. Health Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC. Kerr, H.D., T.J. Kulle, M.L. McIlhany, and P. Swidersky. 1979. Effects of nitrogen dioxide on pulmonary function in human subjects: An environmental chamber study. Environ. Res. 19(2):392-404.
Toxicity end point:	Slight burning of the eyes, slight headache, chest tightness, or labored breathing with exercise in 7/13 asthmatic subjects exposed to NO_2 at 0.5 ppm for 2 h
Time scaling:	Not applied
Uncertainty factors:	None
Modifying factor:	None
Calculations:	0.50 ppm applied across AEGL-1 exposure durations AEGL values were developed on the basis of data on NO_2, the predominant form of nitrogen oxide, and values are considered applicable to all nitrogen oxides. Values for N_2O_4 in units of ppm have been calculated on a molar basis. 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 emergency planning for NO. However, that short-term exposures to NO below 80 ppm should not constitute a health hazard.

Derivation of AEGL-2 for Nitrogen Oxides

Key Study:	Henschler, D., A. Stier, H. Beck, and W. Neumann. 1960. The odor threshold of some important irritant gasses (sulfur dioxide, ozone, nitrogen dioxide) and the manifestations of the effect of small concentrations on man [in German] Arch. Gewerbepathol. Gewerbehyg. 17:547-570.
Toxicity end points:	Burning sensation in nose and chest, cough, dyspnea, and sputum production in normal volunteers exposed to NO_2 at 30 ppm for 2 h
Time scaling:	$C^{3.5} \times t = k$; the value of n was calculated by ten Berge et al. (1986) from the data of Hine et al. (1970). $k = (30 \text{ ppm}/3)^{3.5} \times 2 \text{ h} = 6{,}324.56 \text{ ppm-h}$
Uncertainty factors:	3 for intraspecies variability
Modifying factor:	None AEGL values were developed on the basis of data on NO_2, the predominant form of nitrogen oxide, and values are considered applicable to all nitrogen oxides. Values for N_2O_4 in units of ppm have been calculated on a molar basis. 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 emergency planning for NO.
Calculations:	
10-min AEGL-2:	$C = (6{,}324.56 \text{ ppm-h}/0.167 \text{ h})^{1/3.5}$ $C = 20 \text{ ppm}$
30-min AEGL-2:	$C = (6{,}324.56 \text{ ppm-h}/0.5 \text{ h})^{1/3.5}$ $C = 15 \text{ ppm}$
1-h AEGL-2:	$C = (6{,}324.56 \text{ ppm-h}/1 \text{ h})^{1/3.5}$ $C = 12 \text{ ppm}$

4-h AEGL-2:	$C = (6{,}324.56 \text{ ppm-h}/4 \text{ h})^{1/3.5}$ $C = 8.2$ ppm
8-h AEGL-2:	$C = (6{,}324.56 \text{ ppm-h}/8 \text{ h})^{1/3.5}$ $C = 6.7$ ppm

Derivation of AEGL-3 for Nitrogen Oxides

Key study:	Henry, M.C., R. Ehrlich, and W.H. Blair. 1969. Effect of nitrogen dioxide on resistance of squirrel monkeys to *Klebsiella pneumoniae* infection. Arch. Environ. Health 18(4):580-587.
Toxicity end point:	Signs of marked irritation, but no deaths in monkeys exposed to NO_2 at 50 ppm for 2 h
Time scaling:	$C^{3.5} \times t = k$; the value of n was calculated by ten Berge et al. (1986) from the data of Hine et al. (1970) $k = (50 \text{ ppm}/3)^{3.5} \times 2 \text{ h} = 37{,}801$ ppm-h
Uncertainty factors:	3 for intraspecies variability; 1 for interspecies variability
Modifying factor:	None
	AEGL values were based on studies of NO_2, the predominant form, and values are considered applicable to all nitrogen oxides. Values for N_2O_4 in units of ppm have been calculated on a molar basis. 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.
Calculations:	
10-min AEGL-3:	$C = (37{,}801 \text{ ppm-h}/0.1667 \text{ h})^{1/3.5}$ $C = 34$ ppm
30-min AEGL-3:	$C = (37{,}801 \text{ ppm-h}/0.5 \text{ h})^{1/3.5}$ $C = 25$ ppm

1-h AEGL-3: $C = (37{,}801 \text{ ppm-h}/1 \text{ h})^{1/3.5}$
$C = 20$ ppm

4-h AEGL-3: $C = (37{,}801 \text{ ppm-h}/4 \text{ h})^{1/3.5}$
$C = 14$ ppm

8-h AEGL-3: $C = (37{,}801 \text{ ppm-h}/8 \text{ h})^{1/3.5}$
$C = 11$ ppm

APPENDIX B

ACUTE EXPOSURE GUIDELINE LEVELS FOR NITROGEN OXIDES

Derivation Summary for Nitrogen Oxides

AEGL-1 VALUES

Chemical	10 min	30 min	1 h	4 h	8 h
NO_2/NO	0.50 ppm	0.50 ppm	0.50 ppm	0.50 ppm	0.50 ppm
N_2O_4	0.25 ppm	0.25 ppm	0.25 ppm	0.25 ppm	0.25 ppm

References:
Kerr, H.D., T.J. Kulle, M.L. McIlhany, and P. Swidersky. 1978. Effects of Nitrogen Dioxide on Pulmonary Function in Human Subjects: An Environmental Chamber Study. EPA/600/1-78/025. Health Effects Research Laboratory, U.S. Environmental Protection Agency, Reserch Triangle Park, NC.
Kerr, H.D., T.J. Kulle, M.L. McIlhany, and P. Swidersky. 1979. Effects of nitrogen dioxide on pulmonary function in human subjects: An environmental chamber study. Environ. Res. 19(2):392-404.

Test species/Strain/Number: Human subjects; sex not given; 13 asthmatic subjects with exercise

Exposure route/Concentrations/Durations: Inhalation of NO_2 at 0.5 ppm for 2 h

Effects: Slight burning of the eyes, slight headache, chest tightness, or labored breathing in 7/13 subjects

End point/Concentration/Rationale: Mild symptoms of discomfort in asthmatic subjects

Uncertainty factors/Rationale:
Total uncertainty factor: 1
Interspecies: Not applied because human data were used
Intraspecies: 1 was applied because asthmatics subjects were the test population

Modifying factor: None

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: Extrapolation was not conducted because adaptation to mild sensory irritation occurs. In addition, animal responses to NO_2 have demonstrated a much greater dependence on concentration than on time; therefore, extending the 2-h concentration to 8 h should not exacerbate the human response.

Data quality and support for the AEGL values: AEGL-1 values are considered conservative and should be protective of the toxic effects of NO_2 outside the expected AEGL-1 effects.

AEGL-2 VALUES

Chemical	10 min	30 min	1 h	4 h	8 h
NO_2/NO	20 ppm	15 ppm	12 ppm	8.2 ppm	6.7 ppm
N_2O_4	10 ppm	7.6 ppm	6.2 ppm	4.1 ppm	3.5 ppm

Reference:
Henschler, D., A. Stier, H. Beck, and W. Neumann. 1960. The odor threshold of some important irritant gasses (sulfur dioxide, ozone, nitrogen dioxide) and the manifestations of the effect of small concentrations on man [in German] Arch. Gewerbepathol. Gewerbehyg. 17:547-570.

Test species/Strain/Number: Human, healthy male, 10-14

Exposure route/Concentrations/Durations: Inhalation, 0.5-30 ppm for up to 2 h

Effects:
0.5 ppm: metallic taste
1.5 ppm: dryness of the throat
4 ppm: sensation of constriction
25 ppm: prickling of the nose
30 ppm: burning sensation in nose and chest, cough, dyspnea, sputum production

End point/Concentration/Rationale: Humans exposed to NO_2 at 30 ppm for 2 h experienced pronounced irritation. The point-of-departure is considered a threshold for AEGL-2 because the effects would not impair the ability to escape and were reversible after cessation of exposure.

Uncertainty factors/Rationale:
Total uncertainty factor: 3
Interspecies: Not applied because human data were used
Intraspecies: 3 applied because the mechanism of action of a direct-acting irritant is not expected to differ greatly among individuals (see Section 4.2 for detailed information regarding the mechanism of respiratory toxicity).

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: $C^n \times t = k$, where n = 3.5 (ten Berge et al. 1986)

Data quality and support for the AEGL values: AEGL-2 values should be protective of the toxic effects of NO_2 outside the expected AEGL-2 effects. The values are supported by occupational monitoring data.

AEGL-3 VALUES

Chemical	10 min	30 min	1 h	4 h	8 h
NO_2/NO	34 ppm	25 ppm	20 ppm	14 ppm	11 ppm
N_2O_4	17 ppm	13 ppm	10 ppm	7.0 ppm	5.7 ppm

Reference:
Henry, M.C., R. Ehrlich, and W.H. Blair. 1969. Effect of nitrogen dioxide on

(Continued)

AEGL-3 VALUES Continued

Chemical	10 min	30 min	1 h	4 h	8 h
NO_2/NO	34 ppm	25 ppm	20 ppm	14 ppm	11 ppm
N_2O_4	17 ppm	13 ppm	10 ppm	7.0 ppm	5.7 ppm

(continued)

resistance of squirrel monkeys to *Klebsiella pneumoniae* infection. Arch. Environ. Health 18(4):580-587.

Test species/Strain/Number: Monkeys, 2-6/group

Exposure route/Concentrations/Durations: Inhalation, 10, 15, 35, or 50 ppm for 2 h

Effects:
50 ppm: marked increase in respiratory rate, decrease in tidal volume, microscopic lesions in lung (determinate for AEGL-3)
35 ppm: increase in respiratory rate, decrease in tidal volume, microscopic lesions in lung 10 and 15 ppm: slight changes in lung function

End point/Concentration/Rationale: 50 ppm resulted in marked effects on lung function but no deaths

Uncertainty factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1 applied because the end point in the monkey study is below the definition of AEGL-3 effects, human data support the AEGL-3 point-of-departure and derived values, the mechanism of action does not vary between species with the target at the alveoli, and because of the similarities of the respiratory tract between humans and monkeys.
Intraspecies: 3 applied because the mechanism of action of a direct-acting irritant is not expected to differ greatly among individuals (see Section 4.2 for detailed information regarding the mechanism of respiratory toxicity).

Modifying factor: Not applicable

Animal-to-human dosimetric adjustment: Not applicable

Time scaling: $C^n \times t = k$, where n = 3.5 (ten Berge et al. 1986)

Data quality and support for the AEGL values: The study is of high quality and the AEGL-3 values are supported by human data.

APPENDIX C

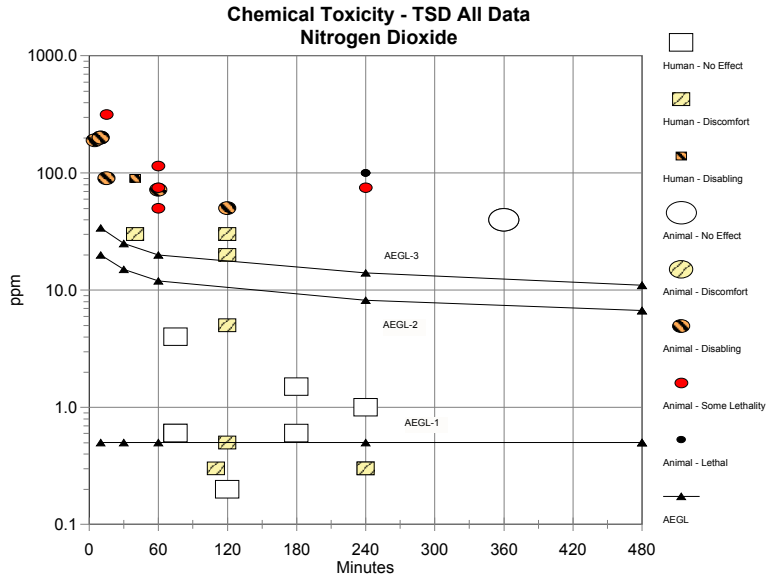

FIGURE C-1 Category plot of toxicity data and AEGLs values for nitrogen dioxide.

TABLE C-1 Data Used in Category Graph

Source	Species	ppm	Minutes	Category
NAC/AEGL-1		0.5	10	AEGL
NAC/AEGL-1		0.5	30	AEGL
NAC/AEGL-1		0.5	60	AEGL
NAC/AEGL-1		0.5	240	AEGL
NAC/AEGL-1		0.5	480	AEGL
NAC/AEGL-2		20	10	AEGL
NAC/AEGL-2		15	30	AEGL
NAC/AEGL-2		12	60	AEGL
NAC/AEGL-2		8.2	240	AEGL
NAC/AEGL-2		6.7	480	AEGL
NAC/AEGL-3		34	10	AEGL
NAC/AEGL-3		25	30	AEGL
NAC/AEGL-3		20	60	AEGL

(Continued)

TABLE C-1 Continued

Source	Species	ppm	Minutes	Category
NAC/AEGL-3		14	240	AEGL
NAC/AEGL-3		11	480	AEGL
Norwood et al. 1966	Human	90	40	2
Morley and Silk 1970	Human	30	40	1
Henschler et al. 1960	Human	30	120	1
Multiple studies	Human	0.6	180	0
Frampton et al. 1991	Human	1.5	180	0
Linn and Hackney 1983, 1984	Human	4.0	75	0
von Nieding et al. 1979	Human	5.0	120	1
Kleinman et al. 1983	Human	0.2	120	0
Sackner et al. 1981	Human	1.0	240	0
Kerr et al. 1978	Human	0.5	120	1
Roger et al. 1990	Human	0.3	110	1
Roger et al. 1990	Human	0.6	75	0
Hine et al. 1970	Dog	75	240	PL
Hine et al. 1970	Rat	100	240	3
Hine et al. 1970	Mouse	100	240	3
Hine et al. 1970	Rabbit	75	60	PL
Henry et al. 1969	Monkey	50	120	2
Hine et al. 1970	Dog	20	1,440	1
Carson et al. 1962	Rat	190	5	2
Carson et al. 1962	Rat	90	15	2
Carson et al. 1962	Rat	72	60	2
Hine et al. 1970	Rat	20	1,440	1
Henschler and Lutge 1963	Human	20	120	1
Bauer et al. 1985	Human	0.3	240	1
Hine et al. 1970	Guinea pig	50	60	PL
Carson et al. 1962	Rabbit	315	15	PL
Carson et al. 1962	Rat	115	60	PL
Meulenbelt et al. 1992	Rat	200	10	2
Hidekazu and Fujio 1981	Mouse	40	720	PL
Henschler and Lutke 1963	Dog	40	360	0
Hine et al. 1970	Guinea pig	20	1,440	1
Hine et al. 1970	Mouse	20	1,440	1