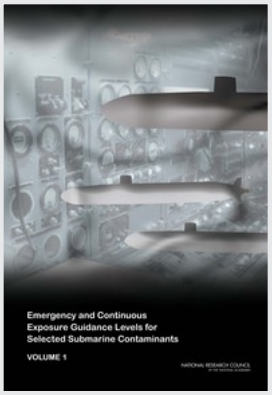

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Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants

VOLUME 1

Subcommittee on Emergency and Continuous
Exposure Guidance Levels for
Selected Submarine Contaminants

Committee on Toxicology

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

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Preface

The submarine is an enclosed and isolated environment when submerged. The crew works, eats, and sleeps in this environment and is exposed to air contaminants 24 hours per day, unlike the typical occupational environment where workers have a respite from workplace exposures at the end of the workday or workweek. To protect the health of the submariners, the U.S. Navy has developed 1-hour and 24-hour emergency exposure guidance levels (EEGLs) and 90-day continuous exposure guidance levels (CEGLs) for a number of chemical contaminants.

In 1995, the Navy began reviewing and updating submarine exposure guidance levels and subsequently asked the Committee on Toxicology (COT) of the National Research Council (NRC) to conduct an independent review of several chemicals. As a result of the Navy's request, the NRC formed the Subcommittee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants. This report is the first of two reports and provides the subcommittee's rationale and recommendations for the following substances: acrolein, carbon dioxide, carbon monoxide, formaldehyde, hydrazine, methanol, monoethanolamine, nitric oxide, nitrogen dioxide, and oxygen.

This report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the Research Council'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 of 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 people for their review of this report: Janice Chambers, Mississippi State University; Rory Conolly, CIIT Centers for Health Research; Dan Costa, Environmental Protection Agency; Darol Dodd, ManTech Environmental Technology, Inc.; Mark Frampton, University of Rochester School of Medicine; Judith Graham, American Chemistry Council; Alan Hall, Toxicology Consulting and Medical Translating Services; and Barry L. Johnson, Emory University Rollins School of Public Health.

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 the report before its release. The review of this report was overseen by Joseph Borzelleca, Virginia Commonwealth University. Appointed by the Research Council, he was responsible for making certain that an independent examination of the report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of the report rests entirely with the subcommittee and the institution.

The subcommittee thanks Commander Warren Jederberg for his support of this project and his assistance in obtaining necessary background materials. The subcommittee also gratefully acknowledges the following people for making presentations: Mr. Rich Hagar (Naval Sea Systems Command), Captain Victoria Cassano (Bureau of Medicine and Surgery), Mr. James Crawl (Naval Environmental Health Center), Dr. Sal DiNardi (Naval Submarine Medical Research Lab), and Dr. Robert Young (Oak Ridge National Laboratory).

In addition, the subcommittee also had the opportunity to visit a nuclear attack submarine, the USS Hartford, in dock at the U.S. Naval Submarine Base New London in Groton, CT. The crew were extremely helpful in providing information about conditions on the submarine. The subcommittee greatly appreciated the tour and found the information useful in its deliberations.

The subcommittee is grateful for the assistance of the NRC staff in preparing this report: Ellen Mantus, project director; James Reisa, director of the Board on Environmental Studies and Toxicology; Kulbir Bakshi, senior program officer for toxicology; Mary Fox, program officer; Jennifer Saunders and Mirsada Karalic-Loncarevic, research associates; Ruth E. Crossgrove, senior editor; Kelly Clark, assistant editor;

Laura Waters and Robert Policelli, project assistants; and Sam Bardley, library assistant.

Finally, I thank the members of the subcommittee for their dedicated efforts throughout the development of this report.

Ernest McConnell, *Chair*
Subcommittee on Emergency and
Continuous Exposure Guidance Levels
for Selected Submarine Contaminants

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Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants

VOLUME 1

Summary

Submariners live in an enclosed and isolated environment when at sea on a submerged submarine. Unlike workers who have respites from occupational exposures at the end of their shifts or workweeks, submariners are potentially exposed to air contaminants 24 hours (h) a day while the submarine is submerged. To protect submariners from potential adverse health effects associated with air contaminants, the U.S. Navy has established 1-h and 24-h emergency exposure guidance levels (EEGLs) and 90-day continuous exposure guidance levels (CEGLs) for a number of those contaminants.

EEGLs are defined as ceiling concentrations (concentrations not to be exceeded) of chemical substances in submarine air that will not cause irreversible harm to crew health or prevent the performance of essential tasks, such as closing a hatch or using a fire extinguisher, during rare emergency situations lasting 1-24 h. Exposures at the EEGLs may induce reversible effects, such as ocular or upper respiratory tract irritation, and are therefore acceptable only in emergencies, when some discomfort must be endured. After 24 h of exposure, the CEGLs would apply. CEGLs are ceiling concentrations designed to prevent immediate or delayed adverse health effects or degradation in crew performance that might result from continuous exposures to chemical substances lasting up to 90 days.

In December 1995, the Navy began reviewing and updating the submarine exposure guidance levels. Because the National Research Council (NRC) Committee on Toxicology (COT) has previously reviewed and provided recommendations for those and other types of exposure guidance levels, the Navy requested that COT review, or develop when necessary, EEGLs and CEGLs for a variety of substances. As a result of the Navy's request, the NRC convened the Subcommittee on Emergency and Continu-

ous Exposure Guidance Levels for Selected Submarine Contaminants in 2002.

STATEMENT OF TASK

Members of the COT subcommittee were selected for their expertise in inhalation toxicology, neurotoxicology, immunotoxicology, reproductive and developmental toxicology, veterinary pathology, pharmacokinetics, epidemiology, and human-health risk assessment. The subcommittee was specifically asked to accomplish the following tasks:

- Evaluate the Navy's current and proposed 1-h and 24-h EEGLs and 90-day CEGLs for the following substances: 2190 oil mist, formaldehyde, acrolein, ozone, monoethanolamine, nitric oxide, nitrogen dioxide, oxygen, carbon dioxide, carbon monoxide, methanol, ammonia, benzene, hydrazine, Freon 12, Freon 114, hydrogen, toluene, and xylene.
- Determine whether the current or proposed guidance levels are consistent with the scientific data and whether any changes to the Navy's exposure levels should be made on the basis of the subcommittee's evaluation.
- For two submarine contaminants for which no guidance levels exist—surface lead and 2,6-di-*t*-butyl-4-nitrophenol—determine whether sufficient data are available to develop EEGLs and CEGLs, and if sufficient data are available, provide recommendations for guidance levels consistent with the data.
- Identify deficiencies in the database relevant to EEGL and CEGL development for the selected chemical substances, and make recommendations for future research, when appropriate.

To accomplish its review, the subcommittee was asked to use the Navy's supporting documentation and other relevant toxicologic and epidemiologic data and publish the results of its evaluations in two separate reports. This is the subcommittee's first report. It contains the EEGL and CEGL recommendations for the following chemicals of concern to the Navy: acrolein, carbon dioxide, carbon monoxide, formaldehyde, hydrazine, methanol, monoethanolamine, nitric oxide, nitrogen dioxide, and oxygen. The remaining chemicals will be addressed in the second report.

APPROACH TO STUDY

In conducting its evaluations, the subcommittee reviewed relevant human and animal data and used data selection criteria described in the NRC report *Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals*.[1] Where possible, primary references were used to derive the exposure guidance levels. Secondary references were used to support the estimates derived and the selection of critical end points. Whenever possible, studies that followed accepted standard scientific methods were selected as key studies (those used to derive the exposure guidance levels). Inhalation exposure studies were used to derive the EEGL and CEGL values. Data on other routes of exposure were considered where appropriate. Human studies were preferred over animal studies. When epidemiologic and human experimental studies were available, a preference typically was given to human experimental studies as these were conducted in a controlled laboratory setting and allowed measurement of personal exposure and end points relevant for derivation of the exposure guidance levels. When appropriate human data were not available, standard laboratory animal studies were used, with preference given to nonhuman primate studies. A weight-of-evidence approach was used to select key studies, thus ensuring that selected data were consistent with the overall scientific database and incorporated what is known about the biologic effects of a chemical on pertinent organ systems.

For derivation of the EEGL and CEGL values, the subcommittee followed basic guidance provided by the NRC report *Criteria and Methods for Preparing Emergency Exposure Guidance Level (EEGL), Short-term Public Emergency Guidance Level (SPEGL), and Continuous Exposure Guidance Level (CEGL) Documents*,[2] but also considered the guidance for

[1]NRC (National Research Council). 2001. Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals. Washington, DC: National Academy Press.

[2]NRC (National Research Council). 1986. Criteria and Methods for Preparing Emergency Exposure Guidance Level (EEGL), Short-term Public Emergency Guidance Level (SPEGL), and Continuous Exposure Guidance Level (CEGL) Documents. Washington, DC: National Academy Press.

developing similar exposure levels provided in more recent NRC reports.[3],[4] The basis for the EEGLs was acute or short-term inhalation and ocular toxicity data, whereas the basis for the CEGLs was repeated inhalation exposure data, and the effects of cumulative exposures were considered. The most sensitive end points were emphasized for derivation of both exposure levels. Also, the subcommittee considered only those health end points relevant to healthy young adult men on the assumption that there are no women serving as permanent crew aboard submarines.

When the key studies, health end points, and exposure levels were identified, the application of uncertainty factors was considered when extrapolating from animals to humans and when extrapolating from lowest-observed-adverse-effect levels to no-observed-adverse-effect levels. When necessary, other factors were applied to account for critical data gaps or for potentially relevant variations in susceptibilities.

CONCLUSIONS AND RECOMMENDATIONS

The subcommittee found substantial differences in the adequacy of the data sets used to derive the EEGLs and CEGLs. For example, formaldehyde has a robust data set that includes both occupational and controlled human studies, whereas monoethanolamine has a paucity of data available for determining effects following inhalation exposure. In fact, there are no human inhalation data for monoethanolamine, and the animal data available are considered incomplete because little information is provided about histologic, hematologic, and enzymatic changes that might occur following repeated or long-term exposure. Few chemicals have substantial data on long-term, low-level exposures. Specific recommendations for research needed to improve the confidence of the derived exposure levels are provided in the individual chemical profiles.

In this report, the subcommittee makes recommendations for 1-h and 24-h EEGLs and 90-day CEGLs for the following chemicals: acrolein, carbon dioxide, carbon monoxide, formaldehyde, hydrazine, methanol,

[3]NRC (National Research Council). 1992. Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. Washington, DC: National Academy Press.

[4]NRC (National Research Council). 2001. Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals. Washington, DC: National Academy Press.

monoethanolamine, nitric oxide, nitrogen dioxide, and oxygen. Those recommendations are listed in Table S-1, and the Navy's current and proposed values have been included in the table for comparative purposes. The bases for the subcommittee's derivations are provided in the individual chemical profiles. Overall, the subcommittee considers the values proposed by the Navy for acrolein, carbon monoxide, formaldehyde, methanol, and nitrogen dioxide to be protective of submariners' health. For carbon dioxide, hydrazine, and monoethanolamine, the subcommittee recommended 1-h EEGL values lower than those proposed by the Navy. The subcommittee considers the other guidance levels for those chemicals to be protective of submariners' health. In the case of oxygen, the subcommittee recommended a higher minimal level for the 90-day CEGL than the one proposed by the Navy; the other minimal levels recommended by the subcommittee are lower than those the Navy has proposed. The subcommittee derived guidance levels for both nitric oxide and nitrogen dioxide, whereas the Navy proposed values for only nitrogen dioxide, assuming that those guidance levels would also be protective in the event of nitric oxide exposure. The subcommittee emphasizes that nitrogen dioxide must be monitored along with nitric oxide, because nitric oxide can combine with oxygen to form nitrogen dioxide, which is more toxic than nitric oxide.

RESEARCH RECOMMENDATIONS

The submarine atmosphere does not appear to be well characterized. In conducting its evaluation, the subcommittee found that few exposure data are available on the Navy's chemicals of concern or other chemicals. This subcommittee agrees with a previous NRC report, *Submarine Air Quality*[5] and recommends again that "the Navy thoroughly survey various classes of submarines for trace contaminants and particulate matter" and that "monitoring on submarines provide complete analysis of submarine air and data on exposure of personnel to contaminants." Furthermore, if the exposure assessments indicate that certain chemicals pose problems (that is, concentrations are higher than 90-day CEGLs), relative source contribu-

[5]NRC (National Research Council). 1988. Submarine Air Quality: Monitoring the Air in Submarines. Washington, DC: National Academy Press.

TABLE S-1 Comparison of Navy's Exposure Guidelines with Those Recommended by the Subcommittee

Chemical	Exposure Level	U.S. Navy Values[a]		NRC Recommended Value[a]
		Current	Proposed	
Acrolein	1-h EEGL	0.05	0.07	0.1
	24-h EEGL	0.01	0.03	0.1
	90-day CEGL	0.01	0.01	0.02
Carbon dioxide	1-h EEGL	40,000	30,000	25,000
	24-h EEGL	40,000	15,000	25,000
	90-day CEGL	5,000	7,000	8,000
Carbon monoxide	1-h EEGL	400	55	180
	24-h EEGL	50	20	45
	90-day CEGL	20	10	9
Formaldehyde	1-h EEGL	3	0.4	2
	24-h EEGL	1	0.1	1
	90-day CEGL	0.5	0.04	0.3
Hydrazine	1-h EEGL	—	4	1
	24-h EEGL	—	0.3	1
	90-day CEGL	—	0.01	0.03
Methanol	1-h EEGL	200	200	600
	24-h EEGL	10	10	50
	90-day CEGL	10	7	10
Monoethanolamine	1-h EEGL	50	6	4
	24-h EEGL	3	3	4
	90-day CEGL	0.5	0.5	0.5
Nitric oxide[b]	1-h EEGL	—	—	130
	24-h EEGL	—	—	50
	90-day CEGL	—	—	3
Nitrogen dioxide	1-h EEGL	1	3	10
	24-h EEGL	1	1	2
	90-day CEGL	0.5	0.5	0.7
Oxygen (min.-max.)	1-h EEGL	130-220 mmHg	—	105 mmHg (min.)
	24-h EEGL	130-160 mmHg	—	127 mmHg (min.)
	90-day CEGL	130-160 mmHg	—	140 mmHg (min.)

[a]All values in parts per million (ppm) unless otherwise noted.
[b]Navy considers the guidance levels for nitrogen dioxide to be also protective of nitric oxide exposure.
Abbreviations: max., maximum; min., minimum; mmHg, millimeters of mercury.

tions should be determined for those chemicals. The subcommittee notes that a few onboard sources, such as cigarette smoking and certain cooking methods, contribute to the formation of multiple compounds considered in this report. Therefore, stricter management or elimination of those sources is likely to solve some exposure problems on board submarines.

The subcommittee did not address exposures to chemical mixtures. When empirical data that characterize mixtures found in submarine air become available, the subcommittee recommends that they be evaluated. The potential for antagonistic, additive, or synergistic interactions between contaminants in the submarine environment is an area of significant uncertainty that remains largely unexamined and needs to be studied.

Several of the chemicals that the subcommittee evaluated for this report are sensory irritants. The derivation of quantitative environmental and occupational exposure limits for sensory irritants is fraught with difficulty because measures of the ocular and respiratory tract irritation experienced by human subjects are often considered subjective. The results of controlled human exposures to many sensory irritants typically use descriptors, such as "mild" or "mild to moderate," and the database for sensory irritation thresholds can be highly variable. Research is needed to quantify the diverse methods and end points used in sensory irritation studies, so that these data can be used in public- and occupational-health risk assessment with greater confidence.

1

Introduction

Submariners live in isolated, confined, and often crowded conditions when at sea. They must adjust to an 18-hour (h) day (6 h on duty and 12 h of training, other related activities, and free time) and are continuously exposed to air contaminants in their environment. To protect submariners from the potential adverse health effects associated with air contaminants, the U.S. Navy has established 1-h and 24-h emergency exposure guidance levels (EEGLs) and 90-day continuous exposure guidance levels (CEGLs) for a number of those contaminants.

In December 1995, the Navy began reviewing and updating submarine exposure guidance levels (Crawl 2003). Because the National Research Council (NRC) Committee on Toxicology (COT) has previously reviewed and provided recommendations for those and other types of exposure guidance levels (NRC 1984a,b,c; 1985a,b; 1986a; 1987; 1988a; 1994; 1996a,b; 2000a,b,c; 2002a,b; 2003), the Navy requested that COT review, or develop when necessary, EEGLs and CEGLs for a variety of chemical substances. Substances were selected for review on the basis of their presence in the submarine atmosphere, the lack of a recent COT review, their toxicity, or their known or suspected concentrations on board (Crawl 2003). As a result of the Navy's request, the NRC convened the Subcommittee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants in 2002.

THE SUBCOMMITTEE'S CHARGE

Members of the COT subcommittee were selected for their expertise in inhalation toxicology, neurotoxicology, immunotoxicology, reproductive

and developmental toxicology, veterinary pathology, pharmacokinetics, epidemiology, and human-health risk assessment. The subcommittee was asked to accomplish the following tasks:

- Evaluate the Navy's current and proposed 1-h and 24-h EEGLs and 90-day CEGLs for the following substances: 2190 oil mist, formaldehyde, acrolein, ozone, monoethanolamine, nitric oxide, nitrogen dioxide, oxygen, carbon dioxide, carbon monoxide, methanol, ammonia, benzene, hydrazine, Freon 12, Freon 114, hydrogen, toluene, and xylene.
- Determine whether the current or proposed guidance levels are consistent with the scientific data and whether any changes to the Navy's exposure levels should be made on the basis of the subcommittee's evaluation.
- For two submarine contaminants for which no guidance levels exist—surface lead and 2,6-di-*t*-butyl-4-nitrophenol—determine whether sufficient data are available to develop EEGLs and CEGLs, and if data are available, provide recommendations for guidance levels consistent with the data.
- Identify deficiencies in the database relevant to EEGL and CEGL development for the selected contaminants, and make recommendations for future research, when appropriate.

To accomplish its charge, the subcommittee was asked to review the Navy's supporting documentation and other relevant toxicologic and epidemiologic data and publish the results of its evaluations in two separate reports. This is the subcommittee's first report, and it contains the EEGL and CEGL recommendations for 10 chemicals of concern to the Navy.

POPULATION CHARACTERISTICS

An estimated 30,000 submariners are on active duty in the U.S. Navy (Cassano 2003). Permanent crew members on U.S. submarines are all male and range in age from 18 to 48 years. Prior to entry into the submarine service, candidates receive a comprehensive physical and psychological examination and are rejected if any major medical problems, such as heart disease, asthma, or chronic bronchitis, are noted (U.S. Navy 1992, 2001). Submariners are also required to undergo a complete physical examination every 5 years (Capt. D. Molé, U.S. Navy, personal commun., May 28, 2003). If any medical problems are noted at that time or during active duty,

submariners may be disqualified from submarine duty (Cassano 2003). Therefore, the population that serves on U.S. submarines represents, in general, an extremely healthy population.

Recent studies that have evaluated mortality patterns in U.S. submariners support the conclusion that submariners represent an extremely healthy population. Charpentier et al. (1993) examined a cohort of 76,160 submariners who served on U.S. nuclear-powered submarines during the period 1969-1982. The study authors compared the mortality rates of the submariners with those of the general adult male population in the United States and found that the standardized mortality ratio (SMR) for total mortality was significantly less than one.[1] The SMR was also significantly lower than the SMR expected for a military population. The SMRs for specific causes of mortality were also less than one. SMRs approached one for only two causes (malignant neoplasms of the brain and central nervous system [SMR = 1.03] and motor-vehicle accidents [SMR = 1.06]). The results reported by the study authors are supported by a study of Royal Navy submariners, who must meet stringent physical requirements similar to those of the U.S. Navy (Inskip et al. 1997).

Morbidity patterns in U.S. Navy submariners also indicate a healthy population. Thomas et al. (2000) evaluated the rates of medical events in crews on 136 submarine patrols over 2 years (1997-1998). Injury was the most common medical-event category, followed by respiratory illness, primarily upper respiratory infections, and then skin problems, such as minor infections and ingrown toenails. Other medical events included ill-defined symptoms, infectious disease, digestive disorders, ear and eye complaints, and musculoskeletal conditions. The categories listed here represent about 90% of the 2,044 medical events reported.

Although recent data indicate that U.S. submariners are a healthy population, some members of this population might be particularly sensitive to certain air contaminants because of either genetic predisposition or conditions arising during active duty. For example, Sims et al. (1999) reported an annual incidence of asthma leading to the disqualification of 0.16% of the active duty personnel serving in the Atlantic Fleet Submarine Force. However, the authors considered the asthma cases to be mild.

Tobacco smokers are another subset of the population that might be more or less sensitive to certain air contaminants. Smoking currently is permitted in restricted areas on U.S. submarines. The percentage of U.S.

[1]An SMR indicates whether the mortality rate for a given population is greater (SMR > 1) or less (SMR < 1) than a comparative population.

submariners who smoke is difficult to estimate, because no broad survey has been conducted. Sims et al. (1999) estimated a prevalence of smoking of 36% on the basis of data from eight submarines. However, Thomas et al. (2000) estimated that the prevalence of smoking might be as low as 22% on the basis of survey data from 1997 collected from one submarine. The Navy has indicated that the percentage of submariners who smoke most likely ranges from 15% to 30% (Cmdr. W. Horn, U.S. Navy, personal commun., August 7, 2003). However, the smoking policies on board submarines vary, as they are determined by the commanding officer.

THE SUBMARINE ENVIRONMENT

The U.S. submarine fleet is composed primarily of two types of submarines (Thomas et al. 2000). Table 1-1 provides some distinguishing characteristics of the crews and patrols for the two submarine types. The nuclear-powered attack submarines have a designated crew of about 130 men who are deployed at irregular intervals for varying lengths of time. The nuclear-powered ballistic missile submarines have two crews that rotate between ship and shore duty on a 90-day cycle.

When submerged, a submarine is an enclosed and isolated environment. Submariners work, eat, and sleep in that environment and potentially are exposed to air contaminants 24 h per day. The submarine differs from typical occupational settings where workers have respites from workplace exposures at the end of their shifts or workweeks.

Operation of a closed vessel can lead to accumulation of air contaminants (NRC 1988b). Major sources of air contaminants on a submarine include cigarette smoking, cooking, and the human body. Other sources include control equipment, the power train, weapons systems, batteries, sanitary tanks, air-conditioning and refrigeration systems, and a variety of maintenance and repair activities.

Several onboard methods are used to maintain a livable atmosphere and remove air contaminants (NRC 1988b). Oxygen generators add oxygen to the air through the electrolysis of seawater. The hydrogen that is generated in the process is discharged to the sea. Monoethanolamine scrubbers are used to remove carbon dioxide from the air. Carbon monoxide that is generated primarily from cigarette smoking and hydrogen that is released while charging the batteries are removed using a carbon monoxide-hydrogen burner that catalytically oxidizes the two components to carbon

TABLE 1-1 Characteristics of Crew and Patrols for U.S. Navy Submarines

Type[a]	Number and Size of Crew	Typical Patrol
Nuclear-powered attack submarines (SSN)	1 designated crew, 130 men	Irregular intervals between patrols; patrols of variable length
Nuclear-powered ballistic missile submarines (SSBN)	2 rotating crews, 160 men per crew	Regularly scheduled patrols; 90-day cycle between ship and shore; patrols >60 days in length

[a]Note that there are three classes of attack submarines—Los Angeles, Seawolf, and Virginia—and one class of ballistic missile submarines—Ohio. There are also two deep-diving specialized research submarines (one nuclear-powered and the other diesel-powered) that are in a class of their own (Capt. D. Molé, U.S. Navy, personal commun., January 15, 2004).
Source: Information from Thomas et al. 2000.

dioxide and water, respectively. Hydrocarbons are also oxidized by this system. Activated carbon filters help remove high-molecular-weight compounds and odorants, and electrostatic precipitators help to remove particles and aerosols. Vent fog precipitators are used in the engine room to remove oil mists generated there. Other means of minimizing air contaminants include restricting the materials that can be brought on board and limiting the types of activities, such as welding, that can be conducted while at sea.

When the submarine is submerged, air is recirculated in a closed-loop system. This system is composed of the forward compartment air-circulation system and the engine room air-circulation system (R. Hagar, Naval Sea Systems Command, personal commun., April 2, 2003). Figure 1-1 provides a generalized schematic of a nuclear-powered attack submarine. The forward compartment air-circulation system contains most of the air-purification equipment and oxygen generators and is designed to condition the air to 80°F and 50% relative humidity. The forward compartment is divided into zones, the fan room serving as the mixing chamber. Stale air from the boat is exhausted to the fan room, and treated air is supplied by the fan room to the boat. The engine room air-circulation system provides heating, cooling, and air distribution within the engine room and is designed to maintain room air temperature below a maximum of 100°F. Electrostatic precipitators and other filters in this compartment treat the engine room air. Air from the engine room is exhausted directly to the fan room, and the fan room supplies air directly to the engine room.

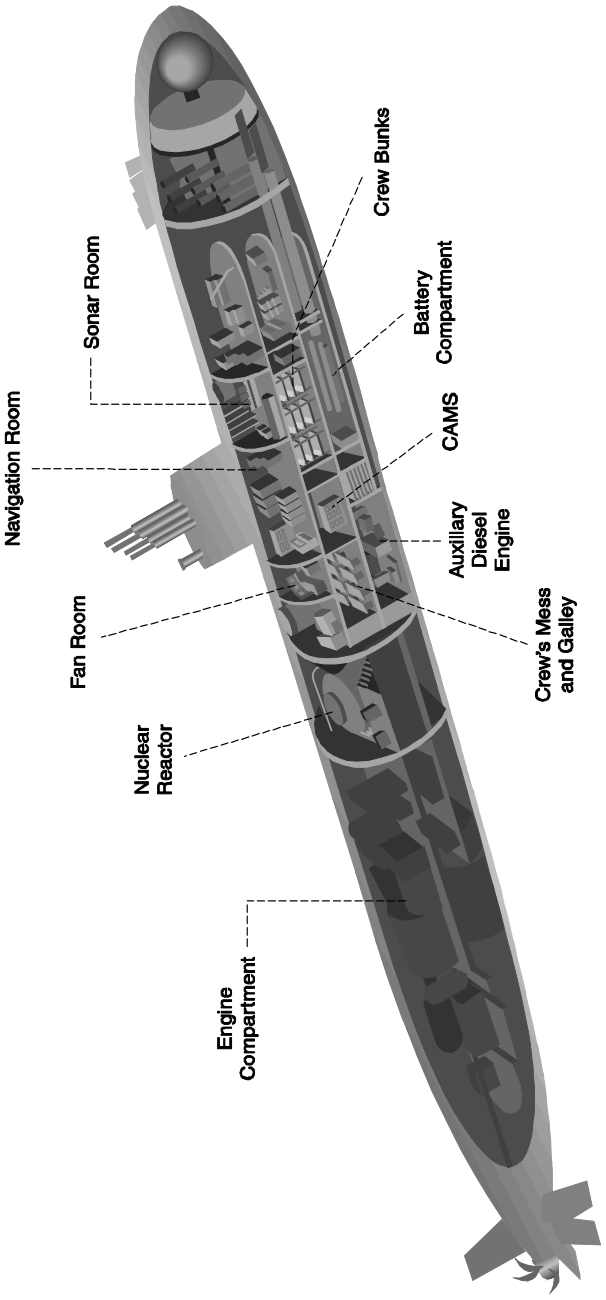

FIGURE 1-1 Generalized schematic of a nuclear-powered attack submarine. Source: Adapted from image courtesy of the Smithsonian/NMAH Transportation.

Special variations in the exhaust airflow path exist (R. Hagar, Naval Sea Systems Command, personal commun., April 2, 2003). Air discharged from the carbon monoxide–hydrogen burners and the carbon dioxide scrubbers is vented directly to the fan room. Many electronic cabinets have fan systems that vent directly to the fan room, and air from the laundry dryers passes through lint screens prior to discharge into the fan room. About 50% of the air vented into the fan room passes through electrostatic precipitators, and air from the galley, scullery, pantry, and water closets go through activated charcoal filters before venting into the fan room. Also, cooking grease is removed from the range and fryer hoods using centrifugal force.

The submarine atmosphere is monitored with the central atmosphere monitoring system (CAMS), which uses an infrared spectrometer to measure carbon monoxide and a mass spectrometer to measure oxygen, nitrogen, carbon dioxide, hydrogen, water vapor, and Freon 11, 12, and 114 (NRC 1988b). A newer version of CAMS also monitors the concentrations of selected trace chemicals in submarine air. Fan room air is monitored continuously, and air in other onboard locations is analyzed on a rotating basis.

Portable devices are routinely used to monitor submarine air (Hagar 2003; NRC 1988b). Photoionization detectors monitor total hydrocarbon levels, although that method is not used in submarines equipped with the newer version of CAMS. A portable oxygen detector verifies oxygen levels weekly. Colorimetric detector tubes are used weekly to measure concentrations of the following compounds: acetone, ammonia, benzene, carbon dioxide, carbon monoxide, chlorine, hydrazine, hydrochloric acid, nitrogen dioxide, ozone, sulfur dioxide, toluene, total hydrocarbons, methyl chloroform, and monoethanolamine. During battery charging operations, portable detectors are also used to monitor hydrogen concentrations. Suspected fluorocarbon or torpedo-fuel leaks are assessed with portable devices that have photoionization detectors. Retrospective passive monitoring of the submarine air provides 30-day time-weighted average concentrations for volatile organic compounds, ozone, acrolein, aldehydes, amines, and nitrosamines. Regardless of the frequency and type of monitoring that is conducted on submarines, NRC (1988b) concluded that "monitoring on submarines does not provide quantitative analysis of all submarine air contaminants and provides only sparse data on exposure."

THE SUBCOMMITTEE'S APPROACH TO ITS CHARGE

In conducting its evaluations, the subcommittee reviewed relevant human and animal data and used data selection criteria described in the NRC (2001) report *Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals*. Specifically, the subcommittee's approach for data selection included the following elements:

- Whenever possible, primary references (published or unpublished study reports) were used to derive the exposure guidance levels. Secondary references were used to support the estimates derived and the selection of critical end points.
- Whenever possible, studies that followed accepted standard scientific methods were selected as key studies (studies used to derive the exposure guidance levels). Evaluation of study quality required the professional expertise and judgement of the subcommittee.
- Inhalation exposure studies were used to derive the exposure guidance levels. Data on other exposure routes were incorporated into the analyses when they provided useful information on pharmacokinetics, metabolism, or mechanisms of toxicity.
- Human studies were preferred for developing the exposure guidance levels. The subcommittee considered human data from accidental exposures, experimental studies, and epidemiologic studies to be valuable in determining the effects of chemical exposure. When epidemiologic and human experimental studies were available, a preference typically was given to human experimental studies because they were conducted in a controlled laboratory setting and allowed measurement of personal exposure and end points relevant for derivation of the exposure guidance levels. To the best of the subcommittee's knowledge, it did not consider data obtained from uninformed subjects or by force or coercion.
- When quality human data were not available, standard laboratory animal studies were used to derive the exposure guidance levels. The animal species used were those that had historical control data and the most relevance to humans. Nonhuman primate studies were generally preferred but often were not available.
- A weight-of-evidence approach was used to select the key studies, thus ensuring that selected data were consistent with the overall

scientific database and incorporated what is known about the biologic effects of a chemical on pertinent organ systems.

- For derivation of the EEGL and CEGL values, the subcommittee followed basic guidance provided by the NRC (1986b) report *Criteria and Methods for Preparing Emergency Exposure Guidance Level (EEGL), Short-term Public Emergency Guidance Level (SPEGL), and Continuous Exposure Guidance Level (CEGL) Documents*, but also considered the guidance for developing similar exposure levels provided in more recent NRC reports (NRC 1992, 2001). The subcommittee evaluated chemicals individually and did not address exposures to chemical mixtures. When empirical data that characterize mixtures found in submarine air become available, the subcommittee recommends that those data be evaluated. The subcommittee considered only those health end points relevant to healthy young adult men on the assumption that women do not serve as permanent crew on board submarines. In deriving the EEGL and CEGL values, the subcommittee assumed that maximal exercise is not achieved due to the confined conditions on the submarine. The subcommittee also assumed that the submarine is operated at or near 1 atmosphere pressure. The specific approaches adopted by the subcommittee for developing EEGLs and CEGLs are outlined in the sections that follow.

Emergency Exposure Guidance Levels

NRC (1986b) defines EEGLs as ceiling concentrations (concentrations not to be exceeded) of chemical substances that will not cause irreversible harm to crew health or prevent the performance of essential tasks, such as closing a hatch or using a fire extinguisher, during rare emergency situations lasting 1-24 h. Exposures at the EEGLs may induce reversible effects, such as ocular or upper respiratory tract irritation, and are therefore acceptable only in emergencies when some discomfort must be endured. After 24 h of exposure, the CEGL would apply.

To develop the 1-h and 24-h EEGLs, the subcommittee reviewed relevant human and animal toxicity data and considered all health end points. The basis for the EEGLs was acute or short-term inhalation and ocular toxicity data, and the most sensitive end points were emphasized. If extrapolation from one exposure duration to another was required, the subcommittee used the available scientific literature or the guidance pro-

vided in *Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals* (NRC 2001).

In deriving EEGLs, the subcommittee used uncertainty factors that ranged in value from 1 to 10. These factors accounted for interspecies differences (extrapolation from animal to human populations, if applicable); intraspecies differences (possible variations or susceptibilities that might be applicable to the healthy male population considered); extrapolations from a lowest-observed-adverse-effect level (LOAEL) to a no-observed-adverse-effect level (NOAEL); and weaknesses or critical gaps in the databases. The subcommittee did strive for consistency; however, its overarching goal was a thorough case-by-case review of available data. Selection of uncertainty factors for each chemical reflects the subcommittee's best judgment of the data on both toxicity and mode of action. Because uncertainty factors of 3 each represent a logarithmic mean (3.16) of 10, the subcommittee considered the product of two uncertainty factors of 3 to equal a composite uncertainty factor of 10, which is consistent with current risk assessment practices (NRC 2001; EPA 2002).

Continuous Exposure Guidance Levels

NRC (1986b) defines CEGLs as ceiling concentrations of chemical substances designed to prevent the immediate or delayed adverse health effects or degradations in crew performance that might result from continuous chemical exposures lasting up to 90 days. To derive CEGLs, the subcommittee used the basic approach outlined for developing EEGLs. Thus, relevant data were reviewed, sensitive end points evaluated, and appropriate uncertainty factors applied. The method differed only in that, when available, inhalation studies with repeated exposures were used as the primary basis for CEGL development. The effects of cumulative exposures over time were taken into account using a weight-of-evidence approach.

Carcinogenic Substances

For known human carcinogens and substances with suspected carcinogenic activity in humans, the U.S. Department of Defense sets military exposure levels to avoid a theoretical excess cancer risk greater than 1 in 10,000 exposed persons (NRC 1986b). For those chemicals that have been

designated as known or suspected human carcinogens by the International Agency for Research on Cancer or by the U.S. Environmental Protection Agency, the subcommittee evaluated the theoretical excess cancer risk resulting from exposures at the 90-day CEGLs. The subcommittee considered deriving the cancer risk resulting from exposures at the 24-h EEGLs, but concluded that such estimates would involve too much uncertainty. Furthermore, the chemicals evaluated in this first report are not suspected of causing cancer after a single exposure from 1-24 h. Additional information regarding cancer risk is provided in individual chapters, when appropriate. The subcommittee notes that COT typically has not proposed CEGLs for carcinogenic substances (NRC 1986b). However, the subcommittee acknowledges that there is value in conducting these evaluations, and it has proposed 90-day CEGLs for compounds with known or suspected carcinogenic activity in human beings.

Comparison to Other Regulatory Standards or Guidance Levels

The subcommittee considered relevant inhalation exposure standards or guidance levels from NRC and other agencies or organizations in its evaluations. However, the subcommittee notes that the EEGLs and CEGLs differ from typical public-health and occupational-health standards in three important ways. First, public-health standards are developed to protect sensitive subpopulations, such as children, the elderly, and others with chronic health conditions who might be particularly sensitive, whereas EEGLs and CEGLs are developed for a healthy adult male population with little variation in physical qualifications. Second, occupational exposure standards are designed for repeated exposure throughout a working lifetime assuming that workers are exposed 8 h per day, 5 days per week for a working lifetime. Submariners can be exposed 24 h per day with no relief from exposure during submergence. In a typical submariner's career, a 10-year assignment to active sea duty would result in about 4.5 to 5 years of cumulative exposure in the enclosed submarine environment (Capt. V. Cassano, U.S. Navy, personal commun., December 16, 2003). Third, EEGLs allow for the development of reversible health effects that would not prevent the performance of essential tasks. Those health effects may not be considered acceptable when setting conventional occupational or public-health exposure standards.

The subcommittee considered the submarine escape action levels

(SEALs) and the spacecraft maximum allowable concentrations (SMACs) to be useful for comparison with EEGLs and CEGLs. However, SEALs are developed for disabled submarines and allow moderate rather than minimal reversible effects (NRC 2002a). SMACs are probably the most comparable to the EEGLs and CEGLs because SMACs are developed with a similar criteria and address adverse effects for a healthy population in an isolated and confined environment. However, SMACs are developed for an older male and female population that experiences the conditions of microgravity during exposure.

ORGANIZATION OF THE REPORT

This report contains the subcommittee's rationale and recommendations for the following substances: acrolein, carbon dioxide, carbon monoxide, formaldehyde, hydrazine, methanol, monoethanolamine, nitric oxide, nitrogen dioxide, and oxygen. Each chapter of this report presents the relevant toxicologic and epidemiologic studies for those substances along with selected chemical and physical properties, toxicokinetic and mechanistic data, and published regulatory and guidance levels for inhalation exposures. The subcommittee's recommendations for exposure guidance levels and the research needed to better define and support those conclusions are provided. The chemical profiles contained in this report are not comprehensive toxicologic profiles. Only those data particularly relevant to the derivation of the EEGLs and CEGLs are discussed. References are provided for recent authoritative reviews of the toxicology for some of the chemicals addressed in this report.

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2

Acrolein

This chapter summarizes the relevant epidemiologic and toxicologic studies on acrolein. Selected chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure limits from the National Research Council (NRC) and other agencies are also presented. The subcommittee considered all of that information in its evaluation of the Navy's current and proposed 1-hour (h), 24-h, and 90-day exposure guidance levels for acrolein. The subcommittee's recommendations for acrolein exposure levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

Acrolein is a reactive, flammable liquid at room temperature that has a pungent odor (Budavari et al. 1989). Amoore and Hautala (1983) reported an odor threshold of 0.16 parts per million (ppm), and Leonardos et al. (1969) reported an odor threshold of 0.21 ppm. Ruth (1986) tabulated odor thresholds ranging from 0.023 to 16.36 ppm and reported a threshold for irritation at 0.55 ppm. Selected physical and chemical properties are summarized in Table 2-1.

OCCURRENCE AND USE

Acrolein primarily is used in the chemical industry as an intermediate in the synthesis of acrylic acid and the synthesis of D,L-methionine, an

animal feed supplement (Etzkorn et al. 1991). Acrolein also exhibits antimicrobial activity and is used as a biocide in a number of process streams, including liquid fuel lines and recirculating process water systems.

Acrolein has been measured in ambient and indoor air (IARC 1995). Ambient air measurements in the United States have detected acrolein at concentrations ranging from 2 parts per billion (ppb) to 7 ppb. Acrolein is a component of tobacco smoke (IARC 1995; EPA 2003). Jones (1999) reported that the acrolein emission factor for mainstream smoke ranges from 10 to 140 micrograms (μg) per cigarette, and the emission factor for sidestream smoke ranges from 100 to 1,700 μg per cigarette. In smoky indoor environments, acrolein concentrations have been reported to range from 1 to 120 ppb (IARC 1995). Acrolein has also been detected in exhaust from gasoline and diesel engines and from the heating of animal fats and vegetable oils, and it is present in a variety of foods (IARC 1995).

Sources of acrolein on submarines include high-temperature paints, motor varnishes, diesel generators, and cigarette smoke (Crawl 2003). ATSDR (1990) noted that acrolein concentrations at 57-85 ppb were measured during system testing conducted on a submarine being overhauled. No other details were provided. Raymer et al. (1994) reported the

TABLE 2-1 Physical and Chemical Properties of Acrolein[a]

Synonyms and trade names	Acraldehyde, acrylaldehyde, acrylic aldehyde, allyl aldehyde, crolean, propenal, 2-propenal, prop-2-en-1-al, 2-propen-1-one
CAS registry number	107-02-08
Molecular formula	CH_2CHCHO
Molecular weight	56.06
Boiling point	52.5°C
Melting point	−88°C
Flash point	−18°C (open cup)
Explosive limits	2.8% to 31% (by volume in air)
Specific gravity	0.8389 at 20°C/4°C
Vapor pressure	210 mmHg at 20°C
Solubility	Soluble in alcohol, ether, and 2 to 3 parts water
Conversion factors	1 ppm = 2.29 mg/m^3; 1 mg/m^3 = 0.44 ppm

[a]Data on explosive limits are from ACGIH (2001); all other data are from Budavari et al. (1989).

Abbreviations: mg/m^3, milligrams per cubic meter; mmHg, millimeters of mercury; ppm, parts per million.

results of air sampling conducted during the missions of two submarines. The fan room, galley, and engine room on each submarine were sampled over 6 h. Sampling indicated acrolein concentrations of 0.39 ppb and 0.14 ppb in the engine rooms of the submarines; no acrolein or "small" concentrations were noted for the other locations. A similar sampling exercise (two submarines, three locations, and a sampling duration of 6 h) was reported by Holdren et al. (1995). Acrolein concentrations ranged from <0.10 to 0.7 ppb on the two submarines. The subcommittee notes that the results presented by Raymer et al. (1994) and Holdren et al. (1995) represent one-time sampling events on four submarines. Whether the reported concentrations are representative of the submarine fleet is not known, particularly as few details were provided about the conditions on the submarines when the samples were taken.

SUMMARY OF TOXICITY

Several reviews of the toxicology of acrolein are available (Beauchamp et al. 1985; ATSDR 1990; IARC 1995; NRC 1996; ACGIH 2001; EPA 2002; EPA 2003). Only data that were directly relevant for deriving the submarine EEGL and CEGL values are discussed.

The adverse health effects of acrolein exposures are defined by the chemical's cytotoxicity at the site of initial contact. Acrolein is a potent lacrimator and respiratory tract irritant. Exposures to airborne concentrations as low as 0.09 ppm for 5 minutes (min) have produced eye irritation. As concentration increases, eye and upper respiratory tract irritation increases. Liquid acrolein is absorbed through intact skin in amounts capable of producing systemic intoxication, and direct contact with the liquid can produce chemical burns.

Effects in Humans

Accidental Exposures

At least three fatalities have been associated with accidental exposure to airborne acrolein. Gosselin et al. (1979) recounted the case of a 4-year-old boy who died after a 2-h exposure to acrolein-containing smoke from an overheated fryer. Autopsy found multiple pulmonary infarcts, desquamation of the bronchial lining, and debris in the bronchiole lumen. A younger

brother was found dead and apparently died of asphyxiation. Prentiss (1937) reported a death that occurred 10 min after a man was exposed to acrolein at 150 ppm. No further details were available.

Mahut et al. (1993) described the case of a 27-month-old boy who was exposed to smoke from burning vegetable oil for 1 h. Acute respiratory failure and respiratory acidosis regressed within a few hours of treatment, but diffuse bronchiectasis developed over the months following the exposure. Bauer et al. (1977) described a similar incident involving a 21-year-old man exposed to kitchen smoke for 6 h. He developed chronic pneumopathy, bronchitis, and emphysema.

Champeix et al. (1966) described the onset of fever, coughing, dyspnea, cyanosis, and acute pulmonary edema with foamy expectoration in a 39-year-old male worker who was accidentally exposed to acrolein vapor. The victim suffered from chronic bronchitis and emphysema 18 months after the accident.

Experimental Studies

Controlled inhalation investigations have demonstrated that even brief exposures to acrolein at concentrations <1 ppm are associated with increased complaints of eye and nose irritation. Those complaints are accompanied by prompt reductions in ventilation rates (Sim and Pattle 1957; Weber-Tschopp et al. 1977).

Lacrimation and evidence of marked eye, nose, and throat irritation developed in 12 adult male volunteers within 20 seconds (s) of exposure to acrolein at 0.8 ppm (Sim and Pattle 1957). Exposure at 0.8 ppm for 10 min was considered "only just tolerable." The subcommittee notes that in this study the description of the method of acrolein administration (mask or chamber) was not clear. When 12 adult male volunteers were exposed at 1.2 ppm, lacrimation and evidence of marked eye, nose, and throat irritation developed within 5 s. Exposure at 1.2 ppm for more than 5 min was considered intolerable.

Stephens et al. (1961) found that 10-35% of humans exposed to acrolein at 0.5 ppm complained of eye irritation within 5 min of initial contact with the chemical. As the duration of exposure increased to 12 min, nearly all of the subjects (91%) complained of eye irritation. Darley et al. (1960) considered the eye irritation associated with a 5-min exposure at 1.3-1.6 ppm to be moderate and the eye irritation associated with a 5-min exposure at 2.0-2.3 ppm to be moderate to severe.

Weber-Tschopp et al. (1977) conducted three controlled acrolein

inhalation trials in healthy young adult male and female volunteers. The first trial studied effects of increasing concentrations, the second studied effects of brief exposures (1.5 min) to progressively increasing concentrations, and the third studied effects of constant exposure. In the first trial, 31 males and 22 females were exposed to acrolein at concentrations that increased gradually from 0 to 0.6 ± 0.02 ppm over a 35-min period. Volunteers were exposed in a chamber. For the final 5 min, the volunteers were exposed at 0.6 ppm. Eye, nose, and throat irritation increased significantly compared with unexposed volunteers at concentrations as low as 0.09, 0.26, and 0.43 ppm, respectively.

In the second trial, 25 female and 17 male volunteers were intermittently exposed to airborne acrolein for up to 1.5 min at increasing concentrations—0, 0.15, 0.3, 0.45, and 0.6 ppm. Questionnaires were provided to volunteers after 1 min of exposure. The volunteers were allowed 8 min of recovery in a well-ventilated room between successive exposures. Discomfort, which was described as a wish to leave the room, was significantly increased at 0.15 ppm. Eye irritation scores increased significantly at 0.3 ppm and higher, and nasal irritation scores increased significantly at 0.6 ppm.

In the third trial, 21 males and 25 females were divided into groups of three and exposed at 0.3 ppm for 1 h. Significant reductions in respiratory rates were noted. Respiratory rates were decreased by 10% in 47% of volunteers after 10 min and in 60% of volunteers after 20 min. Doubling of eye-blink rate was reported in 66% and 70% of volunteers after 10 min and 20 min, respectively. Moderate eye irritation was reported in 18% and 35% of volunteers after 10 min and 20 min, respectively, and severe to very severe eye irritation was reported in 3% and 18% of volunteers after 10 min and 20 min, respectively.

Weber-Tschopp et al. (1977) found that increased complaints of "annoyance" and eye irritation began at concentrations as low as 0.09 ppm. Complaints of nasal irritation increased as acrolein concentrations reached 0.15 ppm or more. A 10% reduction in respiration was evident at 0.3 ppm within 10-20 min of exposure. Complaints of throat irritation increased at acrolein concentrations of 0.43 ppm or more. Thus, the eyes were most sensitive to airborne acrolein exposure.

Occupational and Epidemiologic Studies

Ott et al. (1989) described six male employees who had been exposed to acrolein in workplace air and were later afflicted with multiple myeloma,

non-Hodgkins lymphoma, or nonlymphocytic leukemia. The odds ratios for the three cancers (1.7-2.6) were elevated in workers exposed to acrolein. However, because none of the lower confidence bounds significantly exceeded 1, the number of affected individuals was small, and the workers were concomitantly exposed to other workplace chemicals, no rigorous conclusions or causal inference could be made regarding the carcinogenic potential of inhaled acrolein.

Effects in Animals

Acute Toxicity

The marked irritant effects of inhalation exposures to acrolein result from its chemical reactivity. Ballantyne et al. (1989) calculated the combined LC_{50} values (concentrations lethal to 50% of subjects) in male and female Sprague-Dawley rats for 1 and 4 h to be 26 and 8.3 ppm, respectively. Catalina et al. (1966) found a 10-min LC_{50} of 375 ppm, and Skog (1950) found a 30-min LC_{50} of 131 ppm. The 6-h LC_{50} in mice was 66 ppm (Philippin et al. 1970).

Steinhagen and Barrow (1984) found that even brief exposures (10 min) to acrolein inhibited respiratory rates in mice. The authors calculated RD_{50} (50% reduction in respiratory rate) concentrations at 1.41 and 1.03 ppm for male $B6C3F_1$ and Swiss-Webster mice, respectively. The authors also found that acrolein was the most potent respiratory tract irritant out of 14 aldehydes evaluated under identical conditions in the mice. RD_{50} concentrations of other aldehydes ranged from 3.53 to 4,167 ppm. Kane and Alarie (1979) reported a slightly higher 10-min RD_{50} value (1.7 ppm) for Swiss-Webster mice than did Steinhagen and Barrow (1984).

Although acrolein is a less potent respiratory tract irritant in rats than in mice, similar reductions in the respiratory rates of rats were evident after exposures at 6 ppm (Babiuk et al. 1985). Acrolein is 5 times more potent a respiratory tract irritant (RD_{50} = 6.0 ppm) than formaldehyde (RD_{50} = 31.7 ppm) in Fischer 344 rats (Babiuk et al. 1985).

Several studies have investigated the effects associated with acrolein exposures in rats. Cassee et al. (1996) exposed male Wistar rats to acrolein at 0.67 ppm for 6 h per day for 3 days and found that acrolein exposure produced respiratory thickening and disarrangement similar to that seen in rats that inhaled formaldehyde at 3.2 ppm over the same duration, although the acrolein-induced changes were more pronounced. Springhall et al.

(1990) exposed Porton strain Wistar rats to acrolein at 22, 81, or 249 ppm for 10 min. The animals exposed at 81 or 249 ppm developed slight pulmonary edema and hemorrhage into the lung parenchyma, which was more commonly observed in those exposed at the highest concentration. Immunohistochemical evaluation of the respiratory tract demonstrated concentration-dependent reductions in tracheal nerve fibers reactive for calcitonin gene-related peptide (CGRP) and substance P. A decrease of substance P-reactive nerve fibers in the lung was observed in rats exposed to 249 ppm. The authors considered the reductions to be reflections of acrolein-induced damage to sensory nerve fibers, in contrast to autonomic nerve fibers, because CGRP and substance P are the primary neuropeptides found in the sensory nerves of the rodent respiratory tract.

When male Sprague-Dawley rats inhaled 0.2 or 0.6 ppm acrolein for 6 h, there was a prompt increase in respiratory tract epithelial cell proliferation (Roemer et al. 1993). In hamsters, similar 4-h exposures to acrolein at 6 ppm resulted in a >50% exfoliation rate in bronchial ciliated cells, and by 96 h after acrolein exposure, there was evidence of irregular epithelia with early stratification, hyperplasia, and loss of cilia (Kilburn and McKenzie 1978).

Kaplan (1987) conducted inhalation studies in juvenile baboons exposed to acrolein for 5 min. The animals were trained to perform an escape and avoidance procedure. When baboons were exposed to acrolein at 12, 25, 100, 250, 505, 1,025, or 2,780 ppm, all of the animals completed the avoidance procedure. Baboons exposed at 1,025 and 2,780 ppm developed severe pulmonary edema and died 24 or 1.5 h after acrolein exposure, respectively.

Murphy et al. (1963) found reduced respiratory rates, increased tidal volume, and increased respiratory flow resistance in guinea pigs within 30-60 min of their initial contact with acrolein at 0.6 ppm. Turner et al. (1993) exposed male Dunkin-Hartley guinea pigs to acrolein at 0 or 1.6 ppm for 7.5 h per day for 2 consecutive days. Pulmonary edema (as assessed by wet-to-dry lung weight ratio and protein levels in bronchoalveolar lavage fluid) was present 1 day after acrolein exposure. Evaluation of pulmonary lavage fluid also revealed increased epithelial cells, inflammatory cells (neutrophils and monocytes), and erythrocytes. Those changes were consistent with pulmonary inflammation and hemorrhage.

Leikauf et al. (1989a,b) evaluated inflammatory cells and inflammatory mediators in bronchial lavage fluid and bronchial airway responsiveness in guinea pigs (5-7 per group) administered an intravenous acetylcholine challenge before and 1, 2, 6, and 24 h after exposures to acrolein at

≤0.01 (control), 0.31, 0.67, 0.94, or 1.26 ppm for 2 h. A 2-h exposure at ≥0.94 ppm produced a doubling in bronchial airway resistance to intravenous acetylcholine that persisted for at least 24 h. In a second set of experiments, guinea pigs were exposed to 1.2-1.4 ppm acrolein for 2 h. Pulmonary neutrophil, thromboxane B2, prostaglandin F2α, and leukotriene C4 concentrations were increased in lavage fluid during the first day post-exposure. Pretreatment with either leukotriene antagonists or 5-lipooxygenase activity inhibitors reduced the hyperresponsive acetylcholine response induced by inhaled acrolein. Because of the increased sensitivity of guinea pigs to irritant gases compared with other rodent models, these data might suggest that asthmatic individuals would be more sensitive to inhaled acrolein (Leikauf 2002).

Repeated Exposures and Subchronic Toxicity

Buckley et al. (1984) found that male Swiss-Webster mice exposed to acrolein at 1.67-1.73 ppm for 6 h per day for 5 consecutive days developed moderate erosion and exfoliation of the respiratory epithelium. The changes were accompanied by epithelial squamous metaplasia and focal blebbing, vacuolization, cell separation, and early exfoliation in the nasal turbinates. The olfactory epithelium was also damaged. The injury was considered minimal to moderate and was characterized by ulceration, necrosis, squamous metaplasia, and serous exudates. Most of the epithelial sensory cells in the dorsal meatus were destroyed, and early transformation to a squamous epithelium was evident. Minimal to moderate recovery was observed in the epithelium 72 h post-exposure.

Costa et al. (1986) reported the results of inhalation studies in male Fischer 344 rats exposed to acrolein at 0, 0.4, 1.4, or 4.0 ppm for 6 h per day, 5 days per week for 62 days. Exposure to 4.0 ppm acrolein was associated with severe peribronchiolar and bronchiolar damage. The associated lesions were noted in only 10% of rats exposed to 1.4 ppm. No adverse changes in pulmonary histology were found among rats exposed at 0.4 ppm. Changes in pulmonary function were observed in rats exposed to 4.0 ppm. Changes in maximal expiratory flow-volume curves were observed in rats exposed to 0.4 and 4.0 ppm but not to 1.4 ppm.

Feron et al. (1978) exposed Syrian golden hamsters, Wistar rats, and Dutch rabbits to acrolein at 0, 0.4, 1.4, or 4.9 ppm for 6 h per day, 5 days per week for 13 weeks. Respiratory tract pathology was observed in acrolein-exposed animals. Nasal injury was the most sensitive end point.

Rats, rabbits, and hamsters exposed to 4.9 ppm developed nasal epithelial injury. The normal nasal epithelium was replaced by stratified squamous epithelium with occasional keratinization. Neutrophilic infiltration was observed in the nasal mucosa. At 1.4 ppm, rats displayed nasal metaplasia and inflammation, hamsters showed only a local inflammatory response in the nose, and rabbits were unaffected. One of 12 rats exposed to 0.4 ppm exhibited metaplastic and inflammatory changes in nasal epithelium. Hamsters and rabbits exposed to 0.4 ppm were unaffected.

In a 90-day continuous (24 h per day) inhalation study, Lyon et al. (1970) exposed Sprague-Dawley rats, Princeton or Hartley-derived guinea pigs, squirrel monkeys (*Saimiri sciurea*), and beagle dogs to acrolein at 0, 0.22, 1.0, or 1.8 ppm. Dogs and monkeys were the most sensitive species. Two of four dogs exposed to 0.22 ppm developed moderate emphysema, bronchiolar pathology, and subcapsular splenic hemorrhage. Dogs and monkeys exposed to 1.0 ppm developed signs compatible with ocular and nasal irritation. Rats and guinea pigs exposed to 1.0 ppm and greater displayed focal liver necrosis, and the guinea pigs developed pulmonary inflammation at concentrations at or above 1.0 ppm.

Chronic Toxicity

When groups of Syrian golden hamsters (18 per gender) inhaled acrolein at 0 or 4 ppm for 7 h per day, 5 days per week for 52 weeks, nasal inflammation and epithelial metaplasia were observed in acrolein-exposed hamsters (Feron and Kruysse 1977). Those lesions primarily were observed in the dorsomedial region and nasomaxillary turbinates. The changes persisted for up to 6 months post-exposure in 20% of treated hamsters. There was only one tumor (tracheal papilloma) found in the respiratory tract of one treated female. When Le Bouffant et al. (1980) exposed 20 female Sprague-Dawley rats to acrolein at 8 ppm for 1 h per day, 5 days per week for 10 or 18 months, no respiratory tract metaplasia or neoplasia was observed.

Reproductive Toxicity in Males

When male SPF OFA rats that had been exposed continuously to acrolein at 0.55 ppm for 4 days were mated to females that were then exposed to acrolein at 0.55 ppm throughout gestation, no adverse effects

were observed on the number of pregnant animals, litter size, or fetal weight (Bouley et al. 1976).

Kutzman (1981) was unable to detect any treatment-related changes to reproductive parameters in male Fischer 344 rats that were exposed to acrolein by inhalation at 0, 0.4, 1.4, or 4.0 ppm for 6 h per day, 5 days per week for 62 days of exposure.

Immunotoxicity

Topical acrolein (0.01%, 0.5%, and 2.5%, volume-by-volume in distilled water) applied to the shaved skin of 15 female guinea pigs failed to elicit any signs of sensitization (Susten and Breitenstein 1990).

Astry and Jakab (1983) found a concentration-dependent increase in *Staphylococcus aureus* survival in Swiss mice that inhaled acrolein at 3.0 or 6.0 ppm for 8 h. When male rats inhaled acrolein at 0, 0.1, 1.0, or 3.0 ppm for 6 h per day, 5 days per week for 3 weeks, no significant changes were observed in the responses of pulmonary or spleen lymph node cells to T-cell mitogen or B-cell mitogen, and no significant changes were observed in resistance to *Listeria monocytogenes* infection (Leach et al. 1987). After male Sprague-Dawley rats inhaled acrolein at 0.1, 1.0, or 3.0 ppm for 6 h per day, 5 days per week for 3 weeks, alveolar macrophage lysozyme activity was increased at 1.0 and 3.0 ppm, but there were no effects on macrophage killing or clearance of inhaled ^{35}S-*Klebsiella pneumoniae* (Sherwood et al. 1986).

When CD-1 mice were inoculated with *S. aureus* and *Proteus mirabilis* 30 min prior to a 4- or 24-h inhalation exposure to acrolein at 1-2 ppm, greater numbers of bacteria survived in the lungs of acrolein-treated mice than in the lungs of control mice (Jakab 1977). Jakab (1993) extended these studies using female Swiss mice in nose-only acrolein exposures for 4 h per day over 4 days at an acrolein concentration of 2.5 ppm to determine pulmonary survival of *S. aureus*, *P. mirabilis*, influenza A virus, and *L. monocytogenes*. Inhaled acrolein failed to alter alveolar macrophage immune response or T-cell mediated immunity against those pathogens.

The percentage of inhaled *K. pneumoniae* that survived in the lungs of female CD-1 mice exposed to acrolein at 0.1 ppm (nominal concentration) for 3 h per day for 1 day was no different from the percentage of bacteria that survived in control mice (Aranyi et al. 1986). However, a 5-day exposure produced a decrease in bactericidal activity in the treated animals versus the air-exposed controls. Thus, available data show that

exposures to airborne acrolein at high concentrations can interfere with the in vivo bactericidal activity of murine alveolar macrophages.

Genotoxicity

No studies of the potential genotoxicity of inhaled acrolein in mammals were available (EPA 2003). The International Agency for Research on Cancer (IARC) (1995) and EPA (2003) tabulated the published data on the effects of acrolein in prokaryotic and eukaryotic systems designed to assess mutagenic activity. Acrolein's potent cytotoxicity was considered to be responsible for the conflicting or equivocal results that provide a mixed picture of its genotoxic potential (IARC 1995).

Acrolein induced DNA-protein cross-links and was clastogenic in cultured human lymphoma cells when administered at near cytotoxic concentrations (Costa et al. 1997), and it induced sister chromatid exchange and increased chromosomal aberrations in cultured Chinese hamster ovary cells (IARC 1995). There was no evidence of dominant lethal mutations in mice after a single parenteral dose (IARC 1995).

Acrolein was mutagenic without metabolic activation in *Salmonella typhimurium* TA104 (EPA 2003). Acrolein was not mutagenic with or without metabolic activation in strains TA1535, TA1537, and TA1538 (EPA 2003). Equivocal results were observed in frameshift tester strain TA98 and base repair tester strain TA100 (EPA 2003). Acrolein induced somatic mutations in *Drosophilia melanogaster* exposed at 500-2,000 ppm in air (Vogel and Nivard 1993), and there was a similar response after feeding acrolein at 5-20 millimolar (mM) (Sierra et al. 1991).

Carcinogenicity

At least two laboratories have conducted oral carcinogenicity bioassays of acrolein in rodents. Lijinsky and Reuber (1987) administered acrolein at 0, 100, 250, or 625 ppm in drinking water for 5 days per week to groups of Fischer 344 rats (20 per gender) starting at 7-8 weeks of age. Exposures lasted up to 124 weeks. Survival was comparable in the treated and control groups, and there was no significant treatment-related increase in tumors at any site. Parent et al. (1991) conducted a study in which groups of CD-1 mice (70-75 per gender) were given acrolein at 0, 0.5, 2.0, or 4.5 milligrams per kilogram of body weight (mg/kg) per day in water by gavage

for 18 months. There were no increases in numbers of tumors in the treated animals at any site. In a companion study, Parent et al. (1992) gave groups of Sprague-Dawley rats (70 per gender) 0, 0.05, 0.5, or 2.5 mg/kg per day in water by gavage for up to 102 weeks. There were no indications of carcinogenic response in any tissues, regardless of dose.

As noted above, Feron and Kruysse (1977) conducted a study in which groups of Syrian golden hamsters (18 per gender) inhaled acrolein at 0 or 4 ppm for 7 h per day, 5 days per week for 52 weeks. Six hamsters (six per gender) were sacrificed at 52 weeks, and the remaining hamsters were sacrificed after 81 weeks. One tumor was observed in the respiratory tract of one acrolein-treated females. The study evaluated the effects of benzo[*a*]-pyrene and diethylnitrosamine treatment on acrolein-induced tumor formation, and the authors combined the results of animals exposed to acrolein with and without saline treatment in their data analysis.

On the basis of the published studies described above, IARC (1995) concluded that there was inadequate evidence to determine the carcinogenicity of acrolein in humans and animals. IARC (1995) assigned acrolein to its Group 3 category—not classifiable as to its carcinogenicity to humans. EPA (2003) also concluded that the carcinogenicity of inhaled acrolein could not be determined.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

Mechanisms of acrolein toxicity have been reviewed (EPA 2003). Acrolein reacts with plasma membranes, produces oxygen radicals, and depletes the respiratory tract neuropeptides associated with vasodilation and bronchoconstriction. Depletion of reduced glutathoine (GSH) is an important initial event because it leaves cellular thiol groups vulnerable to oxidation. Acrolein exposures also cause reduced pulmonary compliance and increased pulmonary resistance.

Because of acrolein's marked chemical reactivity, distribution to tissues other than the site of initial contact is very limited (EPA 2003). Acrolein reacts with respiratory tract tissues (Egle 1972). The respiratory tract retention of very high concentrations of inhaled acrolein (>170 ppm) approaches about 80% in dogs (Egle 1972). Studies with anesthetized male Fischer 344 rats exposed to lower acrolein concentrations (0.9-9.1 ppm) showed that nasal absorption of acrolein is significantly dependent on the concentration and ranged from 28% to 62% depending upon acrolein concentrations and inspiratory flow rates used (Morris 1996).

Acrolein reacts with thiols and sulfhydryl groups, and it quickly reacts with protein and nucleic acid primary and secondary amines (EPA 2003). Acrolein depletes rat nasal epithelial GSH (McNulty et al. 1984) and human bronchial epithelial GSH (Grafstrom et al. 1990), and it initiates cell proliferation in the rat respiratory tract (Roemer et al. 1993). When male Wistar rats inhaled acrolein at 1 or 2 ppm, pulmonary lipid peroxidation was extensive (Arumugam et al.1999).

INHALATION EXPOSURE LEVELS FROM THE NRC AND OTHER ORGANIZATIONS

A number of organizations have established or proposed inhalation exposure limits or guidelines for acrolein. Selected values are summarized in Table 2-2.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendations for EEGL and CEGL values for acrolein are summarized in Table 2-3. The current and proposed U.S. Navy values are provided for comparison.

1-Hour EEGL

Sufficiently high concentrations of acrolein in air can induce pronounced eye and upper respiratory tract irritation. Acrolein-induced sensory irritation is prompt (Steinhagen and Barrow 1984; Babiuk et al. 1985) and is characterized by a steep concentration-response relationship (Babiuk et al. 1985). Healthy adult volunteers (18%) complained that exposures to airborne acrolein at 0.3 ppm for 20 min produced "severe to very severe" ocular irritation (Weber-Tschopp et al. 1977). Those complaints were corroborated by a doubling of the eye-blink rate in 66% of subjects after exposure at 0.3 ppm for 10 min and in 70% of subjects after exposure at 0.3 ppm for 20 min. Eye irritation was classified as "moderate" in 35% of the volunteers after 20 min of exposure at 0.3 ppm. Complaints of "a little" eye irritation began after exposure at 0.09 ppm. Complaints of "a little" nasal irritation were reported after exposure at 0.3 ppm for 20-30 min. Weber-Tschopp et al. (1977) identified ocular and nasal irritation thresholds of 0.09 and 0.15 ppm, respectively.

TABLE 2-2 Selected Inhalation Exposure Levels for Acrolein from NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-Ceiling	0.1 (skin)	ACGIH 2000
NIOSH	REL-TWA	0.1	NIOSH 2004
	REL-STEL	0.3	
OSHA	PEL-TWA	0.1	29 CFR 1910.1000
Spacecraft			
NASA	SMAC		NRC 1996
	1 h	0.075	
	24 h	0.035	
	30 days	0.015	
	180 days	0.015	
Submarine			
NRC	EEGL		NRC 1984
	1 h	0.05[b]	
	24 h	0.01[b]	
	CEGL		
	90 days	0.01	
General Public			
ATSDR	Acute MRL	0.00005	ATSDR 1990
	Intermediate MRL	0.000009	
NAC/NRC	AEGL-1 (1 h)	0.03	EPA 2004
	AEGL-2 (1 h)	0.1	
	AEGL-1 (8 h)	0.03	
	AEGL-2 (8 h)	0.1	

[a]The comparability of EEGLs and CEGLs with occupational and public health standards or guidance levels is discussed in Chapter 1, section "Comparison to Other Regulatory Standards or Guidance Levels."

[b]The 1984 NRC subcommittee designated the value as "tentative" on the basis of its review of the data. The 2004 NRC subcommittee provides a qualitative description of uncertainties and identifies data gaps in the final section of this profile rather than qualifying a recommendation with terms, such as "tentative."

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL, acute exposure guideline level; ATSDR, Agency for Toxic Substances and Disease Registry; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; h, hour; MRL, minimal risk level; NAC, National Advisory Committee; NASA, National Aeronautics and Space Administration; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; ppm, parts per million; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

TABLE 2-3 Emergency and Continuous Exposure Guidance Levels for Acrolein

Exposure Level	U.S. Navy Values (ppm)		NRC Recommended Values (ppm)
	Current	Proposed	
EEGL			
1 h	0.05	0.07	0.1
24 h	0.01	0.03	0.1
CEGL			
90 days	0.01	0.01	0.02

Abbreviations: CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level; h, hour; NRC, National Research Council; ppm, parts per million.

On the basis of controlled human exposure studies (Weber-Tschopp et al. 1977), complaints of "a little" eye irritation—the most sensitive acute effect—begin at about 0.09 ppm, which is effectively 0.1 ppm. Therefore, the subcommittee recommends 0.1 ppm as the 1-h EEGL. That level can be considered a minimal lowest-observed-adverse-effect level (LOAEL) and is one-third the concentration at which a doubling of eye-blink rate was noted in about 70% of subjects after exposure for 20 min.

24-Hour EEGL

A concentration-dependent increase in eye-blink rate was observed at concentrations ≥0.17 ppm when human subjects were exposed continuously to increasing concentrations of acrolein over 35 min (Weber-Tschopp et al. 1977). The increase became significant at 0.26 ppm with the rate doubled at 0.3 ppm. Exposures to airborne acrolein described as irritating by most volunteers ranged from 0.3 ppm for 20-30 min (Weber-Tschopp et al. 1977) to 0.5 ppm for 5 min (Stephens et al. 1961). Anecdotal accounts of human inurement to sensory irritants are common, and some degree of adaptation to airborne acrolein can be expected (Bouley et al. 1976). A threshold of 0.09 ppm for ocular irritation in humans has been identified, and the degree of ocular and mucous membrane irritation associated with exposure to acrolein at 0.1 ppm for 1 h is not anticipated to increase over a 24-h period. Therefore, the 1-h EEGL of 0.1 ppm was considered appropriate for use as the 24-h EEGL.

90-Day CEGL

No human studies appropriate for use in deriving the 90-day CEGL were identified. Acute pulmonary inflammation was a consistent consequence of inhalation exposures to acrolein in animals; exposure durations as brief as 5 min (Kaplan 1987) or 10 min (Springhall et al. 1990) have induced concentration-dependent pulmonary edema. Subchronic whole-body inhalation studies in rodents, rabbits, dogs, and nonhuman primates demonstrated excessive salivation, ocular and nasal irritation, nasal inflammation, nasal or tracheal squamous metaplasia, basal cell hyperplasia, and acute pulmonary congestion (Lyon et al. 1970; Feron et al. 1978). Subchronic LOAELs of 0.4 ppm in rats and 1.4 ppm in hamsters were identified on the basis of nasal inflammation observed after exposures lasting 6 h per day, 5 days per week (Feron et al. 1978). Two of four beagles that inhaled acrolein at 0.22 ppm for 24 h per day for 90 days developed emphysema (Lyon et al. 1970). Recognizing the irreversible nature of emphysema and the appropriateness of the 90-day continuous-exposure inhalation protocol of Lyon et al. (1970), the subcommittee selected the beagle LOAEL of 0.22 ppm as the basis for the 90-day CEGL. An interspecies uncertainty factor of 3 was applied on the basis of acrolein's similar irritant action in rodent and human target tissues and the steep acrolein concentration-response relationships seen in both laboratory animals and human volunteers. An uncertainty factor of 3 for extrapolation from a LOAEL to a no-observed-adverse-effect level (NOAEL) was also applied to yield a 90-day CEGL of 0.02 ppm. The subcommittee concludes that application of a total uncertainty factor of 10 is appropriate as the resulting 90-day CEGL is below the exposure concentrations at which any irritation was reported in human volunteers.

DATA ADEQUACY AND RESEARCH NEEDS

Although responses to airborne acrolein are well documented, there remain considerable difficulties and uncertainties associated with the quantification of sensory irritation. Several animal systems, such as rat and mouse RD_{50} calculations, have been designed to predict and quantify sensory irritation (ASTM 1991). Some investigators have designed assays to separate perception of chemical odor from nasal pungency using anosmic and normosmic volunteers (Cometto-Muniz and Cain 1993, 1994). Others (Abraham et al. 1996, 1998; Hau et al. 1999) have devised algorithms for

nasal pungency to rank volatile organic materials. However, none of those methods have received the review and critical evaluation necessary to achieve the level of confidence required for regulatory acceptance.

Derivation of quantitative environmental and occupational exposure limits for sensory irritants is fraught with difficulty, because the reports of ocular and respiratory tract irritation experienced are considered by some to be subjective. That view can lead to points of considerable contention (Paustenbach 2001). The results of controlled human exposures to acrolein use typical descriptors, such as "mild" or "mild to moderate," and the databases of sensory irritation thresholds for acrolein and related materials can be highly variable. Considerable research should be done to quantify the diverse sensory irritation methods for use in public- and occupational-health risk assessment (Dalton 2001). Thus, the subcommittee concludes that additional studies on the irritant effects of acrolein are needed to better define the exposure guidance levels for the short-term durations.

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3

Carbon Dioxide

This chapter summarizes the relevant epidemiologic and toxicologic studies on carbon dioxide (CO_2). Selected chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure levels from the National Research Council (NRC) and other agencies are also presented. The subcommittee considered all of that information in its evaluation of the Navy's current and proposed 1-hour (h), 24-h, and 90-day exposure guidance levels for CO_2. The subcommittee's recommendations for CO_2 exposure levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

CO_2 is a colorless, noncombustible gas with a faint acid taste (Budavari et al. 1989). It is reported to have either no odor (Budavari et al. 1989) or a faintly pungent odor (Ballou 1985). CO_2 is heavier than air, and that contributes to the development of toxic exposure situations in enclosed spaces. Selected physical and chemical properties are listed in Table 3-1.

OCCURRENCE AND USE

CO_2 has numerous applications. It is used in food freezing and chilling, beverage carbonation, chemical manufacture, fire prevention and extinction, metal working, and oil and gas recovery (Ballou 1985). It is produced on combustion of all carbonaceous fuels and is a product of animal metabolism. The annual average atmospheric concentration of CO_2 is 372 parts per million (ppm) (Blasing and Jones 2003).

TABLE 3-1 Physical and Chemical Properties of Carbon Dioxide[a]

Synonyms and trade names	Carbonic acid gas, carbonic anhydride, dry ice
CAS registry number	124-38-9
Molecular formula	CO_2
Molecular weight	44.01
Boiling point	—
Melting point	Sublimes at −78.48°C
Flash point	—
Explosive limits	—
Specific gravity	1.527 with respect to air
Vapor pressure	569.1 mmHg at −82°C
Solubility	Solubility in H_2O at 20°C, 760 mmHg = 88 mL CO_2/100 mL H_2O; less soluble in alcohol and other neutral organic solvents
Conversion factors	1 ppm = 1.80 mg/m^3; 1 mg/m^3 = 0.56 ppm

[a]Data were taken from Budavari et al. (1989).
Abbreviations: mg/m^3, milligrams per cubic meter; mL, milliliters; mmHg, millimeters of mercury; ppm, parts per million; —, not available or not applicable.

Submarine crew are reported to be the major source of CO_2 on board submarines (Crawl 2003). Data collected on nine nuclear-powered ballistic missile submarines indicate an average CO_2 concentration of 3,500 ppm with a range of 0-10,600 ppm, and data collected on 10 nuclear-powered attack submarines indicate an average CO_2 concentration of 4,100 ppm with a range of 300-11,300 ppm (Hagar 2003).

SUMMARY OF TOXICITY

The information below was taken largely from a more comprehensive review, *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Volume 2* (NRC 1996). The studies discussed represent those most relevant to submariners and the submarine environment.

CO_2 is a simple asphyxiant and lethal asphyxiations have been reported at concentrations as low as 110,000 ppm (Hamilton and Hardy 1974). Loss of consciousness can occur within a minute of exposure at 300,000 ppm and within 5-10 minutes (min) of exposure at 100,000 ppm (HSDB 2004). The effects of concentrations of CO_2 between 7,000 and 300,000 ppm in humans and animals are discussed below and include

tremor, headaches, chest pain, respiratory and cardiovascular effects, and visual and other central nervous system (CNS) effects.

The respiratory, cardiovascular, and CNS effects of CO_2 are related to the decreases in blood and tissue pH that result from exposures (Eckenhoff and Longnecker 1995; Yang et al. 1997; HSDB 2004). Changes in pH act directly and indirectly on those systems. The pH changes also trigger various compensatory mechanisms, including increased ventilation to reduce excess CO_2 in the bloodstream, increased renal acid excretion to restore acid-base balance, and sympathetic nervous system stimulation to counteract the direct effects of pH changes on heart contractility and vasodilation (Eckenhoff and Longnecker 1995; HSDB 2004). The key effects for setting EEGL and CEGL values are tremor, headache, hyperventilation, visual impairment, and CNS impairment.

Effects in Humans

Accidental Exposures

In a case report of two men who lost consciousness in a wellhead chamber as a result of exposure to a "high concentration" of CO_2 in the atmosphere, one man exhibited constricted visual fields, enlarged blind spots, photophobia, loss of convergence and accommodation, deficient dark adaptation, headaches, insomnia, and personality changes (Freedman and Sevel 1966). The other man died of asphyxia. In a similar incident, one overexposed man died after 11 months in a coma; he exhibited retinal atrophy and gliosis as well as loss of all ganglion cells (Sevel and Freedman 1967). In addition to that delayed fatality, three men died immediately from asphyxia. These studies are not relevant to establishing EEGL and CEGL values, but they are consistent with the findings that CO_2 affects vision.

Experimental Studies

Experimental studies have shown that CO_2 causes a variety of effects, ranging from nonspecific signs and symptoms, such as tremor, dyspnea, intercostal pain, and headache, to cardiovascular and CNS effects. Each of the effects mentioned is addressed in the following discussion.

Tremor was noted at a CO_2 concentration of 60,000 ppm after several hours of exposure in a review that lacked documentation of specific methods (Schulte 1964). It was also noted in 10 of 12 subjects exposed at 7,000-

14,000 ppm for 10-20 min (Sechzer et al. 1960). Tremor has not been reported at lower concentrations and, in fact, was specifically absent in many of the detailed neurobehavioral protocols discussed below.

Dyspnea is a commonly reported end point and can be induced by acute exposures to CO_2 at >30,000 ppm (NRC 1996). Hyperventilation without dyspnea occurs at exposure concentrations as low as 10,000 ppm (NRC 1996). Dyspnea attributable to CO_2 is aggravated by increasing the level of exertion. Studies at rest will be discussed, followed by discussion of those that included exercise protocols.

White et al. (1952) studied humans exposed to CO_2 at 60,000 ppm for 16 min and reported that 19 of 24 subjects exhibited slight or moderate dyspnea and 5 of 24 exhibited severe dyspneic sensations. At 40,000-50,000 ppm for 17-32 min, 16 subjects reported dyspnea (Schneider and Truesdale 1922). In contrast, no dyspnea was reported in five subjects exposed at 32,000 ppm or at 25,000-28,000 ppm for several hours (Brown 1930).

In the most modern protocol to examine dyspnea, Menn et al. (1970) reported that eight subjects exposed to CO_2 at 11,000 ppm exhibited no increase in dyspnea or intercostal pain during 30 min of maximal exercise. The same study reported that exposure to CO_2 at 28,000 ppm during 30 min of maximal exercise produced increased dyspnea in three of eight subjects and intercostal pain in two of eight subjects, but subjects did not show increased dyspnea at one-half or two-thirds maximal exercise. Sinclair et al. (1971) reported that a 1-h exposure to CO_2 at 28,000 ppm in four subjects caused no dyspnea or intercostal pain during steady strenuous exercise. Thus, the bulk of the data indicate a no-observed-adverse-effect level (NOAEL) for CO_2 of about 28,000 ppm on the basis of the findings on dyspnea and intercostal pain.

Neither dyspnea nor intercostal pain occurred in four subjects exposed to CO_2 at 28,000 ppm for 15-20 days and made to do 45 min of exercise twice daily at up to a heavy level, although the chronic portion of this protocol was not fully described (Sinclair et al. 1971). Similarly, there were no symptoms reported in six subjects exposed to CO_2 at 20,000 ppm for 30 days or 29,000 ppm for 8 days and made to do 10 min of exercise twice a week at a workload of 150 watts (Guillerm and Radziszewski 1979; Radziszewski et al. 1988). Thus, 28,000 ppm is an appropriate chronic NOAEL for dyspnea and intercostal pain.

Headaches are commonly associated with increased CO_2 concentrations in inspired air, but there is conflicting data on the concentrations reliably associated with that end point. There may also be an effect of exertion, because CO_2 seems to cause more headaches at lower concentra-

tions during exercise than it does during rest. In particular, Schneider and Truesdale (1922) reported that for 16 resting subjects exposed to CO_2 at 10,000-80,000 ppm for 17-32 min, headaches developed only at concentrations that were ≥50,000 ppm; however, the headache could be intense. At 28,000 ppm for 1 h of strenuous steady-state exercise, occasional mild headaches were noted among four subjects (Sinclair et al. 1971). At 39,000 ppm for 30 min of exercise at two-thirds maximal exertion, Menn et al. (1970) found mild-to-moderate frontal headaches in six of eight subjects near the end of the exposure period. The headaches resolved after about an hour. At 28,000 ppm and 11,000 ppm for 30 min of exercise, no headaches were reported (Menn et al. 1970). Thus, there is inconsistent modern evidence for mild headaches resulting from CO_2 exposures at 28,000 ppm during exercise. Some level of increased exertion among submarine crew might be likely during 1-24 h emergency episodes; however, headaches induced by CO_2 are both mild and reversible and therefore were not used as a primary end point for setting the 1-h and 24-h EEGLs.

Subchronic CO_2 exposures at 30,000 ppm or higher are known to produce headaches. Glatte et al. (1967) reported that CO_2 at 30,000 ppm for 5 days led to mild to moderate throbbing frontal headaches on the first day in four of seven subjects. The headaches disappeared on day 3 and were not severe enough to interfere with normal activities, including 1 h of moderate exercise daily, although three of the four subjects with headaches requested analgesics. During 30-day exposures at 20,000 ppm, six subjects rarely developed headaches, and exposures at 29,000 ppm led to slight headaches (Radziszewski et al. 1988). Eight subjects, four exposed to CO_2 at 28,000 ppm for 15-30 days and four exposed to CO_2 at 39,000 ppm for 11 days, reported occasional mild headaches during exertion that disappeared after the first day of exposure (Sinclair et al. 1969, 1971). Thus, 20,000 ppm is an appropriate subchronic NOAEL for headaches.

CO_2 is known to increase alveolar ventilation (hyperventilation), not as a toxic effect, but to maintain acid-base homeostasis. Concentrations as low as 10,000 ppm acutely increased ventilation by 32% in one study of 16 subjects exposed for 17-32 min (Schneider and Truesdale 1922). Increases in ventilation are thought to occur mainly through tidal volume increases, although increased respiratory rates have been reported in some studies. Chemoreceptors in brain and carotid bodies probably mediate the response, which sometimes persists after exposure is terminated to blow off CO_2 and restore pH. The hyperventilation is also an adaptive response for preventing hypoxia in lower oxygen environments.

CO_2 exposures at 50,000 or 75,000 ppm for 2 h resulted in decreased specific airway conductance, although exposures at 25,000 ppm did not

(Tashkin and Simmons 1972). Clinical indices of airway impairment were absent in this and other studies.

In summary, it takes an exposure concentration of at least 10,000 ppm to increase minute-volume after a plateau in the hyperventilatory response has been reached, usually after a few hours. It is not clear from the data whether the hyperventilatory response diminishes with time, although in a study at 10,000 ppm, it resolved completely after 8 days of a 44-day exposure (Pingree 1977). Data from Radziszewski et al. (1988) showed a 60% increase in minute-volume during a 2-h exposure at 20,000 ppm. The increase was reduced to 45% after 24 h. There is no indication in the literature that hyperventilation constitutes an adverse response.

Exposures to CO_2 at concentrations much higher than those in ambient air lead to increased partial pressure of CO_2 in alveoli and blood. That causes a lowering of blood pH, which is eventually buffered by blood proteins and bicarbonate. During a 1-h exposure at 28,000 ppm, rapid acidosis occurred after 45 min of mild to moderate exercise, but the acidosis did not impair function in this study, even with prolonged exposures of up to 20 days with 45 min of exercise twice daily (Sinclair et al. 1971). Guillerm and Radziszewski (1979) reported similar results in a study of a 30-day exposure at 20,000 ppm that included twice weekly 10-min exercise periods. Thus, acidosis does not seem to be an end point of concern for setting 1-h and 24-h standards.

CO_2 exposures as low as 7,000 ppm can lower blood pH by up to 0.05 units, but even at high exposures, renal compensation seems to occur in healthy subjects. In a 30-day exposure to CO_2 at 20,000 ppm, there was an average pH change of only 0.01 units (Guillerm and Radziszewski 1979). Compensation occurs over a variable period of time, but effects of lowered pH on clinical status or performance have not been reported either experimentally or operationally (Schaefer et al. 1964a).

Exposures to CO_2 at 10,000-20,000 ppm for 17-32 min were reported to cause slight increases in systolic and diastolic blood pressure (Schneider and Truesdale 1922). Exposures at 50,000 ppm or 70,000 ppm for 15-30 min caused increases in blood pressure but no changes in cardiac output (Kety and Schmidt 1948). Grollman (1930) reported increases in cardiac output and heart rate during 4-25 min exposures at 75,000 ppm.

Electrocardiograph changes resulting from CO_2 exposures have received much attention. A number of changes, including atrial tachycardia and increased QT intervals, were found during exposures at about 300,000 ppm (in 70% oxygen) (MacDonald and Simonson 1953; McArdle 1959). In the more moderate range of 70,000-140,000 ppm, various clinically unimportant rhythm changes, such as premature nodal contractions and rare

premature ventricular contractions, have been reported (Sechzer et al. 1960). Glatte et al. (1967) found no electrocardiograph problems in individuals exposed to CO_2 at 30,000 ppm for 5 days, during which they exercised for 1 h daily. Sinclair et al. (1971) found no increases in premature ventricular contractions at 28,000 ppm for 15-20 days during which subjects were made to engage in moderate and heavy exercise, although this chronic protocol was not adequately described. On the basis of data from Glatte et al. (1967) and Sinclair et al. (1971), subchronic exposures at 30,000-40,000 ppm appear to be free of significant arrhythmia effects. At lower concentrations, chronic exposures resulted in only minor electrocardiograph changes without any rhythm disturbances (Radziszewski et al. 1988). Thus, cardiovascular end points are not of primary concern for setting the EEGL or CEGL values for CO_2.

Guillerm and Radziszewski (1979) reported a 10% reduction in hematocrit and a 9% reduction in red blood cell count in subjects exposed to CO_2 at 20,000 ppm for 30 days. These changes were not observed by the same investigators at 40,000 ppm, which makes them of dubious significance. The investigators attributed them to prolonged confinement rather than to CO_2 exposure. Wilson and Schaefer (1979) found that on Polaris submarine patrols that had measured CO_2 concentrations between 7,000 and 12,000 ppm and carbon monoxide concentrations of 15-20 ppm, the hematology of smokers differed from that of nonsmokers. In the nine smokers examined, red blood cell count increased by a statistically significant 12% on day 6, but returned to near baseline by day 52. However, in 11 nonsmokers, there was no statistically significant change in red blood cell count on any of the 3 examination days. Although the findings might suggest a differential response in smokers exposed to CO_2, they cannot be used to set CO_2 standards.

While on active submarine patrol for 57 days, 7 out of 15 crewmen exposed to CO_2 at 8,000-12,000 ppm developed decreased plasma calcium and increased erythrocyte calcium (Messier et al. 1976). There were no changes in parathyroid hormone or calcitonin (Messier et al. 1976). Some observational data from submarine patrols documented increased urinary calculi in crewmen when CO_2 was present at >10,000 ppm most of the time, instead of at <10,000 ppm (Tansey et al. 1979). There are several physiologic reasons why that is not thought to be causal; in particular, the incidence rate of urinary calculi observed in submariners does not seem to exceed the general population rate. Exposure to CO_2 at 50,000 ppm for 30 min led to increased renal blood flow, glomerular filtration rate, and renal venous pressure, as well as increased renal vascular resistance (Yonezawa 1968). These physiologic changes related to renal compensation for CO_2-

induced acidosis are considered to be innocuous. Thus, electrolyte, bone, and kidney effects are not appropriate end points for developing exposure standards.

It is well established that CO_2 acutely impairs vision and hearing at concentrations exceeding about 50,000 ppm (Yang et al. 1997). Exposures to CO_2 at 61,000-63,000 ppm for 6 min led to 3-8% increases in the hearing threshold for six subjects (Gellhorn and Spiesman 1935). The same authors noted slight impairment after 5-22 min of exposure at 30,000-40,000 ppm and identified a NOAEL of 25,000 ppm (Gellhorn and Spiesman 1934, 1935).

Sun et al. (1996) studied the effects of CO_2 exposures at 25,000 ppm on stereoacuity (depth) perception in three adult subjects—two males and one female—using a two-alternative forced-choice procedure. The exposure duration was not stated, but the subcommittee estimated the duration to be about 1 h on the basis of the 30-min acclimatization period followed by the testing session. The psychometric functions curves of all three subjects shifted to the right, but returned to baseline after 2 h of breathing fresh air. Stereoacuity values, the reciprocal of stereoscopic thresholds, were statistically significantly reduced in all three subjects. In a second study, Yang et al. (1997) investigated the effects of CO_2 exposures at 25,000 ppm on perception of coherent motion in three subjects with normal or corrected-to-normal vision using a two-interval forced-choice psychophysical procedure. The exposure duration appears to have been 1 h. Motion detection thresholds were statistically significantly different during exposure in all three subjects, but the subjects showed complete recovery in fresh air. Although the number of subjects was small, these two studies are considered to be methodologically rigorous and involve more sensitive end points than those discussed previously. These studies suggest an acute lowest-observed-adverse-effect level (LOAEL) for visual effects of 25,000 ppm.

Exposures at 50,000-67,500 ppm in 19.2% oxygen for 37 h caused decreased hand-arm steadiness but caused no changes in computing, translating, number checking, or discrimination of pitch or loudness in four subjects (Consolazio et al. 1947). Among fighter pilots, exposures to CO_2 at 50,000 ppm degraded performance in multiple aspects of simulated landing (Wamsley et al. 1969), clearly indicating that CO_2 at 50,000 ppm impairs neurobehavioral performance.

Brown (1930) studied five subjects exposed to CO_2 concentrations that ranged from 41,000 ppm to 53,000 ppm for 8 h. Results showed a statistically significant 24% decrease in number cancellation in a number cancellation test. The same exposures did not affect performance on the Army Alpha intelligence and arithmetic tests, attention, or muscular coordination,

leaving open the possibility that the 24% decrease in number cancellations from nonspecific responses was the result of confinement in the chamber (Brown 1930). The Brown study did not include any control subjects.

A number of studies suggest that CO_2 exposures in the range of 15,000-40,000 ppm do not impair neurobehavioral performance. Schaefer (1961) reported that 23 crewmen exposed to CO_2 at 15,000 ppm for 42 days in a submarine showed no psychomotor testing effects but showed moderate increases in anxiety, apathy, uncooperativeness, desire to leave, and sexual desire. In a 5-day exposure of seven subjects at a CO_2 concentration of 30,000 ppm, Glatte et al. (1967) reported no effects on hand steadiness, vigilance, auditory monitoring, memory, or arithmetic and problem solving performance. Storm and Giannetta (1974) studied the effects of 2 weeks of exposure to CO_2 at 40,000 ppm on psychomotor performance in a 6-week protocol that included a 2-week pre-exposure baseline period and 2 weeks of recovery. Twenty-four volunteers, ages 18-23, were selected for their motivation and their excellent health. Two experimental groups and two control groups of six subjects each were formed. One exposure group and one control group were on bedrest to simulate weightlessness; the others were active. The primary outcome measure was a tracking task with a joy stick and rudder. Acute effects were not measured. All measurements and practice were done in the pre-exposure and recovery periods. An additional outcome measure, the repetitive psychometric measures (RPM) test, was used. It is a six-part pencil and paper test that measures complex cognitive tasks, such as aiming, flexibility of closure, perceptual speed, visualization, number facility, and speed of closure. Using 20 versions of the subtests minimized practice effects, and the longitudinal design sought to avoid memorization.

CO_2 exposure did not affect performance on the tracking task or any of the six RPM subtests (Storm and Giannetta 1974). There was a learning effect for the tracking task during both pre-exposure and recovery, but the authors still thought it was appropriate to conclude the absence of a performance impairment. The authors considered it especially likely because previous papers had suggested that impairment is easier to detect during skill reacquisition, which occurred following the 2 weeks of exposure without practice, rather than at an asymptotic skill level (Storm and Giannetta 1974). Thus, CO_2 at 40,000 ppm for 2 weeks did not affect performance on multiple tests of cognitive function in physically fit young airmen, a population probably not unlike submariners.

Based on the work of Storm and Giannetta (1974) and Glatte et al. (1967), a NOAEL of 30,000 ppm for general CNS effects could be proposed. However, the subcommittee considers the subtler, if less relevant,

visual effects reported by Sun et al. (1996) and Yang et al. (1997) at 25,000 ppm to be a minimal LOAEL.

Occupational and Epidemiologic Studies

In a review by Schulte (1964), exposure to CO_2 at 30,000 ppm caused dyspnea at rest. Exposures at 20,000-30,000 ppm for several hours caused headaches on mild exertion, and the headaches at 30,000 ppm were more severe than those at 20,000 ppm (Schulte 1964). CNS depression developed after several hours of exposure at 50,000 ppm (Schulte 1964). A CO_2 exposure at 30,000 ppm for 8 days in a working submarine crew (unknown number of subjects) led to clinical observations of euphoria and troubled sleep on day 1 and poor attention, erratic behavior, confusion, and motor skill impairment on days 2-8 (Schaefer 1949a,b). Schaefer (1958, 1959, 1963) reported that some crewmen of a German submarine exposed to CO_2 at 30,000-35,000 ppm in 15-17% oxygen during a 2-month underwater patrol in World War II suffered from impaired attentiveness. The authors did not consider confounding contaminants or the low oxygen concentrations aboard the submarines, and thus it is problematic to rely on these uncontrolled studies.

In the only nonmilitary study, brewery workers exposed during an 8-h work shift to a time-weighted average concentration of CO_2 at 11,000 ppm and to excursions at up to 80,000 ppm for 3 min had blood bicarbonate levels that were no different from controls, consistent with the relatively mild impact of chronic CO_2 exposures on electrolytes and acid-base balance (NIOSH 1976).

Effects in Animals

Changes in lung histopathology, changes in liver and heart, and gastrointestinal bleeding associated with CO_2 exposures were reported in animal studies; however, the quality and relevance of those data is questionable. Those effects are discussed below for the purpose of completeness.

Schaefer et al. (1964b) found lung effects in guinea pigs exposed to CO_2 at 150,000 ppm for durations ranging from 1 to 24 h. The effects observed included subpleural atelectasis, increased lamellar bodies in alveolar lining cells, congestion, edema, hemorrhage, increases in phagocytic pneumocytes, increased lung-to-body weight ratios, increased surface tension of lung extracts, and increases in hyaline membranes. However, in

a 14-day experiment at 150,000 ppm, no changes in lung-to-body weight ratios or surface tension of lung extracts, and no subpleural atelectasis, edema, hemorrhage, or abnormal lamellar bodies in alveolar lining cells were observed (Schaefer et al. 1964b). At 30,000 ppm for 2 days, subpleural atelectasis and edema, but no hyaline membranes, were found in guinea pigs, and at 15,000 ppm for 6 months, only subpleural atelectasis was found (Niemoller and Schaefer 1962). In addition to having somewhat mixed findings and extremely high exposures, the studies had inadequate numbers of control animals (NRC 1996) and were not further considered.

Douglas et al. (1979) reported the proliferation of type II pneumocytes during a 4-week exposure to CO_2 at as little as 10,000 ppm. Gas exchange was not impaired, and the effect was considered to be a metabolic adaptation of the lungs to CO_2 (Douglas et al. 1979). The subcommittee thought that these changes were functionally insignificant, especially given that they are inconsistent with the body of literature on human experiences. Thus, these studies were not used for setting the CEGL.

In an uncontrolled study by Meessen (1948), rabbits exposed at 45,000 ppm in 21% oxygen for 13 days were found to have scattered necrotic areas throughout the liver lobules. Pepelko (1970) did not observe histologic effects in liver, lungs, kidneys, spleen, thyroid, adrenals, or heart in rats exposed to CO_2 at 80,000 ppm for 32 days. Schaefer et al. (1971) found a decrease in glycogen granules and an increase in fat granules in the livers of guinea pigs exposed to CO_2 at 30,000 ppm for 7 days. These observed changes were thought to reflect functional changes in liver metabolism but were not considered liver damage.

Increased fat deposition was seen in the myocardia of guinea pigs exposed to CO_2 at 150,000 ppm for 7 days (Schaefer et al. 1971). Gastrointestinal bleeding was observed in dogs exposed to CO_2 at 150,000 ppm for 3 h and in guinea pigs exposed at 150,000 ppm for 24 h (DeBellis et al. 1968; Schaefer et al. 1971). These results are not considered to be relevant for setting exposure guidelines because of the extremely high exposure concentrations used.

Reproductive Toxicity in Males

Exposures to CO_2 at 25,000 ppm, 50,000 ppm, and 100,000 ppm for 4-8 h in rats produced a concentration-dependent disappearance of mature spermatids attributed to sloughing of mature spermatids and Sertoli cells in the seminiferous tubules (Vandemark et al. 1972). There were no effects after a 1- or 2-h exposure, and there was complete structural recovery

within 36 h, so this end point was not used to set the EEGL and CEGL values. Mukherjee and Singh (1967) exposed mice to CO_2 at 360,000 ppm in 13.4% oxygen alternating 2 h of exposure with 30 min of fresh air for 6 h in one experiment and exposing the mice for 4 h per day for 6 days in another experiment. They found spermatozoa with smaller heads and midpieces in the vas deferens in the short-term experiment and reduced fertility in the 6-day experiment. The very high CO_2 concentrations and low oxygen concentrations make this study inappropriate for setting EEGL and CEGL values.

Immunotoxicity

No relevant information was found regarding the potential immunotoxicity of CO_2.

Genotoxicity

No relevant information was found regarding the potential genotoxicity of CO_2.

Carcinogenicity

No relevant information was found regarding the potential carcinogenicity of CO_2.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

CO_2 freely penetrates cell membranes and diffuses from lungs to blood at a rate 20 times faster than oxygen because CO_2 has much greater solubility (West 1979). CO_2 undergoes catalysis by the enzyme carbonic anhydrase in red blood cells to form carbonic acid, which is then ionized to bicarbonate (Baggott 1982). Thus, 90% of the CO_2 in the body is carried in blood as bicarbonate ion. Of the remaining CO_2, 5% forms carbamino compounds in the reaction of CO_2 with uncharged amino groups on hemoglobin and 5% is dissolved in serum and cytoplasm (Baggott 1982). At rest, CO_2 is exhaled at about 220 milliliters per minute (mL/min), increasing to 1,650 mL/min during moderate exercise (Cotes 1979).

Dyspnea occurs as a result of higher alveolar CO_2 concentrations, leading to reduced exchange capacity, increased blood CO_2, and acidosis. Chemoreceptors in the carotid body and CNS respond to the pH change, which leads to increased ventilation, a homeostatic response, associated with an increased sense of dyspnea analogous to that produced by increased CO_2 production during exercise. The kidneys respond to acidosis by adjusting excretion of hydrogen and bicarbonate ions.

CO_2-induced pH changes directly dilate blood vessels and decrease cardiac contractility. CO_2 also stimulates the sympathetic nervous system, increasing blood concentrations of epinephrine, norepinephrine, and angiotensin to counteract the direct cardiovascular effects (Staszewska-Barczak and Dusting 1981). The sympathetic response does not fully compensate for the direct vasodilation (Eckenhoff and Longnecker 1995).

The subtle visual effects of CO_2 exposure might be related to the changes in neurotransmitter levels and activity caused by CO_2-related pH changes (Yang et al. 1997). Excess CO_2 in blood depresses the excitability of the cerebral cortex (Eckenhoff and Longnecker 1995). At CO_2 concentrations of 250,000 ppm and greater, subcortical areas that have cortical projections are activated, overcoming the depressant effect and sometimes resulting in convulsions (Eckenhoff and Longnecker 1995). Concentrations at 500,000 ppm or greater produce marked cortical and subcortical depression similar to the action of anesthetics (Eckenhoff and Longnecker 1995).

INHALATION EXPOSURE LEVELS FROM THE NRC AND OTHER ORGANIZATIONS

A number of organizations have established or proposed acceptable inhalation exposure limits or guidelines for CO_2. Selected values are summarized in Table 3-2.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendations for EEGL and CEGL values for CO_2 are summarized in Table 3-3. The current and proposed U.S. Navy values are provided for comparison.

TABLE 3-2 Selected Inhalation Exposure Levels for Carbon Dioxide from NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-Ceiling	5,000	ACGIH 2002
	TLV-STEL	30,000	
NIOSH	REL-TWA	5,000	NIOSH 2004
	REL-STEL	30,000	
OSHA	PEL-TWA	5,000	29 CFR 1910.1000
Spacecraft			
NASA	SMAC		NRC 1996
	1 h	13,000	
	24 h	13,000	
	30 days	7,000	
	180 days	7,000	

[a]The comparability of EEGLs and CEGLs with occupational and public health standards or guidance levels is discussed in Chapter 1, section "Comparison to Other Regulatory Standards or Guidance Levels."

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; h, hour; NASA, National Aeronautics and Space Administration; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; ppm, parts per million; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

1-Hour EEGL

Visual impairment and CNS effects are the most appropriate end points for determining the 1-h EEGL. Two studies, Glatte et al. (1967) and Storm and Giannetta (1974), used the repetitive psychometric measures (RPM) tests on multiple subjects in experiments ranging from 5 to 14 days duration and found subchronic NOAELs for visual effects and tremors of 30,000 ppm and 40,000 ppm, respectively. Studies at exposure concentrations of 28,000-29,000 ppm for 30 min to 1 h and up to 8 days reported no CNS effects (Menn et al. 1970; Sinclair et al. 1971; Radziszewski 1988). However, Sun et al. (1996) and Yang et al. (1997) found asymptomatic decrements in stereoacuity and motion perception in a total of six subjects exposed at 25,000 ppm for durations of about 1 h. The effects were fairly subtle, the number of subjects was small, the effects were rapidly reversible, and the toxicologic and operational significance is questionable.

TABLE 3-3 Emergency and Continuous Exposure Guidance Levels for Carbon Dioxide

Exposure Level	U.S. Navy Values (ppm)		NRC Recommended Values (ppm)
	Current	Proposed	
EEGL			
1 h	40,000	30,000	25,000
24 h	40,000	15,000	25,000
CEGL			
90 days	5,000	7,000	8,000

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; h, hour; NRC, National Research Council; ppm, parts per million.

Storm and Giannetta (1974) could be used to establish 40,000 ppm as a NOAEL for clinically apparent CNS effects (visual disturbances, tremor, and neurobehavioral impairment). The 40,000-ppm exposure was well tolerated, and the study included many tests relying on visual function. However, the Sun et al. (1996) and Yang et al. (1997) studies reported a LOAEL at 25,000 ppm on the basis of subtle and asymptomatic errors of visual tracking and depth perception. Because the exposure guidance must be protective in the low-oxygen atmospheres of submarines, the minimal LOAEL of 25,000 ppm is recommended for the 1-h EEGL.

24-Hour EEGL

For the 24-h EEGL, it is noted that the exposure studies discussed above for determining the 1-h EEGL, including the Storm and Giannetta (1974) study, included exposure durations of up to 2 weeks and reported no significant adverse effects at concentrations up to 40,000 ppm. For that reason, no reduction in the 1-h EEGL is warranted for extrapolation to 24 h, and 25,000 ppm also is recommended for the 24-h EEGL.

90-Day CEGL

For a 90-day exposure, the Sun et al. (1996) and Yang et al. (1997) visual function findings are of greater concern, especially given that there was no available 90-day study of neurobehavioral effects of CO_2 exposures. Thus, using the Sun et al. (1996) and Yang et al. (1997) 25,000-ppm

LOAEL as the basis for the CEGL and adjusting it with an uncertainty factor of 3 for limited data on the effects of longer-term exposure yields a 90-day CEGL of 8,000 ppm. The subcommittee does not expect that a 8,000-ppm level will cause long-term neurobehavioral changes given the work of Storm and Giannetta (1974), which included exposures at up to 40,000 ppm for durations of up to two weeks and reported no significant adverse effects on several psychometric tests. The subcommittee also considers a 8,000-ppm level to be protective against other end points, such as headache and metabolic and acid-base changes, that have been studied.

The subcommittee considered the potential secondary effects of hyperventilation associated with CO_2 inhalation. The research cited above offers little information directly pertinent to that issue. There is no evidence that dyspnea and intercostal pain are time dependent. Although the NOAEL for 30 days is 28,000 ppm, hyperventilation and associated symptoms occur at lower levels. Under most conditions, CO_2-induced hyperventilation is not harmful, and it is an adaptive response when oxygen is displaced by CO_2 at an abnormally high level. The secondary toxic effects of CO_2-induced hyperventilation that should be considered include (1) discomfort associated with extreme hyperventilation, (2) impairment of ability to exercise or to take on an extreme workload, and (3) increased inhalation of toxicants.

Sinclair et al. (1969) reported that four subjects exposed to CO_2 at 28,000 ppm for 30 days and another four subjects exposed to CO_2 at 39,000 ppm for 11 days tolerated hyperventilation "without apparent difficulty." Radziszewski et al. (1988) and Guillerm and Radziszewski (1979) found no symptoms in six subjects exposed to CO_2 at 20,000 ppm for 30 days, although minute-volumes increased about 40% during the first several days of the study. Sinclair et al. (1971) found that four male subjects could perform 45 min of light, moderate, and heavy steady-state exercise twice daily during a 15-20 day exposure to CO_2 at 28,000 ppm.

The possibility of increased inhalation of other toxicants as a result of CO_2-induced hyperventilation must be addressed. In Pingree (1977), minute-volumes rose 30% by day 4 and returned to baseline by day 8 of a 44-day study in 15 subjects exposed to CO_2 at 10,000 ppm. A 30% increase in ventilation is modest in comparison with increases normally associated with exercise (for example, about 180% increase with light exercise) (Sinclair et al. 1971). Because CO_2-induced changes disappear within a few days, no significant increases in toxicant exposures are expected (NRC 1996).

DATA ADEQUACY AND RESEARCH NEEDS

The neurobehavioral studies on which the 1-h EEGL is based were conducted no more recently than the 1970s except for the small studies of Sun et al. (1996) and Yang et al. (1997). More sensitive tests and tests specifically designed to evaluate the skills required for high technology equipment use and onboard decision making might be available. It is important to validate the Sun et al. (1996) and Yang et al. (1997) findings, because they suggest significantly lower acceptable concentrations than do previous studies. Also, subchronic studies should be repeated to evaluate more sensitive end points and should include complete lung function tests with diffusing capacity as well as neurobehavioral tests.

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4

Carbon Monoxide

This chapter summarizes the relevant epidemiologic and toxicologic studies on carbon monoxide (CO). Selected chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure levels from the National Research Council (NRC) and other agencies are also presented. The subcommittee considered all of that information in its evaluation of the Navy's current and proposed 1-hour (h), 24-h, and 90-day exposure guidance levels for CO. The subcommittee's recommendations for CO exposure levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

CO is a colorless, odorless gas (Budavari et al. 1989). Selected physical and chemical properties are summarized in Table 4-1.

OCCURRENCE

CO primarily is produced by partial oxidation of carbon-containing materials (Pierantozzi 1995). In the outdoor environment, major sources of CO are motor vehicles and fires (EPA 2000). In the indoor environment, sources include tobacco smoking, combustion engines, and combustion appliances, such as furnaces and gas stoves. On submarines, the primary sources of CO are tobacco smoking, diesel generators, and high-temperature paints (Crawl 2003). Data collected on nine nuclear-powered ballistic missile submarines indicate an average CO concentration of 5 parts per

TABLE 4-1 Physical and Chemical Properties of Carbon Monoxide[a]

Synonyms	Carbonic oxide, carbon oxide, flue gas
CAS registry number	630-08-0
Molecular formula	CO
Molecular weight	28.01
Boiling point	−191.5°C
Melting point	−205.0°C
Flash point	—
Explosive limits	12.5% to 74.2% (volume % in air)
Specific gravity	0.968 with respect to air
Vapor pressure	>1 atm at 20°C
Solubility	Sparingly soluble in water; appreciably soluble in ethyl acetate, chloroform, and acetic acid
Conversion factors	1 ppm = 1.15 mg/m^3; 1 mg/m^3 = 0.87 ppm

[a]Data on vapor pressure are from HSDB (2004); data on explosive limits are from IPCS (2001); all other data are from Budavari et al. (1989).
Abbreviations: atm, atmosphere; mg/m^3, milligram per cubic meter; ppm, parts per million; —, not available or not applicable.

million (ppm) and a range of 0-14 ppm, and data collected on 10 nuclear-powered attack submarines indicate an average CO concentration of 3 ppm and a range of 0-14 ppm (Hagar 2003).

SUMMARY OF TOXICITY

The toxicology of CO in humans was reviewed by the World Health Organization (WHO) (1999), the U.S. Environmental Protection Agency (EPA) (2000), and the NRC (2002). Only human and animal data directly relevant to derivation of the EEGL and CEGL values are discussed in this chapter.

CO interferes with the oxygenation of blood and the delivery of oxygen to tissues because it has about 245 times more affinity for hemoglobin than does oxygen (Roughton 1970). The formation of carboxyhemoglobin (COHb) reduces the oxygen-carrying capacity of blood and shifts the oxygen dissociation curve, reducing the release of oxygen to tissues. Hypoxemia and subsequent tissue hypoxia comprise the best understood mechanism of CO toxicity. The cytotoxic effects of CO independent of oxygen are subjects of current research. CO also binds to muscle myoglobin, cytochrome *c* oxidase, and cytochrome P-450, and many of the adverse effects of CO might be associated with those reactions (WHO 1999;

EPA 2000; Raub et al. 2000). Endogenous production of CO accounts for a background COHb level of about 1% (Radford et al. 1981; Doherty 2000). The log-log plot of CO uptake and COHb saturation, as computed from the Coburn-Foster-Kane equation, is shown in Figure 4-1 (Peterson and Stewart 1975).

The brain and cardiovascular system are the primary targets of CO toxicity. The adverse effects of CO exposures range from subtle vascular and neurologic changes to more serious conditions, such as loss of consciousness and death. Even when CO-intoxicated patients receive treatments, more than 10% of survivors might experience permanent brain damage, and in many cases, the onset of adverse effects is delayed as long as 1 week or more. The primary cause of neurologic injury might be hypotension leading to impaired tissue perfusion (Varon et al. 1999). CO intoxication causes hypotension by myocardial depression, peripheral vasodilation, and ventricular dysrhythmia (Varon et al. 1999).

Morbid complications of CO intoxication are greatly affected by a variety of factors related to cardiovascular health, including the degree and duration of hypotension, and the presence of pre-existing cardiac or pulmonary disease, anemia, or cardiac dysfunction (arrhythmias or other conditions) (Ehrich et al. 1944; Stewart et al. 1975). COHb concentrations in smokers average 4% and range from 3% to 8%; heavy smokers could have COHb concentrations as high as 15% (Raub et al. 2000; Omaye 2002). Submariners who smoke theoretically might be subject to additional health risks from environmental exposure given their already elevated COHb levels.

A number of short- and long-term adaptations to compensate for reduced oxygenation of blood and tissues related to CO exposure have been identified. Those changes are found in humans and animals and include increased coronary and brain blood flow in the short-term and increased hematopoiesis over time (WHO 1999). However, cardiovascular disease might reduce or eliminate the body's ability to compensate for CO-related hypoxemia and tissue hypoxia (WHO 1999).

Effects in Humans

Accidental Exposures

In their review of U.S. mortality records from 1979-1988, Cobb and Etzel (1991) identified 56,133 (0.3%) of the total death records (NCHS

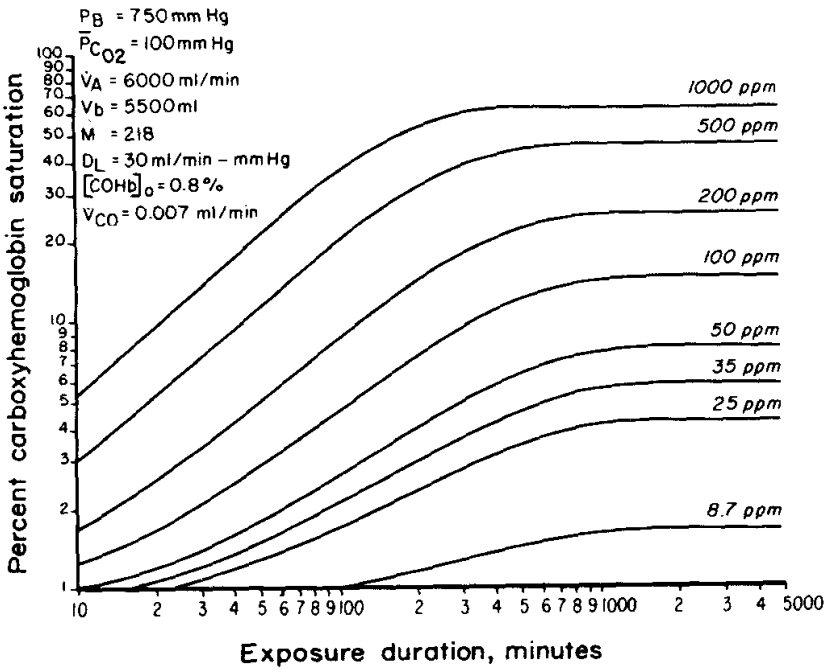

FIGURE 4-1 Carbon monoxide concentrations reached in blood (percent saturation at various durations of exposure) in a normal human subject as a function of inspired CO. Abbreviations: P_B, barometric pressure; PCO_2, average partial pressure of carbon dioxide in lung capillaries; V_A, alveolar ventilation rate; V_b, blood volume; M, equilibrium constant; D_L, diffusing capacity of the lungs; $[COHb]_o$, control value of carboxyhemoglobin prior to carbon monoxide exposure; V_{CO}, rate of endogenous carbon monoxide production. Source: Peterson and Stewart 1975. Reprinted with permission from the *Journal of Applied Physiology*; copyright 1975, the American Physiology Society.

2002) that indicated CO toxicity as a contributing cause of death. Acutely fatal CO poisoning is likely due to hypoxia and its adverse effects on the heart, as suggested by the large number of patients who exhibited marked hypotension and lethal arrhythmias prior to CO-induced death.

Sokal and Kralkowska (1985) provided an analysis of 39 patients (18-78 years of age) intoxicated by CO produced from the combustion of household gas or coal-stove gas. Of the 39 patients exposed to CO, 16 showed mild intoxication and 12 showed moderate intoxication exhibiting symptoms, such as headache, vomiting, tachycardia, and breathing problems, after exposures that lasted about 5 h. COHb concentrations averaged

27%. Eight patients presented with symptoms of severe intoxication, including loss of consciousness and pathologic neurologic signs, tachycardia, and tachypnea after exposures that lasted about 9 h. COHb concentrations averaged 34%. Four subjects exhibited very severe effects, including central nervous system (CNS) damage, and circulatory and respiratory disturbances after exposures that lasted about 10 h. COHb concentrations averaged 31%. The subcommittee notes the lack of agreement between the total number of patients in the study and the number of patients categorized by clinical degree of intoxication.

Ely et al. (1995) reported adverse effects of CO exposures in employees of a sewing company located in a warehouse where a propane-fueled forklift was in operation. Thirty people were exposed to CO concentrations at up to 386 ppm. The five workers who exhibited the most severe symptoms had an average estimated COHb concentration of 35%. One of those workers had seizures. The majority of people exposed reported CNS, behavioral, gastrointestinal, and cardiovascular abnormalities, including headache (93%), dizziness (63%), nausea (60%), chest pain (57%), difficulty breathing (23%), visual changes (20%), and confusion (17%). Eleven of 25 patients contacted 2 years after exposure reported seeking medical care for persistent symptoms.

Hassan et al. (2003) reported two cases of CO poisoning that resulted in sensorineural hearing loss. The subject of the acute poisoning case, a 30-year-old man, presented with a COHb concentration of 29.9%. That subject showed only partial recovery from hearing loss. A 61-year-old woman reported to have endured chronic CO exposure presented with bilateral hearing loss that improved with time. Overall findings indicate that CO affects high-frequency hearing (1-8 kilohertz).

Experimental Studies

The adverse clinical effects of CO have been evaluated extensively in both healthy and high-risk individuals (WHO 1999; EPA 2000); however, only the studies that are most relevant to the safety of submarine crew members (healthy adult males) are discussed here. Table 4-2 summarizes the relevant experimental studies in humans. Chiodi et al. (1941) conducted controlled exposure studies in which four male subjects were exposed to CO at 1,500-3,500 ppm repeatedly for durations of 70 minutes (min) or longer. The subjects had COHb concentrations at up to 52%. There were no adverse effects on basal oxygen consumption, ventilation, pulse rate, blood pressure, or arterial blood pH in that study. The only adverse effect

TABLE 4-2 Human Toxicity Summary

Concentration (ppm)	Exposure Duration	COHb %	Number of Subjects	Effects	Reference
NS	NS	NS	NS	Linear relationship described between decline in VO_2-max and increasing COHb	EPA 1979; Horvath 1981
NS	NS	4.5	NS	Decrements in brightness discrimination in trained subjects	MacFarland et al. 1944
NS	NS	6-7	50	Deficit in "careful driving" skills	Wright et al. 1973
NS	NS	8-12	20	No adverse effects on visual discrimination or depth perception	Ramsey 1973
NS	NS	9	18	No decrement in night vision	Luria and McKay 1979
NS	NS	10 and higher	3	Increased reaction time; decreased precision in maintenance of separation distance between cars; decrease in estimation of time	Ray and Rockwell 1970
700	Time needed to reach target COHb	11 and 17	27	Driving not "seriously" affected; statistically significant increase in roadway viewing time	MacFarland 1973
100	NS	0-20	49	Numbers of errors and completion time increased with increasing COHb concentrations for several but not all tests of cognitive ability beginning at COHb concentrations <5%; no subjective symptoms occurred at COHb concentrations <20%	Schulte 1963
NS	NS	40-45	4	Inability to perform tasks requiring minimal exertion	Chiodi et al. 1941

NS	5-60 min	15-20	NS	Oxygen uptake in tissues unchanged during submaximal exercise	Chevalier et al. 1966; Pirnay et al. 1971; Vogel et al. 1972
NS	15 min	~5-21	5	Maximal physical performance was reduced with increasing concentrations of COHb	Ekblom and Huot 1972
300	45 min	~5	20	Increased reaction time to visual stimuli; light detection sensitivity and depth perception unaffected	Ramsey 1972
50	1 h	2.1	9	"No untoward subjective symptoms or objective signs of illness"	Stewart et al. 1970
100	1 h	~2.5	10	"No untoward subjective symptoms or objective signs of illness"	Stewart et al. 1970
~10,000 "booster dose," 225 maintenance	~1 h	~18-20	8	Reduced maximal oxygen uptake; during submaximal exercise, oxygen delivery to tissues is maintained by increased cardiac output but smaller arteriovenous oxygen concentration difference	Vogel and Gleser 1972
50	1.5-2.5 h	~2	3, 5, or 9, depending on the test	Observed impaired vigilance; no effects on response latency, short-term memory, and ability to subtract numbers mentally	Beard and Grandstaff 1975
250	1.5-2.5 h	~7	3, 5, or 9, depending on the test	No effects on vigilance, response latency, short-term memory, and ability to subtract numbers mentally	Beard and Grandstaff 1975

(*Continued*)

TABLE 4-2 Continued

Concentration (ppm)	Exposure Duration	COHb %	Number of Subjects	Effects	Reference
27-100 to maintain target COHb	2 h	5, 10, 15, and 20	16	Cardiovascular system compensated for reduced oxygen carrying capacity of blood by augmenting heart rate, cardiac contractility, and cardiac output for submaximal upper and lower body exercise; compensatory mechanisms began to fail at moderate exercise and CO exposure	Kizakevich et al. 2000
11,569 initially, 142 maintenance	2.25 h	~17	21	Visual function not affected	Hudnell and Benignus 1989
500	2 h-2 h and 20 min	~26 (after 2 h and 20 min)	6	"Increase in heart rate with minimal exertion;" frontal headaches after 1 hr of exposure; minimal exertion intensified headache pain; headache pain peaked 3.5 h post-exposure; changes in visual evoked response at COHb >20%, returned to normal at COHb <15%	Stewart et al. 1970
100	2.5 h	7	NS	Decrements in two learning tasks; no changes in several other measures of intellectual performance	Bender et al. 1971
100	2.5 h	5.7	16	Increased response times noted in the secondary task of a dual-task procedure in which the primary task was tapping a board	Mihevic et al. 1983

				with a stylus, and the secondary task was announcing or subtracting numbers appearing on a display	
0-1,000; gradually rising concentration reached 1,000 after 2 h and was maintained for 30 min	2 h 30 min	~32 (peak at 2.5 h)	2	Headaches noted during exposure became incapacitating 6 h post-exposure and were not ameliorated with a night's sleep; clinical chemistries and electrocardiograms remained normal; changes in visual evoked response at COHb >20%, returned to normal at COHb <15%; performance impairment noted for manual coordination and hand reaction time tests	Stewart et al. 1970
2, 50, 100, 200, 500	2.5 h	Up to 20	27 (in groups of 2-8)	No impairment in ability to perform time estimation tests	Stewart et al. 1973
2, 50, 100, 200, 500	2.5 h	Up to 20	27 (3 sessions with 1 subject, 47 sessions with 2-8)	Time estimation ability, manual coordination, inspection, and arithmetic performance not impaired	Stewart et al. 1975
0, 50, 125, 200, 250	3 h	1, 3, 6.6, 10.4, 12.4	10	No symptoms and no effects on time perception and tracking performance; subjects exposed at 200 and 250 ppm were not blinded regarding exposure	Mikulka et al. 1970; O'Donnell et al. 1971

(*Continued*)

TABLE 4-2 Continued

Concentration (ppm)	Exposure Duration	COHb %	Number of Subjects	Effects	Reference
5, 35, 70	4 h	1, 3, 5	30	Dual-task conditions included hand-controlled tracking with low- and high-frequency conditions and monitoring lights and responding with a button press to indicate brighter lights; differences in tracking performance noted in the 70-ppm group after 3 h of exposure and in the 35- and 70-ppm exposure groups after 4 h in the high-frequency condition; reaction times on the light detection task increased in the 35- and 70-ppm exposure groups in the final hour of exposure	Putz 1979
70	4 h	5	12	Dual-task conditions as in Putz (1979) and auditory vigilance; statistically significant differences in tracking, response time on light monitoring, and auditory vigilance after 1.5-2 h of exposure	Putz et al. 1979
200	4 h	~16 (after 4 h)	11	Three subjects reported "mild sinus" headaches in the 4th h; headaches vanished 30 min to 2 h post-exposure; no impairment of coordination, reaction time, and visual acuity	Stewart et al. 1970

0, 50, 100, 175, 250	4 h	NS	18	Ability to estimate the length of an auditory signal, compared with a standard signal, reduced at all CO-exposure concentrations; time to onset of performance deficit decreased with increasing CO exposure	Beard and Wertheim 1967
<2, 50, 100, 200, 500	5 h	Up to 20	27 (in groups of 2-8)	No impairment in ability to perform time estimation tests	Stewart et al. 1973
<2, 50, 100, 200, 500	5 h	Up to 20	27 (3 sessions with 1 subject, 47 sessions with 2-8)	Time estimation ability, manual coordination, inspection, and arithmetic performance not impaired	Stewart et al. 1975
100	8 h	11-13	2	"No impairment"	Stewart et al. 1970
50	24 h	~8	3	"No untoward subjective symptoms or objective signs of illness"	Stewart et al. 1970
50	5 d	7	15	No effects on visual functions	Davies et al. 1981
0, 15, 50	24 h/d, 8 d	0.5, 2.4, 7.1	30	Electrocardiographic P-wave changes in 3 of 15 subjects in 15-ppm group and in 6 of 15 subjects in 50-ppm group	Davies and Smith 1980

Abbreviations: COHb, carboxyhemoglobin; d, day; h, hour; min, minute; NS, not stated; ppm, parts per million; VO_2-max, maximal oxygen consumption.

observed was increased cardiac output (20-50% over baseline) at COHb concentrations greater than 40%.

COHb concentrations at 40% caused drastic reductions in the ability of subjects to perform tasks requiring even minimal strength (Chiodi et al. 1941); however, COHb concentrations at 15-20% did not appear to elicit that effect (Chevalier et al. 1966; Pirnay et al. 1971; Ekblom and Huot 1972; Vogel and Gleser 1972; Vogel et al. 1972; Kizakevich et al. 2000). Some controlled experimental studies have found a linear relationship between COHb concentrations at 5-20% and decrements in human exercise performance, measured as maximal oxygen uptake (EPA 1979, 1984, 1991; Horvath 1981; Shephard 1983, 1984). However, the decrements were not considered clinically significant.

From a pool of 18 healthy men (24-42 years of age), Stewart et al. (1970) exposed groups of 2-11 to CO concentrations ranging from 25 to 1,000 ppm for periods of 30 min to 24 h. The exposures took place in sedentary exposure chambers. The study evaluations included measurements of hand and foot reaction time in a driving simulator, Crawford collar and pin tests, Crawford screw tests, a hand steadiness test, the Flanagan coordination test, a complete audiogram, a resting 12-lead electrocardiogram, and measurements of visual evoked response. CO was well tolerated at concentrations up to 100 ppm (COHb at 12.5%) for up to 8 h, eliciting no subjective signs or visual or performance impairments. During a 4-h exposure at 200 ppm, 3 of 11 subjects developed mild sinus-like symptoms during the last hour of exposure when COHb concentrations were their highest (about 16%). Mild headaches occurred at the end of the first hour of a 2-h exposure at 500 ppm (COHb at 25.5%) and were followed by excruciatingly severe occipitofrontal headaches at 3.5 h post-exposure.

Alterations in visual evoked response and other neurobehavioral end points have been inconsistently reported by investigators. For example, some studies reported no adverse effects on vision, visual evoked response, visual discrimination, depth perception, tracking, or manual coordination in subjects who had COHb concentrations ranging from 3% to 20% (MacFarland et al. 1944; Stewart et al. 1970, 1975; Ramsey 1972, 1973; Putz 1979; Putz et al. 1979; Luria and McKay 1979; Davies et al. 1981; Hudnell and Benignus 1989).

Putz (1979), Putz et al. (1979), and Mihevic et al. (1983) used dual-task procedures to evaluate neurobehavioral performance at COHb concentrations up to 5.7% for exposure durations of 2.5-4 h. The primary manual tasks tested were tracking with a hand control (Putz 1979; Putz et al. 1979), and tapping targets on a board with a stylus (Mihevic et al. 1983). Second-

ary tasks were detecting light brightness and responding with a button press (Putz 1979; Putz et al. 1979) and "digit manipulation" that entailed calling out a number on a display or calling out the result of subtracting the number from 100 (Mihevic et al. 1983). In all three studies, the reaction times for the secondary tasks increased after exposure. For example, in Putz (1979) and Putz et al. (1979), reaction times for the highest-exposure condition increased about 70-80 milliseconds. The potential adverse effects described by Putz (1979), Putz et al. (1979), and Mihevic et al. (1983) were considered clinically insignificant.

Beard and Wertheim (1967) reported decrements in time-estimation ability in a study of 18 subjects exposed to CO at 0, 50, 100, 175, and 250 ppm for 4 h. The authors saw a dose-dependent decrease in correct responses. Decrements occurred within 25 min of exposure at 250 ppm, within 30 min of exposure at 175 ppm, within 50 min of exposure at 100 ppm, and within 90 min of exposure at 50 ppm (Beard and Wertheim 1967). COHb concentrations were not reported; however, on the basis of Figure 4-1, 4-h exposures at 50, 100, 175, and 250 ppm would result in COHb concentrations of about 3%, 5%, 8%, and 10%, respectively. In contrast, Mikulka et al. (1970) and O'Donnell et al. (1971) exposed 10 subjects to CO at 0, 50, 125, 200, and 250 ppm for 3 h and found no consistent differences in tracking, time estimation, or the Pensacola Ataxia Battery at any exposure concentration. COHb concentrations averaged 1%, 3%, 6.6%, 10.4%, and 12.4%, respectively. The authors noted that the subjects in the 200- and 250-ppm trials were not blinded regarding exposure (Mikulka et al 1970; O'Donnell et al. 1971). Stewart et al. (1973, 1975) could not replicate the Beard and Wertheim (1967) findings using three different time-estimation tasks, including the one employed by Beard and Wertheim (1967).

Beard and Grandstaff (1975) exposed groups of three, five, or nine subjects to CO concentrations at 0, 50, 175, and 200 ppm for 2 h. COHb concentrations corresponding to the exposures were <2% in the control groups, about 2% at 50 ppm, 5-6% at 175 ppm, and about 7% at 200 ppm. The authors found performance decrements related to CO exposure in the vigilance and perceptual tracking tests, and in a time-estimation task, but not in problem-solving, digit span, and spatial perception tasks. The results of the problem-solving task were confounded by learning. The spatial perception and digit span tasks were not consistently affected by CO. The authors concluded that the interactive nature of the digit span task and the difficulty of the spatial perception task contributed to increased arousal, which mitigated the effects of CO exposure (Beard and Grandstaff 1975).

Putz et al. (1979) observed performance decrements in auditory vigilance after 1.5-2 h exposure to CO at 70 ppm (COHb at 5%). Christensen et al. (1977) exposed 10 subjects for 2 h under four conditions: no exposure, exposure to low oxygen (17%), exposure to CO at 114 ppm, and exposure to CO at 113 ppm in a low-oxygen environment. The authors noted a vigilance performance deficit under low-oxygen conditions (0.5% COHb), but they noted no differences from controls during the CO or CO in low-oxygen exposures. COHb concentrations during those exposures were 2.5% and 2.6% at 50 min and 4.8% and 5.1% at 120 min, respectively.

CO exposures associated with COHb concentrations as low as 6% and as high as 17% have been found to affect performance on driving measures, such as time required to respond to a velocity change in a lead car, glare recovery, hand steadiness, and roadway viewing time (Ray and Rockwell 1970; MacFarland 1973; Wright et al. 1973). However, no serious decrements in driving ability occurred at COHb concentrations $\leq$17% (MacFarland 1973).

CO exposures associated with COHb concentrations at 7% affected subjects' ability to learn 10 nonsense syllables and decreased subjects' ability to recite a series of digits in reverse order; however, subjects showed no changes in ability to perform other tasks involving calculations, analogies, shape selection, dot counting, and letter recognition (Bender et al. 1971).

Benignus (1994) conducted a meta-analysis of the neurobehavioral effects of CO exposures that included data from a number of the studies described above (Ramsey 1973; Stewart et al. 1970, 1973; Wright et al. 1973; Christensen et al. 1977; Putz et al. 1979). Data on how CO exposure affected vigilance, reaction time, hand steadiness, visual threshold, time discrimination, and reasoning were included. The resulting dose-response curves indicated that COHb concentrations of 18-25% are required to produce 10% deficits in neurobehavioral functions in healthy, sedentary adults (Benignus 1994).

Davies and Smith (1980) conducted an 18-day experiment in an enclosed environment where 8 days of exposure were preceded by a 5-day control period and followed by a 5-day recovery period. Fifteen naval serviceman were exposed to CO at 15 ppm, another 15 were exposed to CO at 50 ppm, and 14 servicemen served as controls. The mean COHb concentrations in the 15- and 50-ppm exposure groups were 2.4% and 7.1%, respectively. Electrocardiographic P-wave changes greater than 0.1 millivolts (mV) were observed in 3 of the 15 subjects exposed to CO at 15 ppm and in 6 of the 15 subjects exposed to CO at 50 ppm after 2 days of expo-

sure. The authors concluded that those changes were the result of a specific toxic effect on conducting tissue (Davies and Smith 1980).

Occupational and Epidemiologic Studies

Seufert and Kiser (1996) investigated the effects of passive smoking on the end-expiratory CO concentrations of the nonsmokers among 126 crewmen aboard a nuclear-powered submarine during a 62-h submergence. Of the men, 40 were smokers, and 86 were nonsmokers. The CO concentration on board increased from 2.6 to 9.2 ppm during the 62-h study period. The average end-expiratory CO concentrations among nonsmokers was 9 ppm at the start of the study and 21 ppm after 62 h of submergence. End-expiratory CO concentrations among smokers averaged 26 ppm at the start of the study and increased an average of 8.4 ppm during submergence. Post-exposure end-expiratory CO concentrations in nonsmokers were similar to the presubmergence levels measured in smokers (Seufert and Kiser 1996).

During 52 days of a submarine tour, Wilson and Schaefer (1979) found increased hematocrit, hemoglobin, and red blood cell counts in smoking and nonsmoking submariners and increased reticulocytes in the smokers. CO concentrations ranged from 15 to 20 ppm, and the average carbon dioxide (CO_2) concentration was 9,000 ppm. Those findings could result from the low-oxygen environment as well as the CO concentrations.

No adverse health effects were observed in Holland Tunnel workers exposed to CO at an average concentration of 70 ppm (COHb at 5-10%) for 2-h periods during their 8-h workshifts for about 13 years (Sievers et al. 1942). In a retrospective study of bridge and tunnel officers exposed to CO who had average COHb levels at <5%, a significant increase in mortality from arteriosclerotic heart disease was reported in the tunnel workers (Stern et al. 1988). The authors suggested that their findings might be the result of long-term continuous CO exposures or acute peak exposures or both. However, their data on duration of employment were not related to heart disease mortality and did not support the long-term exposure hypothesis (Stern et al. 1988). Smith and Steichen (1993) reviewed the human and animal literature and concluded that CO is not atherogenic.

Many epidemiologic studies of the general population have shown positive correlations between short-term exposures to ambient air pollutants and increased mortality and exacerbation of pre-existing illness, as assessed by daily counts of deaths or hospital admissions. Studies of particulate matter and mortality are perhaps the most well known and convincing

example of this literature. The evidence for ambient CO exposure and health effects is less well established as illustrated below. Air pollution studies are often hampered by biologic, epidemiologic, and statistical uncertainties and data gaps. Selected studies with CO findings, reviewed below, highlight some of the key issues including the confounding effects of other air pollutants and weather, and the lack of information on long-term health effects.

Studies in North America and Europe suggest that there are associations between short- and long-term exposures to CO, hospital admissions in general, and admissions for cardiovascular and respiratory diseases. Morris et al. (1995) reported that for the period 1986-1989, the increases in relative risk (RR) of hospital admissions associated with 10-ppm increases in ambient CO concentrations ranged from 10% in New York City (RR = 1.1) to 37% in Los Angeles (RR = 1.37); other cities like Chicago, Houston, Milwaukee, and Philadelphia showed increases within that range. In a single pollutant model, CO had the greatest effect on RR. Burnett et al. (1997) evaluated the adverse effects of daily measures of ambient air pollution during summertime in Toronto, Canada, on the basis of unscheduled hospital admissions on the same day for cardiac and respiratory diseases. The mean daily 1-h maximum CO concentration was 1.8 ppm. There were no significant correlations between CO exposure concentrations and cardiovascular or respiratory admissions. Poloniecki et al. (1997) reported that ambient CO concentrations in London were positively associated with hospital admissions on the following day for cardiovascular diseases and myocardial infarctions over all seasons when CO was modeled alone. In a multiple-pollutant model with black smoke and ozone, CO was associated with myocardial infarction admissions during the cool months (Poloniecki et al. 1997). Although Burnett et al. (1997) did not find any correlation between CO concentrations and hospital admissions at a mean daily 1-h maximum CO concentration of 1.8 ppm, Poloniecki et al. (1997) found associations at lower CO concentrations. Poloniecki et al. (1997) reported that their 90th-percentile CO concentration was 1.8 ppm. On the basis of the studies by Burnett et al. (1997) and Poloniecki et al. (1997), it appears that ambient CO concentrations have stronger effects on cardiovascular risk during cool months than they do during summertime.

The average daily 1-h maximum CO concentrations in these studies have been as low as 0.2 ppm; however, the CO metrics employed in the studies are difficult to justify because of the variability in endogenous CO production. In addition, it is difficult to mechanistically and pathophysiologically explain associations between low-level CO exposures and the exacerbation of heart disease.

The air pollution studies were not considered in deriving the EEGLs and CEGL for CO. Outcomes in the general population are not relevant to the healthy submariner population, and the studies lack precise exposure measures.

Effects in Animals

A large number of acute and repeated-dose animal toxicity studies have been conducted and reported. Given the large amount of available human data on the effects of CO exposures, only relevant animal studies are discussed here. Table 4-3 summarizes the studies discussed below.

Acute Toxicity

In unrestrained male Crl:CD rats, the LC_{50} values (concentrations lethal to 50% of subjects) for the 5-, 15-, 30-, and 60-min exposure periods were 10,151 ppm (95% confidence interval [CI] = 9,580-10,953 ppm), 5,664 ppm (95% CI = 5,218-6,078 ppm), 4,710 ppm (95% CI = 4,278-5,254 ppm), and 3,954 ppm (95% CI = 3,736-4,233 ppm), respectively (E.I. du Pont de Nemours and Co. 1981). COHb concentrations at 60% or higher are lethal in unrestrained rats. Acute effects are more severe in restrained rats (NAC 2004). Thirty-minute LC_{50} values for Swiss-Webster and ICR mice were 3,570 and 8,000 ppm, respectively (Hilado et al. 1978). In guinea pigs, the LC_{50} for acute 4-h exposure was 5,718 ppm (95% CI = 4,809-6,799 ppm) (Rose et al. 1970).

In monkeys exposed to CO at 1,000 ppm for several hours, severe intoxication was observed at 25 min of exposure and was followed by observed deficits in behavioral task performance, physical activity, and coordinated movements (Purser and Berrill 1983). The threshold for ventricular fibrillation induced by an electrical shock was reduced in monkeys by exposures to CO at 100 ppm for 6 h (COHb at 9.3%) (DeBias et al. 1976). In dogs, exposures to CO at 100 ppm for 2 h (COHb at 6.3-6.5%) increased the susceptibility to induced ventricular fibrillations (Aronow et al. 1979). COHb concentrations at 13-15% increased the severity and extent of ischemic injury and the magnitude of ST-segment elevation in myocardially infarcted dogs.

TABLE 4-3 Animal Toxicity Summary

Concentration (ppm)	Exposure Duration	COHb %	Species	Effects	Reference
10,151 5,664 4,710 3,954	5 min 15 min 30 min 60 min	60 or higher in rats that died	Crl:CD rats	LC_{50}s for unrestrained rats	E.I. du Pont de Nemours and Co. 1981
3,570	30 min	NS	Swiss-Webster	LC_{50}s for Swiss-Webster and ICR mice	Hilado et al. 1978
8,000	30 min	NS	ICR		
1,000	30 min	NS	Male cynomolgus monkeys	Monkeys became less active after 20 min of exposure and appeared severely intoxicated lying or rolling on the floor after 25 min; behavioral task performance was unaffected for the initial 15 min of exposure, slowing at the first signs of intoxication	Purser and Berrill 1983
100	2 h	~6	Dogs	Decreased ventricular fibrillation threshold	Aronow et al. 1979
5,718	4 h	NS	Male Hartley strain guinea pigs	LC_{50}	Rose et al. 1970
100	6 h	~9.3	40 monkeys, 9 with myocardial infarction	Voltage necessary to induce fibrillation was highest for normal, control monkeys and lowest for infarcted monkeys breathing CO; CO exposure alone lowered fibrillation thresholds as did myocardial infarction; effects of CO and infarction together were additive	DeBias et al. 1976

5,000, 10,000	3 min, 6-12 times per day for 3-4 wk	NS	Guinea pigs	After immunization with sheep red blood cells, three of four exposure groups had increased numbers of pulmonary alveolar macrophages, and all had increased polymorphonuclear leukocytes; reduced numbers of plaque-forming cells in spleen and lungs	Snella and Rylander 1979
50	6 wk	NS	15 dogs	Pathologic electrocardiograms in 10 dogs, pathology of heart in 7 dogs, and pathology of brain in 6 dogs	Lindenberg et al. 1962
100	6 wk	NS	Dogs	Pathologic changes in heart and brain	Lindenberg et al. 1962
50, 100	6 wk	NS	Dogs	Abnormal electrocardiograms appeared in second week and persisted through study period	Preziosi et al. 1970
100	5.75 h/d, 6 d/wk for 11 wk	21	Dogs	Electrocardiograph changes; degenerative changes in individual muscle fibers	Ehrich et al. 1944
100	5.75 h/d, 6 d/ wk for 11 wk	~ 20	Dogs	Gait and posture anomalies were observed, and cerebral cortical damage found at autopsy	Lewey and Drabkin 1944
100	5.75 h/d, 6 d/wk for 11 wk	~20	1 dog with ligated posterior coronary artery	Severe cerebral damage and myocardial alternations	Lewey and Drabkin 1944

(*Continued*)

TABLE 4-3 Continued

Concentration (ppm)	Exposure Duration	COHb %	Species	Effects	Reference
50	24 h/d for 3 mon	NS	Dogs	No electrocardiographic or heart rate changes observed	Musselman et al. 1959
100	23 h/d for 14 wk	~14	Dogs, normal and with myocardial infarction	Animals remained in good health, no obvious untoward signs could be attributed to exposure	DeBias et al. 1972
100	23 h/d for 24 wk	~12	Monkeys, normal and with myocardial infarction	Electrocardiograms of infarcted and noninfarcted animals exposed to CO displayed increased P-wave amplitudes; a greater degree of myocardial ischemia, signified by higher incidence of T-wave inversion, observed in infarcted animals exposed to CO	DeBias et al. 1973
Up to 462	12 h/d for 14 mon	~20	Female monkeys, standard or cholesterol added diet	No myocardial infarctions observed; no differences in plasma cholesterol or aortic or coronary atherosclerosis could be attributed to exposure	Malinow et al. 1976

Abbreviations: COHb, carboxyhemoglobin; d, day; h, hour; LC_{50}, concentration lethal to 50% of subjects; min, minute; month, mon; NS, not stated; ppm, parts per million.

Repeated Exposures and Subchronic Toxicity

Several investigators examined the cardiovascular toxicity of subchronic and chronic CO exposures in nonrodent models. Pathologic changes in the heart and brain have been noted in animals subchronically exposed to CO concentrations capable of generating COHb concentrations in excess of 20%. Lindenberg et al. (1962) evaluated the effects of CO in eight dogs exposed to CO at 100 ppm. Four dogs were exposed continuously for 24 h per day, 7 days per week for 6 weeks, and another four dogs were exposed intermittently for 6 weeks. All dogs had abnormal electrocardiograms, and some of their hearts showed histologic evidence of muscle degeneration. In that study, Lindenberg et al. (1962) also exposed dogs to CO at a concentration of 50 ppm for 24 h per day, 7 days per week for 6 weeks. The exposures produced COHb concentrations of 2.6-5.5%. CO exposures caused no changes in hemoglobin levels or hematocrit; however, electrocardiographic changes similar to, but less severe than, those observed in the 100-ppm exposure group were noted in the third week of exposure.

Preziosi et al. (1970) reported that dogs exposed both intermittently (6 h per day, 5 days per week) and continuously at 50 and 100 ppm for 6 weeks had abnormal electrocardiograms in the second week of exposure and continuing through the exposure period. Heart and brain pathology were observed in some dogs in all exposure groups. Heart pathology included right and left heart dilation and myocardial thinning, which was accompanied by older scarring in some cases and fatty degeneration of heart muscle in others. Brain findings included mobilization of glial cells and thinning of the white matter in the central semi ovale. Four dogs exposed to CO at 100 ppm for 5.75 h per day, 6 days per week for 11 weeks showed electrocardiographic changes, degenerative changes in heart muscle fibers, and histopathologic damage to the brain (Lewey and Drabkin 1944). Ehrich et al. (1944) exposed four dogs to CO at 100 ppm on the same schedule for 11 weeks. Electrocardiographic changes occurred at variable times during the exposure. Although the gross pathologies of the hearts were normal, marked degenerative changes in individual fibers were observed. Dogs were exposed by Musselman et al. (1959) to CO at 50 ppm for 24 h per day, 7 days per week for 3 months. No changes in electrocardiograms or heart rates were observed.

DeBias et al. (1972) did not observe electrocardiographic or hematologic changes in normal and cardiac-infarcted dogs exposed to CO at 100 ppm (COHb at 14%) for 23 h per day for 14 weeks. DeBias et al. (1973) observed increased P-wave amplitudes in the myocardia of both normal and cardiac-infarcted cynomologus monkeys during exposures to CO at 100

ppm for 23 h per day for 24 weeks. T-wave inversions were more common in infarcted animals. Characteristic increases in hematocrit, hemoglobin, and red blood cell counts were observed in both normal and infarcted animals. Four monkeys with P-wave changes were selected for histopathology. Nuclear hyperplasia of the atria was observed in all four, suggesting atrial hypertrophy. No pathologic changes in brain, spleen, muscle, lungs, kidneys, or adrenal glands were found.

Chronic Toxicity

Malinow et al. (1976) found no myocardial infarctions or electrocardiographic abnormalities in normal or cholesterol-fed cynomologus monkeys exposed to pulses of CO at up to 462 ppm for 30 min per hour, 12 h per day for 14 months.

Reproductive Toxicity in Males

No reports on the potential reproductive toxicity of CO in males were available.

Immunotoxicity

Snella and Rylander (1979) exposed one group of guinea pigs to CO at 5,000 ppm and three groups of guinea pigs to CO at 10,000 ppm for 3 min, 6 or 12 times per day for 3-4 weeks. The animals were injected with sheep red blood cells one week prior to cessation of exposure and sacrifice. All exposed groups showed increased polymorphonuclear leukocytes in pulmonary lavage fluid, and three of the four groups exhibited increased numbers of pulmonary alveolar macrophages. The numbers of plaque-forming cells among spleen and lung cells were reduced compared with controls, but the changes were not statistically significant. This study was included for completeness, but it is not relevant to setting the EEGL and CEGL values because of the high CO concentrations used and the unusual exposure regimen.

Genotoxicity

There are no reports on the genotoxic potential of CO.

Carcinogenicity

There are no reports on the carcinogenic potential of CO.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

The information in this section was obtained from reviews by WHO (1999), EPA (2000), and Raub et al. (2000). CO absorption occurs through the lungs at the respiratory bronchioles and alveolar ducts and sacs. The rate of uptake of CO is largely a function of the rate of COHb formation, and that relationship is linear at lower CO concentrations. CO is transferred from a gas phase to a liquid phase, across the air-blood barrier. CO diffuses across the alveolar-capillary membrane, through plasma, across the red blood cell membranes, and finally into the red blood cell stroma to bind to hemoglobin. Because of the rapid binding of CO with hemoglobin in the red blood cells, there is a high pressure differential between red blood cells and air, favoring rapid diffusion of CO into blood. CO uptake is substantially faster than CO elimination because of the low blood-to-air CO gradient and the tight binding of CO to hemoglobin. CO diffusion capacity increases with physical exercise, and there are significant diurnal variations that result from factors, such as variations in hemoglobin concentrations, blood flow, oxygen consumption, and ventilatory pattern (Forster 1987; Frey et al. 1987).

CO has about 245 times more affinity for hemoglobin than does oxygen. In humans, the vast majority of CO is in the vascular compartment, and about 10-15% of CO is in extravascular tissues. There are considerable amounts of CO bound to myoglobin in cardiac and skeletal muscles. During exercise, CO will diffuse from blood to skeletal muscle because the relative rate of CO binding increases more for myoglobin than for hemoglobin. Brain concentrations of CO are about 30-40 times lower than blood concentrations.

The factors that govern CO uptake also control CO elimination. The elimination half-lives of CO in blood show considerable interhuman and

concentration-related variabilities. At 2-20% COHb, the elimination half-life of CO is 3-5 h and is slightly higher at higher COHb concentrations. During sleep, the elimination half-life of CO is increased to about 8 h, and smokers eliminate CO much more slowly than do nonsmokers. Health status has a significant influence on CO elimination. Endogenous production of CO also affects the release of CO.

Formation of COHb reduces the oxygen-carrying capacity of blood and shifts the oxygen dissociation curve, reducing the release of oxygen to tissues. CO hypoxia and intracellular hypoxia caused by smoking and cardiovascular diseases are additive. Pathologic conditions, such as anemia, polycythemia, and coronary artery disease, are known to enhance the adverse hypoxic effects of CO significantly. CO does not accumulate in the body with chronic exposure; however, the anoxia associated with chronic exposure can cause central nervous system damage (Omaye 2002). CO also binds to myoglobin, cytochrome P-450, and other hemoproteins. CO binding to hemoproteins is favored at low intracellular pressure of oxygen, particularly in brain and myocardial tissues. Other potential biochemical mechanisms of action of CO include inhibition of hemoprotein function, free radical production (increase in nitric oxide production), and stimulation of guanylate cyclase. At the physiologic level, the mechanisms involve alterations in blood flow, mitochondrial dysfunction, altered production of high-energy intermediates, and vascular damage (arterial damage, leakage of albumin, and leukocyte sequestration) (WHO 1999; EPA 2000; Raub et al. 2000).

INHALATION EXPOSURE LEVELS FROM THE NRC AND OTHER ORGANIZATIONS

A number of organizations have established or proposed inhalation exposure limits or guidelines for CO. Selected values are summarized in Table 4-4.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendations for EEGL and CEGL values for CO are summarized in Table 4-5. The current and proposed U.S. Navy values are provided for comparison.

TABLE 4-4 Selected Inhalation Exposure Levels for Carbon Monoxide from NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	25	ACGIH 2002
NIOSH	REL-Ceiling	200	NIOSH 2004
	REL-TWA	35	
OSHA	PEL-TWA	50	29 CFR 1910.1000
Spacecraft			
NASA	SMAC		NRC 1994
	1 h	55	
	24 h	20	
	30 days	10	
	180 days	10	
Submarine			
NRC	EEGL		NRC 1985
	1 h	400	
	24 h	50	
	CEGL		NRC 1985
	90 days	20	
	SEAL 1 (10 days)	125	NRC 2002
	SEAL 2 (24 h)	150	NRC 2002
General Public			
NAC	Proposed AEGL-1 (1 h)	NR	66 Fed. Reg. 21940 (2001)
	Proposed AEGL-2 (1 h)	83	
	Proposed AEGL-1 (8 h)	NR	
	Proposed AEGL-2 (8 h)	27	

[a]The comparability of EEGLs and CEGLs with occupational and public health standards or guidance levels is discussed in Chapter 1, section "Comparison to Other Regulatory Standards or Guidance Levels."

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL, acute exposure guideline level; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; h, hour; NAC, National Advisory Committee; NASA, National Aeronautics and Space Administration; NIOSH, National Institute for Occupational Safety and Health; NR, not recommended; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissable exposure limit; REL, recommended exposure limit; SEAL, submarine escape action level; SMAC, spacecraft maximum allowable concentration; TLV, Threshold Limit Value; TWA, time-weighted average.

TABLE 4-5 Emergency and Continuous Exposure Guidance Levels for Carbon Monoxide

Exposure Level	U.S. Navy Values (ppm)		NRC Recommended Values (ppm)
	Current	Proposed	
EEGL			
1 h	400	55	180
24 h	50	20	45
CEGL			
90 days	20	10	9

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; h, hour; NRC, National Research Council; ppm, parts per million.

1-Hour EEGL

In deriving the 1-h EEGL, the subcommittee focused on neurobehavioral impairments to hand coordination, driving and tracking tasks, and cognitive functions and physical performance deficits resulting from acute CO exposures. Scientific reviews and a statistical meta-analysis of the neurobehavioral effects of CO exposure concluded that even moderate impairments (>10% decrements in performance) are not expected at COHb concentrations below 20% (Benignus 1994; WHO 1999; EPA 2000). Also, Kizakevich et al. (2000) showed that healthy young men can perform submaximal upper and lower body exercise without overt cardiovascular impairment after 1-2 h of CO exposure and at COHb concentrations of up to 20%. Therefore, the subcommittee set the 1-h EEGL with the goal of keeping COHb concentrations below the 20% COHb threshold.

Recognizing possible differences between COHb concentrations in smokers and nonsmokers, the subcommittee started with a CO concentration of 200 ppm, which would result in COHb concentrations of about 5% on the basis of Figure 4-1. Adjusting for the low-oxygen atmosphere (see Box 4-1), the subcommittee calculated a 1-h EEGL of 180 ppm. That guidance level should be protective against severe headaches according to the research of Stewart et al. (1970) and be tolerated by both smokers and nonsmokers without critical neurobehavioral performance impairments. However, the subcommittee notes that heavy smokers with a baseline COHb of 15% could attain a COHb of 20% in 1 h (Raub et al. 2000). No additional uncertainty factors were applied, because the value is based on a large body of human research, and the subcommittee considers a 1-h

exposure at 180 ppm to be a no-observed-adverse-effect level (NOAEL).

The subcommittee acknowledges that the experimental human literature on the acute effects of CO exposures includes a number of studies that report subtle deficits in visual detection thresholds and impaired performance on vigilance, time-estimation, and driving performance tasks at low COHb concentrations (MacFarland et al. 1944; Beard and Wertheim 1967; MacFarland 1973; Wright et al. 1973; Beard and Grandstaff 1975; Putz et al. 1979). However, Beard and Grandstaff (1975) and other authors also reported that higher cognitive functions typically were unaffected (Schulte 1963; Bender et al. 1971). Attempts to replicate the findings of MacFarland et al. (1944) and Beard and Wertheim (1967) were unsuccessful (Mikulka et al. 1970; Stewart et al. 1970, 1973, 1975; O'Donnell et al. 1971). Furthermore, the magnitudes of the changes reported in those neurobehavioral studies were considered to be mild to moderate. For example, the subcommittee concluded that decrements in crew members' ability to detect subtle changes in the brightness of lights would not impair the crew's performance of essential tasks. In addition, the test conditions used to evaluate vigilance did not reflect emergency conditions aboard submarines. Other performance-related findings, such as the deficits in driving skills demonstrated by MacFarland (1973) and Wright et al. (1973), were not of sufficient magnitude to be of concern in deriving the 1-h EEGL.

24-Hour EEGL

The effects of concern for setting the 24-h EEGL were cardiovascular effects and impaired neurobehavioral performance. The subcommittee identified a NOAEL of 50 ppm on the basis of a lack of neurobehavioral findings in the 24-h exposure study by Stewart et al. (1970) and a minimal lowest-observed-adverse-effect level (LOAEL) of 50 ppm on the basis of the electrocardiographic (P-wave) changes observed after 2 days of exposure in the Davies and Smith (1980) study. By applying an adjustment factor for the low-oxygen environment, the subcommittee calculated a 24-h EEGL of 45 ppm. The subcommittee does not expect that a 24-h exposure at 45 ppm would result in lasting cardiovascular effects, and therefore, no LOAEL-to-NOAEL uncertainty factor was applied. No uncertainty factors were applied because the key studies employed healthy male subjects.

90-Day CEGL

The cardiovascular effects of CO exposures are of primary concern in setting the 90-day CEGL. There are no experimental human studies of appropriate duration. The one long-term observational study (Wilson and Schaefer 1979) did not evaluate cardiovascular effects, and environmental conditions on board submarines have changed since that study was conducted. Therefore, the subcommittee selected the DeBias et al. (1973) study of normal and cardiac-infarcted cynomolgus monkeys exposed to CO at 100 ppm for 23 h per day for 24 weeks as the starting point for the derivation of the CEGL. The infarcted monkeys exposed to CO exhibited a higher incidence of electrocardiographic T-wave inversions, and both the normal and infarcted monkeys exposed to CO at 100 ppm developed increased P-wave amplitudes, making the 100-ppm concentration a minimal LOAEL. By adjusting the LOAEL for the low-oxygen atmosphere, the subcommittee arrived at a value of 90 ppm.

An intraspecies uncertainty factor of 3 was applied on the basis of the work by Davies and Smith (1980) in which 3 of 15 healthy subjects exhibited electrocardiographic P-wave changes after 2 days of exposure to CO at 15 ppm. A LOAEL-to-NOAEL uncertainty factor of 3 brought the total uncertainty factor to 10. The subcommittee therefore recommends a 90-day CEGL of 9 ppm.

DATA ADEQUACY AND RESEARCH NEEDS

Although the literature on the effects of CO exposures in humans and animals is extensive, a number of data gaps remain. The conflicting results of studies on the neurobehavioral and cardiovascular effects of low-level CO exposures are of concern for submariners. There is little experimental or epidemiologic information available on the potential for increased health risks in smokers exposed to CO. Subchronic and chronic low-level exposure studies and long-term follow-up studies in submariners, including those who smoke, are needed to reduce uncertainty in the derivation of the 90-day CEGL.

BOX 4-1 Adjustment for the Low-Oxygen Atmosphere

The subcommittee used the Haldane equation to determine the adjustment for low-oxygen atmospheres applied to the EEGLs and the CEGL for CO. The Haldane equation relates the partial pressure of carbon monoxide (PCO) in air, the partial pressure of oxygen in air (PO_2), and the concentrations of COHb and oxyhemoglobin (O_2Hb) in blood (Douglas et al. 1912). The Haldane constant, *M*, is equal to about 200 (Douglas et al. 1912).

Haldane Equation:

$$\frac{[\text{COHb}]}{[\text{O}_2\text{Hb}]} = \frac{M\,[\text{PCO}]}{[\text{PO}_2]}.$$

COHb is inversely related to PO_2. In a lower-oxygen atmosphere, less CO would be required to reach a certain COHb level.

The average PO_2 in the submarine atmosphere is 148-149 millimeters of mercury (mmHg) (Hagar 2003), or about 90% that of the outside atmosphere at sea level. Under those conditions, it would take about 10% less CO to reach a certain COHb concentration over a defined exposure period. Therefore, the subcommittee applied a factor of 0.9 to the starting CO concentrations identified in the experimental literature when deriving the EEGL and CEGL values. Adjustments to the EEGL and CEGL values may be required when oxygen concentrations are outside the range cited above.

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5

Formaldehyde

This chapter summarizes the relevant epidemiologic and toxicologic studies on formaldehyde. Selected chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure levels from the National Research Council (NRC) and other agencies are also presented. The subcommittee considered all of that information in its evaluation of the Navy's current and proposed 1-hour (h), 24-h, and 90-day exposure guidance levels for formaldehyde. The subcommittee's recommendations for formaldehyde exposure levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

Formaldehyde is a flammable, colorless gas at room temperature and has a pungent, suffocating odor (Budavari et al. 1989). Odor thresholds ranging from 0.5 to 1.0 parts per million (ppm) (ATSDR 1999) and 0.06 to 0.5 ppm (Gerberich et al. 1994) have been reported. Formaldehyde reacts readily with many substances and polymerizes easily, making it one of the world's most important industrial chemicals (Gerberich et al. 1994). Selected chemical and physical properties are listed in Table 5-1.

OCCURRENCE AND USE

Formaldehyde is an important industrial chemical because of its versatility as a chemical intermediate (Gerberich et al. 1994). It primarily is used in the production of urea-formaldehyde, phenol-formaldehyde, and

TABLE 5-1 Physical and Chemical Properties of Formaldehyde[a]

Synonyms and trade names	Formic aldehyde, methanal, methyl aldehyde, methylene oxide, oxomethane, oxymethylene
CAS registry number	50-00-0
Molecular formula	HCHO
Molecular weight	30.03
Boiling point	−19.5°C
Melting point	−92°C
Flash point	83°C (closed cup)
Explosive limits	7% to 73%
Specific gravity	1.067 with respect to air
Vapor pressure	3,890 mmHg at 25°C
Solubility	Very soluble in water; soluble in alcohol and ether
Conversion factors	1 ppm = 1.23 mg/m^3; 1 mg/m^3 = 0.81 ppm

[a]Flash point and explosive limits from ACGIH (2001), vapor pressure from HSDB (2003), and all other data from Budavari et al. (1989).
Abbreviations: mg/m^3, milligrams per cubic meter; mmHg, millimeters of mercury; ppm, parts per million.

melamine-formaldehyde resins, which are used as adhesives in the production of particle board, fiber board, and plywood. Formaldehyde is also used in the manufacture of plastics, insulation, fertilizers, fungicides, biocides, corrosion inhibitors, embalming fluids, disinfectants, and household cleaners, and it is used in the textile industry in the production of permanent press and fire-retardant fabrics.

Formaldehyde occurs naturally in the environment and is emitted from vegetation, forest fires, and animal wastes (ATSDR 1999). It is a natural component of fruits and other foods and is an essential intermediate in human metabolism (IARC 1995; ATSDR 1999). Although naturally occurring, formaldehyde also enters the environment from many anthropogenic sources. In fact, combustion sources, such as power plants, incinerators, refineries, wood stoves, kerosene heaters, and cigarettes, are typically the largest contributors of formaldehyde emitted to the environment (ATSDR 1999). Other sources of formaldehyde emissions include motor vehicles, construction materials, textiles, paper, and cosmetics.

Formaldehyde has been monitored in both ambient and indoor air; concentrations are typically higher in indoor air (ATSDR 1999). Ambient measurements in urban and rural areas in the United States indicate a range

of 1 to 68 parts per billion (ppb) (ATSDR 1999). Kelly et al. (1994) reported a median concentration of 2.5 ppb after a survey of 58 locations. Indoor concentrations of formaldehyde are highly dependent on building construction (ATSDR 1999). For example, a range of 20 to 800 ppb was found in mobile homes, homes containing urea-formaldehyde foam insulation, and homes where residents had reported adverse symptoms. Average concentrations at 76 ppb and 50 ppb were reported in newer homes and older conventional homes, respectively. Although emissions from pressed-wood products might be the largest contributors of formaldehyde in indoor air, ATSDR (1999) noted that 10-25% of exposures might result from environmental tobacco smoke.

Sources of formaldehyde on submarines include high-temperature paints, motor varnishes, diesel generators, and cigarette smoke (Crawl 2003). A few measurements of formaldehyde have been made on board submarines. Raymer et al. (1994) reported the results of air sampling conducted over 6 h during the missions of two submarines. Sampling indicated formaldehyde concentrations at 24 ppb and 8.1 ppb in the fan rooms, 17 ppb and 9.0 ppb in the engine rooms, and 24 ppb and 6.9 ppb in the galleys of two submarines. A similar sampling exercise (two submarines, three locations, and a sampling duration of 6 h) was reported by Holdren et al. (1995). Formaldehyde concentrations ranged from 5.1 to 20.2 ppb on the two submarines. The subcommittee notes that the results presented by Raymer et al. (1994) and Holdren et al. (1995) represent one-time sampling events on four submarines. Whether the reported concentrations are representative of the submarine fleet is not known, particularly as few details were provided about the conditions on the submarines when the samples were taken.

SUMMARY OF TOXICITY

Formaldehyde is one of the most well-studied chemicals used today, and its toxic effects have been the subject of several comprehensive reviews (NRC 1981; NRC 1994; IARC 1995; Paustenbach et al. 1997; ATSDR 1999; ACGIH 2001; Health Canada 2001; Bender 2002; WHO 2002; Liteplo and Meek 2003; NAC 2003). This review relies on those documents, which conclude that irritation of the eyes and upper respiratory tract is the primary human health effect of concern for setting exposure limits for both acute and chronic inhalation exposures to formaldehyde. Formaldehyde irritation does not appear to follow Haber's law (concentration [C] ×

exposure time [t] = response [k]) for extrapolating between short-term and long-term toxicity levels. Generally, concentrations that do not produce short-term sensory irritation also do not produce sensory irritation after repeated exposure. Accommodation to low concentrations that cause short-term irritation has been reported; in such cases, irritation subsides with exposure duration. Risk of cancer and other chronic health effects appears to be negligible at concentrations that do not produce chronic irritation and overt target tissue damage.

Formaldehyde is widely used in industry, agriculture, and commercial products, and a wealth of clinical toxicology and epidemiologic data are available from workplace, community, and controlled exposures. Thus, the subcommittee placed more emphasis on reviewing adverse health effects in humans than in animals.

Effects in Humans

Accidental Exposures

No reports of deaths in humans resulting from inhaled formaldehyde were mentioned in the literature, and only a few case reports of accidental inhalation exposures resulting in human intoxication were found in the reviews consulted (IARC 1995; ATSDR 1999; ACGIH 2001; Health Canada 2001; WHO 2002; Liteplo and Meek 2003; NAC 2003). Effects of formaldehyde at high but unreported concentrations include tracheobronchitis and spasms and edema of the larynx (ACGIH 2001). Pulmonary edema, inflammation, and pneumonia occurred after exposure to airborne formaldehyde at concentrations of 50 to 100 ppm (ACGIH 2001). Allergic reactions and asthma-like conditions also have been reported following occupational exposures.

Experimental Studies

A number of controlled-exposure studies have been conducted in human volunteers. These studies are generally short-term (for example, 90 minutes [min] or less), but unlike occupational studies, they are not confounded by simultaneous exposures to other chemicals that might affect the reports of irritation attributed to formaldehyde. Some controlled chamber studies have focused on potentially more sensitive individuals, such as asthmatic individuals, nonsmokers, and people who previously have re-

sponded adversely to formaldehyde. Other studies have examined the consequences of continuous versus discontinuous formaldehyde exposures and exercise during exposures. Thus, data from 22 clinical studies involving over 500 subjects form the most reliable basis for estimating health-protective short-term exposure levels for airborne formaldehyde (see Table 5-2).

As summarized by NAC (2003), the most sensitive end point identified in the study literature is ocular and upper respiratory tract irritation. A concentration of 1 ppm appears to be the approximate threshold between complaints of symptoms ranging from none to mild to moderate with no clear concentration-response relationship or increase in complaints among exposed subjects compared with controls (subjects exposed to clean air) and definite symptoms of discomfort in a number of exposed subjects. For example, a controlled study in asthmatic subjects (Harving et al. 1990) found no association between subjective ratings of sensory irritation and increasing formaldehyde exposures at concentrations of 0, 0.01, 0.1, and 0.69 ppm. The "clean air" control groups in the chamber studies are important for distinguishing between the background occurrence of irritation symptoms in subjects and the effects related to formaldehyde exposures. IARC (1995) noted irritation thresholds of 0.5-1 ppm in those studies. NAC (2003) identified 0.9 ppm as the highest exposure concentration at which the responses of subjects whose eyes were sensitive to formaldehyde were not significantly different from controls. Even at 3 ppm, however, the majority of subjects reported only mild (typically defined as present but not annoying) to moderate (annoying) irritation. In only one study at that concentration did any subject rate the eye irritation as severe (1 of 180 subjects) (Sauder et al. 1987; NAC 2003). Although many studies do not report the ranges of individual irritation scores, the small variation in scores indicates that a score of severe is very unlikely.

In the study with the subject reporting severe eye irritation (Sauder et al. 1987), "severe" was defined by the investigators as debilitating, but scoring depended on the interpretations of the participants, who rated their own symptoms. In that study, 22% of subjects exposed to clean air reported eye irritation, and 33% reported nose or throat irritation. The overall difference between the eye-irritation responses to exposure at 3 ppm and exposure to clean air was not statistically significant until 1 h into the exposure. In addition to the person who reported severe eye irritation, another person reported no irritation, and according to group means, the rest of the subjects rated their eye irritation as mild (defined as present but not annoying) at 1 h and at 180 min. At 120 min, one of the subjects may have reported eye irritation as between mild and moderate. All subjects in the Sauder et al. (1987) study were clinically diagnosed with asthma, and the

subject who reported severe eye irritation was a female who remained in the chamber for the full 3 h of the study and successfully completed the spirometry measurements at 15, 30, 60, 120, and 180 min during the study period. Spirometry measurements showed little change in forced expiratory volume at 1 second (FEV_1). Thus, this subject appears to be an outlier, and it is doubtful whether the severe eye irritation reported was actually debilitating.

Many of the controlled inhalation studies included potentially sensitive individuals. These studies either excluded less sensitive individuals (for example, those without complaints of eye irritation at 1.3-2.2 ppm or smokers) or focused on potentially sensitive individuals (for example, asthmatic individuals and those with formaldehyde-related contact dermatitis or previous formaldehyde sensitivity) (see Table 5-2). As summarized by NAC (2003), Bender (2002), and Paustenbach et al. (1997), the results of those studies indicate that sensitive individuals might experience moderate ocular irritation at 1 ppm. Below 3 ppm, formaldehyde appears to be largely scrubbed in the upper airways, because asthmatic individuals (who normally react to mid- and lower-respiratory airway irritants) engaging in moderate exercise showed no decrements in several pulmonary function parameters when exposed at up to 3 ppm. Thus, asthmatic individuals exposed to airborne formaldehyde at exposure concentrations at or below 3 ppm do not appear to be at greater risk of suffering airway dysfunction than nonasthmatic individuals. In addition, the short-term chamber studies indicate that adaptation or accommodation to irritation can develop with time.

Changes in pulmonary function (described as mild and reversible changes in FEV_1 and midexpiratory flow) can occur in individuals sensitized to formaldehyde at concentrations approaching 2 ppm (Bender 2002). Only five studies investigated the effects of airborne concentrations above 3 ppm (see Table 5-2). One study noted severe irritation symptoms at 5 ppm; however, another study reported no complaints of ocular irritation at 8 ppm in four out of five subjects. Mild lacrimation was noted at 13.8 ppm in another study, but adaptation occurred within 30 min. A concentration of formaldehyde at 20 ppm was described as objectionable.

Occupational and Epidemiologic Studies

Occupational and epidemiologic studies involve longer, more continuous exposure durations and a greater number of subjects but are less controlled for simultaneous exposures to other substances, such as irritants, solvents, or particulates. Many of these investigations suffer from uncertain

exposure concentrations (Paustenbach et al. 1997; ATSDR 1999; ACGIH 2001; Bender 2002). Some of the occupational studies also involved exposures to formaldehyde in particulates because paraformaldehyde or powered resins were being used. The mean airborne concentrations of formaldehyde reported in many of these studies do not adequately represent peak excursions, which would be more likely to be associated with adverse health effects. In several studies, documentation of health complaints relied on self-reporting and recall via surveys. Thus, these studies are useful as supporting evidence with regard to limits for sensory irritation and pulmonary function, particularly over longer exposure periods, but they lack the precision of the controlled inhalation studies.

Studies of occupational formaldehyde exposures involve workers in the manufacture of formaldehyde, formaldehyde-based resins, and other chemical products; wood products and paper; textiles and garments; and metal products and mineral wool. Some studies involve workers exposed to formaldehyde used in their occupational settings, which included mortuaries, hospitals, and laboratories. In general, studies in workers associate irritation with lower concentrations of formaldehyde than those reported in the controlled human studies. Eye irritation has been reported in occupational studies at concentrations as low as 0.01 ppm, although ACGIH (2001) notes that those exposures occurred in association with other chemicals that may have been acting synergistically. Most studies reported increased eye, nose, or throat irritation beginning at about 0.3 ppm and above (Paustenbach et al. 1997; ACGIH 2001). As found in the experimental chamber studies, exposure concentrations at about 1 ppm and above result in more consistent reports of eye and mucous membrane irritation in major percentages of workers, such as 40-50%.

Some level of background irritation is apparent in workers and the general population, and irritation is often reported by control subjects in studies evaluating the effects of formaldehyde exposure. A study of workers exposed to formaldehyde from particle board or molded product found that 21% of workers exposed to workplace concentrations at 0.4-1 ppm reported sore throat as compared with 8% of those exposed at 0.05-0.4 ppm (Horvath et al. 1988). In the control group of that same study, sore throat occurred in 4% of workers, which was not statistically different from the percentage of workers experiencing sore throat exposed at 0.05-0.4 ppm. The control group also had occurrences of nose irritation in 2% of subjects and burning or watering eyes in 9% of subjects. In a study of funeral workers (Holness and Nethercott 1989), half of the exposed workers reported eye irritation at an average airborne concentration of 0.4 ppm; however, half of the control workers also complained of eye irritation. Thus, complaints of sensory

irritation at the lowest-reported study concentrations are not necessarily indicative of a response above background attributable to formaldehyde exposure.

Overall, the occupational studies support the results of the chamber studies—they show that formaldehyde is a concentration-dependent irritant of the eyes and mucous membranes that has little or no adverse effects on pulmonary function in workers exposed at concentrations below 3 ppm. Even after long exposure durations (for example, mean exposure times of 10-12 years), there is no consistent evidence of permanent impairment resulting from those low exposure concentrations.

In addition to irritation, histopathologic changes in the nasal epithelium of workers was examined in some studies. Some studies have noted changes in nasal histology (typically mild dysplasia) in workers exposed at average concentrations of 0.5-2.4 ppm; peak exposures in those studies, when noted, were considerably higher (5-18.5 ppm) (IARC 1995). WHO (2002) noted that the available data are consistent with the hypothesis that formaldehyde induces histopathologic lesions in the nose; however, the weight of evidence for causality is weak because of limitations in the number of studies, study sizes, and study designs that did not allow for evaluation of exposure-response relationships.

Several studies have been conducted in residential populations exposed to formaldehyde in their homes (Paustenbach et al. 1997; ACGIH 2001; Bender 2002). Unlike occupational exposures, residential exposures potentially involve continuous exposures more similar to those experienced by submariners (that is, 24 h per day rather than 8 h per day). Unfortunately, studies of residential exposures typically lack sufficient control for the presence of other irritants and the many confounding factors that could affect subject responses. Many of the studies also rely on self-reported health status. One of the largest studies involved nearly 2,000 residents of 397 mobile homes and 494 conventional homes (Ritchie and Lehnen 1987). Participants were not selected randomly; they responded to a free testing service for formaldehyde, which was offered to individuals by the state of Minnesota when an examining physician made a written request. Thus, those recruited in the study had complained of symptoms thought to be related to airborne formaldehyde exposures. Over 60% of the residents reported eye, nose, and throat irritation or headache at airborne concentrations above 0.3 ppm; 12-32% reported eye irritation at 0.1-0.3 ppm; and only 1-2% reported eye irritation at 0.1 ppm, which was the background rate. The background rate of nose and throat irritation was 20%. Nevertheless, Bender (2002) notes that the symptoms reported at formaldehyde

TABLE 5-2 Irritant Effects of Formaldehyde in Controlled Human Studies

Concentration (ppm)	Time	Subjects (no.) and Effects	Reference
0, 0.41	2 h	Healthy, occupationally exposed (5) and contact dermatitis (13) subjects No effect on pulmonary parameters (VC, FEV_1); immune response in subjects with contact dermatitis (increased chemi-luminescense of neutrophils)	Gorski et al. 1992
0, 0.41	2 h	Healthy (11) and patients with skin hypersensitivity to formaldehyde (9) (all nonsmokers) No differences in response between groups; transient increase in symptoms of sneezing, rhinorrhea, or itchy eyes; nasal washings showed increases in eosinophils, albumin, and total protein, but not neutrophil, basophil, or mononuclear cells	Pazdrak et al. 1993
0, 0.41	2 h	Healthy, nonoccupationally exposed subjects (10) and occupationally exposed asthmatic subjects (10) No differences in response between groups; transient increase in nasal symptoms of sneezing, rhinorrhea, edema, or itchy eyes; increases in leucocytes and eosinophils in nasal washings; no allergic response; no clinical symptoms of bronchial irritation or effects on pulmonary function parameters (FEV_1, PEF)	Krakowiak et al. 1998
0, 0.10, 0.69	90 min	Asthmatic nonsmoking subjects (15) No significant change in pulmonary function parameters (FEV_1 and airway resistance) or in bronchial reactivity; no association of subjective ratings of asthmatic symptoms, if any, with increasing air concentration	Harving et al. 1986, 1990

(*Continued*)

TABLE 5-2 Continued

Concentration (ppm)	Time	Subjects (no.) and Effects	Reference
0, 0.17, 0.39, 0.9	5.5 h	Formaldehyde exposed workers (32); controls (29) Subjective symptoms (headache, tiredness) did not correlate with exposure; no clear effect of concentration on memory—some concentration-related effect on a few tests (addition speed, response time) but limitations in experimental design and control issues	Bach et al. 1990
0, 0.35, 0.56, 0.7, 0.9, 1.0	6 min	Healthy subjects (groups of 5-28), excluded those reporting eye irritation in clean air or nonresponders at 1.3 or 2.2 ppm Eye irritation evaluated—average scores of none to slight at 0.35 to 0.9 ppm; slight to moderate at 1.0 ppm; slight adaptation with time	Bender et al. 1983
1	90 min	Healthy (9) and formaldehyde-sensitive (9) subjects (previously complained about nonrespiratory effects of urea-formaldehyde foam insulation) No effects on pulmonary function parameters (FVC, FEV_1, max and midexpiratory flow rate); complaints of eye irritation, nasal congestion, tearing, and throat irritation	Day et al. 1984
0, 1.0	3 h	Control asthmatic subjects (4); subjects with asthma attributed to urea-formaldehyde foam (23) No differences between groups in immunologic parameters, either before or after exposure; minor immunologic changes in both groups post-exposure	Pross et al. 1987

0, 0.2, 0.4, 0.8, 1.6	5 h	Healthy subjects (16) No differences in nasal airway resistance or pulmonary function parameters; decrease in nasal mucus flow at all concentrations; no discomfort at 0.2 or 0.4 ppm for 2 h, some slight discomfort reported in the 3-5 h period (conjunctival irritation, dryness of nose and throat), but discomfort rated higher at 0.2 ppm than at 0.4 ppm, and only five or fewer subjects reported any discomfort; average discomfort scored as slight during exposure at 1.6 ppm and first noted in the latter part of the first h but decreased somewhat after 3 h; no effect on performance on mathematical tests or number-transfer tasks	Andersen and Molhave 1983
0, 2.0 (at rest) 0, 2.0 (exercise)	40 min	Healthy (15) and asthmatic (15) nonsmoking subjects No significant decrement in pulmonary function parameters (flow-volume parameters and airway resistance) or bronchial reactivity both at rest and with exercise; subjective symptoms ranged up to severe (but not incapacitating) for odor for some individuals, but median scores for nose, throat, and eye irritation were ≤moderate; no increase in symptomology with exercise	Witek et al. 1986; 1987; Schachter et al. 1985; 1986
0, 0.1, 1.0, 3.0	20 min	Asthmatic patients who suspected formaldehyde as the cause (13) No significant difference in pulmonary function parameters (FEV_1, VC); no asthmatic response to formaldehyde challenge	Frigas et al. 1984

(*Continued*)

TABLE 5-2 Continued

Concentration (ppm)	Time	Subjects (no.) and Effects	Reference
0, 0.5, 1.0, 2.0, 3.0 at rest; 2.0 with exercise	3 h	Healthy nonsmoking subjects (19; only 9 exposed at 3 ppm and 10 exposed at 0.5 ppm) No significant decrements in pulmonary function parameters (FVC, FEV_1, $FEF_{25-75\%}$, SGaw) or increases in bronchial reactivity (methacholine challenge) at any concentration; nasal flow resistance increased at 3 ppm; significant dose-response relationship for odor sensation and eye irritation, but eye irritation scored mild or mild (in five of nine) to moderate (in four of nine) at 3 ppm; eye irritation beginning at 1 ppm	Kulle et al. 1987; Kulle 1993
0, 3.0 with heavy exercise (healthy subjects); moderate exercise (asthmatic subjects)	1 h	Healthy (22) and asthmatic (16) nonsmoking subjects No difference in symptoms between groups; eye, nose, and throat irritation scored mild to mild-moderate (group means); small decreases in some pulmonary function parameters (FEV_1, FVC, FEV_3, but not $FEF_{25-75\%}$) in healthy individuals engaging in heavy exercise; no change in specific conductance or nonspecific airway reactivity of either group	Green et al. 1987
0, 3.0 with heavy exercise (15 min each 0.5 h)	2 h	Healthy nonsmoking subjects (24) Increase in subjective symptoms of eye, nose, and throat irritation, rated mild to moderate on average; small, but statistically significant increase in two ($FEF_{25-75\%}$, specific airway resistance) of several pulmonary function measurements at some time intervals (no effect on FEV_1, FVC, FEV_3); no increase in cough	Green et al. 1989

0, 3.0 with intermittent exercise	3 h	Healthy nonsmoking subjects (9) Nonbiologically significant, transient change in some pulmonary function parameters (FEV_1, $FEF_{25-75\%}$); increase in nose, throat, and eye irritation, rated mild to moderate by individuals; only one subject rated eye irritation as moderate	Sauder et al. 1986
0, 3.0	3 h	Asthmatic nonsmoking subjects (9) No significant group change in pulmonary function parameters (FEV_1, FVC, $FEF_{25-75\%}$, SGaw, or FRC) or airway reactivity; significant increase in nose, throat (at 30 min), and eye irritation (at 60 min), rated as none to mild-moderate except for one subject who reported severe eye irritation	Sauder et al. 1987
0, 1, 3	10 min	Asthmatic nonsmoking subjects (7) Similar responses in airway resistance following exposure at 0, 1, or 3 ppm with and without exercise (exercise increased all responses)	Sheppard et al. 1984
0.03 to 3.2; 0, 1.0, 2.0, 2.9, 4.0; or 1.2, 2.1, 2.8, 4.0	37 min (n = 33); 1.5 min (n = 48)	Healthy subjects (two exposure groups of 33 and 48) Poorer air quality and greater nose irritation reported during the short exposures than during the 37-min exposure, whereas the opposite was true for eye irritation; with increasing concentrations, both eye and nose irritation increased from none to "a little"; eye blinking not affected at 1.2 ppm but was statistically significantly increased at 2.1 ppm	Weber-Tschopp et al. 1977
0, 1, 2, 4, 5	5 min except for 2 ppm (12 min)	Healthy students (groups of 7 to 75) Assessed eye irritation only: 1 ppm considered threshold for detection; 5 ppm produced severe eye irritation	Stephens et al. 1961

(*Continued*)

TABLE 5-2 Continued

Concentration (ppm)	Time	Subjects (no.) and Effects	Reference
8, 12	≤15 seconds	Healthy, atopic subjects (1-6) Eye irritation for 5 of 6 subjects at 12 ppm but only for 1 of 5 at 8 ppm; irritation of the throat at both concentrations; changes in airway resistance	Douglas 1974
13.8	30 min	Healthy male subjects (12) Nasal and eye irritation (not severe) with mild lacrimation; adaptation to the eye irritation	Sim and Pattle 1957
20	Several min	Healthy subjects (2) Lacrimation within 15-30 seconds; eye, nose, and throat irritation considered objectionable	Barnes and Speicher 1942

Source: Adapted from NAC (2003).

Abbreviations: $FEF_{25-75\%}$, forced expiratory flow rate between 25% and 75% of forced vital capacity; FEV_1, forced expiratory volume at 1 second; FEV_3, forced expiratory volume at 3 seconds; FRC, functional residual capacity ; FVC, forced vital capacity; ppm, parts per million; h, hour; min, minute; PEF, peak expiratory flow; SGaw, specific airway conductance; VC, vital capacity.

concentrations below 0.3 ppm could in part be attributed to smoking or passive smoke exposures because below that concentration, rates of reported symptoms were highest in smokers, were intermediate in passive smokers, and were lowest in nonsmokers. Only above 0.3 ppm did the frequency of reported symptoms become similar among smokers, passive smokers, and nonsmokers. In addition, only formaldehyde was measured, and because the methodology underestimated the actual exposure concentrations, the reported concentrations could be 10-20% too low (Paustenbach et al. 1997). Past exposures also could have been higher than those reported given the length of time between the filing of health complaints and the actual survey of formaldehyde in the residential structures (Bender 2002).

Effects in Animals

As summarized in other reviews (Paustenbach et al. 1997; ATSDR 1999; ACGIH 2001; Health Canada 2001; WHO 2002; Liteplo and Meek 2003; NAC 2003), a number of studies have been conducted in animals. Many of those studies are not directly useful for evaluating exposure limits in humans because of the very high exposure concentrations used. The studies in animals, however, included more continuous exposures under controlled conditions over longer time periods, such as 26 weeks, and provided supporting evidence for the nature of toxic effects, the target tissues, and the concentration-response relationship observed in human studies.

Some important physiologic differences among animals that affect the extrapolation of results to humans must be noted. Unlike nonhuman primates and humans, rats are obligate nose-breathers. Because of their nasal anatomy and physiology, rats have greater localized tissue damage in the anterior nasal passages but less penetration to lower airways when exposed at the same air concentrations as primates (Monticello et al. 1991; Kimbell et al. 2001a). Thus, formaldehyde reaches a greater range of respiratory tissues in monkeys, as measured by DNA-protein cross-link formation (Casanova et al. 1991). The middle turbinates of rhesus monkeys were found to be the major target site; very little formaldehyde reached extranasal tissues (the larynx, trachea, and proximal portions of major intrapulmonary airways) at 2 and 6 ppm. No detectable DNA-protein cross-links were reported in extranasal tissues of monkeys exposed to 0.7 ppm.

Data from lower air exposure concentrations in animal studies are generally consistent with experimental studies in humans in the following ways:

- Adverse effects at the lowest airborne-formaldehyde exposure concentrations involve sensory irritation.
- The degree of sensory and irritant effects at lower exposure levels depends on concentration rather than duration.
- Formaldehyde is well scrubbed by nasal and upper respiratory passages at exposure concentrations below 3 ppm and relatively little reaches the lungs at those low concentrations.
- Few to very slight signs of irritation occur below 1 ppm, and those signs are relatively mild; acute effects are reversible at below 3 ppm.

Reflex reductions in respiration rate via trigeminal nerve stimulation in the nasal passages have been used to quantify sensory irritation in rodents (Paustenbach et al. 1997; ATSDR 1999; ACGIH 2001). That measure is a more sensitive indicator in mice than in rats, because rats are less able to reduce their respiration rates. Ten-minute exposures at 3 ppm produced 50% decreases in the respiration rates (RD_{50}) of mice (Kane and Alaire 1977). Repeated 3-h exposures on 4 consecutive days produced progressively greater decreases in the respiration rates of mice at 1 ppm (decreases of about 15% on day 1 to about 35% on day 4) and at 3 ppm (decreases of about 45% on day 1 to about 70% on day 4) (Kane and Alaire 1977). During the exposures, the degree of response reached a plateau in the first 10 min and then decreased over the remainder of the 3-h period, although that decrease in response was delayed on day 4 during exposures at 3 ppm.

Guinea pigs appear to be susceptible to changes in lower airway resistance and hyperreactivity of the lungs resulting from acute formaldehyde exposure. However, there are apparent inconsistencies among the studies with regard to the concentrations and exposure periods that induced those changes (that is, whether concentrations as low as 0.3 ppm or as high as 9.4 ppm are required) (ATSDR 1999).

Studies in rodents and a few monkeys (ATSDR 1999; WHO 2002) indicate that at concentrations beginning at about 3 ppm for short-term exposures (for example, 6-22 h per day for 3 days) and above 2 ppm for longer-term exposures (for example, $\geq$13 weeks), cellular lesions, such as cytolethality and hyperplasia, might develop in the nasal passages and, with increasing concentrations, in other areas of the respiratory system. Epithelial lesions of the upper respiratory tract observed in rats and monkeys were histologically similar, although regional differences in occurrence were evident. Site-specific damage in the nasal epithelium of rats also was correlated in a concentration-dependent manner with the degree of inhibi-

tion of mucociliary function at 2, 6, and 15 ppm (6 h per day for 1-3 weeks). Overall, studies in animals have demonstrated that the adverse effects of formaldehyde exposures, including nasal tumors (discussed below in the section on carcinogenicity), are associated with the overt cytotoxicity and tissue hyperplasia caused by the chemical's potent irritant properties (Connolly et al. 2003).

Reproductive Toxicity in Males

No compelling evidence of male reproductive toxicity due to formaldehyde exposure was noted in the literature (ATSDR 1999). One human study (Ward et al. 1984) examined sperm samples from 11 workers exposed at time-weighted average air concentrations of 0.6-1.3 ppm. The mean sperm count of the exposed workers was lower, but not significantly lower, than controls. No differences were found in the frequency of abnormal sperm.

Several studies in rodents exposed to formaldehyde concentrations at up to 10-40 ppm reported no effects on male reproductive organs (ATSDR 1999).

Immunotoxicity

Although exposures to airborne formaldehyde have been associated with occupational asthma, consistent evidence of a formaldehyde-induced allergic respiratory syndrome is lacking (ATSDR 1999). Formaldehyde resin dust appears to be more likely to induce asthma than gaseous formaldehyde (Lemiere et al. 1995).

A few studies show limited evidence of increased IgE antibody activity in a small fraction of formaldehyde-exposed adult subjects, but allergen-specific IgE against formaldehyde have not been identified. Even in a case that reported probably the most compatible history (occupational exposure to formaldehyde and symptoms of bronchial spasms) and immunologic findings (positive skin test results to formaldehyde-human serum albumin [F-HAS] and positive IgE and IgG titers to F-HAS), the subject had a negative methacholine challenge at 25 mg per milliliter (mL) and negative formaldehyde inhalation challenges at 0.3, 1, 3, and 5 ppm for 20 min (Grammar et al. 1993). In general, investigations in formaldehyde-

exposed populations have not found an immunologic basis for respiratory or conjunctival reactions to formaldehyde (Patterson et al. 1987; Grammar et al. 1990, 1993; IARC 1995; ATSDR 1999; Kim 2001). Evidence from animal studies does not indicate that repeated inhalation exposures to formaldehyde have a direct effect on the immune system, although suggestive evidence indicates that formaldehyde might indirectly facilitate sensitization of the nasal tissues to high-molecular-weight allergens (ATSDR 1999).

Formaldehyde solutions are irritating to the skin and can induce allergic contact dermatitis. It has been estimated that 8.4% of the U.S. population have positive patch-test reactions; however, that percentage probably is considerably inflated (IARC 1995). Maibach (1983) notes that more than 40% of patch-test results are not reproducible, especially for chemicals such as formaldehyde that cause an allergic or irritant response at similar concentrations.

Genotoxicity

Various mutagenicity studies—for example, tests of frequency of chromosomal aberrations and sister chromatid exchange in peripheral lymphocytes or micronuclei in oral and respiratory nasal mucosa cells and peripheral lymphocytes—have been conducted in worker populations and have yielded both positive and negative results (IARC 1995). There is uncertainty in interpreting these studies because of small sample sizes, inconsistencies in findings, and the lack of dose-response for the increased frequency of micronuclei in the nasal mucosa cells of exposed groups compared with controls (IARC 1995). Occupational exposures included other chemicals and substances in addition to formaldehyde. Urinary fractions from hospital autopsy service workers that were assayed for mutagenicity in *Salmonella typhimurium* showed no evidence of increased mutagenicity compared with control samples (Connor et al. 1985).

Formaldehyde has been demonstrated to be genotoxic in a wide variety of experimental systems both in vitro in animal and human cells and in vivo in animals (IARC 1995). As summarized by IARC (1995), formaldehyde reacts readily with DNA, and formaldehyde exposures have been associated with DNA-protein cross-links in the nasal respiratory mucosa of rats and monkeys. The frequency of cross-links increases in a linear fashion with increasing formaldehyde concentration, then inflects upward around 2-3 ppm and above. In human and rodent cells in vitro, formaldehyde

induces DNA-protein cross-links, DNA single-strand breaks, chromosomal aberrations, sister chromatid exchange, and gene mutation. Cell transformation also has been demonstrated in rodent cells in vitro.

As noted by Casanova et al. (1991), DNA-protein cross-link formation is more related to the delivered dose (the concentration of formaldehyde at the tissues) to various regions of the upper respiratory tract than to the administered dose (the ambient concentration of formaldehyde initially inhaled). Cross-links can occur in rat nasal tissues at exposure concentrations that are not associated with demonstrable cytolethality or carcinogenicity. Species differences in DNA-protein cross-link formation are thus related to anatomic and physiologic differences that affect the delivered dose. Although DNA-protein cross-link formation might not be directly related to gene mutations at subcytotoxic doses, it has been used as a predictor of the probability of procarcinogenic mutation per cell division and has been incorporated in models for low-dose carcinogenicity in animals and in humans (CIIT 1999; Conolly et al. 2003).

Carcinogenicity

A large number of studies (>40 epidemiologic studies) have examined the carcinogenic potential of formaldehyde in animals and humans. The findings from those studies have been evaluated by a number of agencies and committees engaged in setting regulatory standards and guidelines (IARC 1995; Paustenbach et al. 1997; ATSDR 1999; ACGIH 2001; WHO 2002; EPA 2003; NAC 2003). The reviews concur that inhaled formaldehyde induces tumors, such as nasal squamous cell carcinoma, in rats and mice exposed at airborne concentrations that are associated with significant irritation and result in hyperplasia and tissue damage after repeated exposures (>6 ppm, typically 10-15 ppm). In humans, the overall evidence for carcinogenicity is less consistent, and any statistically significant associations are generally weak. Some epidemiologic studies (ATSDR 1999) have found an excess number of nasopharyngeal cancers, and two meta-analyses (Blair et al. 1990; Partanen 1993) reported a significantly higher risk of such cancers among workers with substantial exposure compared with those with low to moderate exposure or no exposure. However, those associations were relatively weak (relative risks [RR] = 2.1 [95% confidence interval (CI) = 1.1-3.5] and 2.7 [95% CI = 1.4-5.6] in Blair et al. [1990] and Partanen [1993], respectively). IARC (1995) considered the two meta-

analyses to be suggestive of a causal relationship between formaldehyde and nasopharyngeal cancer but noted that its "conclusion is tempered by the small numbers of observed and expected cases in the cohort studies."

A more recent meta-analysis by Collins et al. (1997) did not find a significant association once the cohort studies were adjusted for under-reporting of nasopharyngeal cancer (RR = 1.0 [95% CI = 0.5-1.8]) and the case-control studies were analyzed separately (RR = 1.3 [95% CI = 0.9-2.1]). Collins et al. (1997) also noted that the exposure information in the case-control studies was less certain than the exposure information in the cohort studies.

Several recent studies have continued to investigate the association between formaldehyde exposure and cancer. In an updated study of 7,000 workers from a U.S. chemical plant, Marsh et al. (2002) reported that although statistically significant excesses in nasopharyngeal and pharyngeal cancer were observed in exposed versus unexposed workers, most of the cancer cases were among workers who had less than 1 year of employment in the earlier years of the plant's history. Nasopharyngeal cancer cases among workers with greater than 1 year of employment had low-average formaldehyde exposure. Thus, Marsh et al. (2002) concluded that the cancer excesses observed were not associated with formaldehyde exposure.

A follow-up study of 14,014 British workers (Coggon et al. 2003) did not find an increase in sinonasal cancer or nasopharyngeal cancer. The study authors concluded that the evidence for formaldehyde carcinogenicity in humans is unconvincing and that although the occurrences of sinonasal or nasopharyngeal cancer cannot be ignored, the small increase in lung cancer mortality (standardized mortality ratio = 1.28 [95% CI = 1.13-1.44]) observed in subjects occupationally exposed to formaldehyde is of greater concern, particularly among the highest-exposure groups (>2 ppm). However, no increases in lung cancer mortality were associated with longer durations of high exposure or the time elapsed since the first high exposure. Another recent follow-up study of a cohort of 25,619 industrial workers from 10 U.S. plants found no associations with lung cancer. However, a significant increasing risk trend for nasopharyngeal cancer was observed with highest peak or cumulative exposure measured in ppm-years, although not for average exposure or duration of exposure measured in years (Hauptmann et al. 2004). The significant trend was based on small numbers. For peak exposures, nasopharyngeal cancers were observed in seven workers in the highest-exposed group (>4 ppm; RR = 1.83), no workers in each of the other two exposure groups (>0 to <2 ppm and >2 to <4 ppm), and two

workers in the control group (RR = 1.00). The trend for cumulative exposure was based on setting the relative risk for the low-exposure group to 1.00. Thus, the number of cancer deaths and relative risks for the cumulative exposure groups were two workers (RR = 2.40) for unexposed controls, three workers (RR = 1.00) for >0 to <1.5 ppm-years, one worker (RR = 1.19) for >1.5 to <5.5 ppm-years, and three workers (RR = 4.14) for >5.5 ppm-years. A cohort study of 11,039 U.S. garment workers in three plants reported no nasal or nasopharyngeal cancers or increases in respiratory cancers with duration of formaldehyde exposure or with historical exposure when concentrations were presumably higher (Pinkerton et al. 2004).

Although the toxicologic and mechanistic evidence is weaker for cancers at locations other than the upper airways, some evidence has indicated an association of formaldehyde exposure with leukemia in workers. Some studies, particularly those involving medical workers and other professionals exposed to formaldehyde, have reported increased risk of leukemia; however, studies in industrial workers who are often exposed at higher concentrations provide less evidence of such increased risk (IARC 1995; ATSDR 1999). Among the recent studies, the study of British workers by Coggon et al. (2003) did not find an increased risk of leukemia in all workers or in those with high exposure (>2 ppm) or with more years at high exposure. Peak exposures were not specifically evaluated. Hauptmann et al. (2003) reported an increased risk for myeloid leukemia with high peak exposures to formaldehyde (peak concentrations >4 ppm compared with 0.1-1.9 ppm; SMR = 3.46, 95% CI = 1.27-9.43) in the same cohort of U.S. industrial workers evaluated by Hauptmann et al. (2004) for solid tumors and respiratory cancers. Risk of myeloid leukemia was not associated with cumulative exposure measured in ppm-years and was described as "weakly," but not significantly, associated with duration of exposure measured in years. Overall risk of leukemia in those workers was lower than that in the U.S. population. Pinkerton et al. (2004) likewise found no significant increase in leukemia or myeloid leukemia in garment workers as compared with the U.S. population but reported significant increases in myeloid leukemia in those employed in the early years when exposures were presumably higher, those with 10 or more years of exposure, and those with 20 or more years since first exposure.

In general, however, the biologic plausibility for formaldehyde exposures to cause leukemia or other nonrespiratory cancers in humans has been considered weaker than that for high-dose formaldehyde exposures to cause nasopharyngeal cancers on the basis of pharmacokinetic and toxicologic

evidence (IARC 1995, 2004; ATSDR 1999; WHO 2002; EPA 2003). IARC (2004) recently changed its previous classification of formaldehyde as "probably carcinogenic to humans (Group 2A)," on the basis of limited evidence in humans and sufficient evidence in animals, to "carcinogenic to humans (Group 1)," on the basis of sufficient evidence in animals and humans. Although the monograph with the full documentation was not available for review, the summary provided by IARC (2004) indicates that the basis for this decision was primarily the trend for increased risk of nasopharyngeal cancers in workers with peak and cumulative exposures reported by Hauptmann et al. (2004)—considered "the largest and most informative cohort study of industrial workers exposed to formaldehyde" and supported by other epidemiological studies (IARC 2004). The U.S. Environmental Protection Agency's (EPA's) review of formaldehyde in their Integrated Risk Information System database, last revised in 1991, rated formaldehyde as a "probable human carcinogen" (Group B1) on the basis of limited evidence in humans and sufficient evidence in animals (EPA 2003).

Several reviews generally agree that the carcinogenicity of formaldehyde observed in animals appears to occur by a mechanism that would result in a practical threshold for significant cancer risk (Paustenbach et al. 1997; CIIT 1999; ACGIH 2001; WHO 2002; Connolly et al. 2003; NAC 2003; Gaylor et al. 2004). Specifically, a high incidence of tumor formation appears to be related to chronic tissue irritation at higher doses. Chronic tissue irritation at those high doses leads to cytotoxicity and regenerative hyperplasia. As a result, exposure limits that are protective against significant irritation and irreversible changes in the nasal mucosa would also be protective against carcinogenic effects. The recent findings from some epidemiologic studies of increased risks for higher-exposure groups and high-peak exposures are consistent with this general theory.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

Consistent with its action as an upper respiratory irritant, formaldehyde is a highly water-soluble vapor that is readily absorbed by the upper respiratory tract and is rapidly metabolized to formic acid or formate (IARC 1995; ATSDR 1999). Because absorption appears to be limited to the respiratory tissues at the point of contact and formaldehyde is rapidly metabolized, little if any formaldehyde is found in the blood streams of

humans or animals (Heck et al. 1985; Casanova et al. 1988). Absorption via the skin is considered to be very limited.

All tissues of the body metabolize formaldehyde to formate via formaldehyde dehydrogenase, a major metabolic enzyme that routinely metabolizes formaldehyde produced endogenously in the body. Formate is oxidized to carbon dioxide and exhaled, and small amounts of formate are excreted in the urine. Sometimes the carbon from metabolized formate is used in cellular structures in the body. Studies on the kinetics of formaldehyde in primates have reported a biologic half-life of about 1.5 min (McMartin et al. 1979). The classic formate toxicity associated with methanol ingestion is not considered an issue for the smaller amounts of formate produced in the body after inhalation exposures to formaldehyde (ATSDR 1999). Therefore, toxicity as a result of accumulation and storage is not an issue for formaldehyde.

INHALATION EXPOSURE LEVELS FROM THE NRC AND OTHER ORGANIZATIONS

A number of organizations have established or proposed inhalation exposure levels or guidelines for formaldehyde. Selected values are summarized in Table 5-3.

Occupational and public criteria for air are based largely on preventing the irritation effects of formaldehyde. Most levels are time-weighted average (TWA) concentrations over a designated period, although the American Conference of Governmental Industrial Hygienists (ACGIH) and the National Institute for Occupational Safety and Health (NIOSH) have specified ceiling values. Those ceiling values have been called into question by a panel of experts convened by the Industrial Health Foundation at the request of the Formaldehyde Institute (Paustenbach et al. 1997). On the basis of the chamber studies, which are more reliable for setting effect levels compared with worker studies, the panel concluded that 0.3 ppm was sufficiently protective as a TWA concentration and recommended a ceiling value of 1.0 ppm.

The NASA spacecraft maximum allowable concentrations (SMAC) values, which may be more analogous to submarine exposure levels than other limits, are based on a Wisconsin mobile home study (1-h and 24-h SMACs) and background concentrations (30-day and 180-day SMACs)

TABLE 5-3 Selected Inhalation Exposure Levels for Formaldehyde from NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-Ceiling	0.3 (A2, suspected human carcinogen)	ACGIH 2001
NIOSH	REL-Ceiling	0.1 (15 min)	NIOSH 2004
	REL-TWA	0.016	
OSHA	PEL-STEL	2 (15 min)	29 CFR
	PEL-TWA	0.75	1910.1048 (c)
Spacecraft			
NASA	SMAC		NRC 1994
	1 h	0.4	
	24 h	0.1	
	30 days	0.04	
	180 days	0.04	
General Public			
ATSDR	Acute MRL	0.04	ATSDR 1999
	Intermediate MRL	0.03	
	Chronic MRL	0.008	
NAC/NRC	Proposed AEGL-1 (1 h)	1	EPA 2004
	Proposed AEGL-2 (1 h)	8	
	Proposed AEGL-1 (8 h)	1	
	Proposed AEGL-2 (8 h)	8	

[a]The comparability of EEGLs and CEGLs with occupational and public health standards or guidance levels is discussed in Chapter 1, section "Comparison to Other Regulatory Standards or Guidance Levels."

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL, acute exposure guideline level; ATSDR, Agency for Toxic Substances and Disease Registry; h, hour; min, minute; MRL, minimal risk level; NAC, National Advisory Committee; NASA, National Aeronautics and Space Administration; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; ppm, parts per million; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

(NRC 1994). In contrast, the National Advisory Committee for Acute Exposure Guideline Levels (AEGLs) (NAC 2003) developed higher short-term levels based on the numerous human experimental studies, which were

considered a more reliable basis for the short-term AEGLs than the single, uncontrolled study and background levels used by NASA.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendations for EEGL and CEGL values for formaldehyde are summarized in Table 5-4. The current and proposed U.S. Navy values are provided for comparison.

1-Hour EEGL

Exposure limits for formaldehyde should be set to prevent moderate irritation effects in the eyes and mucous membranes of submariners. Unfortunately, it is difficult to quantify a threshold for irritation because at the lowest levels the symptoms are subjective, individuals vary in sensitivity, and variable rates of background irritation occur in the population. Bender (2002) stated that even with the wealth of human and animal studies, a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) for irritation could not be determined for setting a reference concentration.

The subcommittee found that the most reliable data set for determining the 1-h and 24-h EEGLs was from the controlled experimental studies in humans rather than the relatively uncontrolled and uncertain Wisconsin mobile home study used to develop the 1-h SMAC of 0.4 ppm (NRC 1994). The 1-h SMAC is identical to the 1-h EEGL proposed by the Navy. On the basis of the controlled experimental studies in humans, the appropriate range for a short-term exposure concentration that would produce only mild to moderate irritation in almost all submariners is 1-3 ppm. The controlled chamber studies indicate that 1 ppm is the concentration at which reports of irritation begin to increase significantly over background and that by 3 ppm most subjects report mild to moderate irritation. Tests have been conducted at up to 3 ppm in a large number of human subjects. The few studies conducted in human subjects at concentrations above that range indicated more severe effects, and the animal data indicate that irreversible respiratory tissue damage can occur at concentrations above that range, although generally with repeated exposures.

A midrange concentration of 2 ppm was selected as the 1-h EEGL. A concentration of 2 ppm allows for some sensory irritation that is reversible

and should not interfere with critical duties, such as opening a hatch, but also protects against moderate eye irritation that could occur in a few individuals and interfere with duties. No uncertainty factors were considered necessary for the 1-h EEGL because of the robust data set from the controlled studies in human subjects, including potentially more sensitive individuals, such as nonsmokers, asthmatic individuals, and formaldehyde-sensitive individuals.

24-Hour EEGL

Because the irritation effects associated with airborne formaldehyde depend on concentration rather than the product of concentration and exposure duration ($C \times t$), the 24-h EEGL should be similar to the 1-h EEGL. However, because a few crew members might experience moderate irritation at 2 ppm, the subcommittee concluded that 2 ppm would not be as tolerable for a 24-h period. Therefore, the recommended 24-h EEGL is 1 ppm. At that concentration, most crew members should experience no irritation to mild irritation, and very few, if any, would experience moderate irritation. It is also likely that adaptation over the 24-h exposure period would further abate any discomfort experienced. The 24-h SMAC of 0.1 ppm, which is the same as the Navy's proposed value, was based on the lower end of the concentration range anticipated to cause eye irritation in 4% of subjects as reported in the Wisconsin mobile home study, in which

TABLE 5-4 Emergency and Continuous Exposure Guidance Levels for Formaldehyde

Exposure Level	U.S. Navy Values (ppm)		NRC Recommended Values (ppm)
	Current	Proposed	
EEGL			
1 h	3	0.4	2
24 h	1	0.1	1
CEGL			
90 days	0.5	0.04	0.3

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; h, hour; NRC, National Research Council; ppm, parts per million.

confounders, such as exposures to other irritants and smoking, were not controlled (NRC 1994). As noted above, the larger database of controlled studies in humans supports a much higher exposure level.

90-Day CEGL

Irritation also is the end point of greatest concern for subchronic and chronic exposures to formaldehyde, and concentrations that cause no irritation to moderate irritation (up to 3 ppm) are not associated with other irreversible adverse health effects. For longer exposure periods, however, it is more important to avoid discomfort from irritation. Although a threshold for irritation is difficult to set, even with the wealth of information on formaldehyde, that level is typically set on the basis of a concentration that would not cause irritation in any of the exposed individuals. Individual susceptibility to formaldehyde appears to be difficult to predict, and typically sensitive groups, such as asthmatic individuals, do not appear to be any more sensitive to irritation effects than healthy subjects at exposure concentrations below 3 ppm. On the basis of the information available, a concentration of 0.3 ppm is unlikely to result in discomfort in the submariner population. Reported symptoms of eye and mucous membrane irritation at that concentration were not increased above control conditions in controlled chamber studies (see Table 5-2). In workers, 0.3 ppm is the lower level at which most occupational studies begin to report increasing irritation in some individuals, most of whom simultaneously are exposed to formaldehyde and other irritant chemicals and substances. In the survey of 2,000 residents, which suffered from potential under-reporting of exposure concentrations, 0.3 ppm is the concentration above which a majority of subjects reported irritation or headache and above which rates of irritation could not be explained by smoking (Richie and Lehnen 1987).

CARCINOGENICITY ASSESSMENT

The EPA cancer unit risk factor for assessing the upper-bound cancer risk associated with inhaled formaldehyde was developed in 1991 (EPA 2003). EPA's slope factor assumes no low-dose threshold for cancer risk and is extrapolated from rates of nasal carcinomas in rats at formaldehyde concentrations above 5 ppm. Use of the EPA unit risk factor of 1.3×10^{-5} per microgram per cubic meter (1.6×10^{-2} per ppm) results in a theoretical

upper-bound excess risk over background of 5×10^{-3} (50 in 10,000) for continuous lifetime (estimated to be 70 years) exposure at the 0.3-ppm 90-day CEGL. Because the maximum length of cumulative exposure is estimated to be 5 years of a submariner's career, the theoretical upper-bound excess risk for a submariner at 0.3 ppm would be 3×10^{-4}.

In reality, the risk is far lower. On the basis of the evidence that the contributory mechanisms of action at high doses in rodents (that is, cytolethality and regeneration) would not occur at lower doses, the EPA unit risk factor for formaldehyde overestimates the risk at doses not associated with cytotoxicity. A two-stage clonal growth model developed by CIIT (1999) that was reviewed by an external scientific review panel convened by Health and Welfare Canada and EPA (Health Canada/EPA 1998) incorporates the scientific evidence in a nonlinear model for formaldehyde risk assessment. As of 2004, EPA has yet to revise the existing unit risk factor for formaldehyde.

The two-stage clonal growth model is based on the available data on rodent carcinogenicity, formaldehyde dosimetry in regions of the nose, pharmacokinetic differences between rodents and primates, and mutagenicity. The model incorporates two separate modes of action for carcinogenicity. At high doses, the dose-response relationship is primarily determined by cytotoxicity and regenerative cellular proliferation. The data suggest a curve shaped like a hockey stick or the letter "J," indicating a lower dose threshold for risk (CIIT 1999; Conolly et al. 2003). On the basis of the genotoxicity of formaldehyde, the model also assumes a low-dose linear mechanism related to direct mutagenicity, which is supported by data on DNA-protein cross-link formation (CIIT 1999; Conolly et al. 2003). Thus, on the basis of mutagenicity, lower doses conservatively are assumed to have a linear dose-response curve.

Without significant cytotoxicity and regenerative cellular proliferation, the slope of the dose-response relationship at low doses is much smaller than at high doses. Consequently, the threshold for zero difference from the control response has been predicted at 5.4 ppm with a 95% lower confidence limit of 2.7 ppm (Gaylor et al. 2004). The model also predicts separate dose-response outcomes for nonsmokers, mixed smoking, and smokers, with smoking resulting in a steeper slope. Research and analysis related to the CIIT (1999) model has been published in separate papers (Kimbell et al. 2001a,b; Overton et al. 2001; Conolly et al. 2003; Georgieva et al. 2003; Schlosser et al. 2003; Conolly et al. 2004; Gaylor et al. 2004). WHO (2002) and Health Canada (2001) relied on the model in their risk assessments for inhaled formaldehyde.

On the basis of the dose-response relationship presented for lower doses by CIIT (1999), the estimated risk for continuous lifetime exposure to formaldehyde at 0.3 ppm is substantially lower than that predicted by the current EPA unit risk factor (that is, lifetime risks on the order of 1×10^{-7} for nonsmokers and 3×10^{-6} for smokers). The risks associated with 5-year cumulative exposures over submariners' careers would be even lower.

In summary, the carcinogenicity assessment based on EPA's unit risk factor for formaldehyde indicates that exposure at the 0.3-ppm 90-day CEGL over a submariner's career would be associated with an upper-bound risk that is 3 times the risk goal of 1 in 10,000. The available evidence, however, strongly suggests that the risk from formaldehyde at high doses demonstrated in animals studies cannot be extrapolated to lower doses using the EPA's approach (Conolly et al. 2003; Gaylor et al. 2004). The more recent CIIT assessment results in a theoretical cancer risk well below the U.S. Department of Defense "acceptable" risk level of 1 in 10,000, even for lifetime exposure at the 0.3-ppm 90-day CEGL. The subcommittee concluded that the CIIT assessment more accurately reflects the scientific weight of evidence for formaldehyde carcinogenicity than does EPA's approach.

DATA ADEQUACY AND RESEARCH NEEDS

Formaldehyde has a relatively robust data set for developing health-protective exposure levels that includes controlled human studies, occupational and nonoccupational studies, and animal studies. Uncertainties for setting exposure levels include the short-term nature of controlled human studies (less than 24 h) and the apparent variation and subjectiveness in individual reporting and rating of irritation associated with formaldehyde. The variation is not related to the typical sensitivities of such subgroups as asthmatic individuals. Because the available evidence indicates that adaptation occurs with time, the lack of longer-term studies is not considered to be a serious data limitation for setting EEGLs. Continued research and publication on the low-dose carcinogenicity of formaldehyde will help support the confidence of the CEGL for protecting submariners from the effects of longer-term exposures to formaldehyde.

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6

Hydrazine

This chapter summarizes the relevant epidemiologic and toxicologic studies on hydrazine. Selected chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure levels from the National Research Council (NRC) and other agencies are also presented. The subcommittee considered all of that information in its evaluation of the Navy's proposed 1-hour (h), 24-h, and 90-day exposure guidance levels for hydrazine. The subcommittee's recommendations for hydrazine exposure levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

Hydrazine is a base slightly weaker in strength than ammonia that can function as a strong reducing agent or as an oxidizing agent under certain conditions (Schiessl 1995). At ambient temperatures, hydrazine is a fuming, colorless, oily, hygroscopic liquid with an ammonia-like odor (NRC 1996). Hydrazine vapors readily condense on surfaces at ambient temperatures. Odor thresholds of 2-3 parts per million (ppm) (Ruth 1986) and 3.7 ppm (Amoore and Hautala 1983) have been reported. Selected physical and chemical properties are summarized in Table 6-1.

OCCURRENCE AND USE

Hydrazine has numerous industrial applications (Schiessl 1995). It is used in the synthesis of many derivatives, including foaming or blowing

TABLE 6-1 Physical and Chemical Properties of Hydrazine[a]

Synonyms and trade names	Diamine, diamide, anhydrous hydrazine, hydrazine base, nitrogen hydride
CAS registry number	302-01-2
Molecular formula	NH_2NH_2
Molecular weight	32.05
Boiling point	113.5°C
Melting point	2.0°C
Flash point	52°C (open cup)
Explosive limits	4.7% to 100%
Specific gravity	1.0036 at 25°C/4°C
Vapor pressure	14.4 mmHg at 25°C
Solubility	Soluble in water and methyl, ethyl, propyl, and isobutyl alcohols
Conversion factors	1 ppm = 1.3 mg/m^3; 1 mg/m^3 = 0.76 ppm

[a]Data on explosive limits and vapor pressure are from ACGIH (2001); all other data are from Budavari et al. (1989).
Abbreviations: mg/m^3, milligrams per cubic meter; mmHg, millimeters of mercury; ppm, parts per million.

agents, polymers, antioxidants, fungicides, herbicides, insecticides, plant growth regulators, and pharmaceuticals, such as the antibiotic isoniazid. Because hydrazine is a strong reducing agent, it is used as an oxygen scavenger to prevent corrosion in boiler water and hot-water heating systems. Hydrazine has been used as a principal component of missile and rocket fuels and as a component of fuel cells used primarily for military applications.

Hydrazine is a component of tobacco smoke. The quantity of hydrazine in mainstream cigarette smoke ranges from 24 to 43 nanograms (ng) per cigarette and averages 32 ng per cigarette (Liu et al. 1974; Hoffmann and Hecht 1990). The quantity in sidestream smoke (smoke emitted from a smoldering cigarette) might be higher than in mainstream smoke (for example, 94 ng) (Liu et al. 1974).

Air samples collected aboard the USS *Cavalla* (USS *Cavalla* 1986) indicated a concentration of hydrazine at 0.5 ppm. No information on sampling protocol, location, operations, or duration was available, and no information concerning the sources of hydrazine aboard the USS *Cavalla* was provided (NRC 1988).

SUMMARY OF TOXICITY

Only data relevant to the derivation of EEGL and CEGL values for hydrazine are discussed below. For comprehensive reviews of hydrazine toxicology, see NRC (1996), the Agency for Toxic Substances and Disease Registry (ATSDR) (1997), the International Agency for Research on Cancer (IARC) (1999), and the American Conference of Governmental Industrial Hygienists (ACGIH) (2001).

Hydrazine vapor is a potent ocular and upper respiratory tract irritant in humans and common laboratory animals and is absorbed readily through intact skin, the lungs, and the gastrointestinal tract (Reinhardt and Brittelli 1981). Hydrazine is a convulsant at high doses (Witkin 1956) but can depress the central nervous system (CNS) at lower doses (Back and Thomas 1970). Tremors, pulmonary edema, and hepatotoxicity have been reported in people poisoned with hydrazine (Choudhary and Hansen 1998). The toxicity of multiple low doses is cumulative (NRC 1996). Hydrazine toxicity is concentration-dependent. As inhaled concentrations increased from 14 ppm to 225 ppm, the median time to death in rats decreased from 27 days to 4.5 days (Comstock et al. 1954).

Hydrazine has been tested in many experimental systems for genotoxicity. Although many of those tests resulted in negative or equivocal results, positive results were observed in gene mutation studies with bacteria, yeast, and *Drosophila melanogaster* (IARC 1999). In vivo studies of gene mutation and chromosomal effects have not resulted in consistent positive genotoxic results; however, DNA adducts were reported in three species following hydrazine exposure (IARC 1999). No genotoxicity studies via inhalation have been conducted in laboratory animals, and no data on genotoxicity are available for humans.

Nasal tumors have been observed in rats after repeated hydrazine inhalation. Duration of exposure was more significant than concentration in the production of hydrazine-induced nasal cancer in rodents (Latendresse et al. 1995). An epidemiologic study of workers in a hydrazine production plant reported no elevation in cancer risk among men exposed to hydrazine (Wald et al. 1984). A follow-up study of the cohort also failed to show significantly increased cancer risk (Morris et al. 1995). Those studies, however, considered only a small population and lacked rigorous industrial hygiene data. An epidemiologic study of men employed in rocket-engine testing jobs exposed to hydrazine and several other hazardous substances suggested an increased risk for lung and possibly other cancers (Ritz et al.

1999; Morgenstern and Ritz 2001). However, the potential cancer risk from inhalation exposures to hydrazine cannot be determined from the available human studies.

Effects in Humans

Accidental Exposures

Frierson (1965) described the consequences of accidental exposure to hydrazine and unsymmetrical dimethyl hydrazine (UDMH). One case involved a 36-year-old man who discovered a high concentration of hydrazine and UMDH while checking for leaks. He obtained an acid suit and respirator and continued to attempt to identify the source of the leak. He later complained of a burning sensation on his face, a sore throat, and a tight chest. He became pale and developed muscle twitching with clonic movements and pulmonary edema. In another case, a 44-year-old male pipe fabricator received a strong inhalation dose of hydrazine and UMDH and developed severe dyspnea, trembling, muscle weakness, and pulmonary edema. A third case involved the exposure of four men after a liquid hydrazine and UMDH spill. All of the men suffered from severe nausea and vomiting.

Sotaniemi et al. (1971) describes the death of a 59-year-old male machinist who handled hydrazine hydrate once each week over a period of 6 months. No account of his work practice or percutaneous hydrazine uptake was provided. He complained of conjunctivitis, tremors, and lethargy after each exposure. On the last day of his employment, he developed gastrointestinal distress and fever. On admission to the hospital, the patient presented with atrial fibrillation; stomatitis; conjunctivitis; upper abdominal pain and enlarged abdomen; jaundice and a tender, palpable liver; elevated bilirubin and creatinine; oligouria with protein and erythrocytes in his urine; and black feces. Chest X-rays revealed pleural effusions and shadowing. He died 15 days after hospitalization. Autopsy revealed tracheitis, bronchitis, and pneumonia; renal tubular necrosis, hemorrhage, and inflammation; and focal hepatocellular necrosis. An enlarged and discolored heart exhibiting degeneration of the cardiac muscle also was noted, but the relationship of that observation to hydrazine exposure was unclear. No empirical hydrazine concentrations were obtained, but subsequent simulations suggested a workplace air concentration of about 0.05 ppm.

Richter et al. (1992) described neurobehavioral impairment in many

parameters, including mood, memory, learning, comprehension, and concentration, in a water treatment technician occupationally exposed to hydrazine-containing mixtures. His condition improved over several years with the cessation of exposure.

There are at least three reports that describe the consequences of hydrazine ingestion (Drews et al. 1960; Reid 1965; Harati and Niakan 1986). Clinical signs and symptoms included vomiting, weakness, dyspnea, confusion, lethargy, ataxia, restlessness, and loss of consciousness.

Experimental Studies

No controlled experimental studies of hydrazine and its potential health effect were identified.

Occupational and Epidemiologic Studies

Contassot et al. (1987) provided an abstract summary of workplace exposures to hydrazine at <0.1, 0.1-1.0, or >1.0 ppm among 130 men. These men had been employed for at least 6 months. Analyses suggested that the standardized incidence ratio (the ratio of the number of cases observed to the number of new cases expected on the basis of age-specific rates) achieved statistical significance for an excess of all cancers in the high-exposure group, but that ratio was reduced when skin cancers were excluded from consideration.

Wald et al. (1984) studied 427 men who experienced varying levels of hydrazine exposure at a plant in the United Kingdom. The facility produced 700 tons of hydrazine per year from 1945 to 1971. Hydrazine exposures were estimated on the basis of simulated spills. Airborne hydrazine concentrations in the general plant environment were estimated to be 1-10 ppm, whereas the concentrations near storage vessels were estimated to be up to 100 ppm. Workers were categorized by severity of exposure. Exposures at 1-10 ppm were considered high, and exposures at <1 ppm were considered moderate to low. There were 1,565 man-years in the high-exposure group and 6,786 man-years in the moderate-to-low exposure group. Overall mortality among hydrazine-exposed employees was lower than expected (49 vs 61.47 expected). Mortality rates from lung cancer (5 vs 6.65 expected), other types of cancer (7 vs 9.27 expected), and all other causes (37 vs 45.55 expected) were similar to the expected values.

Morris et al. (1995) provided a follow-up of 95% of the 427 workers initially evaluated by Wald et al. (1984) and Roe (1978), adding 10 years of observation time for this population. There were no increases in mortality from all causes (86 total deaths; standardized mortality ratio [SMR] = 0.75), from lung cancer (8 deaths; SMR = 0.66), from digestive tract cancers (9 deaths; SMR = 0.95), or from cancers at other sites (8 deaths; SMR = 0.76) compared with rates for England and Wales. Among workers with the highest levels of exposure, there were three deaths from lung cancer (SMR = 1.08) and 20 total deaths (SMR = 0.74). Of the three lung cancer deaths, two occurred in workers who were exposed to hydrazine for less than 2 years. None of the SMR values were significantly different from 1.

Morgenstern and Ritz (2001) and Ritz et al. (1999) described an occupational cohort of 6,107 men involved in rocket-engine fueling and testing who were potentially exposed to hydrazine, 1-methylhydrazine, and 1,1-dimethylhydrazine for at least 2 years. Mean follow-up time was 29 years; only 23% of the cohort died in that time. Workplace exposures might also have included asbestos, beryllium, chlorine, fluorine, hydrogen peroxide, isopropyl alcohol, kerosenes, nitric acid, rocket-engine exhaust, and chlorinated solvents (Ritz et al. 1999). Workers were assigned to exposure groups on the basis of exposure severity. Propulsion or test mechanics or technicians involved in hydrazine pumping were in the high-exposure category; propulsion or test inspectors, test or research engineers, and instrumentation mechanics were in the medium-exposure category; and workers with little opportunity for direct hydrazine exposure were in the low-exposure category. All subjects had engaged in at least 6 months of service in their job category. The data showed reduced mortality rates from all cancers and from all causes compared with rates for white males in the United States. The all-cause SMR also was consistent with that observed for other high-socioeconomic-status workers. No excess lung cancer mortality was seen in the medium-exposure group, but the lung cancer rate ratio (RR) for the high-exposure group compared with unexposed workers ranged from 1.68 (95% confidence interval [CI] = 1.12-2.52) to 2.10 (95% CI = 1.36-3.25) depending on exposure duration and lag time for hydrazine exposure. Rate ratios for lymphopoietic cancers and urinary tract cancers increased with exposure. When examined by decade of employment, lung (RR = 2.01; 95% CI = 1.21-3.33) and lymphopoietic (RR = 2.45; 95% CI = 0.91-6.58) cancer risks were increased for those who were working during the 1960s, a time when rocket-engine test firings and hydrazine fuel consumption were at their highest levels. Despite the study limitations (collapsing heterogeneous cancers by organ system, exposure estimated by job title,

possible exposure to solvents and other materials, reliance on mortality instead of cancer incidence data, and the inability to control completely for tobacco smoking), Ritz et al. (1999) reached the following conclusion: "occupational exposure to hydrazine or other chemicals associated with rocket-engine testing jobs increased the risk of dying from lung cancer and possibly other cancers."

Effects in Animals

Acute Toxicity

The 1-h LC_{50} (concentration lethal to 50% of subjects) for hydrazine in hamsters (whole-body exposure) was 2,585 ppm (Back et al. 1978). Hydrazine exposures induced alopecia and lung, liver, and kidney damage in the exposed animals. The 4-h LC_{50} for hydrazine in rats (570 ppm) was somewhat greater than that in mice (252 ppm) (Jacobson et al. 1955). After a single 1-h exposure at an average concentration of 80 ppm, male Wistar rats salivated, some developed convulsions, and one of six rats died (Comstock et al. 1954). However, the subcommittee questions the accuracy of the reported hydrazine concentration because Latendresse et al. (1995) found a maximum 1-h nonlethal concentration in five male and five female Fischer 344 rats at 750 ppm. When 10 adult male hamsters and five adult male and five adult female Fischer 344 rats inhaled hydrazine at 750 ppm for 1 h, the transitional, respiratory, and olfactory epithelium in the anterior nasal passages showed bilateral necrosis and exfoliation (Latendresse et al. 1995). Apoptosis in the posterior olfactory epithelium was also noted in some rats.

CMA (1993) exposed Sprague-Dawley rats to an aqueous aerosolized 64% solution of hydrazine for 1-h. The 1-h LC_{50} values estimated for hydrazine alone were 4,420 ppm for males and 2,590 ppm for females.

Topical hydrazine can produce chemical burns, and it is absorbed through the skin in amounts sufficient to precipitate systemic intoxication and death (Smith and Clark 1972). The rat single-dose oral LD_{50} (60 milligram per kilogram [mg/kg]) (Witkin 1956) is similar to a lethal dose observed in dogs following dermal exposure (96 mg/kg) (Smith and Clark 1972) and the dermal LD_{50} values reported in rabbits and guinea pigs (93-190 mg/kg) (Rothberg and Cope 1956). Thienes et al. (1948) investigated the ocular toxicity of hydrazine and reported that one drop instilled in rat or rabbit eyes caused permanent damage. Six drops of an aqueous 25% solu-

tion applied at a rate of 1 drop per 10 minutes (min) also caused permanent damage, but a 1% solution produced no visible reaction.

Repeated Exposures and Subchronic Toxicity

Weatherby and Yard (1955) exposed eight male guinea pigs to hydrazine at 2-5 ppm for 6 h per day, 5 days per week for 9 days. During that time, no signs of hydrazine intoxication were observed. From day 10 until day 69, hydrazine concentrations were increased to 3-6 ppm. Six of the eight guinea pigs survived and appeared in good health at study termination. Necropsy revealed pulmonary lymphoid hyperplasia, diffuse atelectasis, and evidence of an inflammatory infiltrate. Weatherby and Yard (1955) exposed two male mongrel dogs to hydrazine at 2-5 ppm for 6 h per day, 5 days per week for up to 7 days. Both dogs became lethargic and lost coordination by day 5. At day 7, one died, and the other was killed in extremis. When one male and one female dog were exposed at 3-6 ppm for 6 h, the male showed signs of hydrazine poisoning within 24 h. After 19 days of hydrazine exposure, the study was terminated and tissues were collected. The dogs' livers showed marked fatty infiltration into the central zone with areas of hepatocellular necrobiotic hyalinization and distention of the biliary canaliculi. The proximal renal cortex was congested, and capillary endothelium showed moderate hyperplasia.

Haun and Kinkead (1973) conducted inhalation studies with 97% anhydrous hydrazine using 50 male Sprague-Dawley rats, 40 female ICR mice, eight male beagles, and four female rhesus monkeys per exposure group. The protocol used two designs: (1) continuous exposures at 0.2 or 1 ppm for 24 h per day, 7 days per week (33.6 or 168 ppm-h per week) for 6 months and (2) intermittent exposures at 1 or 5 ppm for 6 h per day, 5 days per week (30 or 150 ppm-h per week) for 6 months. Minimal eye irritation was noted in monkeys continuously exposed at 1 ppm and intermittently exposed at 5 ppm during the first few weeks of the study and periodically thereafter. None of the primates died under either protocol. One of the dogs continuously exposed at 1 ppm developed tonic convulsions—one episode after 3 months of exposure and two episodes on the same day after 5 months of exposure. Two dogs died after 16 weeks of continuous exposure at 1 ppm. Within 8 weeks, 2-7% of the mice exposed intermittently at 1 ppm or continuously at 0.2 ppm died, and 35-40% exposed intermittently at 5 ppm or continuously at 1 ppm died. No clinical signs or hydrazine-related mortality was reported for the rats. Body weights

of exposed monkeys were not significantly different from those of controls. Body weights were depressed in dogs continuously exposed at 1 ppm, and a dose-dependent decrease in the body weights of rats was observed. In rats exposed continuously at 0.2 ppm, the decrease was not statistically significant after 10 weeks of exposure. Mice were not weighed. Hematologic and clinical chemistry measurements were normal in monkeys and rats; reduced erythrocyte count, hematocrit, and hemoglobin developed within 8 weeks in dogs exposed either continuously at 1 ppm or intermittently at 5 ppm but returned to normal within 2 weeks post-exposure in the two dogs evaluated from those exposure groups. Hematology and clinical chemistry parameters were not evaluated in the mice. Hepatic fatty infiltration was evident in mice at all exposure concentrations, which is consistent with observations of dogs that inhaled hydrazine at 3-6 ppm for 19 days reported in Weatherby and Yard (1955). Slight to moderate hepatic fat accumulation was found in exposed monkeys, but that accumulation was also observed to some degree in the control monkeys. Dogs exposed continuously at 1 ppm or intermittently at 5 ppm also developed fatty livers. Bronchopneumonia was reported in rats exposed intermittently at 5 ppm. It was not clear whether the finding had any relationship to hydrazine exposure.

House (1964) conducted a 90-day continuous (24 h per day, 7 days per week) inhalation study with hydrazine (95%) in 10 male rhesus monkeys, 50 male Sprague-Dawley rats, and 100 male ICR mice. Animals were exposed to an average hydrazine concentration of 0.78 ppm (range, 0.25-1.38 ppm). Separate groups of equal numbers of animals were housed in an adjacent room and served as concurrent controls throughout the exposure period. After the first day, the treated monkeys developed reddish faces and swollen eyes. They became weak and thin throughout the exposure period and exhibited reduced food and water consumption. Two of the treated monkeys died, one on day 30 and the other on day 85; a control monkey died on day 30. The body weights of the treated monkeys began to decrease after day 45. Clinical chemistry and urinalysis parameters appeared normal. Necropsy demonstrated hepatic fatty infiltration in 7 of the 10 treated monkeys; two of the concurrent controls showed similar changes. Two treated monkeys also exhibited mild congestion of the liver, and one of them developed fatty liver. Calcification in the adrenal glands (2 of 10), kidney (3 of 10), and heart (3 of 10) were observed in treated monkeys. Renal congestion and nephritis, adrenal calcification, and cardiac dilatation were observed similarly in both exposed and control monkeys. Whole-body exposures of male Sprague-Dawley rats and male ICR mice killed 98-99% of those animals; the majority of the rats died between days 46 and 64, and

the majority of the mice died within the first 30 days of exposure. Animals were "weak and sick early in the test," and water and food consumption declined obviously until death.

Ten weeks of 1-h exposures, one per week at 750 ppm produced a significant reduction in body weights in adult male and female Fischer 344 rats and male Syrian hamsters (Latendresse et al. 1995). No hydrazine-induced mortality occurred in rats or hamsters. Inhaled hydrazine induced acute inflammation, exfoliation, desquamation, necrosis, and squamous metaplasia in the nasal transitional epithelia. At 28-30 months after cessation of exposure, polypoid adenomas (in 4 of 99 males and 6 of 95 females) and a squamous cell carcinoma (in 1 of 99 males) were found in rats. The authors considered the apoptosis found in the olfactory epithelium and the squamous metaplastic transitional epithelium to be an adaptive response. Hamsters exhibited nasal transitional hyperplasia in 2 of 94 and frank neoplastic transformation (polypoid adenomas) in 3 of 94. No such changes were observed in any of the concurrent controls.

Latendresse et al. (1995) also exposed adult male and female Fischer 344 rats and male Syrian hamsters to hydrazine at 75 ppm for 1 h each week for 10 weeks and held them post-exposure for 24 to 30 months. The body weights of female rats were significantly reduced during the exposure period. Proliferative lesions (focal squamous epithelial hyperplasia and squamous cell carcinoma) were observed in 2.2% of male rats (2 of 93). A nasal polypoid adenoma was observed in one hamster.

Chronic Toxicity

MacEwen et al. (1981) and Vernot et al. (1985) published the results of a hydrazine inhalation study conducted in male and female Fischer 344 rats, female C57BL/6 mice, male Syrian hamsters, and 6-month-old male and female beagle dogs.

Groups of 100 rats were exposed to hydrazine at 0.05, 0.25, 1.0, or 5.0 ppm for 6 h per day, 5 days per week for 52 weeks and were maintained for an additional 18 months post-exposure. Rat mortality at termination of the study was similar in all groups. Body weights of rats were decreased compared with controls; the most significant effect was observed in male rats exposed at 5 ppm. Significantly increased incidences of non-neoplastic lesions primarily were observed in the nasal cavity (squamous metaplasia and epithelial hyperplasia), larynx (squamous metaplasia and inflammation), and trachea (squamous metaplasia and inflammation) of male and

female rats exposed at 5 ppm; however, squamous metaplasia of the nasal cavity was not significantly increased in the females. Lymph node hyperplasia was significantly increased in females exposed at 5 ppm, and hepatic focal cell hyperplasia was significantly increased in females exposed at 1 and 5 ppm. Statistically significant changes in tumor incidence included increases in nasal adenomatous polyps in females exposed at 5 ppm and in males exposed at 1 and 5 ppm; increases in nasal villous polyps in males exposed at 5 ppm; and increases in thyroid carcinomas in males exposed at 5 ppm. The nasal tumors were associated with chronic local irritation, and most of the rat nasal tumors were seen at 12 months post-exposure. The first appearance of nasal tumors in male and female rats occurred at 20 and 23 months, respectively, following initiation of exposure.

Groups of 400 female C57BL/6 mice were exposed to hydrazine at 0.05, 0.25, or 1.0 ppm and maintained for 15 months post-exposure. No non-neoplastic pathology could be detected after hydrazine exposure. An increase in pulmonary adenomas observed in mice exposed at the highest concentration was marginal compared with concurrent controls. When those results were compared with data from an additional control group of 385 female C57BL/6 mice, there were no significant differences. Vernot et al. (1985) noted that the historical control incidence of pulmonary adenomas in C57BL/6 mice was 2-3% and considered the 3.2% increase observed at 1.0 ppm to be consistent with the background rate in that strain. Higher concentrations could not be assessed due to the high mortality indicated in previous studies (Haun and Kinkead 1973; MacEwen et al. 1974).

Groups of 200 hamsters exposed to hydrazine at 0.25, 1.0, or 5.0 ppm experienced increased mortality during the early phase of the study. All groups exhibited decreased body weights compared with controls; however, only animals exposed at 5 ppm showed significantly decreased body weights in the final months of the study. Hepatic, renal, and adrenal amyloidosis were increased significantly in all exposed groups, but 22-23% of the concurrent controls exhibited the same conditions. Amyloidosis was also significantly increased in the spleens of animals exposed at 1 and 5 ppm and in the thyroids of animals exposed at 0.25 and 5 ppm. Exposure-related amyloidosis, hemosiderosis, testicular senile atrophy, and bile duct hyperplasia appeared to reflect accelerated age-related degeneration. The only significant increase in tumors was restricted to the 16 hamsters in the 5.0-ppm exposure group (n = 160) that had nasal adenomatous polyps.

Dogs were exposed to hydrazine by inhalation at 0.25 and 1.0 ppm and were maintained for 38 months post-exposure. Hematology and clinical chemistry parameters appeared normal during the exposures. Although liver

function tests indicated no exposure-related hepatic effects, one of the eight dogs exposed at 1.0 ppm developed increased serum glutamic-pyruvic transaminase after exposure. At necropsy—36 months after cessation of exposure—swollen, vacuolated hepatocytes were observed in the dog. One dog exposed at 0.25 ppm developed tumors (hemangioma of the splenic capsule and papillary carcinoma of the anus); however, the authors considered those findings to be unrelated to hydrazine exposure.

Reproductive Toxicity in Males

No relevant studies were identified.

Immunotoxicity

Allergic contact dermatitis related to hydrazine exposure is common among people who do not take precautions to prevent skin contact (ATSDR 1997; ACGIH 2001). Also, Reidenberg et al. (1983) described the case of an adult woman intermittently exposed to hydrazine who developed a photosensitive rash, fatigue, antinuclear antibodies, and antibody to DNA and showed a positive reaction to a hydrazine exposure that simulated occupational exposure. Reidenberg et al. (1983) concluded that hydrazine exposures can induce a lupus erythematosus-like disease, possibly similar to that seen in patients ingesting hydralazine. Bigazzi (1997) listed hydrazine as a xenobiotic associated with systemic lupus erythematosus-like syndrome.

Genotoxicity

Assessing the genotoxicity of highly reactive chemicals can be particularly problematic. Hydrazine is a very strong reducing agent, and under certain conditions it can be an oxidant. Hussain and Frazier (2002) demonstrated that 4 h of hydrazine exposure (25 millimolar [mM]) in rat hepatocytes in vitro depleted reduced glutathione, increased oxidized glutathione, increased reactive oxygen species generation, increased lipid peroxidation, and reduced catalase, suggesting that hydrazine induced intracellular oxidative stress. Because intracellular oxidative stress has been associated with oxidative damage to DNA, it should not be surprising that exposures

at oxidant concentrations that exceed the capacity of antioxidant systems are likely to result in oxidant DNA damage. Highly reactive chemicals also might interact with the DNA in bacterial systems differently than they do with DNA in mammalian systems. Because bacterial DNA is not isolated and enclosed by a nuclear membrane, it is more susceptible to reactive chemicals than mammalian DNA. In mammalian cells, reactive chemicals are more likely to interact with the cytoplasmic constituents, which provide a buffer between the point-of-entry and the nucleoplasm. Isolating alkylated or oxidized DNA from animals treated with reactive chemicals also can be problematic. For chemicals that cause cell necrosis at the concentrations being studied for alkylation or oxidation, the presence of DNA from necrotic cells can confound the isolation of DNA from viable cells and yield false positive results. Confounding of results also can occur when DNA is collected from cells that contain residual reactive chemical. In this case, confounding can be reduced by isolating and washing intact nuclei before isolating the DNA from treated cells (English et al. 1994).The highly reactive nature of hydrazine makes all of these potential problems applicable when interpreting genotoxicity study results.

The Agency for Toxic Substances and Disease Registry (ATSDR) (1997) and the International Agency for Research on Cancer (IARC) (1999) compiled reports on hydrazine and hydrazine hydrate mutagenicity in *Salmonella typhimurium*, *Photobacterium leiognathi*, *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *D. melanogaster* and in cultured mouse lymphoma cells. Although the study results for bacterial systems in *E. coli* and *S. cerevisiae* are consistently positive for genotoxicity, study results in *S. typhimurium* are highly variable and often show no dose-response relationship when examined across studies (IARC 1999). Somatic mutation studies with *D. melanogaster* are positive for mutagenicity, although studies for sex-linked recessive lethal mutations are negative or equivocal (IARC 1999). Hydrazine treatment increased sister chromatid exchange in cultured Chinese hamster ovary cells and induced alkylation of calf thymus DNA. As the hydrate or sulfate, hydrazine induced unscheduled DNA synthesis in cultured mouse hepatocytes and increased DNA strand breaks and unscheduled DNA synthesis in cultured rat hepatocytes. In vivo, hydrazine exposure resulted in a positive mouse spot test and in DNA strand breaks in mouse liver and lung cells (IARC 1999). In vivo, hydrazine failed to induce sister chromatid exchanges in mouse bone marrow or liver cells, weakly induced bone marrow cell micronuclei in one of three studies, did not induce dominant lethal mutations in mice, and did not induce sperm abnormalities in mice (IARC 1999).

Oral or parenteral hydrazine administration to hamsters, rats, and mice, typically at acutely toxic doses, resulted in the formation of N7-methylguanine and O^6-methylguanine adducts in hepatic DNA (IARC 1999). The significance of these results must be viewed in terms of the doses, because as Leakakos and Shank (1994) demonstrated in neonatal rats, hydrazine induced methylguanines in liver DNA only when the dose was necrogenic.

Carcinogenicity

At least three studies quantify the carcinogenic potential of inhaled hydrazine in rodents. MacEwen et al. (1974) presented the results of the long-term post-exposure observation of rats (10 per group) and mice (10 per group) exposed to hydrazine by inhalation for 6 months by Haun and Kinkead (1973). Because of chronic brochopneumonia, an insufficient number of rats survived to assess the carcinogenic potential of inhaled hydrazine. The numbers of mice with alveolargenic carcinomas increased with exposure concentration (one of eight at 0 ppm; two of six at 1 ppm, intermittent; three of eight at 0.2 ppm, continuous; five of six at 5 ppm, intermittent; and five of nine at 1 ppm, continuous). Invasion of the pleura and pleural space and metastases occurred with some of the alveolargenic carcinomas. In mice exposed at 5 ppm intermittently, the tumors exhibited an increased frequency of metastatic activity. Tumors were found in the liver and in the intercostal muscle. Lymphosarcoma of the spleen with invasion of the capsule (in two of nine) and a hepatoma (in one of nine) were found in a few mice exposed at 1 ppm continuously. In the mouse that had the hepatoma, the spleen was replaced almost entirely by neoplastic tissue. The authors considered the data to be consistent with the induction of murine lung tumors observed after oral exposures to hydrazine sulfate. The authors also noted the similarity of the single hepatoma observed after hydrazine inhalation to those reported after oral exposures to hydrazine sulfate.

Rats and hamsters chronically exposed by inhalation to hydrazine at 5 ppm for 6 h per day, 5 days per week for 1 year (Vernot et al. 1985) or subchronically exposed at 750 ppm for 1 h each week for 10 weeks (Latendresse et al. 1995) developed proliferative lesions, including epithelial hyperplasia and nasal tumors. Hydrazine-induced nasal tumors were principally polyploid adenomas arising from the nasal turbinates in the proximal nasal airways, which receive high inspiratory airflows resulting in high regional deposition of many reactive xenobiotics (Kimbell et al.

1993). Because of the intranasal location of these nasal tumors and the morphologic characteristics of the adenomas, Latendresse et al. (1995) concluded that the tumors arose from the nasal transitional epithelium. Because the investigators found only mild or minimal rhinitis in exposed rodents, they also suggested that hydrazine-induced local inflammation likely played a minimal role, if any, in the promotion and progression of the tumors. This conclusion is unlike that made in the case of rats and mice chronically exposed to formaldehyde. In that case, ongoing nasal inflammation and epithelial cytotoxicity were closely associated with the induction of nasal tumors (Liteplo and Meek 2003). The nasal tumors induced in rodents by chronic inhalation of formaldehyde also were morphologically different from those induced by hydrazine; they were principally squamous cell carcinomas rather than adenomas, and they stemmed from regions of the nasal cavity that are normally lined by both transitional and respiratory epithelium. Therefore, given the apparent differences in nasal epithelial and inflammatory responses elicited by hydrazine and formaldehyde, it has been suggested that hydrazine might induce rodent nasal tumors by a somewhat different mechanism than formaldehyde.

Hydrazine and formaldehyde produce cytotoxicity and regenerative hyperplasia at the doses associated with tumors, although these tumors potentially differ in location and type, and the role of inflammation in tumor development also might differ. For hydrazine, mechanistic studies to establish the role of acute cytotoxicity in the development of tumors in the nasal transitional epithelium of rodents are not available. The relationship between the early cytotoxic changes observed in rodents acutely or subchronically exposed to hydrazine (epithelial degeneration, necrosis and exfoliation, and subsequent regenerative hyperplasia and metaplasia) and the later development of nasal tumors was not determined by Latendresse et al. (1995).

Chronic oral bioassays in mice, rats, and hamsters have demonstrated the unequivocal carcinogenic activity of hydrazine in rodents (IARC 1999; ACGIH 2001). Dose-dependent hepatocellular carcinomas in hamsters (Bosan et al. 1987); hepatocellular adenomas, carcinomas, and cholangiomas in rats (IARC 1999); and pulmonary adenomas, pulmonary carcinomas, hepatocarcinomas, myeloid leukemia, lymphomas, and reticulum cell sarcomas in mice (ACGIH 2001) are typical consequences of chronic hydrazine ingestion. On the basis of the animal bioassay data, hydrazine was classified by IARC (1999) as possibly carcinogenic to humans (Group 2B) and by the U.S. Environmental Protection Agency (EPA 1991) as a probable human carcinogen (B2).

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

Hydrazine kinetics and metabolism have not been fully characterized. Hydrazine is eliminated in rat urine as acetylhydrazine, diacetylhydrazine, and 1,4,5,6-tetrahydro-6-oxo-3-pyridazine carboxylic acid (Sanins et al. 1992). Hydrazine and acetylhydrazine have been measured in the urine of humans exposed to hydrazine (Koizumi et al. 1998). The fate of hydrazine depends on dose and route of administration. For example, Llewellyn et al. (1986) administered hydrazine to rats by inhalation at 10-500 ppm for 1 h and found that 2-10% of the absorbed dose was eliminated as urinary hydrazine, 1.7-4% was eliminated as acetyl hydrazine, and 4.5-11.4% was eliminated as diacetylhydrazine. Springer et al. (1981) administered an oral dose of [^{15}N]hydrazine at 32 mg/kg to rats and found that about 30% appeared in urine as hydrazine and about 20% appeared as an acid-hydrolyzable derivative. Rats exhaled 25% of the dose as nitrogen within 30 min of dosing. Exhaled ^{15}N increased only slightly with dose. Hydrazine elimination appeared to be biphasic, with half-lives of 0.74 and 26.9 h. Hydrazine, acetyl, diacetyl and hydrazone metabolites, and 1,4,5,6-tetrahydro-6-oxo-3-pyridazine carboxylic acid were detected in the urine of rats administered hydrazine at 427 mg/kg (Preece et al. 1991). There was evidence of the production of ammonia and urea, suggesting enzymatic hydrolysis of the N-N bonds by N-oxidation.

There are distinct, quantifiable pharmacogenetic differences in the rates at which humans metabolize hydrazine (Koizumi et al. 1998). People who are slow acetylators have less functional polymorphic *N*-acetyltransferase (NAT2), and they accumulate higher concentrations of circulating hydrazine than do those capable of rapid acetylation (Blair et al. 1985). Some 29 different allelic variants of NAT2 have been identified (Mitchell and Warshawsky 2003). The frequency of each acetylation phenotype is race-dependent. For example, Japanese are generally fast acetylators; slow acetylators account for about 10% of Japanese and about 50% of Caucasians (Deguchi et al. 1990; Hickman and Sim 1991).

Koizumi et al. (1998) evaluated 297 Japanese hydrazine workers and found the rapid acetylation phenotype in 45%, the intermediate in 45%, and the slow in the remaining 10%. The authors then calculated the biological half-life of hydrazine in 12 Japanese hydrazine workers (age 44 ± 14 years): four rapid acetylators, four intermediate acetylators, and four slow acetylators. All 12 were employed at the same Shikoku district plant, and personal sampling found 8-h time-weighted average (TWA) hydrazine exposures

ranging from 0.07 to 0.12 ppm. There were no significant differences in inhaled hydrazine concentrations between the groups. Prior to work shifts, urinary concentrations of hydrazine and acetylhydrazine in urine were not detectable. Acetylhydrazine accounted for 3-5% of total urinary hydrazine immediately after and up to 36 h after hydrazine exposure ceased. Elimination half-times were significantly different between the three phenotypes: 3.9 ± 1.7 h for slow acetylators, 2.3 ± 0.4 h for intermediate acetylators, and 1.9 ± 0.7 h for the rapid acetylators.

The mechanism by which inhaled hydrazine results in nasal tumors in rodents has not been investigated. The relationships between hydrazine cytotoxicity, cell degeneration, and necrosis—factors that are fundamental in rodent nasal cancers caused by formaldehyde (Liteplo and Meek 2003)—have not been studied in detail (Latendresse et al. 1995). Because Latendresse et al. (1995) found only mild or minimal rhinitis in hydrazine-exposed rodents, they suggested that hydrazine-induced local inflammation probably played a minimal role, if any, in the promotion and progression of nasal tumors. Positive in vitro genotoxicity and DNA methylation studies suggest that hydrazine might act through a genotoxic mechanism, although evidence for in vivo genotoxicity at doses lower than those that cause overt tissue damage is lacking. There are no studies of hydrazine genotoxicity following inhalation exposure. There are no genotoxicity data available for humans via any exposure route, and epidemiologic data are considered inadequate for determining the carcinogenic potential of hydrazine in humans.

INHALATION EXPOSURE LEVELS FROM THE NRC AND OTHER ORGANIZATIONS

A number of organizations have established or proposed exposure limits or guidelines for inhaled hydrazine. Selected values are summarized in Table 6-2.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendations for EEGL and CEGL values for hydrazine are summarized in Table 6-3. The proposed U.S. Navy values are provided for comparison.

1-Hour EEGL

NRC (1996) discussed the difficulties of deriving a 1-h hydrazine exposure limit. No rigorous exposure data from workplace studies (Morris et al. 1995; Ritz et al. 1999) or accounts of human intoxication (Frierson 1965; Sotaniemi et al. 1971) are available. Percutaneous uptake of hydrazine through intact dog skin is rapid (30 seconds) (Smith and Clark 1972) and confounds the estimation of total dose in reports of acute inhalation toxicity. No physiologically based pharmacokinetic (PBPK) model for hydrazine is available, and no pulmonary absorption data are available to assist in determining absorbed dose. Therefore, the 1-h EEGL was by necessity, based on chamber air concentrations used in controlled inhalation studies in animals.

House (1964) indicated that the eyelids of adult male rhesus monkeys became swollen during the initial 24 h of exposure to hydrazine at 0.4 ppm (the mean concentration for the first 10 days of a 90-day study that had an overall concentration range of 0.25-1.38 ppm). A similar inhalation study involving continuous exposure at 1 ppm for 24 h per day, 7 days per week for 6 months or intermittent exposure at 5 ppm for 6 h per day, 5 days per weeks for 6 months in four female rhesus monkeys resulted in minimal ocular irritation during the first few weeks of the study (Haun and Kinkead 1973).

In the absence of controlled ocular and nasal irritation data for human beings, the data from controlled inhalation studies in monkeys were considered to be the most relevant. The House (1964) study describes difficulties in maintaining constant exposure concentrations, and the controlled conditions used by Haun and Kinkead (1973) are considered more reliable than those reported by House (1964). Thus, the continuous hydrazine exposure concentration of about 1 ppm associated with eye irritation in rhesus monkeys was considered appropriate for deriving the 1-h EEGL. Although the 1-ppm concentration was associated with ocular irritation, those changes were reversible and were not life-threatening. No uncertainty factors were applied because the primary acute effect of hydrazine is the result of direct contact irritation, which should be similar for both monkeys and humans, and because this irritation is not likely to vary among individuals of the same species. Therefore, the recommended 1-h EEGL is 1.0 ppm.

TABLE 6-2 Selected Inhalation Exposure Levels for Hydrazine from NRC and Other Organizations[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	0.01	ACGIH 2000
NIOSH	REL-Ceiling	0.03	NIOSH 2004
OSHA	PEL-TWA	1	29 CFR 1910.1000
Spacecraft			
NASA	SMAC		NRC 1996
	1 h	4	
	24 h	0.3	
	30 days	0.02	
	180 days	0.004	
General Public			
ATSDR	Intermediate MRL	0.004	ATSDR 1997
NAC/NRC	Proposed AEGL-1 (1 h)	0.1	EPA 2004
	Proposed AEGL-2 (1 h)	13	
	Proposed AEGL-1 (8 h)	0.1	
	Proposed AEGL-2 (8 h)	1.6	
NRC	SPEGL		NRC 1985
	1 h	0.12	
	24 h	0.005	

[a]The comparability of EEGLs and CEGLs with occupational and public health standards or guidance levels is discussed in Chapter 1, section "Comparison to Other Regulatory Standards or Guidance Levels."

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL, acute exposure guideline level; ATSDR, Agency for Toxic Substances and Disease Registry; h, hour; MRL, minimal risk level; NAC, National Advisory Committee; NASA, National Aeronautics and Space Administration; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Health and Safety Administration; PEL, permissible exposure limit; ppm, parts per million; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; SPEGL, short-term public emergency guidance level; TLV, Threshold Limit Value; TWA, time-weighted average.

24-Hour EEGL

Continuous 24-h hydrazine exposures at 0.2 or 1.0 ppm in groups of four female rhesus monkeys, lasting 24 h per day 7 days per week for 6 months failed to increase mortality (Haun and Kinkead 1973; MacEwen et

TABLE 6-3 Emergency and Continuous Exposure Guidance Levels for Hydrazine (ppm)

Exposure Level	U.S. Navy Values		NRC Recommended Values
	Current	Proposed	
EEGL			
1 h	—	4	1
24 h	—	0.3	1
CEGL			
90 days	—	0.01	0.03

Abbreviations: CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level; h, hour; NRC, National Research Council; ppm, parts per million.

al. 1974). Minimal eye irritation was observed in monkeys exposed at 1.0 ppm. There were no significant differences in hematologic parameters (hemocrit, hemoglobin, erythrocyte and leukocyte counts, differential count and reticulocyte counts), clinical chemistry profiles (sodium, potassium, cholesterol, calcium, phosphorus, total bilirubin, alubumin:globulin ratios, total protein, blood urea nitrogen [BUN], serum glutamic-oxaloacetic transaminase [SGOT], serum glutamic-pyruvic transaminase [SGTP], chloride, creatinine, triglycerides, glucose, alkanine phosphatase), or body weights between control and hydrazine-treated monkeys.

At termination of the 6-month study, the livers of the control monkeys showed "some degree of fatty liver change" (MacEwen et al. 1974). The livers from hydrazine-treated monkeys showed slight to moderate hepatic fat infiltration. Organ weights (organs not specified) from monkeys exposed to hydrazine were not statistically different from those of the controls (Haun and Kinkead 1973).

The 24-h EEGL was based on the results of the 6-month continuous exposure study in rhesus monkeys conducted by Haun and Kinkead (1973). The primary treatment-related consequence of continuous exposure to hydrazine at 1.0 ppm was ocular irritation judged to be "minimal" by the authors. The process of identifying the inter- and intraspecies uncertainty factors for the 24-h EEGL was identical to that described for the 1-h EEGL. Therefore, the recommended 24-h EEGL is 1.0 ppm.

90-Day CEGL

A 90-day CEGL for hydrazine exposure for noncancer effects can be derived from the hydrazine inhalation data from monkeys. The subcommit-

tee found that the size, anatomy, and physiology of the respiratory tract of the monkey made it an appropriate model for humans. Continuous exposure, as would occur during a 3-month operational tour aboard an Ohio class submarine, is best represented by the 90-day (House 1964) to 180-day (Haun and Kinkead 1973; MacEwen et al. 1974) exposures employed in the monkey studies. The 1.0-ppm concentration from the 6-month continuous-exposure study by Haun and Kinkead (1973) was considered to be a minimal lowest-observed-adverse-effect level (LOAEL) for the monkeys because the slight to moderate fat accumulation in the livers of exposed animals was also observed to some degree in the controls, and there were no abnormal findings in clinical chemistry, hematology, or organ or body weights. The authors did not specify any difference in hepatic fat accumulation between rhesus monkeys that inhaled hydrazine at 0.2 ppm compared with those that inhaled hydrazine at 1.0 ppm.

Application of an interspecies uncertainty factor of 3 to the monkey 6-month continuous exposure no-observed-adverse-effect level (NOAEL) or possibly minimal LOAEL of 0.2 ppm was considered appropriate. An intraspecies uncertainty factor of 2 was applied on the basis of the differential rates of hydrazine acetylation measured in slow and fast acetylators in a group of Japanese hydrazine workers (Koizumi et al. 1998). Thus, a total uncertainty factor of 6 was applied to yield a 90-day CEGL of 0.03 ppm.

CARCINOGENICITY ASSESSMENT

The current EPA (1991) inhalation unit risk factor used in estimating the theoretical excess cancer risk of hydrazine (4.9×10^{-3} per micrograms per cubic meter [$\mu g/m^3$]) is based on Global 82 linearized multistage fitting to the combined incidence of nasal adenoma and adenocarcinoma in male Fischer 344 rats (MacEwen et al. 1981). Assuming that the EPA unit risk value is an accurate reflection of hydrazine carcinogenic potency in humans, theoretical excess cancer risk at the 90-day CEGL of 0.03 ppm exceeds 1×10^{-4}. EPA qualified the inhalation value: "The unit risk should not be used if the [hydrazine] air concentration exceeds 2 $\mu g/m^3$ [2 ppb], since above this concentration the unit risk may not be appropriate." However, no explanation is supplied to support that statement.

NRC (1986) recognized that assuming all carcinogenic responses are directly proportional to the total dose over the entire exposure range "is likely not to hold for all materials and all tissues that these materials affect. Knowledge of mechanisms that produce different dose-response curves should, in the future, lead to better material/mechanism-specific risk assessment computations."

The EPA calculation from 1991 does not directly consider the mode of action of hydrazine. Hydrazine is a reliable rodent carcinogen at chronic oral doses, producing hepatic and lung tumors. Chronic hydrazine inhalation studies in rats and mice yield concentration-related nasal and lung tumors. Hydrazine is clearly genotoxic in rats, mice, hamsters, and guinea pigs, inducing dose-dependent increases in hepatic O^6-methylguanine and 7-methylguanine (Bosan and Shank 1983; Lambert and Shank 1988). Hydrazine-induced rodent liver tumors arise only after repeated exposures sufficient to produce hepatocellular necrosis (Leakakos and Shank 1994). It must be noted that methylguanines are detectable in rat hepatic DNA only after necrogenic doses and that hydrazine-induced DNA alkylation at cytotoxic doses is directed at or near specific genes (Leakakos and Shank 1994; Zheng and Shank 1996).

Jenner and Timbrell (1994) reported the initial depletion of reduced hepatic glutathione (GSH) after hydrazine exposure. That finding was later confirmed by Hussain and Frazier (2002) who also reported the commensurate increase in oxidized GSH. Those changes are followed by generation of free methyl, acetyl, hydroxyl, and hydrogen radicals; increased reactive oxygen species; and inhibition of catalase activity sufficient to overwhelm cellular antioxidant defense mechanisms (Hussain and Frazier 2002). Increased cellular lipid peroxidation follows. Hydrazine cytotoxicity appears to be inextricably linked with the oxidative stress and damage observed after exposures sufficient to saturate and overwhelm normal defense mechanisms. Given the dependence of the response on exposure time, Hussain and Frazier (2002) concluded that hydrazine heptocellular toxicity is a highly nonlinear function of dose. Latendresse et al. (1995) found that the induction of rodent nasal tumors by hydrazine also is a nonlinear function of dose.

Vernot et al. (1985) described the similarities between hydrazine-induced and formaldehyde-induced rodent nasal cancers. Although the rigorous molecular dosimetry available for formaldehyde does not exist for hydrazine, the available data support the conclusion that the carcinogenic potential of inhaled hydrazine, like that of formaldehyde (Conolly et al. 2003), is a threshold phenomenon that is associated with local cytotoxicity and regenerative hyperplasia. The subcommittee concluded that preventing upper respiratory tract irritation and associated cytotoxicity should eliminate, for all practical purposes, any excess carcinogenic risk posed by occupational hydrazine exposures. Although theoretical risk values can be generated using published linear potency factors, the subcommittee concluded that those values are unreliable given the uncertainty associated with them. Because the 90-day CEGL is far lower than the hydrazine concentra-

tions associated with respiratory tract irritation and its consequences in primates, the health risk to submariners posed by hydrazine exposures less than or equal to the 90-day CEGL of 0.03 ppm can be considered de minimis.

DATA ADEQUACY AND RESEARCH NEEDS

Sufficient data were available for deriving the submarine guidance levels for hydrazine. However, fundamental mechanistic studies of hydrazine tumorigenesis in the rat nasal epithelium recommended by Latendresse et al. (1995) have not been conducted. Although similarities to formaldehyde carcinogenesis have been noted in this profile, such as the association with pronounced necrosis and regenerative hyperplasia, the subcommittee concludes that data are needed to determine the relationship between overt cytotoxicity induced in rodent respiratory tract tissues and carcinogenic response. Data are also needed to elucidate the contribution of the genotoxic activity of hydrazine at doses and exposures that elicit a significant carcinogenic response, given the overt tissue damage observed at those doses. These data would improve the confidence of the 90-day CEGL value and its protectiveness for longer-term exposures to hydrazine.

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7

Methanol

This chapter summarizes the relevant epidemiologic and toxicologic studies on methanol. Selected chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure levels from the National Research Council (NRC) and other agencies are also presented. The subcommittee considered all of that information in its evaluation of the Navy's current and proposed 1-hour (h), 24-h, and 90-day exposure guidance levels for methanol. The subcommittee's recommendations for methanol exposure levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

Methanol is a colorless, flammable liquid at ambient temperatures (Budavari et al. 1989; English et al. 1995). Selected physical and chemical properties are provided in Table 7-1.

OCCURRENCE AND USE

Methanol is used as a solvent, as an ethanol denaturant, as an antifreeze in windshield washer fluid, and as an intermediate in the synthesis of formaldehyde, methyl tertiary-butyl ether, and other chemicals (Hawley 1977; English et al. 1995). Methanol also can be used in automotive fuel and is sold in a blend of 85% methanol and 15% unleaded premium gasoline ("M85") (IPCS 1997). Methanol can be used for fuel cells and is used

in the treatment of wastewater and sewage (NTP 2003). Methanol is produced naturally as a by-product of anaerobic metabolism in many varieties of bacteria. Likewise, methanol is a by-product of mammalian carbon metabolism (IPCS 1997). Methanol is a natural component of fruits, vegetables, and fermented spirits (Soffritti et al. 2002). Ingestion of the food additive aspartame results in human exposures to methanol (Soffritti et al. 2002).

The uses or sources of methanol on board submarines are unknown. NRC (1988) listed methanol as a possible air contaminant on board submarines and reported a concentration of 6 parts per million (ppm). No information was provided on sampling protocol, location, operations, or durations. More recent analyses of air samples from submarines did not report methanol as an air contaminant (Raymer et al. 1994; Holdren et al. 1995).

TABLE 7-1 Physical and Chemical Properties of Methanol[a]

Synonyms and trade names	Methyl alcohol, wood alcohol, carbinol, methylol, colonial spirits, columbian spirits, methyl hydroxide, monohydroxymethane, pyroxylic spirits, wood naphtha, and wood spirits
CAS registry number	67-56-1
Molecular formula	CH_3OH
Molecular weight	32.04
Boiling point	64.7°C
Melting point	−97.8°C
Flash point	12°C (closed cup)
Explosive limits	6.0% to 36.5%
Specific gravity	0.7915 at 20°C/4°C
Vapor pressure	127 mmHg at 25°C
Solubility	Miscible with water, ethanol, ether, benzene, and most organic solvents
Conversion factors	1 ppm = 1.31 mg/m^3 ; 1 mg/m^3 = 0.76 ppm

[a]Data on vapor pressure are from HSDB (2004); all other data are from Budavari et al. (1989).

Abbreviations: mg/m^3, milligrams per cubic meter; mmHg, millimeters of mercury; ppm, parts per million.

SUMMARY OF TOXICITY

The primary reviews used in this section include those prepared by the International Programme on Chemical Safety (IPCS) (1997), Kavet and Nauss (1990), and the National Toxicology Program (NTP) (2003). Methanol is an endogenously produced by-product of metabolism and is a natural constituent in animal blood, urine, saliva, and expired air. Exogenous exposures to methanol can occur by ingestion, inhalation, or dermal contact. Methanol is absorbed readily by those routes and is rapidly distributed to tissues. Methanol is metabolized sequentially, primarily in the liver, to formaldehyde, formic acid, and carbon dioxide. Formic acid dissociates to formate and hydrogen ions. Nearly all of the available information on methanol toxicity in humans relates to the consequences of acute, rather than chronic, exposures and is based on clinical cases of methanol poisoning. Those cases, as well as experimental studies in animals, have established that formate is the toxic metabolite of methanol. Formate accumulation accounts for the metabolic acidosis (reduced blood pH) and blindness observed in people diagnosed with methanol poisoning. Hepatic folate status governs the rate of formate detoxification. Central nervous system (CNS) depression, weakness, headache, vomiting, severe metabolic acidosis, optic disc edema, and bilateral necrosis of the putamen also occur in people during methanol intoxication. Other adverse effects of methanol exposure in humans include minor skin and eye irritation.

Effects in Humans

Accidental Exposures

Some of the case studies reporting acute methanol intoxication in humans date back to the late 1800s. Historically, the majority of human poisoning cases involved adults and were related to the accidental or intentional consumption of alcoholic beverages in which methanol was substituted for ethanol. Although those types of exposures continue to occur, most contemporary poisoning cases in the United States involve children who have ingested methanol-based windshield wiper fluids or other automotive products (Davis et al. 2002). The minimum lethal oral dose in humans is about 0.3-1 gram per kilogram of body weight (g/kg) (Kavet and Nauss 1990). The oral methanol doses that have produced toxicity vary

widely and might reflect concurrent ingestion of ethanol, inadequate dietary folate intake, or other factors.

Acute methanol poisoning occurs in three distinct clinical phases. The first phase resembles ethanol intoxication and initially is characterized by CNS depression, ataxia, and difficulty breathing (Tephly 1991). Intoxicated patients also might present with acute gastritis or pancreatitis, anorexia, intense abdominal pain, vomiting, and diarrhea. Toxicity observed during the first phase primarily is the result of direct toxicity from the parent alcohol. The second phase is usually a short (12-24 h), asymptomatic period. The third phase primarily is associated with the effects of formate accumulation. Visual disturbances and metabolic acidosis represent classical clinical findings associated with methanol intoxication. Visual disturbances can include blurred vision, altered visual fields, impaired pupil response to light, and permanent or temporary blindness. Ophthalmoscopic examinations of people with visual dysfunction often reveal the initial presence of optic disc hyperemia followed by persistent peripapillary edema. Optic disc pallor can occur 1-2 months after poisoning and is a sign of irreversible eye damage (Sharpe et al. 1982; Tephly 1991). Other signs and symptoms observed during the third phase include headache, dizziness, nausea, abdominal pain, vomiting, and dyspnea.

Severe methanol intoxication might cause damage to the putamen, a brain structure linked with motor control (Ley and Galli 1983; Koopmans et al. 1988; Finkelstein and Vardi 2002). Other complications of severe acute methanol intoxication include coma, seizures, blindness, oliguric renal failure, cardiac failure, cerebral edema, cerebral or subarachnoid hemorrhage, and pulmonary edema (Davis et al. 2002). Death can be rapid or can occur several hours after coma. Death is associated with apnea and convulsions. Autopsies conducted on victims of methanol poisoning revealed gross pathologic effects consisting of edematous, hemorrhagic, and degenerative changes in visceral organs, liver, kidneys, lungs, and the CNS (McLean et al. 1980).

Although methanol poisoning by ingestion is well documented, fewer cases of poisoning by inhalation exposure have been reported. The following case report illustrates important clinical features of the inhalation toxicity of methanol in humans. Frenia and Schauben (1993) described seven cases of suspected methanol poisoning in adults who intentionally inhaled a carburetor-cleaning fluid that contained about 23% methanol mixed with other solvents. Affected individuals developed CNS depression, nausea, vomiting, dyspnea, photophobia, and visual dysfunction. They also developed markedly elevated blood methanol (>50 milligrams per deciliter

[mg/dL]) and formate (2.6 millimoles [mmol]) concentrations and severe metabolic acidosis.

Downie et al. (1992) reported a case of ocular toxicity following percutaneous exposure to methanol. The case involved a 31-year-old male worker who used methanol to clean a tanker. The cleaning took several hours to complete. The worker wore a positive-pressure breathing apparatus but no protective clothing during the activity. His clothes became saturated with methanol. About 8-10 h after the exposure, the worker developed CNS depression, blurred vision, dyspnea, metabolic acidosis, and semi-coma. Ophthalmoscopic examination revealed optic disc pathology consistent with methanol poisoning. This and the preceding case report confirm that the clinical signs and clinical pathology changes observed following methanol inhalation or dermal exposures are identical to those observed after methanol ingestion.

Experimental Studies

Several experimental human chamber studies and dermal absorption studies have been performed with methanol. Many of those studies exposed healthy men of an age range comparable to that of submariners. Limitations inherent in each of those studies include small sample size, limited numbers of exposure concentrations, relatively short exposure durations, and an inability to completely mask the odor of methanol from subjects and the experimenters. Most of the studies controlled for dietary sources of methanol and limited pre-exposure intake of ethanol. The studies are useful sources of data on background blood methanol and formate concentrations, and they provide important data on blood methanol and formate concentrations measured after methanol-exposure scenarios relevant to submariners (Table 7-2). Some of the studies also provide information about symptom reporting and neurobehavioral effects.

Inhalation Exposure

Cook et al. (1991) exposed 12 healthy nonsmoking young men (22-32 years of age) to methanol at 191 ppm for 75 minutes (min). The subjects did not describe any symptoms related to methanol exposure. End-of-exposure plasma formate concentrations were unaffected by methanol exposure, despite an about 3.3-fold increase in the mean plasma methanol concentra-

tion (Table 7-2). Subjects also were tested for an array of neurobehavioral end points. The majority of results were negative. Statistically significant effects and trends were found in brain wave patterns, particularly in response to light flashes and sounds (P-200 and N1-P2 components of event-related potentials); performances on the Sternberg memory task; and subjective measures of fatigue and concentration. The study authors noted that the effects were mild and did not exceed normal ranges.

Chuwers et al. (1995) examined the neurobehavioral effects of 4-h inhalation exposures to either water vapor or methanol at 200 ppm in healthy men (n = 15) and women (n = 11), 21-51 years of age. Blood samples were collected before and after exposure, and a battery of neurobehavioral and neurophysiologic tests was performed. In general, there were no significant effects on visual, neurophysiologic, or neurobehavioral end points. Slight effects on P-300 brain wave amplitudes (in response to sensory stimuli) and performances on the Symbol Digit test (a test examining information processing and psychomotor skills) were observed. The study authors concluded that methanol exposure at 200 ppm had little effect on neurobehavioral performance. The authors also reported that subjects could not detect an odor during the methanol exposures. It should be noted that data reported by Osterloh et al. (1996) and d'Alessandro et al. (1994) describe similar subject characteristics and largely duplicate the findings reported by Chuwers et al. (1995). Thus, they do not represent new studies but different facets of a single study.

Muttray et al. (2001) exposed 12 healthy subjects to methanol at either 20 or 200 ppm for 4 h. Electroencephalograph (EEG) activity was recorded before and after each exposure—once with the subjects' eyes closed, once with the subjects' eyes opened, and once during a choice reaction test (color word stress test). Subjective symptoms were assessed via questionnaires. Exposures at 200 ppm did not result in significant symptoms of narcosis or irritation compared with the reports from exposures at 20 ppm in the same subjects. Changes in the EEG theta-band at 200 ppm suggested a slight excitatory effect; however, the authors reported these effects to be weaker than those elicited by human circadian cycles.

Lee et al. (1992) exposed six healthy male volunteers (29-55 years of age) to methanol at 200 ppm for 6 h. Subjects were either at rest or engaged in mild physical exercise (sessions consisted of 20 min at a work load of 50 watts on a bicycle ergometer, followed by 20 min at rest, repeated for 6 h) during the exposures. Pre- and post-exposure blood methanol and formate concentrations were determined. Blood methanol concentrations were increased following methanol inhalation (Table 7-2). Exercise did not

TABLE 7-2 Blood Methanol and Formate Concentrations Observed in Humans Following Experimental Methanol Exposures

Methanol Concentration (ppm)	Exposure Duration (h)	Plasma/Serum Methanol Concentration (mg/L)[a]		Plasma/serum formate Concentration (mmol/L)		Reference
		Pre-exposure	Post-exposure	Pre-exposure	Post-exposure	
190	1.25	0.57 ± 0.31	1.88 ± 0.47	0.08 ± 0.03	0.08 ± 0.02	Cook et al. 1991
200	4	1.8 ± 2.6	6.5 ± 2.7	0.24 ± 0.18	0.30 ± 0.19	Chuwers et al. 1995
200	6	1.82 ± 1.21 (rest) 1.93 ± 0.93 (exercise)	6.97 ± 1.24 (rest) 8.13 ± 1.49 (exercise)	0.20 ± 0.03 (rest) 0.19 ± 0.04 (exercise)	0.19 ± 0.05 (rest) 0.21 ± 0.02 (exercise)	Lee et al. 1992
400	8	2.65 ± 1.8 (rest)	13.4 ± 4.8 (rest)	ND	ND	Franzblau et al. 1995
800	1	1.3 ± 0.6	6.6 ± 1.2	ND	ND	Batterman et al. 1998
800	8	1.8 ± 0.9	30.7 ± 6.9	ND	ND	Batterman et al. 1998

[a]Mean ± standard deviation.

Abbreviations: h, hours; mg/L, milligrams per liter; mmol/L, millimoles per liter; ND, not determined; ppm, parts per million.

influence end-of-exposure blood methanol concentrations, even though pulmonary ventilation was increased (10.5 L/min at rest vs 18.6 L/min while exercising). Methanol exposures did not result in altered blood formate concentrations (Table 7-2). The authors of this study did not report symptoms; thus, the study could not be used to establish submarine exposure guidance levels for methanol.

Batterman et al. (1998) examined the relationships between methanol concentrations in blood, urine, and exhaled breath in people exposed to methanol vapor. Exposure scenarios and group characteristics are provided in Table 7-3. Periodic breath, blood, and urine samples were collected. Pre- and post-exposure blood methanol and formate concentrations were determined (Table 7-2). Because the study authors failed to report whether any symptoms were observed, this study could not be used to establish exposure guidance levels for methanol.

Franzblau et al. (1995) exposed three men (31-55 years of age) and one woman (49 years of age) to methanol at 0, 100, 200, 400, and 800 ppm for 8 h. Subjects completed each exposure either at rest or while performing light exercise on a bicycle ergometer that served to increase minute ventilation by about 50% over baseline. Blood and breath samples were collected before exposure and after 6 and 8 h of exposure. Blood methanol concentrations were significantly increased after 6 and 8 h of exposure (Table 7-2). Franzblau stated that none of the methanol-exposed subjects reported symptoms (A. Franzblau, University of Michigan, personal commun., June 14, 1999, and October 3, 2000).

Dermal Exposure

For 60 min, Dutkiewicz et al. (1980) exposed six human volunteers (ages not specified) to methanol at 0.19-0.21 mL applied to an area of the forearm 11.2 square centimeters (cm^2) large. Dermal absorption was examined 15-60 min after application, and the mean calculated absorption rate was 11.5 $mg/cm^2/h$. The absorption rate peaked between 20 and 30 min after application. The authors estimated that immersion of one hand in liquid methanol for 2 min would result in a body burden of about 170 mg, which is similar to the body burden that results from inhaling methanol at about 40 ppm for 8 h.

Franzblau et al. (1995) used the three men (31-55 years of age) and one woman (49 years of age) who participated in their aforementioned inhalation study and four additional men (26-33 years of age) in a dermal

TABLE 7-3 Experimental Parameters of Batterman et al. (1998)

Exposure Concentration (ppm)	Duration (h)	Gender	Age (years)	Sample Size
800	0.5, 1, 2	Female	41-60	4
800	8	Male	Unknown	12
800	8	Female	Unknown	7

Abbreviations: h, hours; ppm, parts per million.

study. One hand of each volunteer was placed in a beaker containing neat methanol for 0, 2, 4, 8, or 16 min. Blood and breath methanol samples were taken immediately after the exposures and at 12 additional time points during the first 8 h after exposure. Blood methanol concentrations peaked at about 45-60 min post-exposure and averaged 11.3 mg/L. Breath methanol concentrations peaked at about 15 min post-exposure and averaged 9.3 ppm. The authors stated that exposure to one hand (440 cm^2, <3% of body surface area) for 16 min resulted in blood methanol concentrations similar to those observed following inhalation at 400 ppm for 8 h.

Batterman and Franzblau (1997) exposed seven men (22-54 years of age) and five women (41-63 years of age) to methanol. One hand of each volunteer was placed in a beaker containing neat methanol for 0, 2, 4, 8, or 16 min. Blood samples were taken immediately after the exposures and at 11 additional time points during the first 7 h post-exposure. Methanol delivery into the blood began during or immediately after exposure and reached a maximum rate at 30 min post-exposure. Peak blood methanol concentrations occurred about 2 h after exposure and ranged from 2.7 ± 0.9 mg/L following the 2-min exposure to 11.5 ± 2.3 mg/L following the 16-min exposure. The area under the curve (AUC) correlated highly with duration of exposure and peak blood methanol concentration. The average derived dermal absorption rate was 8.1 ± 3.7 mg/cm^2/h. The authors reported that the exposed hand was often temporarily whitened in color and appeared very dry. That effect was most marked after the longer exposures.

Occupational and Epidemiologic Studies

Several studies have examined human exposures to methanol in occupational settings. One of the first studies was conducted by Tyson and Schoenberg (1914) who described about 100 cases of methanol intoxication resulting from occupational inhalation exposures. A much more recent

study of occupational inhalation exposures by Frederick et al. (1984) was considered by Kavet and Nauss (1990) to be the most definitive. In that study, 84 teachers' aides working near paper duplicators reported headaches, dizziness, blurred vision, and nausea. The aides used a 99% methanol fluid for 1 h per day, 1 day per week or for 8 h per day, 5 days per week over a period of 3 years. Methanol concentrations measured in the breathing zone of a subset of the workers (n = 21) ranged from 365 to 3,080 ppm. Dermal exposures to methanol also might have occurred; however, those exposures were not determined.

A study by Kawai et al. (1991) examined subjective complaints and clinical findings in 22 workers exposed to high concentrations of methanol (mean = 459 ppm) and 11 workers exposed to lower concentrations (mean = 31 ppm). Breathing-zone exposures to methanol (time-weighted averages) were determined during representative shifts. The most common complaints in workers exposed at the highest concentration included dimmed vision (not considered to be related to retinal toxicity), nasal irritation, headache, forgetfulness, and increased skin sensitivity. Three workers exposed to methanol at 119-3,577 ppm exhibited slow pupil response to light or mild mydriasis. The optic discs were unaffected, and there were no indications of permanent eye damage in those individuals. The study authors did not believe that the ocular effects were the result of formate poisoning.

Effects in Animals

Several animal models have been used to evaluate methanol toxicity. The animal studies have clearly demonstrated that nonhuman primates are the most appropriate model for humans. Like humans, monkeys exposed to methanol develop increased blood formate concentrations, metabolic acidosis, and blindness (Roe 1982; Tephly and McMartin 1984). Conversely, rats, mice, dogs, and other resistant species exposed to methanol neither accumulate formate nor develop metabolic acidosis or blindness (Roe 1982; Tephly and McMartin 1984). Therefore, the subcommittee focused its review on studies conducted in nonhuman primates.

Acute Toxicity

No relevant studies on the acute toxicity of methanol in monkeys or sensitive animal species were available.

Repeated Exposures and Subchronic Toxicity

The Japanese New Energy Development Organization (NEDO) conducted inhalation toxicity studies in cynomolgus monkeys (*Macaca fascicularis*) (NEDO 1986). The monkeys were exposed to air (n = 1) or to methanol vapor at 3,000 (n = 1), 5,000 (n = 2), 7,000 (n = 1), or 10,000 (n = 1) ppm for 21 h per day for 21, 21, 14, 6, and 6 days, respectively. Exposures at >5,000 ppm were associated with metabolic acidosis, reduced movement, vomiting, and dyspnea. Clinical signs were severe enough to warrant early termination of the study. The monkey exposed at 3,000 ppm exhibited astrocyte hyperplasia in the basal ganglia, fatty hepatic degeneration, and a transient decrease in food consumption. The study report indicates that 3,000 ppm represented a lowest-observed-adverse-effect level (LOAEL). It should be noted that this study had inadequate sample sizes for statistical evaluations.

NEDO also exposed cynomolgus monkeys to methanol at 1,000 (n = 3), 2,000 (n = 3), 3,000 (n = 3), or 5,000 (n = 2) ppm for 21 h per day for 7 months and 20, 20, and 12 days, respectively (NEDO 1986). Animals were held for various times to evaluate whether recovery occurred. Exposures at 5,000 ppm were associated with metabolic acidosis, reduced movement, vomiting, dyspnea, and mild optic nerve atrophy. Exposures at 2,000 ppm were associated with metabolic acidosis.

Andrews et al. (1987) exposed three male and three female cynomolgus monkeys per group to either air or to methanol vapors (99.85% purity) at 500, 2,000, or 5,000 ppm for 6 h per day, 5 days per week for 4 weeks. Weekly measurements of body weight revealed no differences between control and treated animals. Absolute adrenal weights were significantly decreased in female monkeys in the 5,000-ppm exposure group, but the authors indicated that the effect was not considered to be biologically significant. Gross and histopathologic examination revealed no other effects in any of the examined organs. No ocular abnormalities were noted during an ophthalmoscopic examination. The study authors concluded that 5,000 ppm represented a no-observed-adverse-effect level (NOAEL) for repeated methanol exposure in monkeys.

Chronic Toxicity

NEDO exposed female cynomolgus monkeys (eight per group) to methanol vapor at 0, 10, 100, or 1,000 ppm for 22 h per day for 7 months (two per group), 19 months (three per group), or 29 months (three per

group). Exposures at up to 1,000 ppm were not associated with any evidence of optic nerve or retinal pathology (NEDO 1986). However, chronic exposures at 1,000 ppm were associated with basal ganglial astrocyte hyperplasia and metabolic acidosis. Chronic exposures at 100 ppm were associated with hematologic changes, altered electrocardiograms, and responsive stellate cells in the cerebral white matter. The shorter-term studies conducted by NEDO in monkeys indicate that the latter response is transient. For example, stellate cells were no longer observed in monkeys 6 months after the end of a 20-day near-continuous exposure to methanol at 3,000 ppm. The NEDO report (1986) indicates that 10 ppm was a NOAEL for chronic exposure.

Burbacher et al. (1999a,b; 2004a,b) conducted an extensive methanol inhalation study in cynomolgus monkeys. The study assessed whether subchronic methanol exposure at 200-1,800 ppm was associated with overt adult toxicity, female reproductive toxicity, or both and whether in utero exposure to methanol affected offspring development. Monkeys were exposed to air (n = 9) or to methanol at 200 (n = 12), 600 (n = 11), or 1,800 (n = 12) ppm for 2.5 h per day, 7 days per week through an initial 4-month exposure period, during breeding (ranging from 3 to 236 days), and throughout pregnancy (ranging from 150 to 178 days). Mean (± standard error of the mean) blood methanol concentrations observed in monkeys at the end of the initial 4-month period were 2.3 ± 0.1, 4.7 ± 0.1, 10.5 ± 0.3, and 35.6 ± 1.0 mg/L for the 0-, 200-, 600-, and 1,800-ppm exposure groups, respectively. Plasma formate concentrations were unaffected by methanol exposure. Blood methanol concentrations observed in the 600- and 1,800-ppm exposure groups did not fit a linear, one-compartment first-order model, suggesting that saturation of methanol metabolism occurred in the highest exposure group. Clinical signs consistent with methanol intoxication, such as CNS depression, ataxia, and blindness, were not observed in any of the exposed monkeys. Body-weight gain was unaffected by methanol exposure. Histopathologic evaluations were not performed in this study.

Reproductive Toxicity in Males

An NTP expert panel judged that there are insufficient human data to evaluate the reproductive toxicity of methanol (NTP 2003). The panel concluded that "adverse reproductive effects would not occur in male rats following inhalation exposure to ≤800 ppm." In reaching that conclusion, the panel cited four studies that examined serum hormone concentrations

in male rats exposed to methanol by inhalation and two studies that included histologic evaluations of reproductive organs. The panel considered a study conducted by Lee et al. (1991) to be the definitive work and had high confidence in the results of that study. Lee et al. (1991) exposed 8-week-old Sprague-Dawley rats to methanol at 200 ppm for 8 h per day for 1-6 weeks and observed no effects on testosterone, weight of androgen-sensitive organs, capability of testes exposed in vivo to produce testosterone in vitro, or pathology. In the second part of the Lee et al. (1991) study, normal and folate-deficient methanol-sensitive Long-Evans rats were exposed to methanol at 800 ppm for 20 h per day, 7 days per week for 13 weeks. A higher incidence, but not severity, of age-related testicular degeneration was observed in the folate-deficient 18-month-old rats. However, the incidence of age-related testicular lesions in the normal 18-month-old treated rats was equal to that in control rats. Poon et al. (1995) found no lesions in the reproductive organs of 4 to 5-week-old male and female Sprague-Dawley rats that inhaled methanol at 2,500 ppm for 6 h per day for 4 weeks. The results of Poon et al. (1995) were consistent with the findings of Lee et al. (1991). The NTP panel concluded that "the blood levels of methanol associated with reproductive toxicity in rodents are 700 mg/L and greater. Blood methanol levels of this magnitude in humans would be associated with frank methanol (formate) toxicity."

Immunotoxicity

No relevant data on methanol immunotoxicity were found by the subcommittee. Natural killer-cell function was reduced in mice given a near lethal oral dose (0.8 times the dose lethal to 50% of subjects [LD_{50}]) of methanol (Zabrodskii et al. 2003). Few details were provided, and the actual dose of methanol was not reported. Thus, that study has little utility in the subcommittee's assessment.

Genotoxicity

IPCS (1997) conducted a thorough review of the genotoxicity information available for methanol. The majority of the findings were negative; however, some positive results were identified. IPCS (1997) stated that "the structure of methanol (by analogy with ethanol) does not suggest that it would be genotoxic." IPCS (1997) reported negative findings in the Ames

test, cultured cell mutation assay in CH-V79 cells, and the micronucleus test performed by NEDO (1986). Negative findings were also reported for chromosome aberrations and sister chromatid exchange (IPCS 1997).

Inhaled methanol had no mutagenic effects in hamsters (Obe and Ristow 1979) or mice (Campbell et al. 1991). Fu et al. (1996) examined micronuclei formation in the reticulocytes of pregnant CD-1 mice fed diets containing adequate or marginal levels of folic acid (1,200 nanomoles per kilogram [nmol/kg] and 400 nmol/kg, respectively) and gavaged with methanol in water at 0 or 5,000 mg/kg per day on gestation days 6-10. Methanol exposure did not increase micronucleated cell frequency. Gattás et al. (2001) reported that methanol exposure was associated with increased incidence of oral mucosa micronuclei in fuel-pump operators in São Paulo, Brazil, after the introduction of a fuel composed of 33% methanol, 60% ethanol, and 7% gasoline.

Carcinogenicity

There is little evidence from animal studies to suggest that inhaled methanol is a carcinogen. The most comprehensive study of the carcinogenicity of methanol was conducted in rodents by NEDO (1986). Male and female Fischer 344 rats and $B6C3F_1$ mice were exposed to methanol vapor at 10, 100, or 1,000 ppm for 20 h per day for 24 months. Increased incidence of papillary adenomas and adrenal pheochromocytomas was observed at the highest dose, but the increases were not statistically significant. The NEDO report indicates that there was no evidence of methanol-induced cancer.

Soffritti et al. (2002) conducted a chronic drinking water carcinogenicity study in male and female Sprague-Dawley rats. For 24 months, rats were exposed to drinking water that contained methanol at 0, 500, 5,000, or 20,000 ppm. Exposures to methanol in drinking water at 5,000 ppm or greater were associated with statistically significant increases in the incidence of carcinomas of the ear ducts. Those data were found to be of limited use to the subcommittee because inhalation was not the route of exposure.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

A lot is known about the chemical and biological behavior of methanol. Methanol is converted to formaldehyde by hepatic alcohol dehydro-

genase (Tephly and McMartin 1984). Formaldehyde is a very reactive compound with a very short life span in blood, and it does not contribute to the ocular toxicity of methanol (McMartin et al. 1979). Formaldehyde is metabolized to formic acid by a glutathione-mediated pathway involving formaldehyde dehydrogenase. Formic acid dissociates to formate and hydrogen ions (Tephly and McMartin 1984). Formate is detoxified to carbon dioxide by a multistep pathway (Jacobsen and McMartin 1986). In all species studied, the detoxification is achieved through a tetrahydrofolate-dependent pathway. When compared with rodents, humans and nonhuman primates have low hepatic tetrahydrofolate concentrations and metabolize formate to carbon dioxide relatively slowly. The rate at which methanol-derived formate accumulates to toxic levels following methanol exposures is primarily influenced by the rate at which formate is metabolized.

Formate is the metabolic product of methanol thought to be responsible for the acute toxic effects of methanol exposure (McMartin et al. 1980; Tephly and McMartin 1984). Blood formate concentrations at 10 mmol/L or greater have been reported in humans diagnosed with neuro-ocular toxicosis following methanol exposures by ingestion (Kavet and Nauss 1990). Formate contributes to metabolic acidosis, and it acts as an inhibitor of cytochrome c oxidase activity in intact rat liver mitochondria (Nicholls 1975). Reduced cytochrome c oxidase can result in decreases in cellular adenosine triphosphate production and might subsequently lead to neurotoxicity.

Methanol is rapidly absorbed after oral, inhalation, or dermal exposure. In a group of 22 human volunteers exposed to methanol at 200 ppm for 4 h, the mean absorption half-life was 0.80 ± 0.55 h (Osterloh et al. 1996). Only a fraction of inhaled methanol is absorbed across the respiratory tract epithelium into the systemic circulation (Perkins et al. 1995; Fisher et al. 2000). Inhalation studies in humans have shown net absorptions of methanol of 60-85% (Sedivec et al. 1981). Studies conducted in nonhuman primates show similar percentages of absorption (Fisher et al. 2000).

Like that of ethanol, methanol metabolism follows saturable zero-order kinetics at low concentrations. Studies conducted in monkeys have provided some indication of when methanol metabolism becomes saturated. In studies conducted by Horton et al. (1992), rhesus monkeys were exposed to methanol concentrations at 200, 1,200, or 2,000 ppm for 6 h. End-of-exposure blood methanol and formate concentrations were determined for up to 12 h after the end of the 6-h exposure, and they were directly proportional at 1,200 and 2,000 ppm. The 200-ppm exposure concentration resulted in blood methanol concentrations that were lower than those that would be proportional to the measurements at the two higher doses. The

nonlinearity observed in the blood methanol concentrations suggested that methanol elimination proceeds by a saturable pathway and that monkeys demonstrate dose-dependent kinetics at exposure concentrations between 200 and 2,000 ppm. Although blood formate concentrations varied considerably among the individual monkeys, the authors were unable to detect any changes in total blood formate concentrations following 6-h methanol exposures at 200, 1,200, or 2,000 ppm.

Clinical case reports have confirmed that blood methanol concentrations poorly predict ocular toxicity. Although maximal blood methanol concentrations following acute lethal methanol exposures in humans are often undetermined, blood methanol concentrations in excess of 1,000 mg/L are commonly reported within 24-48 h of methanol ingestion (Kostic and Dart 2003).

Methanol is excreted unchanged in urine or expired air or as formate in urine. Methanol elimination is dose dependent. The half-life of methanol elimination in blood in highly exposed humans who do not receive ethanol treatment or dialysis treatment ranges from 17 to 27 h, and the half-life of methanol elimination in expired air after moderate oral or dermal exposure is 1.5 h. The amount of formate excreted in the urine varies greatly among species and ranges from 1% in rabbits to 20% in dogs; humans excrete formate in intermediate amounts (Kavet and Nauss 1990).

Rodent studies have indicated that folate deficiency might alter the rate at which formate is metabolized. Rats maintained on a folate-deficient diet develop increased blood formate concentrations following high-dose (4 g/kg) intraperitoneal methanol exposures (Makar and Tephly 1976). Dorman et al. (1994) examined the pharmacokinetics of inhaled [^{14}C]methanol in normal and folate-deficient cynomolgus monkeys. Four normal female monkeys were initially exposed to [^{14}C]methanol at 10, 45, 200, or 900 ppm for 2 h. Average (± standard deviation) peak blood [^{14}C]formate concentrations were 0.07 ± 0.02, 0.25 ± 0.09, 2.3 ± 2.9, and 2.8 ± 1.7 micromolar (μM) following methanol inhalation at 10, 45, 200, and 900 ppm, respectively. The monkeys were then fed a folate-deficient diet supplemented with 1% succinylsulfathiozole for 6-8 weeks to reduce their serum and erythrocyte folate concentrations to <3 nanograms per milliliter (ng/mL) and 120 ng/mL, respectively. Finally, the monkeys were exposed to [^{14}C]methanol at 900 ppm for 2 h. End-of-exposure methanol concentrations, AUC, and total amounts of [^{14}C]methanol and [^{14}C]CO_2 exhaled were linearly and significantly related to inhaled methanol concentrations indicating that dose-dependent methanol metabolism and pharmacokinetics did not

occur. The average (± standard deviation) peak blood [^{14}C]formate concentration was 9.5 ± 4.7 μM. Despite folate deficiency, peak [^{14}C]formate concentrations remained a small fraction of the endogenous formate blood concentrations seen in the monkeys (0.28-0.56 millimolar [mM]) or in healthy humans (see Table 7-2).

Conditions that can predispose humans to folate deficiency include gastrointestinal disorders that reduce folate absorption (such as Crohn's disease and adult gluten enteropathy), chronic alcoholism, pernicious anemia, and psychiatric disorders, such as depression. Smoking and use of methotrexate, sulfasalazine, trimethoprim, or other medications that are folic acid antagonists also increase susceptibility to folate deficiency. Those factors are unlikely to be of concern in the healthy submariner population. The methylenetetrahydrofolate reductase polymorphism 677T mutation that decreases folate activity is common among the general population. Homozygosity was found in 21% of a Hispanic population sampled in California and in 12% of Caucasians sampled in the United States (Botto and Yang 2000). Genetic differences in folate receptor activity and in enzymes involved in folic acid metabolism are, at this time, theoretical causes of folate deficiency (Antony and Hansen 2000).

Polymorphisms in human alcohol dehydrogenase and P450 2E1 (CYP2E1) genes have been described (Fairbrother et al. 1998; McCarver et al. 1998) and could influence the rates at which methanol is metabolized. Polymorphisms in the alcohol dehydrogenase allele might lead to greater susceptibility to the direct effects of methanol in affected individuals, and decreased metabolism could result in higher blood methanol concentrations. Conversely, those individuals would be expected to exhibit lower blood formate concentrations.

INHALATION EXPOSURE LEVELS FROM THE NRC AND OTHER ORGANIZATIONS

A number of organizations have established or proposed inhalation exposure limits or guidelines for methanol. Selected values are summarized in Table 7-4.

TABLE 7-4 Selected Inhalation Exposure Levels for Methanol from NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	200	ACGIH 2002
	TLV-STEL	250	
NIOSH	REL-TWA	200 (skin)	NIOSH 2004
	REL-STEL	250 (skin)	
OSHA	PEL-TWA	200	29 CFR 1910.1000
Spacecraft			
NASA	SMAC		NRC 1994
	1 h	30	
	24 h	10	
	30 days	7	
	180 days	7	
Submarine			
NRC	EEGL		NRC 1985
	1 h	200	
	24 h	10	
	CEGL		
	90 days	50[b]	
General Public			
NAC/NRC	Proposed AEGL-1 (1 h)	530	EPA 2004
	Proposed AEGL-2 (1 h)	2100	
	Proposed AEGL-1 (8 h)	270	
	Proposed AEGL-2 (8 h)	510	

[a]The comparability of EEGLs and CEGLs with occupational and public health standards or guidance levels is discussed in Chapter 1, section "Comparison to Other Regulatory Standards or Guidance Levels."
[b]Proposed in 1968. No value was proposed in 1985.
Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL, acute exposure guideline level; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; h, hour; NAC, National Advisory Committee; NASA, National Aeronautics and Space Administration; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; ppm, parts per million; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendations for EEGL and CEGL values for methanol are summarized in Table 7-5. The current and proposed U.S. Navy values are provided for comparison.

1-Hour EEGL

Health effects of concern following an acute 1-h inhalation exposure to methanol include narcosis, delayed neuro-ocular toxicity, headaches, and mucous membrane irritation. Andrews et al. (1987) reported that cynomolgus monkeys developed neither narcosis nor histopathologic evidence of optic nerve or retinal pathology following inhalation exposures to methanol at 5,000 ppm for 6 h per day, 5 days per week for 4 weeks. Studies conducted by Burbacher et al. (1999a,b; 2004a,b) in cynomolgus monkeys failed to produce any evidence of visual toxicity or narcosis in monkeys exposed to methanol at 1,800 ppm for 2.5 h per day, 7 days per week through an initial 4-month exposure period, during breeding, and throughout pregnancy. The weight of evidence from the two studies suggests that short-term (1 h) exposure to methanol vapors at 1,800 ppm does not produce neuro-ocular toxicity in monkeys and that 1,800 ppm represents an acute NOAEL in that species. A 3-fold uncertainty factor to account for intraspecies differences was applied. That uncertainty factor accounts for possible differences in either methanol or formate metabolism among people. Metabolic differences are most pronounced at high exposure concentrations, such as 1,800 ppm, where metabolism can become saturated. Application of the intraspecies uncertainty factor to the monkey NOAEL of 1,800 ppm yields a 1-h EEGL of 600 ppm.

The recommended 1-h EEGL is supported by methanol exposure studies conducted in humans. Franzblau et al. (1995) exposed four people to methanol at 800 ppm for 8 h. The authors indicated that none of the subjects developed symptoms (A. Franzblau, University of Michigan, personal commun., June 14, 1999, and October 3, 2000). Batterman et al. (1998) reported a mean peak blood methanol concentration of 6.6 ± 1.2 mg/L for the subjects in the Franzblau et al. (1995) study. That concentration is about 150-fold lower than the mean blood methanol concentration seen in humans poisoned with methanol (1,000 mg/L) (Kostic and Dart 2003).

TABLE 7-5 Emergency and Continuous Exposure Guidance Levels for Methanol (ppm)

Exposure Level	U.S. Navy Values		NRC Recommended Values
	Current	Proposed	
EEGL			
1 h	200	200	600
24 h	10	10	50
CEGL			
90 days	10	7	10

Abbreviations: CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level; h, hour; NRC, National Research Council; ppm, parts per million.

24-Hour EEGL

Health effects of concern following a 24-h inhalation exposure to methanol would include narcosis, delayed neuro-ocular toxicity, headaches, mucus membrane irritation, and impaired neurobehavioral function. Several chamber studies conducted in humans have evaluated whether a 4-h exposure to methanol at 200 ppm could result in neurobehavioral or electrophysiologic changes (Chuwers et al. 1995; Muttray et al. 2001). The authors of those studies concluded that methanol exposure at 200 ppm for 4 h had little effect on neurobehavioral performance or EEG activity. Exposures to methanol at 200 ppm did not result in increased symptoms of narcosis or irritation compared with exposures at 20 ppm in the same subjects (Muttray et al. 2001). Moreover, human exposures at 200 ppm for 4 h (Chuwers et al. 1995) or 6 h (Lee et al. 1992) did not result in statistically or toxicologically significant increases in blood formate concentrations. Peak blood methanol concentrations observed in the studies were <10 mg/L and were much lower than blood methanol concentrations measured in humans poisoned with methanol (1,000 mg/L) (Kostic and Dart 2003). The weight of evidence from these studies (Chuwers et al. 1995; Muttray et al. 2001) suggests that short-term (4-6 h) exposure to methanol vapors at 200 ppm does not produce headaches, irritation, neuro-ocular toxicity, or significant neurobehavioral toxicity in humans. A 4-fold adjustment to account for the shorter duration was applied to the 6-h data to yield a 24-h EEGL of 50 ppm. That adjustment was reasonable because the average end-of-exposure blood methanol concentration observed in people exposed at 200 ppm for 4 h (6.5 ± 2.7 mg/L; Chuwers et al. 1995) was similar to that seen in people

exposed at 800 ppm for 1 h (6.6 ± 1.2 mg/L; Batterman et al. 1998) and was about 2-fold lower than that seen in people exposed at 400 ppm for 8 h (13.4 ± 4.8 mg/L; Franzblau et al. 1995). The 24-h EEGL value is further supported by the results of repeated-exposure studies that failed to produce any evidence of visual toxicity, narcosis, or metabolic acidosis in monkeys exposed to methanol at 1,000 ppm for 21 h per day for 21 days (NEDO 1986).

The subcommittee did not apply uncertainty factors to account for differences in methanol or formate metabolism among humans. Alcohol dehydrogenase activity measurements can differ up to about 3-fold among people (Norberg et al. 2003). However, even susceptible people would not be expected to develop blood methanol concentrations high enough to result in neurotoxicity following exposure at the recommended 24-h EEGL of 50 ppm. Data collected in cynomolgus monkeys—a species that, like humans, metabolizes formate more slowly than rodents—indicate that toxicologically significant accumulations of formate did not occur in folate-deficient monkeys exposed to [^{14}C]methanol at 900 ppm for 2 h (Dorman et al. 1994). Therefore, no adjustment was deemed necessary to account for differences in formate metabolism due to folate deficiency. Moreover, many foods have been fortified with folate to reduce the incidence of birth defects. Thus, the incidence of folate deficiency has decreased during the past decade.

90-Day CEGL

Health effects of concern following a continuous 90-day inhalation exposure to methanol would include ocular toxicity, hepatotoxicity, and neurotoxicity. The subcommittee used the NEDO (1986) study in cynomolgus monkeys to determine the recommended 90-day CEGL. The monkeys were exposed to methanol vapor at 0, 10, 100, or 1,000 ppm for 22 h per day for 7 months (two monkeys per group), 19 months (three monkeys per group), or 29 months (three monkeys per group). Exposures at up to 1,000 ppm were not associated with any evidence of optic nerve or retinal pathology (NEDO 1986). The NEDO report (1986) indicated that 10 ppm was a study NOAEL, whereas exposures at 100 ppm were associated with reversible hematologic changes, altered electrocardiograms, and subtle neuropathology (increased numbers of responsive stellate cells in the cerebral white matter) that might represent adverse responses. Chronic exposures at 1,000 ppm were associated with astrocyte hyperplasia in the basal ganglia

and metabolic acidosis. The subcommittee used the study NOAEL of 10 ppm as the 90-day CEGL. The subcommittee did not apply uncertainty factors to account for interspecies differences or time extrapolation because monkeys are a sensitive animal species and the exposure duration was much longer than 90-days. Application of uncertainty factors to account for differences in methanol and formate metabolism among people was likewise unnecessary because subtle differences in metabolism are unlikely to be a factor at 10 ppm.

Pharmacokinetic considerations can be used to support the subcommittee's decisions and the proposed 90-day CEGL value. A 24-h exposure at 10 ppm (13.1 milligrams per cubic meter [mg/m^3]) would yield an aggregate daily exposure of about 2.2 mg/kg per day assuming a 70-kg human breathing at a rate of 20 m^3 per day (twice the resting ventilation rate) and an absorption fraction of 60% (Sedivec et al. 1981; Kavet and Nauss 1990; Fisher et al. 2000). That upper-limit burden results in an estimated tissue concentration of about 0.05 mM, a value that is nearly 100-fold lower than the Michaelis-Menten constant (Km)[1] observed by Makar et al. (1968) in monkeys (8.7 mM). Actual tissue concentrations would be lower than that because of ongoing metabolism and pulmonary and renal excretion. Makar et al. (1968) estimated that primates metabolize methanol at a rate (V_{max}) of about 48 mg/kg/h. The hourly rate at which an individual would metabolize methanol is greater than the anticipated rate of intake from inhalation exposures at 10 ppm. The margin between elimination and intake rates at 10 ppm is also greater than the anticipated variability in elimination rates among humans.

The 90-day CEGL is also supported by repeated-exposure studies that failed to produce any evidence of visual toxicity, narcosis, or metabolic acidosis in monkeys exposed to methanol at 1,000 ppm for 21 h per day for 21 days (NEDO 1986). Studies by Andrews et al. (1987) and Burbacher et al. (1999a,b; 2004a,b) further support the subcommittee's recommended 90-day CEGL.

[1]The Km, or Michaelis-Menten constant, describes the affinity of an enzyme for a substrate and is defined as the substrate concentration that produces one-half the enzyme's maximum velocity.

DATA ADEQUACY AND RESEARCH NEEDS

The data available on methanol toxicity were deemed sufficient to derive EEGL and CEGL values. The subcommittee in part relied on the subchronic inhalation studies in monkeys performed by NEDO. The subcommittee recognizes that there were some weaknesses in those studies. The report produced by NEDO (1986) is often fragmentary and lacks full descriptions of the raw data from the experiments. Moreover, some of the histologic descriptions in that report are incomplete and inadequately documented. Other studies were available to support the validity of the NEDO studies. When considered collectively, the relevant studies provided an adequate database for the subcommittee's deliberations.

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8

Monoethanolamine

This chapter summarizes the relevant toxicologic studies on monoethanolamine (MEA). Selected chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure levels from the National Research Council (NRC) and other agencies are also presented. The subcommittee considered all of that information in its evaluation of the Navy's current and proposed 1-hour (h), 24-h, and 90-day exposure guidance levels for MEA. The subcommittee's recommendations for MEA exposure levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

MEA is a viscous, hygroscopic liquid (Budavari et al. 1989). Selected physical and chemical properties are summarized in Table 8-1.

OCCURRENCE AND USE

MEA has a variety of uses. It is used as a scrubbing agent to remove carbon dioxide and hydrogen sulfide from gases; as a reagent in the synthesis of surface active agents and antibiotics; as a component in polishes and emulsifiers; as a softening agent in the tanning industry; and as an agent to disperse agricultural chemicals (Budavari et al. 1989). MEA is used along with diethanolamine in cosmetic products as an emulsifier, thickener, wetting agent, detergent, and alkalizing agent (CIR 1983). Ethanolamines, including MEA, are used in synthetic and semisynthetic machining and

TABLE 8-1 Physical and Chemical Data on Monoethanolamine[a]

Synonyms	Aminoethanol, β-aminoethanol, 2-amino-1-ethanol, β-aminoethyl alcohol, 1-amino-2-hydroxyethane, ethanolamine, colamine, β-ethanolamine, ethylolamine, glycinol, 2-hydroxyethanamine, β-hydroxyethylamine, 2-hydroxyethylamine, olamine
CAS registry number	141-43-5
Molecular formula	$HOCH_2CH_2NH_2$
Molecular weight	61.08
Boiling point	170.8°C
Melting point	10.3°C
Flash point	195°C
Explosive limits	5.5% to 17%
Specific gravity	1.0117 at 25°C/4°C
Vapor pressure	0.404 mmHg at 25°C
Solubility	Soluble in water, methanol, and acetone
Conversion factors	1 ppm = 2.5 mg/m^3; 1 mg/m^3 = 0.4 ppm

[a]Data on explosive limits and vapor pressure were taken from HSDB (2003); all other data were taken from Budavari et al. (1989).
Abbreviations: mg/m^3, milligrams per cubic meter; mmHg, millimeters of mercury; ppm, parts per million.

grinding fluids as corrosion inhibitors or to adjust pH (Kenyon et al. 1993). Analysis of a selection of machining and grinding fluids identified MEA at concentrations generally less than the detection limit (0.2 micrograms per milliliter [μg/mL]) to 2%; one product contained 11% MEA (Kenyon et al. 1993).

On board submarines, MEA is used in the ventilation system scrubbers to remove carbon dioxide from the air. No atmospheric measurements of MEA on board submarines have been reported.

SUMMARY OF TOXICITY

At high concentrations, airborne MEA is an irritant to the skin, eyes, and respiratory tract of laboratory animals. Continuous exposure to high concentrations of MEA for long periods of time has been reported to pro-

duce lethargy in laboratory animals. High oral doses of MEA have been reported to result in organ weight and histopathologic changes in the liver and kidneys of laboratory animals; the effects observed suggest that MEA may interfere with lipid metabolism. The liver is the primary site of metabolism for MEA, and metabolites of MEA are found in the urine of laboratory animals. MEA is an intermediate in the formation of phospholipids and choline, and it is formed endogenously from serine. MEA is excreted in the urine of unexposed humans and animals. Genotoxicity studies with MEA have been largely negative for mutagenic and clastogenic effects. Unlike diethanolamine, MEA has not been found to form a stable nitrosamine. No medical case reports or epidemiologic studies were identified during this review of MEA. Data are lacking for several toxicity end points, including chronic exposure effects, carcinogenicity, and male reproductive effects.

Effects in Humans

Accidental Exposures

No relevant information was identified.

Experimental Studies

Weeks et al. (1960) reported that volunteers who smelled MEA vapor described the odor as ammoniacal, musty, or foreign; some volunteers were unable to characterize the odor. The MEA concentration detectable for 50% of a group of human volunteers (n = 12) was 2.6 parts per million (ppm) (95% confidence interval [CI] = 2-3.3 ppm) (Weeks et al. 1960). Volunteers detected the MEA by means of sensation rather than odor; a describable odor was noted at about 25 ppm (Weeks et al. 1960).

Occupational and Epidemiologic Studies

In a National Institute for Occupational Safety and Health (NIOSH) health hazard evaluation report (NIOSH 1993), the authors noted in a summary paragraph about MEA that "no systemic effects from industrial exposure have been reported." A similar observation was made by Beard and Noe (1981). No other relevant information was identified.

Effects in Animals

Acute Toxicity

The LC_{50} (concentration lethal to 50% of subjects) of MEA is estimated to be greater than its theoretical saturated vapor concentration (520 ppm) on the basis that no mortality was found in rats exposed to saturated MEA vapor for 6 h (Knaak et al. 1997). The acute oral toxicity (LD_{50}) of MEA in rats is in the range of 1,720-2,740 milligrams per kilogram (mg/kg) (Smyth et al. 1951; CIR 1983). The Cosmetic Ingredient Review (CIR 1983) provided the following oral LD_{50} values for other species: mouse, 700-1,500 mg/kg; rabbit, 1,000-2,900 mg/kg; and guinea pig, 600 mg/kg. The dermal LD_{50} for MEA in rabbits was reported to be 1 g/kg (ACGIH 2001).

MEA at concentrations of 1%, 5%, and 10% was tested in vivo and in vitro for histopathologic evidence of irritation using the skin of C3H mice (Helman et al. 1986). Although no skin lesions were observed, lactate dehydrogenase (LDH) values were elevated in the culture medium for skin discs exposed to MEA at 5% or 10% in vitro. Leakage of LDH from the skin discs was interpreted by the authors to suggest mild toxicity to mouse skin (Helman et al. 1986). Aqueous solutions at 25% were corrosive to rabbit skin in vivo (Knaak et al. 1997).

Undiluted MEA is considered severely irritating to the eye (Knaak et al. 1997). No animal studies for skin sensitization or allergenicity have been reported.

Repeated Exposures and Subchronic Toxicity

Treon et al. (1957) exposed dogs, cats, guinea pigs, mice, and rats to MEA vapor and aerosol using a number of exposure scenarios that included 793 mg per cubic meter (m^3) (primarily as an aerosol) for 7 h per day for 5 days or 126 mg/m^3 (primarily as a vapor) for 7 h per day for 25 days over a 30-day period. The authors noted that the only effect observed was difficult breathing in guinea pigs exposed at 260 mg/m^3 or greater.

Timofievskaya (1962) exposed rats to MEA at 80-160 ppm via inhalation for 5 h per day for up to 6 months. Decreased body weights, altered hematology, altered urine chemistries, and altered hippuric acid synthesis were observed. The authors concluded that the liver and kidneys were target organs.

Continuous inhalation exposure studies (24 h per day, 7 days per week) were conducted with MEA using dogs, guinea pigs, and rats (Weeks et al. 1960). Groups of three male beagle dogs were continuously exposed to MEA at 6, 12, 26, or 102 ppm for 60, 90, 90, or 30 days, respectively (Weeks et al. 1960). Because no attempt was made to prevent carbon dioxide (CO_2) in the exposure chambers from binding to MEA, CO_2 concentrations in the test chambers were slightly lower than in the control chambers. At 102 ppm, clinical signs were marked and included evidence of skin irritation, rales, and tremors; MEA condensed on all surfaces, causing the haircoats of the dogs to become wet, matted, and greasy. On the first day of MEA exposure, the dogs showed their immediate discomfort through behaviors, including an uneasy demeanor, scratching at the chamber door, panting, muzzle licking, and vigorous head shaking, which were followed by salivation and vomiting within a few hours. Within 24 h of initiation of exposure, the dogs responded poorly to attempts to attract their attention, and they were lethargic by 48 h. Head shaking during the first 4 days of exposure resulted in hematomas at the base of the ears. Skin irritation involving the scrotum and sternum was observed by the fourth day of exposure and became more generalized and more severe as the exposure period progressed. One of the dogs in the treatment group died after 25 days of exposure. At necropsy, skin irritation was the only consistent change. Microscopic examination of various tissues showed cloudy swelling of hepatocytes, increased Kupffer cell pigmentation, and vascular congestion in the liver. Kidney tubules also showed evidence of cloudy swelling and hyaline droplet formation. The nasal turbinate mucosa was eroded in some areas, and plasma cell infiltrates were present in other areas. The lungs of the dog that died had small foci of hemorrhage and pneumonitis. Clinical chemistry changes included decreases in albumin and increases in globulin in the three exposed dogs. Hematologic changes included increases in white blood cell count, changes in white blood cell ratios, and decreases in hemoglobin and hematocrit values in the three exposed dogs. At 26 ppm, dogs showed immediate signs of restlessness and discomfort. They were more irritable than the controls, and after a few days they were less alert and appeared lethargic. Their haircoats became wet, greasy, and matted. The dogs' skin became irritated and developed small ulcers at floor contact points in less than a week's time. At 12 ppm, there were no behavioral effects observed immediately at the beginning of the exposure period or after 1 h or 24 h of exposure to MEA. However, skin irritation was observed, and animals became lethargic after 3 weeks of exposure. At 6 ppm,

there were slight decreases in alertness and activity, and there was some skin irritation after 2-3 weeks of exposure. No significant pathology, clinical chemistry, or hematology changes were seen at exposure levels below 102 ppm.

A group of 22 male guinea pigs was exposed to MEA at 75 ppm continuously for 24 days, and a group of 30 male guinea pigs was exposed at 15 ppm continuously for 90 days (Weeks et al. 1960). At 75 ppm, the animals were restless, irritable, and obviously uncomfortable. They initially showed increased activity, but as the exposure period lengthened, they showed decreased activity and increased water consumption. Evidence of skin irritation was observed. An unstated level of mortality was seen. Microscopic examination of tissues showed fatty changes in the liver, slight cloudy swelling in the liver and kidneys, increased lymphocyte infiltration in the lungs, and decreased spermatogenesis. At 15 ppm, animals became less active after about 3 days of exposure and were definitely lethargic after 10 days. Weight gain was decreased about 10%, and water consumption increased 40%. No other changes were reported for animals in the 15-ppm group.

A group of 45 female rats was continuously exposed to MEA at 66 ppm for 30 days, a second group of 45 female rats was continuously exposed at 12 ppm for 90 days, and a third group of 20 male and female rats was continuously exposed at 5 ppm for 40 days (Weeks et al. 1960). The effects observed in rats exposed at the high and intermediate exposure concentrations were similar to those observed in guinea pigs similarly exposed. Microscopic examination of the tissues of rats exposed to MEA at 66 ppm showed fatty changes in the liver, slight cloudy swelling in the liver and kidneys, increased lymphocyte infiltration of the lungs, and patchy pneumonitis. At 5 ppm, all rats showed pelt discoloration after 12 days and transitory hair loss over the head and back after 3 weeks. Slowness in movement also was observed after 3 weeks of exposure at 5 ppm. The body-weight gains of the rats exposed at 5 ppm were not different from those of the control group. No gross or microscopic changes were observed in the organs of rats exposed at 5 or 12 ppm.

In a study reported by Smyth et al. (1951), rats were not affected by MEA in their diets when fed 320 mg/kg per day for 90 days. However, liver and kidney weights were increased at 640 mg/kg per day, and mortality was observed in animals that consumed 1,280 mg/kg per day.

Chronic Toxicity

No relevant studies were identified.

Reproductive Toxicity in Males

The testes of two dogs that survived exposure to MEA at 102 ppm for 30 days showed decreased spermatogenesis on histologic examination (Weeks et al. 1960). The testes of a third dog that died after exposure at 102 ppm also showed decreased sperm formation (Weeks et al. 1960). Spermatogenesis appeared to be decreased in an unspecified number of guinea pigs exposed to MEA at 75 ppm (Weeks et al. 1960). Due to the limited amount of information available, the significance of the findings is uncertain.

Immunotoxicity

Repeated-insult skin patch tests for allergenicity in human volunteers have shown negative results (Knaak et al. 1997). The results provided no evidence of hypersensitivity to MEA. No other immunotoxicity studies were identified in the literature.

Genotoxicity

MEA provided no evidence of mutagenicity when tested with strains TA98, TA100, TA1535, TA1537 of *Salmonella typhimurium* with or without activation by Aroclor 1254 stimulated rat or hamster S9 liver fractions (Mortelsmans et al. 1986). The Hazardous Substances Data Bank (HSDB 2003) cites a Russian study indicating that MEA is a weak inducer of chromosome breaks in cultured human lymphocytes. Dean et al. (1985) found no mutagenic or clastogenic effects in studies of MEA in *Escherichia coli* WP2 tyr, *Sacchraromyces cerevisiae*, and a rat liver cell chromosomal aberration assay. Inoue et al. (1982) found no evidence of genotoxicity in an in vitro hamster embryo transformation assay.

Carcinogenicity

No relevant studies were identified.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

MEA is the only endogenously occurring ethanolamine formed by mammalian metabolic systems. MEA is formed from serine and is an intermediate in the formation of phospholipids and choline. MEA is naturally excreted in the urine of unexposed humans (Dent and Walshe 1953; HSDB 2003). The average urinary excretion rates in men and women are 0.162 mg/kg per day and 0.492 mg/kg per day, respectively (HSDB 2003).

The systemic distribution and metabolism of [^{14}C]-labeled MEA was studied in athymic nude mice following application to their skin or application to human skin grafted onto the mice (Klain et al. 1985). Extensive metabolism of the MEA that penetrated the skin was observed; 24% of the applied radioactive dose was found in the liver, which was a major site of metabolism. Radiolabel was found in all organs examined, including the kidneys (2.53% of dose), lungs (0.55% of dose), brain (0.27% of dose), and heart (0.15% of dose). Hepatic ethanolamine, choline, and serine were highly radioactive, as were hepatic proteins. Of the topical radioactive dose, 18% was metabolized to $^{14}CO_2$, and 4.6% was excreted in the urine over 24 h. Urinary metabolites included glycine, serine, choline, and uric acid. Radiolabel was found in expired air 5 minutes after intraperitoneal injection of [^{14}C]-labeled MEA (Klain et al. 1985). The distribution and metabolism of MEA following intraperitan eal injection was similar to that seen following dermal application (Taylor and Richardson 1967).

INHALATION EXPOSURE LEVELS FROM THE NRC AND OTHER ORGANIZATIONS

Several organizations have established or proposed inhalation exposure limits or guidelines for MEA. Selected values are summarized in Table 8-2.

TABLE 8-2 Selected Inhalation Exposure Levels for Monoethanolamine from NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	3	ACGIH 2002
	TLV-STEL	6	
NIOSH	REL-TWA	3	NIOSH 2004
	REL-STEL	6	
OSHA	PEL-TWA	3	29 CFR 1910.1000
Submarine			
NRC	EEGL		NRC 1984
	1 h	50	
	24 h	3	
	CEGL		
	90 days	0.5	

[a]The comparability of EEGLs and CEGLs with occupational and public health standards or guidance levels is discussed in Chapter 1, section "Comparison to Other Regulatory Standards or Guidance Levels."

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level; h, hour; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; ppm, parts per million; REL, recommended exposure limit; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendations for EEGL and CEGL values for MEA are summarized in Table 8-3. The U.S. Navy values are provided for comparison.

1-Hour EEGL

In recommending exposure guidance levels for MEA, the subcommittee considered several issues. There is a limited amount of information about the effects associated with the inhalation of MEA. The information

TABLE 8-3 Emergency and Continuous Exposure Guidance Levels for Monoethanolamine (ppm)

Exposure Level	U.S. Navy Values		NRC Recommended Values
	Current	Proposed	
EEGL			
1 h	50	6	4
24 h	3	3	4
CEGL			
90 days	0.5	0.5	0.5

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; h, hour; NRC, National Research Council; ppm, parts per million.

that is available is incomplete when compared with modern protocols used for inhalation toxicity studies. Because MEA was deposited on the skin of test animals during inhalation exposures, absorption of MEA through the skin or ingestion of MEA due to grooming might have contributed to the systemic effects observed in some of the studies. Data on MEA exposures in humans via inhalation are not available for use in determining exposure guidance levels. Consequently, there is a significant amount of uncertainty in identifying exposure guidance levels for MEA in submarine atmospheres.

Uncertainty in interpreting clinical sign information provided in the critical study further complicates the extrapolation of animal data for developing exposure levels. The only study that provides relevant information for extrapolation is Weeks et al. (1960). In that study, MEA vapor apparently condensed on the inside of the inhalation chamber walls and other surfaces and was deposited in sufficient concentration on the haircoats of the test animals to make the hair and skin wet, greasy to the touch, and matted. The deposition of MEA on the test animals was associated with skin irritation affecting dogs, rats, and guinea pigs. As the test concentrations of MEA decreased, the onset time to grossly observable deposition and skin irritation increased. At 26 ppm, dogs showed immediate skin and behavioral changes. At 12 ppm, dogs and rats showed no effects at 1 and 24 h, but showed skin and behavioral changes similar to those seen at 26 ppm after 2-3 weeks of continuous exposure. It is unlikely that surface deposition of MEA would occur in the submarine environment or that submarine crew would tolerate the accumulation of MEA on their skin. The clothing worn by the crew would most likely protect the skin from significant MEA

deposition, and the crew would most likely remove MEA deposited on their skin by washing. Along with the reported deposition of MEA on the skin and the signs of skin irritation, test animals were observed to be less alert and to have reduced activity levels. Because the test animals were exposed nearly continuously, observations of behavioral effects were presumably made through the windows in the inhalation chamber walls. It is difficult to know precisely how to interpret the results reported by Weeks et al. (1960), because no follow-up behavioral examinations were conducted. The behavioral effects may have been a secondary consequence of the skin irritation observed; affected animals might have reduced their activity levels because of fatigue associated with chronic dermal irritation. Alternatively, the behavioral changes might have been primary effects. The subcommittee made the conservative assumption that reduced alertness and reduced activity levels were primary effects that could be used for determining exposure guidance levels.

To determine the 1-h EEGL for MEA, the 12-ppm continuous exposure in dogs conducted by Weeks et al. (1960) was chosen as the most appropriate exposure scenario for extrapolation. Although the mode of action of MEA is unknown, no effects were observed after exposure at 12 ppm for 1 or 24 h. At 12 ppm, effects associated with MEA were observed after 3 weeks of continuous exposure. Because there was little variation in the responses of dogs, rats, and guinea pigs at similar MEA exposure levels, an interspecies uncertainty factor of 3 was applied. No intraspecies uncertainty factor was applied, because little variability is expected in the responses of submarine crew members to deposition of MEA on the skin. Also, dermal allergenicity studies in human volunteers did not result in sensitization (Knaak et al. 1997), indicating that the effects of skin contact with MEA are unlikely to vary among humans. Application of the uncertainty factor of 3 to the no-effect concentration of 12 ppm results in a 1-h EEGL for MEA vapor of 4 ppm.

24-Hour EEGL

Because the 12-ppm continuous-exposure study in dogs did not result in effects until after 2-3 weeks of exposure (Weeks et al. 1960), there should not be any differences in the toxicologic results of exposures to MEA lasting 1 or 24 h. Therefore, extrapolating for the 24-h EEGL results in the same concentration (4 ppm) as was recommended above for the 1-h EEGL.

90-Day CEGL

Weeks et al. (1960) did not provide a no-effect level for repeated inhalation exposures of greater than 2-3 weeks duration. To determine a 90-day CEGL, the exposure scenario that resulted in the lowest-effect level was chosen for extrapolation. Rats exposed to MEA at 5 ppm via inhalation for 40 days showed pelt discoloration after 12 days of exposure. Transitory hair loss over the head and back and slowness in movement were observed after 3 weeks of exposure. The interspecies uncertainty factor was set at 3. Because exposure at 5 ppm resulted in minimally adverse effects, a LOAEL-to-NOAEL uncertainty factor of 3 was used for calculating the CEGL. The 90-day CEGL resulting from the application of a total uncertainty factor of 10 to the rat low-effect level of 5 ppm is 0.5 ppm. That value is an approximate order of magnitude less than that associated with behavioral changes in rats and dogs and is thus considered protective.

DATA ADEQUACY AND RESEARCH NEEDS

There is a paucity of data available for determining the effects of MEA following inhalation exposure. The available studies are considered incomplete because little information is provided about histologic, hematologic, and enzymatic changes that might be produced systemically or in the nasal turbinates following repeated or long-term exposure to MEA. None of the inhalation studies provide a no-effect level useful for direct extrapolation to human exposure conditions. Although MEA does not appear to be genotoxic, no data on carcinogenicity are available for review. Additional short-term studies would be helpful for developing 1- and 24-h exposure limits with greater confidence, because there is insufficient resolution within the available data set to support the development of different values for those time points. Well-designed continuous 90-day and lifetime studies would provide information for developing 90-day exposure limits and for determining the carcinogenic potential of MEA.

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9

Nitric Oxide

This chapter summarizes the relevant epidemiologic and toxicologic studies on nitric oxide (NO). Selected chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure levels from the National Research Council (NRC) and other agencies are also presented. No exposure levels for NO currently exist for submarines or have been proposed by the Navy. However, exposure levels for nitrogen dioxide (NO_2) have been established by the Navy and are considered to be protective against the adverse effects that might result from NO exposure. The subcommittee's recommendations for NO exposure levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

NO is a colorless gas that combines with oxygen to form NO_2 (Budavari et al. 1989). The rate of NO_2 formation depends on the concentration of oxygen and the square of the concentration of NO (NIOSH 1976). At an NO concentration of 100 parts per million (ppm), NO_2 forms at a rate of 2.8 ppm per minute (min) under normal atmospheric conditions (NIOSH 1976). An odor threshold of 0.3-1 ppm has been reported (ACGIH 2001). Selected physical and chemical properties are summarized in Table 9-1.

OCCURRENCE AND USE

NO is unstable in air and converts to NO_2. The low-oxygen conditions on board submarines will slow NO_2 formation. Because NO in air converts

TABLE 9-1 Physical and Chemical Data on Nitric Oxide[a]

Synonyms	Nitrogen monoxide
CAS registry number	10102-43-9
Molecular formula	NO
Molecular weight	30.01
Boiling point	−151.7°C
Melting point	−163.6°C
Flash point	—
Explosive limits	—
Specific gravity	1.04 with respect to air
Vapor pressure	45,600 mmHg at −94.8°C
Solubility	4.6 mL/100 mL of water at 20°C
Conversion factors	1 ppm = 1.23 mg/m^3; 1 mg/m^3 = 0.81 ppm

[a]Data on vapor pressure were taken from HSDB (2003); all other data were taken from Budavari et al. (1989).
Abbreviations: mg/m^3, milligrams per cubic meter; mL, milliliter; mmHg, millimeters of mercury; ppm, parts per million; —, not available or not applicable.

to NO_2, which is more toxic, the two chemicals should be monitored simultaneously (ACGIH 2001).

NO primarily is used as an intermediate in the production of nitric acid (Budavari et al. 1989). In recent years, NO has been recognized for its role as a regulator of cardiovascular, immune, and nervous system functions (Kiss 2000; Weinberger et al. 2001). It has been investigated and used as a treatment for various pulmonary diseases (Troncy et al. 1997).

NO is a component of smog. Sources of NO include exhaust from internal-combustion engines, smoke from fires, and tobacco smoke (ACGIH 2001). The Navy has indicated that the primary sources of nitrogen oxides on submarines are the diesel generator, the vent fog precipitator, and cigarette smoking (Crawl 2003).

SUMMARY OF TOXICITY

NO relaxes vascular smooth muscle making it an effective treatment for persistent pulmonary hypertension in newborns (Channick and Yung 1999; INO Therapeutics 2001). High NO exposures produce methemoglobinemia, a reversible event. Seger (1992) describes the clinical signs and symptoms at increasing concentrations of methemoglobin. Clinical cyanosis and "chocolate brown" blood occur in humans at about 15-20% methemo-

globin; anxiety, exertional dyspnea, weakness and fatigue, dizziness, lethargy, headache, syncope and tachycardia occur at methemoglobin concentrations between 20% and 45%; loss of consciousness begins at between 45% and 55%; and stupor, seizures, coma, bradycardia, and cardiac arrhythmias occur between 55% and 70% methemoglobin. Methemoglobin concentrations at greater than 70% lead to heart failure and death (Seger 1992).

The toxicity of air pollutants, notably NO and NO_2, may be influenced by the pattern of exposure as well as concentration and duration in that cyclical peak exposures, such as those associated with rush-hour traffic, have been shown to enhance the toxic effects of NO and NO_2 in animals (EPA 1993; Mercer et al. 1995). No information on pattern of exposure on submarines was provided to the subcommittee. The influence of exposure pattern on toxicity highlights the critical importance of continuous monitoring to characterize the submarine atmosphere.

Effects in Humans

Accidental Exposures

Two women anesthetized with nitrous oxide and oxygen became cyanotic and developed respiratory distress (Clutton-Brock 1967). One of the women developed severe pulmonary edema and died of cardiac arrest. The other developed respiratory distress but recovered completely after oxygen and steroid therapy. Later it was discovered that the nitrous oxide cylinder was contaminated with NO, and it was estimated that the NO concentration was at least 10,000 ppm (Greenbaum et al. 1967).

Experimental Studies

Clinical studies have been conducted in patients administered therapeutic doses of NO to treat various respiratory diseases. A lung transplant patient treated with NO at 80 ppm for 8 hours (h) developed a circulating methemoglobin concentration of 9.4% (Adatia et al. 1994). When the NO concentration was reduced to 40 ppm for the ensuing 4 h, the methemoglobin concentration decreased to 6.6%. The methemoglobin concentration returned to nearly normal (0.9% increase) when the inhaled NO concentration was reduced to 20 ppm for an additional 12 h. There were no adverse health effects associated with NO treatments in patients with acute respira-

tory distress syndrome who were administered NO at 20 ppm for 48 h followed by an exposure at 10 ppm for an additional 8 days (Manktelow et al. 1997). No clinical signs were noted in patients who were treated for pulmonary hypertension and cardiac disease with NO at 40 ppm for 5 min (Pepke-Zaba et al. 1991) or in patients treated for bronchial asthma and chronic obstructive pulmonary disease with NO at 80 ppm for 10 min (Hogman et al. 1993). The methemoglobin concentration of one patient treated with NO at 80 ppm for 6 h increased to 9.4%. A second patient administered NO at 80 ppm developed a methemoglobin concentration of 14% after 18 h, and a third patient developed a concentration of 9.6% after 108 h (Wessel et al. 1994). In nine infants who had congenital heart disease and were treated 21 h postsurgery with NO at 50 ppm for 41 h, the average circulating methemoglobin concentration was 1.4%, and the NO_2 concentrations were less than 2.4 ppm (Schulze-Neick et al. 1997).

Five healthy volunteers (four males and one female, ages 30-36 years) were exposed to NO at 32, 64, and 128 ppm for 3 h, and 512 ppm for 50 min (Young et al. 1994). The 512 ppm exposure was stopped when the mean methemoglobin concentration reached 5%. Six healthy male volunteers (ages 30-38 years) were exposed to NO at 100 ppm for 3 h (Young et al. 1996). It was suggested in Young et al. (1994) that maximum methemoglobin concentrations are likely reached 3-5 h after inhalation begins. Exposure to NO at up to 128 ppm for 3 h did not result in clinically significant methemoglobinemia (Young et al. 1994, 1996).

Occupational and Epidemiologic Studies

NO has been studied in association with various diseases as a component of air pollution. However, these studies often include other pollutants and lack precise measures of exposure. Further, outcomes in the general population are not relevant to the healthy submariner population. Thus, no relevant occupational and epidemiologic information was located.

Effects in Animals

Acute Toxicity

Mice exposed to NO at 350 ppm for up to 8 h all died; however, exposure at 320 ppm resulted in 50% mortality, and complete survival occurred following exposure at 310 ppm (Pflesser 1935; EPA 1993). There

was no evidence of lung injury or pulmonary edema in the mice that died, and death was thought to have occurred as a result of methemoglobin formation.

Rats exposed to NO at 10 or 50 ppm for 180 min were evaluated for changes in discrimination learning using a delayed-response operant-conditioning technique (Groll-Knapp et al. 1988). The 50-ppm dose significantly decreased the number of correct trials and the total number of lever presses. Methemoglobin concentrations did not exceed 3.98%. In another study (Garat et al. 1997), no NO-related toxic effects were noted in the lungs of rats exposed to NO at 10 or 100 ppm for 40 h while breathing either 21% or 100% oxygen.

Although 11 of 20 adult male Fischer 344 rats exposed to NO at 1,000 ppm for 30 min died 30 min post-exposure, lesions were not observed in the lungs by histopathology in the rats that lived or the rats that died (Stavert and Lehnert 1990). No effects were noted in guinea pigs exposed to NO at 175 ppm for 120-150 min (Paribok and Grokholskaya 1962). No evidence of lung injury was reported in newborn lambs with persistent pulmonary hypertension when treated with NO at 80 ppm for 23 h (Zayek et al. 1993). The average methemoglobin concentration was 3%.

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). One dog in the 640-ppm group died. No deaths occurred in the other groups. The circulating methemoglobin concentrations for the treatment groups were 3%, 6.6%, 24%, and 78%, respectively. Lung compliance increased during the 640-ppm exposure but remained stable in all other treatment groups. Lung resistance and peak inspiratory and expiratory flow rates were not affected. In a follow-up study to investigate potential electrocardiographic effects, conscious sling-restrained beagles were exposed to NO at 40, 80, 160, and 320 ppm via tracheal fistula. Cardiac conduction, rate, and rhythm were not affected at any concentration (Mihalko et al. 1998).

Repeated Exposures and Subchronic Toxicity

Waters et al. (1998) exposed rats by nose-only inhalation to NO at 0, 80, 200, 300, 400, or 500 ppm for 6 h per day for 1, 3, or 7 days. Exposures >300 ppm were lethal to rats after 1.5 h of exposure. Methemoglobin concentrations were elevated in groups receiving >200 ppm. Ultrastructural examination of terminal bronchioles and adjacent alveoli identified increased incidence and severity of interstitial edema in the 200-ppm group as compared with controls after 1 and 7 days of treatment. The findings

were attributed to a contaminating concentration of NO_2 (2.6 ppm) produced during the exposure. In a follow-up study, rats were exposed by nose-only inhalation to NO at 0, 40, 80, 160, 200, or 250 ppm for 6 h per day for 4 weeks (Waters et al. 1998). No treatment-related effects were found by light microscopy in lung tissue or major organs. No treatment-related ultrastructural changes were observed in animals exposed at 200 ppm.

Rats exposed to NO at 0.5 ppm with two daily peak exposures at 1.5 ppm for 9 weeks showed an increased number of fenestrations in the alveolar septa of the lungs, a reduced number of interstitial cells, and a thinning of the interstitial space (Mercer et al. 1995). Although NO exposure resulted in alterations of the interstitial septa of the lungs, morphological alterations of the epithelial cells were not evident. The authors considered these lesions to be the initial stages in an emphysema-like destruction of the alveolar septa. Others have reported enlargement of air spaces in rats exposed continuously to NO at 2 ppm for 6 weeks (Azoulay et al. 1977) and many large air spaces in the periphery of the lungs of mice exposed to NO at 10 ppm for 30 weeks (Holt et al. 1979). However, similar lesions were not reported in rats exposed to NO at up to 250 ppm for 6 hrs per day for 4 weeks (Waters et al. 1998) or in mice exposed to 2.4 ppm for 23-29 months (Oda et al. 1980).

No increase in death rate was observed compared with controls in mice exposed to NO at 10 ppm for 6.5 months (Oda et al. 1976) or at 2.4 ppm NO for a lifetime (23-29 months) (Oda et al. 1980). Furthermore, the blood nitrosylhemoglobin concentration remained steady at 0.01% for lifetime exposure at 2.4 ppm and at 0.13% for 6.5-month exposure at 10 ppm. The average circulating methemoglobin concentration was 0.2% for the 6.5-month exposure, and the maximum methemoglobin concentration was 0.3% in the longer study.

Chronic Toxicity

No relevant information was found regarding chronic toxicity with the exception of the study noted above (Oda et al. 1980).

Reproductive Toxicity in Males

No relevant information was found regarding the potential reproductive toxicity of NO in males.

Immunotoxicity

No relevant information was found regarding the potential immunotoxicity of NO.

Genotoxicity

There is a paucity of information on the genotoxicity of NO. Chromosome aberrations were not observed in Sprague-Dawley rats exposed to NO at 9, 19, or 27 ppm for 3 h (Isomura et al. 1984). A dose-related increase in the number of revertants of *Salmonella typhimurium* was observed in cultures exposed to atmospheres containing NO at 0-20 ppm for 30 min, but 50 ppm was cytotoxic (Arroyo et al. 1992).

Carcinogenicity

No relevant information was found regarding the potential carcinogenicity of NO.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

NO is unstable in air and oxidizes to form NO_2. However, the mechanisms of toxicity for NO and NO_2 differ. NO binds to hemoglobin, resulting in reversible methemoglobinemia. NO_2 exposure results in initial irritation with mild dyspnea followed by a delayed onset of pulmonary edema and, ultimately, interstitial fibrosis (Hine et al. 1970; NIOSH 1976). Thus, if an NO exposure is not sufficient to cause death, recovery can be complete. Exposures to NO_2 that are not rapidly lethal might result in persistent effects and even delayed death. The conversion of NO to NO_2 in medicinal applications is highly dependent on the concentration of oxygen at room temperature. Treatment of infants with NO at 50 ppm yielded NO_2 concentrations <2.4 ppm (Schulze-Neick et al. 1997). Furthermore, at NO concentrations <80 ppm, there were neither significant increases in NO_2 nor clinical levels of NO_2 toxicity (Wessel et al. 1997; Davidson et al. 1998; Finer and Barrington 2000). In addition, exposures <100 ppm usually do not form significant amounts of methemoglobin in children or adults (Winberg et al. 1994; Roberts et al. 1997; Finer and Barrington 2000).

Inhaled NO is absorbed into the blood stream and binds to hemoglobin

to form 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). The binding and formation of methemoglobin is NO concentration- and time-dependent (Ripple et al. 1989). The oxygen dissociation curve of methemoglobin is shifted markedly to the left, so oxygen is not easily released (Weinberger et al. 2001). Methemoglobin concentrations greater than 70% result in lethal hypoxia in humans (Seger 1992).

NO binding to hemoglobin in rats (Maeda et al. 1987), mice (Oda et al. 1980), and rabbits (Sharrock et al. 1984) is rapidly reversible and has a half-life of 15-20 min when the animals are placed in clean air. It is interesting to note that about 85-92% of NO is absorbed into the bodies of healthy humans during inhalation exposures at 0.33-5.0 ppm (Yoshida and Kasama 1987). However, only about 35% of inhaled NO is absorbed by the lungs in patients with acute lung injury who are exposed to NO at 5-40 ppm as ongoing therapy (Westfelt et al. 1997).

INHALATION EXPOSURE LEVELS FROM THE NRC AND OTHER ORGANIZATIONS

Several agencies have established or proposed inhalation exposure levels for NO. Selected values are summarized in Table 9-2.

TABLE 9-2 Selected Inhalation Exposure Levels for Nitric Oxide from Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	25	ACGIH 2002
NIOSH	REL-Ceiling	25	NIOSH 2004
OSHA	PEL-TWA	25	29 CFR 1910.1000

[a]The comparability of EEGLs and CEGLs with occupational and public health standards or guidance levels is discussed in Chapter 1, section "Comparison to Other Regulatory Standards or Guidance Levels."

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; ppm, parts per million; REL, recommended exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendations for EEGL and CEGL values for NO are summarized in Table 9-3.

1-Hour EEGL

Five healthy human volunteers (four males and one female, ages 30-36 years) did not manifest clinically significant methemoglobinemia when exposed to NO at 128 ppm for 3 h (average maximum methemoglobin concentration was 3.75%) (Young et al. 1994). The study suggested that maximum methemoglobin concentrations are not likely to be reached until 3-5 h of exposure. On the basis of Young et al. (1994), the subcommittee recommends a 1-h EEGL value of 130 ppm. Because Young et al. (1994) employed healthy human subjects, no uncertainty factors were applied. The recommended 1-h EEGL is supported by studies in rats and dogs demonstrating an absence of effects on methemoglobin concentrations and lung or cardiac functions during exposures at 200 ppm or less for 6 h (Mihalko et al. 1998; Waters et al. 1998).

24-Hour EEGL

At about 15-20% methemoglobin, clinical cyanosis, and "chocolate brown" blood begin to appear (Seger 1992). Thus, an increase in methemo-

TABLE 9-3 Emergency and Continuous Exposure Guidance Levels for Nitric Oxide (ppm)[a]

	U.S. Navy Values		
Exposure Level	Current	Proposed	NRC Recommended Values
EEGL			
1 h	—	—	130
24 h	—	—	50
CEGL			
90 days	—	—	3

[a]The subcommittee makes these recommendations with the understanding that NO_2 will be monitored concurrently with NO.

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; h, hour; NRC, National Research Council; ppm, parts per million.

globin formation of 10-15% could be used as a point of departure for adverse health effects resulting from NO exposure. In three patients treated for respiratory disease with NO at 80 ppm, methemoglobin concentrations were 9.6% after 108 h exposure, 14% after 18 h, and 9.4% after 6 h (Wessel et al. 1994). When the 80-ppm exposure was decreased, the percent of methemoglobinemia also decreased. In another study, a lung transplant patient treated with NO at 80 ppm for 8 h developed a circulating methemoglobin concentration of 9.4% (Adatia et al. 1994). Average methemoglobin concentrations were 1.4% in infant patients exposed to NO at 50 ppm for a mean of 41 h (Schulze-Neick et al. 1997). In healthy subjects exposed at 128 ppm for 3 h there was no clinical evidence of significant methemoglobinemia (the average methemoglobin concentration was 3.75%) (Young et al. 1994). On the basis of this information in both healthy adults and sensitive patient populations, exposures to NO at 50 ppm for 24 h would not be expected to cause any adverse health effects in a noncompromised adult human population.

90-Day CEGL

No long-term human exposure studies that used sufficiently high concentrations of NO to identify a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) for disease or lethality were found. However, in mice exposed to NO at 10 ppm for 6.5 months or at 2.4 ppm for 23-29 months, the average methemoglobin concentration was 0.2% at 10 ppm, and the maximum methemoglobin concentration was 0.3% at 2.4 ppm. There were no significant signs of disease or difference in death rates compared with controls (Oda et al. 1976; Oda et al. 1980). The subcommittee selected 10 ppm as the NOAEL to develop a 90-day CEGL. Applying an interspecies uncertainty factor of 3, the 90-day CEGL is 3 ppm. Because there is minimal variation in methemoglobin concentrations in the animal data and in the human data available for shorter durations, no additional uncertainty factors were applied.

DATA ADEQUACY AND RESEARCH NEEDS

Sufficient data are available to develop 1-h exposure limits. Additional nonlethal exposure data would assist in deriving 24-h exposure limits, because the present recommendations primarily rely on limited data in

respiratory-compromised patients who might be less sensitive to NO than normal humans. There are no supporting long-term studies on NO available to determine 90-day exposure limits or to determine the carcinogenicity of NO. Thus, well-designed, continuous 90-day and lifetime studies would provide needed information to develop 90-day exposure limits and to determine the carcinogenic potential of NO.

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Nitrogen Dioxide

This chapter summarizes the relevant epidemiologic and toxicologic studies on nitrogen dioxide (NO_2). Selected chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure levels from the National Research Council (NRC) and other agencies are also presented. The subcommittee considered all of that information in its evaluation of the Navy's current and proposed 1-hour (h), 24-h, and 90-day exposure guidance levels for NO_2. The subcommittee's recommendations for NO_2 exposure levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

NO_2 is a reddish-brown gas that decomposes in water to form nitric acid and nitric oxide (NO) (Budavari et al. 1989). The odor threshold for recognition of NO_2 in air is 0.1 to 0.4 parts per million (ppm) (NIOSH 1976). Most individuals become tolerant of or desensitized to the odor as exposure duration is increased. Selected physical and chemical properties are summarized in Table 10-1.

OCCURRENCE AND USE

NO_2 has a number of industrial applications (Lewis 1993). It is an intermediate in nitric acid production, a nitrating agent in explosives, a polymerization inhibitor for acrylates, and an oxidizing agent in rocket fuels. It has been used to bleach flour (Budavari et al. 1989).

TABLE 10-1 Physical and Chemical Data on Nitrogen Dioxide[a]

Synonyms	—
CAS registry number	10102-44-0
Molecular formula	NO_2
Molecular weight	46.01
Boiling point	21.15°C
Melting point	−9.3°C
Flash point	—
Explosive limits	—
Specific gravity	1.448 at 20°C/4°C (liquid)
Vapor pressure	908 mmHg at 25°C
Solubility	Soluble in concentrated sulfuric and nitric acids
Conversion factors	1 ppm = 1.88 mg/m^3; 1 mg/m^3 = 0.53 ppm

[a]Data on vapor pressure were taken from HSDB (2003); all other data were taken from Budavari et al. (1989).
Abbreviations: mg/m^3, milligrams per cubic meter; mmHg, millimeters of mercury; ppm, parts per million; —, not available or not applicable.

NO_2 is a component of smog and a precursor of ozone (Costa and Amdur 1996). Motor-vehicle exhaust and emissions from other commercial and industrial combustion processes are the major anthropogenic sources of NO_2 (HSDB 2003). Natural sources include forest fires and atmospheric lightning discharges (HSDB 2003). The Navy has indicated that the primary sources of NO_2 on board submarines are the vent fog precipitator, the diesel generator, and cigarette smoking (Crawl 2003).

SUMMARY OF TOXICITY

NO_2 irritates mucous membranes, inciting cough and dyspnea. Higher concentrations of NO_2 produce changes in lung function in healthy subjects and lesions in the pulmonary tract of animals. Increased airway resistance has been reported to occur when exposures to NO_2 exceed 2.5 ppm (Beil and Ulmer 1976; von Nieding et al. 1979, 1980; von Nieding and Wagner 1979). However, other investigators have not observed any NO_2-induced changes in airway resistance or spirometry at concentrations between 2 and

4 ppm (Linn et al. 1985; Mohsenin 1987, 1988; Sandström et al. 1990). Below 1 ppm, the evidence for changes in lung volumes, flow-volume characteristics of the lungs, or airway resistance in healthy subjects is very weak. Asthmatic patients and individuals with respiratory disease are considered to be more sensitive to inhaled NO_2 at concentrations greater than 1-2 ppm than are healthy individuals.

The following information comes from a comprehensive review by the U.S. Environmental Protection Agency (EPA 1993). NO_2 appears to have its primary pulmonary effects on the distal bronchioles, proximal alveolar ducts, and alveolar parenchyma. Sufficiently high concentrations of NO_2 produce subtle to major changes in pulmonary function depending on concentration and duration of exposure. The terminal conducting airways and adjacent alveolar ducts and alveoli are most sensitive to the toxic effects of NO_2. The ciliated cells of the bronchiolar epithelium and the type I cells of the alveolar epithelium are highly susceptible to NO_2-induced injury. The ciliated bronchioles can become denuded of cilia, and nonciliated bronchiolar cells (in rodents) lose their dome-like luminal surface projections. The type I cells in the alveoli become necrotic and slough, and type II cells proliferate to replace them. Pulmonary edema is the hallmark of severe NO_2 toxicosis. Death results from bronchospasm or pulmonary edema. NO_2 is not considered to be a directly acting carcinogen in animals or humans.

The immune system appears to be a secondary target of repeated exposures to NO_2 (EPA 1993). Animals treated with NO_2 and subsequently challenged with either pathogenic bacteria or viruses were less resistant to infection compared with untreated animals. Humoral immune responses were also affected. In NO_2-treated animals, there was a reduction in circulating antibody and antibody producing cells. The cellular (T-cell) immune response appeared to be less affected by NO_2 than the humoral (B-cell) response.

The toxicity of air pollutants, notably NO and NO_2, may be influenced by the pattern of exposure as well as concentration and duration in that cyclical peak exposures, such as those associated with rush-hour traffic, have been shown to enhance the toxic effects of NO and NO_2 in animals (EPA 1993; Mercer et al. 1995). No information on pattern of exposure on submarines was provided to the subcommittee. The influence of exposure pattern on toxicity highlights the critical importance of continuous monitoring to characterize the submarine atmosphere.

Effects in Humans

Accidental Exposures

Inhaled NO_2 can produce a syndrome known as silo-filler's disease. Gas that accumulates above silage in silos contains a mixture of nitrogen oxides that can attain NO_2 concentrations of 200-4,000 ppm within 2 days (Lowry and Schuman 1956; Douglas et al. 1989). Silo-filler's disease can progress from an immediate cough, dyspnea, and a choking sensation to a 2-3 week period of apparent remission, followed by fever, progressively severe dyspnea, cyanosis, cough, inspiratory and expiratory rales, neutrophilic leukocytosis, and discrete nodular densities in the lungs. Seventeen patients examined after being exposed to silo gas developed similar symptoms. Autopsy of one patient who died revealed diffuse alveolar damage with hyaline membranes, hemorrhagic pulmonary edema, and acute edema of the airways (Douglas et al. 1989).

An accidental acute exposure of hockey players and spectators to NO_2 from a malfunctioning motor on an ice resurfacer resulted in onset of cough, hemoptysis, or dyspnea during or within 48 h of the exposure (Hedberg et al. 1989). No spirometry effects were identified in the hockey players at 10 days or 2 months following exposure. NO_2 concentrations were not measured in the arena.

Experimental Studies

Healthy individuals exposed to NO_2 at <1.5 ppm generally show no symptoms or effects on pulmonary function (Folinsbee et al. 1978; Adams et al. 1987; Frampton et al. 1991; Kim et al. 1991; Hazucha et al. 1994). Although exposure at 1.5 ppm for 3 h did not significantly affect pulmonary function, there were slight but significant decreases in forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) when subjects were challenged with carbachol (Frampton et al. 1991). However, no changes were observed in the pulmonary airway reactivity or in symptoms of irritation in healthy adults exposed to NO_2 at 1 ppm for 2 h, at 2 ppm for 3 h (Hackney et al. 1978), at 2 ppm for 4 h (Devlin et al. 1992), at 3 ppm for 2 h (Goings et al. 1989), or at 2.3 ppm for 5 h (Rasmussen et al. 1992). When normal subjects were exposed to NO_2 at 2 ppm for 1 h and challenged with methacholine, there was an increase in airway reactivity, but there were no changes in lung volume or spirometry (Mohsenin 1988).

Biochemical effects have also been noted in healthy adults exposed to NO_2. Exposures at 1-4 ppm for 3-4 h have caused (1) increases in recovery of polymorphonuclear leukocytes in bronchoalveolar lavage fluid (Devlin et al. 1992; Frampton et al. 1992), (2) decreases in serum glutathione peroxidase activity (Rasmussen et al. 1992), (3) decreases in red blood cell membrane acetylcholinesterase activity and increases in peroxidized red blood cell lipids and glucose-6-phosphate dehydrogenase activity (Posin et al. 1978), (4) decreases in alpha-1-protease inhibitor activity (Mohsenin and Gee 1987), and (5) small reductions in red blood cell counts (Frampton et al. 2002).

Healthy volunteers exposed to NO_2 at 10 ppm for 6 h or at 20 ppm for 2 h noted an odor upon entering the exposure chamber (Henschler and Lütge 1963). At the 20-ppm concentration, minor scratchiness of the throat was reported by all subjects after 50 minutes (min), and three of the eight volunteers experienced a slight headache towards the end of the 2-h exposure (Henschler and Lütge 1963). Methemoglobin levels were unaffected in the 10-ppm exposure group and increased 1% on average in the 20-ppm exposure group. In another study involving exposures to 10-14 healthy volunteers, no symptoms of irritation occurred in those exposed to NO_2 at 20 ppm for 2 h, if they had been exposed to several lower concentrations of NO_2 during the preceding days (Henschler et al. 1960). An exposure at 30 ppm for 2 h, however, caused definite discomfort. Subjects experienced a burning sensation and an increasingly severe cough for most of the second hour of exposure, although the cough began to improve near the end of the exposure period. As the exposure continued, the burning sensation migrated into the lower airways and deep into the chest and was accompanied by marked sputum secretion and dyspnea. Near the end of 2 h, the exposure was described as barely tolerable.

Several studies have been conducted to assess the effects of NO_2 on pulmonary function in asthmatic individuals and patients with chronic lung disease or bronchitis. However, most of the results from studies on pulmonary function and airway hyperactivity in asthmatic humans have been inconclusive and conflicting. Nevertheless, humans with asthma appear to be at greater risk for the respiratory effects of NO_2 exposure than healthy individuals are. For example, it has been reported that asthmatic individuals exposed to NO_2 at 0.3 or 0.5 ppm for 2-4 h exhibited slight reductions in FEV_1 and specific airway conductance and experienced wheezing and tightness of the chest (Kerr et al. 1979; Bauer et al. 1985). However, in several other studies, exposures of asthmatic subjects to concentrations of NO_2 at 0.13-1.0 ppm did not significantly affect pulmonary function in

adolescents or adults during exercise or rest (Sackner et al. 1981; Kleinman et al. 1983; Linn and Hackney 1984; Koenig et al. 1985, 1987; Mohsenin 1987; Morrow and Utell 1989; Roger et al. 1990; Rubinstein et al. 1990; Vagaggini et al. 1996).

Occupational and Epidemiologic Studies

As mentioned above, silo-filler's disease is an occupational hazard to farmers (Lowry and Schuman 1956; Douglas et al. 1989). Welders are exposed to a mixture of fumes, gases, and NO_2. An acetylene-torch welder developed shortness of breath and chest discomfort while welding for about 30 min in a confined space. Eighteen hours after the incident, chest X-rays revealed pulmonary edema. Simulation of the incident produced a concentration of NO_2 of at least 90 ppm within 40 min and total oxides of nitrogen in excess of 300 ppm (Norwood et al. 1966). Morley and Silk (1970) measured NO_2 at 30 ppm during a 40-min welding job. No adverse effects were observed in the six people present. Morley and Silk (1970) also described 11 cases, including one resulting in death, of "nitrous fume gassing" of workers in the chemical, engineering, and shipbuilding industries whose symptoms included choking, cough, dyspnea, cyanosis, headache, chest pain and tightness, nausea, and pulmonary edema. Similar signs and symptoms occurred in four firemen who were exposed to an unknown amount of NO_2 (Tse and Bockman 1970).

In a review of epidemiologic studies, the U.S. Environmental Protection Agency (EPA 1993) determined that there was insufficient evidence to make a conclusion about the long- or short-term health effects of exposure to NO_2. The studies reviewed included investigations of (1) lung function, respiratory symptoms, and various respiratory diseases in relation to gas-stove use in the home (a surrogate for NO_2 exposure) and (2) lung function, respiratory symptoms, various respiratory diseases, and mortality in relation to both indoor and outdoor NO_2 concentrations. The majority of the studies did not include individual exposure measurements or estimates. The literature indicates that infants and adults respond similarly to NO_2, but children 5-12 years of age and people with pre-existing disease appear to be more sensitive to low-level NO_2 exposures (EPA 1995). Recent investigations of similar design and type have yielded similar results (Farrow et al. 1997; Pilotto et al. 1997; Schindler et al. 1998; Peters et al. 1999a,b; Fusco et al. 2001; Brunekreef and Holgate 2002; Wong et al. 2002). Epidemiologic studies, by design, identify associations between health effects and expo-

sures but are usually inadequate for defining continuous-exposure concentrations pertinent to the setting of EEGLs and CEGLs for submariners.

Effects in Animals

Acute Toxicity

Hine et al. (1970) studied the effects of NO_2 at varying concentrations and exposure durations in mice, rats, guinea pigs, rabbits, and dogs. At 40 ppm for varying durations, lacrimation, conjunctivitis, and increased respiration occurred in all five species. When all species were exposed to NO_2 at 20 ppm for 24 h, they exhibited minimal signs of irritation and changes in behavior, and histologic examination revealed lung congestion and interstitial inflammation. Lethality was first noted in guinea pigs exposed at 50 ppm for 1 h, in rats and mice exposed at 50 ppm for 24 h, and in rabbits and dogs exposed at 75 ppm for 1 and 4 h, respectively.

Wistar rats were exposed to NO_2 at 25, 75, 125, 175, or 200 ppm for 10 min (Meulenbelt et al. 1992a,b). No signs of toxicity were observed at 25 ppm. At 75 ppm, there were significant increases in lung weights and in subpleural hemorrhages accompanied by pale discoloration of the lung. Histopathology revealed atypical pneumonia, edema, focal desquamation of the terminal bronchiolar epithelium, and increased numbers of macrophages and neutrophilic lymphocytes. The lesions increased in severity at the higher concentrations, and interstitial thickening of the centriacinar septa was present in the 175-ppm and 200-ppm exposure groups. When the rats were exposed at 175 ppm for 20 min, five of six of them died.

The lung weights of male Fischer 344 rats were increased significantly following exposures to NO_2 at 150 ppm for 5 min, 100 ppm for 15 min, and 75 ppm for 30 min (Lehnert et al. 1994). Rats exposed at 90 ppm for 15 min or 72 ppm for 60 min showed severe signs of respiratory distress and eye irritation lasting about 2 days, and they showed significantly increased lung-to-body weight ratios during the first 48 h after exposure (Carson et al. 1962). Histopathology revealed pulmonary edema and an increased incidence of chronic murine pneumonia. Rats exposed at 65 ppm for 15 min or 28 ppm for 60 min had mild signs and symptoms, whereas those exposed at 33 ppm for 15 min had no adverse clinical signs of toxicity or pathologic changes. Histopathologic changes have been noted in the type I and type II cells of the lungs of Wistar rats exposed to NO_2 at 20 ppm for 20 h (Hayashi et al. 1987). Other studies have noted similar changes as well as alveolar

and interstitial edema, bronchiolitis, bronchiolar epithelial cell hyperplasia, and loss of cilia 1-3 days following exposure at 26 ppm for 24 h (Schnizlein et al. 1980; Hillam et al. 1983) or at 20 ppm for 24 hours (Rombout et al. 1986).

When Sprague-Dawley rats were exposed to NO_2 at 14 ppm for 24 h, 48 h, or 72 h, Stephens et al. (1978) observed minor loss of cilia from the epithelial cells lining the terminal airways. In another study of Wistar rats exposed to NO_2 at 2 or 10 ppm for 3 days, the tracheal and bronchiolar epithelium were sporadically deciliated and fibrinous deposits were observed in the alveoli at the 10-ppm concentration (Azoulay-Dupuis et al. 1983).

Changes in minute-ventilation have been evaluated in Fischer 344 rats exposed to NO_2 at 100, 300, or 1,000 ppm for 1-20 min (Lehnert et al. 1994) or at 200 ppm for 15 min (Elsayed et al. 2002). As concentrations increased, there were decreases in the minute-ventilation, which were considered to be the result of declines in tidal volume but not in breathing frequency.

Respiratory function was monitored in squirrel monkeys exposed to NO_2 at 10-50 ppm for 2 h (Henry et al. 1969). Only slight effects on respiratory function and mild histopathologic changes in the lungs were noted at the 10 and 15 ppm concentrations. At the 35- and 50-ppm concentrations there were marked increases in respiratory rate and decreases in tidal volume. Histopathologic changes in the lungs were severe.

Repeated Exposures and Subchronic Toxicity

Alveolar macrophages have an immunosurveillance role in the lungs. The effects of NO_2 on resident alveolar macrophages have been inconsistent. Wistar rats exposed to NO_2 at 10 ppm for 28 days exhibited inhibition of the immunosuppressive activity of alveolar macrophages (Koike et al. 2001). In a study of New Zealand rabbits exposed to NO_2 at 0.3 ppm for 2 h per day for 13 days, there was a decrease in macrophage phagocytic capacity, although exposure at 1.0 ppm for 2 days increased phagocytic capacity (Schlesinger 1987). No effects were observed after 6 days of exposure at 1.0 ppm. In two studies with Fischer 344 rats, there was a trend toward increased numbers of alveolar macrophages and increased cell volume when the rats were exposed to base concentrations of NO_2 at 0.5 ppm and 2.0 ppm for 22 h per day, 7 days per week and to two 1-h peak concentrations of 1.5 ppm and 6.0 ppm on 5 of 7 days for 6 weeks (Crapo et al.

1984; Chang et al. 1986). An increase in alveolar macrophages was noted in the lungs of Wistar rats exposed to NO_2 at 2.7 ppm for 4 weeks, but not in rats exposed at 1.3 or 0.5 ppm (Rombout et al. 1986). An increase in alveolar macrophages was also observed in Fischer 344 rats exposed to NO_2 at 5 ppm for up to 15 weeks (Gregory et al. 1983) and in Wistar rats exposed at 10 ppm for 21 days (Hooftman et al. 1988). Phagocytic activity was reduced in the alveolar macrophages after exposure to NO_2 at 25 ppm for 14 and 21 days (Hooftman et al. 1988). However, in Fischer 344 rats, suppression of phagocytic activity occurred after 7 days of exposure at 4 ppm and 5 days of exposure at 8 ppm, but activity returned to normal following 10 days of exposure at those concentrations (Suzuki et al. 1986). In Fischer 344 rats exposed to NO_2 at 10 ppm for 1, 3, and 20 days, the numbers of inflammatory cells and the total protein concentrations were increased in the bronchoalveolar lavage. Tumor necrosis factor-alpha was markedly reduced, and interleukin-10 and interleukin-6 were increased in alveolar macrophages (Garn et al. 2003).

In mice treated with NO_2 at either 1 or 5 ppm for 6 h on 2 consecutive days, a concentration-dependent decrease in alveolar macrophage phagocytosis was observed in the lower respiratory tract (Rose et al. 1989). However, when the exposure concentration was increased to 15 ppm for the same duration, no further affect on alveolar macrophage phagocytosis was noted. A significant increase in vital capacity of the lung occurred in rats exposed to NO_2 at 0.5 ppm for 6 h per day, 5 days per week for 4 weeks, but no effects were noted at the 1-ppm concentration (Evans et al. 1989). When Wistar rats were exposed to NO_2 at 5.4 ppm for 3 h per day for 30 days, there was a tendency toward increased lung volume (Yokoyama et al. 1980), although a definite increase in lung volume was observed in Fischer 344 rats exposed at 9.5 ppm for 24 months (Mauderly et al. 1990). Decreases in tidal volume and increases in respiratory rate were observed in squirrel monkeys exposed continuously (24 h per day) to NO_2 at 5 ppm for 2 months (Henry et al. 1970).

There was mild loss of bronchiolar cilia and fibrin deposition in the alveoli of Wistar rats exposed to NO_2 at 10 ppm for 3 days (Azoulay-Dupuis et al. 1983). No lesions were observed at the 2-ppm concentration for 3 days or when Sprague-Dawley rats were exposed at 5 ppm for 3 days (Messiha et al. 1983). Hypertrophy and hyperplasia of type II cells were noted in the lungs of Swiss-Webster mice exposed to NO_2 at 0.34 ppm for 6 h per day, 5 days per week for 6 weeks (Sherwin and Richters 1982). No pathology was noted in five species of animals (guinea pig, rabbit, dog,

squirrel monkey, or rat) continuously exposed to NO_2 at 0.53 ppm for 90 days (Steadman et al. 1966).

No pulmonary pathology was seen in Wistar rats continuously exposed at up to 1.3 ppm for 28 days (Rombout et al. 1986). At 2.7 ppm, there was focal thickening of the centriacinar septa, progressive loss of cilia in the trachea and main bronchi, and hypertrophy of the bronchiolar epithelium and epithelial cells. Those lesions were more severe at the 10.6-ppm concentration and included extensive shortening and loss of cilia in the trachea and bronchioles, necrosis of type I cells, an increase in the number of type II cells, thickening of the proximal alveolar septa, alveolar dilatation, and increased numbers of macrophages in the bronchioles (Rombout et al. 1986). Wistar rats exposed to NO_2 at 10.6 ppm for 4 days had significantly increased pulmonary activities of glucose-6-phosphate dehydrogenase, glutathione reductase, and glutathione peroxidase and increased numbers of type II cells (van Bree et al. 2000).

No lesions were noted in the nasal cavities or lungs of Wistar rats exposed to NO_2 at 4 ppm for 6 h per day, 5 days per week for up to 21 days (Hooftman et al. 1988). At 10 ppm, there were increases in the cellularity of the bronchiolar walls, alveolar ducts, and adjacent alveoli. Hypertrophy or hyperplasia of small bronchi and bronchiolar epithelium was observed. These lesions were exacerbated at the 25-ppm concentration. Three other studies revealed lesions in the lungs of Wistar rats (Hayashi et al. 1987), JCL:SD rats (Kyono and Kawai 1982), and guinea pigs (Yuen and Sherwin 1971) continuously exposed to NO_2 at 10 ppm for 14 days, 1 month, and 6 weeks, respectively.

Chronic Toxicity

In several studies in which rats were exposed to NO_2 at 2 ppm continuously for 360-763 days, no inflammation was observed, but the rats exhibited loss of cilia in bronchioles, decreased numbers of ciliated cells, hypertrophy and hyperplasia of bronchiolar epithelium, increased thickness of collagen fibrils, alveolar distention, and variability of alveolar sizes (Freeman et al. 1968; Stephens et al. 1971a,b, 1972; Evans et al. 1972). Rats exposed continuously at 0.8 ppm during their natural lifetimes grew normally but showed elevated respiratory rates and occasional minimal changes in the morphology of bronchiolar epithelial cells (Freeman et al. 1966).

Bronchial epithelial hyperplasia was observed in Sprague-Dawley

rats continuously exposed to NO_2 at 4 ppm for 16 weeks (Haydon et al. 1965). Fischer 344 rats exposed to NO_2 at 9.5 ppm for 7 h per day, 5 days per week for 6 months had no histologic changes (Mauderly et al. 1987; Mauderly 1989). However, by 24 months of exposure, mild hyperplasia of the epithelium in terminal bronchioles and extension of bronchiolar epithelial cell types into proximal alveoli were observed (Mauderly et al. 1989; Mauderly et al. 1990). An occasional alveolus contained a slight mixed inflammatory-cell infiltrate.

Monkeys (*Macaca* species) exposed to NO_2 at 2 ppm continuously for 14 months revealed bronchiolar epithelial hypertrophy and changes to cuboidal cells in the proximal bronchiolar epithelium (Furiosi et al. 1973). Several monkeys exposed at 5 ppm continuously for 2 months had normal minute respiratory volumes but had depressed tidal volumes and a compensatory increase in respiratory rate (Henry et al. 1970). There were mild effects in tidal volume, minute-volume, and respiration rates in squirrel monkeys exposed at 1.0 ppm for 493 days (Fenters et al. 1973).

EPA (1993) includes a discussion of the potential for chronic NO_2 exposures to cause emphysema in animals. Intermittent or continuous exposures to NO_2 ranging from 1 to 90 ppm for 12-33 months have yielded positive or equivocal results in squirrel monkeys, Wistar rats, hamsters, and guinea pigs (Gross et al. 1968; Freeman et al. 1972; Fenters et al. 1973). In Fenters et al. (1973), only monkeys challenged with influenza virus in addition to the NO_2 exposure developed histopathologic changes indicating slight emphysema. Studies with negative results for emphysema from exposures to NO_2 ranged from 0.5 to 30 ppm with intermittent or continuous exposures from 12-25 months to mongrel dogs, mice, rats, rabbits, hamsters, and guinea pigs (Wagner et al. 1965; Freeman et al. 1968; Blair et al. 1969; Kleinerman et al. 1985; Mauderly et al. 1989, 1990). The relevance of some of the studies to human emphysema was questioned because of differences in the clinical definitions of emphysema for humans and animals (EPA 1993).

Reproductive Toxicity in Males

No information was found regarding the reproductive toxicity of NO_2 in humans. In animals, no effects on spermatogenesis or germinal or interstitial testicular cells were observed in male LEW/fmai rats exposed to NO_2 at 1.0 ppm for 7 h per day, 5 days per week for 21 days (Kripke and Sherwin 1984).

Immunotoxicity

Several studies have investigated the effects of NO_2 on the immune system and on host resistance to infectious agents. Mice exposed to NO_2 at concentrations ranging from 0.5 to 10 ppm continuously for 15 days to 12 months exhibited increased mortality when challenged with *Streptococcus* species (Ehrlich et al. 1979; Gardner 1980; Gardner et al. 1982; Graham et al. 1987; Miller et al. 1987). Similar results were observed in mice exposed at 0.5 ppm continuously for 3 months and challenged with *Klebsiella pneumoniae* (Ehrlich and Henry 1968) or exposed to the viral agents A/PR/8 (Henry et al. 1970; Ito 1971) or cytomegalovirus (Rose et al. 1988, 1989).

Two studies evaluating humoral immunity in the CD-1 mouse and the squirrel monkey reported reduced serum neutralizing antibody titers, but there was no change in hemagglutination inhibition titers (Fenters et al. 1971; Fenters et al. 1973; Ehrlich et al. 1975). The mice were exposed to a 0.5-ppm base concentration of NO_2 plus a 1-h daily 2-ppm peak for 3 months, and the monkeys were exposed to NO_2 at either 1 or 5 ppm continuously for 16 months or 169 days, respectively. Several other studies did not report significant effects on antibody responses (Antweiler et al. 1975; Lefkowitz et al. 1986; Fujimaki 1989; Rose et al. 1989). A decrease in the plaque-forming cell response was observed in mice exposed at 1.5 ppm for 14 days (Lefkowitz et al. 1986), at 1.6 ppm continuously for 4 weeks (Fujimaki et al. 1982), or at 20 ppm for 48 h (Azoulay-Dupuis et al. 1985).

When BALB/c mice were exposed to NO_2 at 10 ppm for 2 h per day for 30 weeks, there was a marked depression in the ability of T cells to respond to nonspecific stimuli (Holt et al. 1979). Others have noted an alteration in T lymphocyte subpopulations and a tendency toward suppression in the percentages of total T cells or subpopulations of T cells (Richters and Damji 1988; Damji and Richters 1989; Selgrade et al. 1991; Frampton et al. 2002).

The role of NO_2 in allergic responses in animals is unclear. In a recent study in which BALB/c mice were challenged with ovalbumin and exposed to NO_2 at 5 or 20 ppm for 3 h, it was determined that NO_2 can both exacerbate and inhibit some features of the development of allergic disease in mice (Proust et al. 2002).

NO_2 has immunosuppressive effects on both the local (pulmonary) and systemic immune responses in laboratory animals. Those effects can occur at relatively low concentrations of NO_2 but usually require long durations of exposure. Although there is sufficient evidence to suggest that NO_2 could

increase the severity of pulmonary infections in animals, it is unclear whether that effect would occur in humans.

Genotoxicity

No information was found regarding the genotoxicity of NO_2 in humans. A concentration-dependent increase in chromosome aberrations was observed in Sprague-Dawley rats exposed to NO_2 at 8, 15, 21, or 27 ppm for 3 h (Isomura et al. 1984). Lung cells isolated from the rats and exposed to concentrations of NO_2 at 15 ppm and greater showed a concentration-related increase in mutation to ouabain resistance.

Carcinogenicity

No relevant information was found regarding the carcinogenicity of NO_2 in humans. There is no evidence that exposure to NO_2 induces neoplasia in laboratory rodents. However, NO_2 was a weak promoter of tumor development in initiation-promotion experiments (Benemanskii et al. 1981; Richters and Richters 1989; Ichinose et al. 1991).

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

About 70-80% of inspired NO_2 is absorbed by the respiratory tract in healthy adult humans (EPA 1993). Pulmonary absorption of NO_2 appears to be regulated by a reaction between inhaled NO_2 and constituents of the pulmonary surface lining layer that forms nitrite (Saul and Archer 1983; Postlethwait and Bidani 1990, 1994). It is thought that NO_2 causes pulmonary injury by lipid peroxidation, either through a reaction that involves hydrogen abstraction by readily oxidizable tissue components to form nitrous acid and an organic radical (Postlethwait and Bidani 1994; EPA 1995) or through reaction with water which forms nitrous and nitric acid (Greenbaum et al. 1967; Goldstein et al. 1977). The peroxide products formed disrupt cellular membranes that are essential for maintaining cellular integrity and function, and they probably account for the epithelial damage to the lungs and the pulmonary edema (EPA 1995).

The reactive products of inhaled NO_2 are distributed throughout the body via the blood stream (Goldstein et al. 1977). When nitrite that is formed deep in the lungs by absorption of NO_2 interacts with red blood cells, the nitrite is oxidized to nitrate (Postlethwait and Mustafa 1981). Thus, inhaled NO_2 produces nitrosylhemoglobin but not methemoglobin (Oda et al. 1980). After exposure, the half-life of nitrite is several minutes, whereas that of nitrate is about 1 h (Oda et al. 1981).

NO_2 is an irritant to the mucous membranes that causes coughing and dyspnea that can persist for a few hours following exposure (NIOSH 1976). More severe effects of exposure to NO_2 include cyanosis, chest pain, moist rales, and pulmonary edema (NIOSH 1976; Douglas et al. 1989). Death results from bronchospasm and pulmonary edema associated with hypoxemia, respiratory acidosis, metabolic acidosis, and a shift of the oxyhemoglobin dissociation curve to the left (Douglas et al. 1989). It is not uncommon in the acute phase of NO_2 intoxication to have an apparent recovery followed by late-onset bronchiolar injury that develops into bronchiolitis fibrosa obliterans (NIOSH 1976; NRC 1977; Douglas et al. 1989).

Acute exposures to high concentrations of NO_2 can result in immediate death, delayed symptoms with pulmonary edema within 48 h, or apparent recovery followed by chronic pulmonary disease of varying severity (NIOSH 1976; NRC 1977). An investigation of the morphologic and biochemical changes in the lungs of mice exposed to NO_2 at 140 ppm for 1 h (Siegel et al. 1989) revealed acute cell death in areas adjacent to the distal terminal bronchioles accompanied by a significant increase in protease inhibitor activity, lung protein content, and lung wet weights. Two days following exposure, hypertrophy and hyperplasia of the epithelial cells, increased numbers of intraalveolar macrophages and neutrophils, complete obliteration of the alveolar structure with progressive congestion and edema of the lungs, and further biochemical changes, including increases in B-glucuronidase, lactate dehydrogenase, and choline kinase activities, were observed. Other lesions included the loss of ciliated cells, disruption of capillary junctions, degeneration of type I cells, and proliferation of type II cells (Siegel et al. 1989; Elsayed 1994).

INHALATION EXPOSURE LEVELS FROM THE NRC AND OTHER ORGANIZATIONS

Several agencies have established or proposed inhalation exposure levels for NO_2. Selected values are summarized in Table 10-2.

TABLE 10-2 Selected Inhalation Exposure Levels for Nitrogen Dioxide from NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	3	ACGIH 2002
	TLV-STEL	5	
NIOSH	REL-STEL	1	NIOSH 2004
OSHA	PEL-Ceiling	5	29 CFR 1910.1000
Submarine			
NRC	SEAL-1 (10 days)	5	NRC 2002
	SEAL-2 (24 h)	10	
General Public			
NAC/NRC	Proposed AEGL-1 (1 h)	0.5	EPA 2004
	Proposed AEGL-1 (8 h)	0.5	
	Proposed AEGL-2 (1 h)	12	
	Proposed AEGL-2 (8 h)	6.7	
NRC	SPEGL (1 h)	1	NRC 1985
	SPEGL (24 h)	0.04	

[a]The comparability of EEGLs and CEGLs with occupational and public health standards or guidance levels is discussed in Chapter 1, section "Comparison to Other Regulatory Standards or Guidance Levels."

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; AEGL, acute exposure guideline level; h, hour; NAC, National Advisory Committee; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; ppm, parts per million; REL, recommended exposure limit; SEAL, submarine escape action level; SPEGL, short-term public emergency exposure guidance level; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendations for EEGL and CEGL values for NO_2 are summarized in Table 10-3. The current and proposed U.S. Navy values are provided for comparison.

1-Hour EEGL

Upon entering the chamber, healthy male volunteers exposed to NO_2 at 30 ppm for 2 h noted an intense odor; however, that odor was unnotice-

TABLE 10-3 Emergency and Continuous Exposure Guidance Levels for Nitrogen Dioxide (ppm)

	U.S. Navy Values		
Exposure Level	Current	Pro posed	NRC Recommended Values
EEGL			
1 h	1	3	10
24 h	1	1	2
CEGL			
90 days	0.5	0.5	0.7

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; h, hour; NRC, National Research Council; ppm, parts per million.

able by 25-40 min postentry (Henschler et al. 1960). For the first hour of exposure, one individual complained of a slight tickling of the mucous membranes of the nose and throat after 30 min, and two others reported the same nose and throat symptoms after 40 min. No other symptoms were experienced until 70 min of exposure, when all subjects experienced a burning sensation and an increasingly severe cough that began to decrease after an exposure duration of 100-120 min. When healthy male volunteers were exposed at either 10 ppm for 6 h or 20 ppm for 2 h, minor scratchiness of the throat was noted after 50 min, and three of eight subjects reported slight headaches toward the end of the exposure period (Henschler and Lütge 1963).

The 2-h, 30-ppm concentration exposure in humans was used to derive the 1-h EEGL (Henschler et al. 1960). During the first hour of exposure in that study, three subjects complained of a slight tickling of the mucous membranes of the nose and throat. An intraspecies uncertainty factor of 3 was applied, resulting in a 1-h EEGL of 10 ppm. The intraspecies uncertainty factor was used because of the small number of human subjects studied and the mild symptoms observed in three subjects within the first hour.

24-Hour EEGL

In the absence of appropriate human studies, the subcommittee relied on the animal toxicology literature to set the 24-h EEGL. Five different species of laboratory animals (mice, rats, guinea pigs, rabbits, and dogs)

exposed to NO_2 at 20 ppm for 24 h exhibited minimal signs of irritation and changes in behavior and some congestion and interstitial inflammation in the lungs (Hine et al. 1970). Histopathologic changes observed in rats exposed at 20 ppm for 20 h included changes in type I and type II cells (Hayashi et al. 1987). Other studies reported changes in type I and II cells, alveolar and interstitial edema, bronchiolitis, bronchiolar epithelial cell hyperplasia, and loss of cilia occurring at 1-3 days following exposure to NO_2 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). In another study (Stephens et al. 1978), Sprague-Dawley rats exposed to NO_2 at 14 ppm for 24, 48, or 72 h exhibited minor losses of cilia from the epithelial cells lining the terminal airways.

Thus, 20 ppm was selected to develop a 24-h EEGL. Applying an interspecies uncertainty factor of 3 and an intraspecies uncertainty factor of 3 resulted in a composite uncertainty factor of 10. The 24-h EEGL is 2 ppm. This value is supported by several observations in healthy human volunteers, indicating that concentrations of NO_2 in excess of 2 ppm can incite functional changes in the lungs (EPA 1993).

90-Day CEGL

In the absence of long-term quantitative human data, the nonhuman primate data was used to develop the 90-day CEGL. Monkeys exposed to NO_2 at 2 ppm continuously for 14 months had bronchiolar epithelial hypertrophy forming cuboidal epithelium in the proximal bronchioles (Furiosi et al. 1973). In squirrel monkeys exposed to NO_2 at 1 ppm for 493 days, there were mild effects in tidal volume, minute-volume, and respiration rates. Slight emphysema was observed but only in monkeys also challenged with influenza virus (Fenters et al. 1973). Thus, minimal effects are observed in nonhuman primates exposed to NO_2 continuously for longer than 1 year. Continuous exposure at 5 ppm for 2 months in squirrel monkeys did not affect minute respiratory volume, but depressed tidal volume, producing a compensatory increase in respiratory rate (Henry et al. 1970). In addition, rats exposed to NO_2 at 2 ppm continuously for a lifetime exhibited slight changes in pulmonary morphology but had normal life-spans (Freeman et al. 1968). No pathology was noted in five species of laboratory animals exposed to NO_2 at 0.53 ppm continuously for 90 days (Steadman et al. 1966).

The subcommittee began with an NO_2 concentration of 2 ppm, which

is a concentration associated with only mild respiratory effects over a 14-month continuous exposure. The subcommittee applied an interspecies uncertainty factor of 3 yielding a 90-day CEGL recommendation of 0.7 ppm. No additional uncertainty factors were applied given research indicating that there is minimal variation in the mild respiratory effects sometimes observed in healthy and asthmatic populations at exposure concentrations <1 ppm.

DATA ADEQUACY RESEARCH NEEDS

Sufficient data were available, having a fairly high degree of confidence, to develop 1-h and 24-h exposure limits for NO_2. The 90-day exposure limit was based on long-term exposures in nonhuman primates, which could result in conservative values. Thus, continuous subchronic and chronic exposure data are needed to improve the subcommittee's confidence in the 90-day exposure limit determined.

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11

Oxygen

This chapter summarizes relevant epidemiologic and biomedical studies associated with exposures to low-oxygen atmospheres. Selected chemical and physical properties and pathophysiologic and mechanistic data are also presented. The subcommittee considered all of that information in its evaluation of the Navy's current and proposed 1-hour (h), 24-h, and 90-day guidance levels for oxygen. The subcommittee's recommendations for minimal oxygen levels are provided at the conclusion of this chapter along with a discussion of the adequacy of the data for defining those levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

Oxygen is a colorless, odorless, tasteless gas that supports combustion (Budavari et al. 1989). Selected physical and chemical properties are listed in Table 11-1.

OCCURRENCE AND USE

Oxygen is a highly combustible gas that is necessary to sustain life (Patty 1963; Ebbing and Wrighton 1996). The principal uses of oxygen stem from its strong oxidizing and life-sustaining properties. It is used in medicine for therapeutic purposes (Ebbing and Wrighton 1996). Oxygen is used in steel production, copper smelting, and coal gasification (Hawley 1977). It also is used in the synthesis of methanol, acetylene, and other chemicals and as an oxidizer for liquid rocket propellants (Hawley 1977).

TABLE 11-1 Physical and Chemical Properties of Oxygen[a]

Synonyms and trade names	Molecular oxygen
CAS registry number	7782-44-7
Molecular formula	O_2
Molecular weight	32
Boiling point	−182.96°C
Melting point	−218.4°C
Flash point	—
Explosive limits	—
Density	1.429 g/L at 0°C
Vapor pressure	760 mmHg at −183.1°C
Solubility	1 volume of gas dissolves in 32 volumes of water or 7 volumes of alcohol at 20°C; soluble in other organic liquids
Conversion factors	1 ppm = 1.31 mg/m^3; 1 mg/m^3 = 0.76 ppm

[a]Data on vapor pressure were taken from HSDB (2004); all other data were taken from Budavari et al. (1989).

Abbreviations: g/L, grams per liter; mg/m^3, milligrams per cubic meter; mmHg, millimeters of mercury; ppm, parts per million; —, not available or not applicable.

Ambient air is composed of 20.9% oxygen, 78.1% nitrogen, 0.03% carbon dioxide, and less than 1% other gases (Lide 1991). Data collected on nine nuclear-powered ballistic missile submarines indicate an average partial pressure of oxygen (PO_2, the product of the barometric pressure and the percentage of oxygen in the ambient atmosphere) of 148 millimeters of mercury (mmHg) and a range of 123-188 mmHg; data collected on 10 nuclear-powered attack submarines indicate an average PO_2 of 149 mmHg and a range of 118-180 mmHg (Hagar 2003). See Box 11-1 for descriptions of terms related to gas pressures and oxygen physiology.

SUMMARY OF TOXICITY AND ADVERSE RESPONSES ASSOCIATED WITH LOW-OXYGEN ENVIRONMENTS

Oxygen is a highly combustible gas that is necessary to sustain animal life. Excessive amounts of oxygen in the system (hyperoxia) can be detrimental to human health. Oxygen toxicity can occur with exposure to hyperoxic conditions at ambient pressure or in hyperbaric environments. Oxygen toxicity is characterized by pulmonary toxicity, neurotoxicity, and

BOX 11-1 Terms Related to Gas Pressures and Oxygen Physiology

Terms related to the pressure of the atmosphere or the partial pressure of oxygen

Normobaric: Denoting a barometric pressure equivalent to sea-level pressure (760 mmHg).

Hyperbaric: Pertaining to pressure of ambient gases above sea-level normal (>760 mmHg).

Hypobaric: Pertaining to pressure of ambient gases below sea-level normal (<760 mmHg).

Partial pressure of oxygen (PO_2): The partial pressure of oxygen is determined by the barometric pressure. At sea level, the atmospheric pressure is 760 mmHg, and oxygen makes up 20.946% of inspired air. Thus, at sea level, oxygen exerts a partial pressure of about 159 mmHg (760 × 0.20946).

Fraction of inspired oxygen (FiO_2): The percent of oxygen in the inspired gas.

Terms related to the partial pressure of oxygen within different sites of the respiratory tract

Partial pressure of inspired oxygen (PiO_2): The partial pressure of oxygen in inspired air is not equivalent to that found in the atmosphere. Water vapor humidifies the inspired air and dilutes the amount of oxygen by reducing the partial pressure by the saturated vapor pressure (47 mmHg). The PiO_2 at sea level is 149 mmHg ([760 − 47] × 0.2094).

Alveolar partial pressure of oxygen (P_AO_2): The partial pressure of oxygen in the alveolus is controlled by the rate of oxygen absorption into the blood and the rate at which oxygen is delivered by ventilatory processes. The P_AO_2 at sea level is about 104 mmHg.

Terms that define oxygen transport within the blood

Arterial oxygen tension (PaO_2): Equivalent to the partial pressure of oxygen in the plasma phase of arterial blood or the amount of dissolved oxygen in the plasma phase. The PaO_2 is determined by P_AO_2 and the interface between the alveolus and the capillaries. Measured by an electrode that senses dissolved oxygen molecules, such as a co-oximeter.

Alveolar-arterial PO_2 difference: The difference between measured PaO_2 and calculated P_AO_2.

(continued)

Oxygen saturation of hemoglobin (SaO_2): The percentage of all the available heme binding sites saturated with oxygen is the hemoglobin oxygen saturation or the SaO_2. SaO_2 is determined mainly by PaO_2 and the relationship between the two variables is the oxygen dissociation curve. An SaO_2 of 97% means that for every 100 hemoglobin binding sites, 97 are occupied with an oxygen molecule, and the other three are either bound to something else or are unbound.

Oxygen saturation curve: The oxyhemoglobin dissociation curve mathematically equates SaO_2 to PaO_2.

Hyperoxia: An excess of oxygen in the system resulting from exposure to high oxygen concentrations, especially at hyperbaric pressures of oxygen.

Hypoxia: A concentration of oxygen in arterial blood that is less than normal. **Anoxia** refers to complete lack of oxygen.

ocular toxicity (Carraway and Piantadosi 1999). Oxygen toxicity in humans is dependent on both the exposure concentration and the duration of exposure. For example, studies in human volunteers have shown that adverse pulmonary effects can occur following 12-16 h of exposure to oxygen at 1.0 absolute atmospheres (ATA), following 8-14 h of exposure at 1.5 ATA, or following 3-6 h of exposure at 2.0 ATA (Clark and Lambertsen 1971; Clark et al. 1999). Oxygen-induced pulmonary toxicity is characterized by substernal and tracheal irritation and cough consistent with tracheobronchitis (Carraway and Piantadosi 1999).

Exposure to abnormally high oxygen concentrations (for example, PO_2 above 1.5 to 2.0 ATA) is also associated with seizures, dizziness, nausea, tunnel vision, blindness, fatigue, anxiety, confusion, ataxia, and other signs of neurotoxicity (Carraway and Piantadosi 1999). Retrolental fibroplasia is a common ophthalmologic effect seen in newborn children exposed to high levels of oxygen under normal barometric (normobaric) conditions (Weinberger et al. 2002). Oxygen-induced retinopathies have been reported to occur under ambient conditions in adults (Nichols and Lambertsen 1969; Kobayashi and Murakami 1972); however, the incidence of that disease is quite low (Carraway and Piantadosi 1999). Some sailors with specialized job duties, such as divers, potentially are at risk for oxygen toxicity; however, oxygen toxicity is not anticipated to be a significant concern among submariners, because submarines are often kept oxygen deficient to decrease the risk of onboard fires. Therefore, this review focuses on hypoxic, rather than hyperoxic, environmental conditions.

Hypoxia may be defined as "any state in which the oxygen in the lung, blood and/or tissues is abnormally low compared with that of normal resting man breathing air at sea level" (Bartels et al. 1973). Environmental hypoxia, as occurs on board submarines, exists when the barometric pressure or the PO_2 is low (Bartels et al. 1973). Exposures to hypoxic conditions can trigger adaptive and adverse responses. Physiologic changes involve the cardiovascular, pulmonary, and hematopoietic systems and include hyperventilation, tachycardia, pulmonary hypertension, cerebral vasoconstriction, systemic vasodilation, hypocapnia, respiratory alkalosis, erythropoietin synthesis, enhanced red blood cell production, and an increased hematocrit. Other changes include altered moods and impaired cognitive or motor performance. People who have not acclimated to hypoxic conditions can develop headaches, fatigue, shortness of breath, nausea, anorexia, sleep disturbances, or vomiting. Severe hypoxia can also result in pulmonary edema, cerebral edema, or retinal hemorrhage, although these lesions are most commonly seen at high altitudes.

Effects in Humans

Environmental Exposures to Low-Oxygen Environments

Although oxygen makes up about 20.9% of the atmosphere at all elevations, PO_2 decreases as elevation increases, which means that there is less oxygen available for respiration at high altitudes. At sea level, the barometric pressure is about 760 mmHg, and the PO_2 in dry air is about 160 mmHg (760 mmHg × 0.209). Inspired air is humidified, thus reducing the PO_2 to 149 mmHg. In a normal human, the PO_2 in the alveolus is similar to that in blood (about 100 mmHg in alveolus vs about 94 mmHg in the arterial blood, where oxygen is carried by hemoglobin). At 5,500 m, the barometric pressure is about 380 mmHg, and the PO_2 is only 80 mmHg (380 × 0.209). As one ascends in altitude, the partial pressure of inspired oxygen (PiO_2, PO_2 corrected for the water vapor that humidifies the inspired air and dilutes the amount of oxygen), the arterial oxygen tension (PaO_2, a measure of the partial pressure of oxygen in the plasma phase of arterial blood), and the arterial oxygen saturation (SaO_2, the percentage of heme binding sites saturated with oxygen) also decrease (see Table 11-2).

Environmental hypoxia is a common occurrence since a significant portion of the human population lives at high altitude (≥2,500 meters [m]). Responses observed in people due to high altitude (hypobaric hypoxia)

TABLE 11-2 Arterial Blood Gas Values Associated with Different Altitudes

Altitude (m)	Barometric Pressure (mmHg)	Partial Pressure of Inspired Oxygen (PiO_2) (mmHg)	Arterial Oxygen (PaO_2) (%)	Arterial oxygen Saturation (SaO_2) (%)
0	760	149	94	97
1,500	630	122	66	92
2,500	564	108	60	89
3,000	523	100	53	85
3,600	483	91	52	83
4,600	412	76	44	75
5,500	379	69	40	71
6,100	349	63	38	65
7,300	280	52	34	50

Abbreviations: m, meters; mmHg, millimeters of mercury.
Source: Hultgren 1997. Reprinted with permission; copyright 1997, Hultgren Publications.

have been extensively studied and may be relevant for submariners. At altitudes above 2,500 m, most people exhibit decreased SaO_2 (Moore 2000). People can live at high altitudes because they acclimate to the decreased PO_2 associated with reduced atmospheric pressure. Physiologic changes in response to high altitudes predominantly involve the cardiovascular, pulmonary, and hematopoietic systems (Hultgren 1997; Moore 2000). A decrease in PaO_2 is a potent stimulus to the carotid and aortic chemoreceptors and results in hyperventilation and tachycardia. Decreased PaO_2 (hypoxia) is also a powerful stimulus for increased cerebral blood flow and vasodilation. However, hypocapnia caused by hypoxic hyperventilation may cause cerebral vasoconstriction, thus offsetting vasodilatory effects of hypoxia. Hypoxic hyperventilation also can result in a respiratory alkalosis that causes a shift of the oxyhemoglobin curve to the left.

Increased ventilation and enhanced maximum oxygen extraction are additional aspects of immediate and chronic high-altitude acclimatization (Moore 2000). Longer-term responses include elevated renal erythropoietin synthesis, which results in enhanced red blood cell production and increased hematocrit. Genetic differences have been recognized in some high-altitude natives, such as Quechaus and Sherpas. Some high-altitude natives demonstrate blunted hypoxic ventilatory responses, which are mediated by the carotid body oxygen sensor; reduced hypoxic pulmonary vasoconstrictor responses; increased blood volumes; elevated red blood cell mass;

and additional modifications to striatal muscle metabolism (Hochachka et al. 1999; Moore 2000). Many of these changes are also found in people adapted for endurance performance (Hochachka et al. 1999).

People who have not acclimated to high altitude before engaging in physical activities can develop acute high-altitude illness, which also is referred to as acute mountain sickness (AMS). Headache is the most common AMS symptom and is often most intense during the night and shortly after arising in the morning. This phenomenon is usually attributed to increased hypoxemia caused by altitude-induced periods of sleep apnea. Other symptoms may include fatigue, shortness of breath, nausea, anorexia, sleep disturbances, or vomiting (Hackett and Roach 2001; Basnyat and Murdoch 2003). Symptoms generally develop within 6-18 h after arrival at high altitude. The incidence of AMS among participants attending North American conferences in locations at altitudes of 1,920-2,956 m was about 25% (Montgomery et al. 1989; Honigman et al. 1993). Rapid ascent to altitudes above 3,600 m causes AMS in many people (34-68%) (Sonna 2002). Rapid ascent to altitudes at or above 5,333 m causes severe, incapacitating symptoms in almost all individuals. Although AMS is usually self-limiting, in rare cases individuals can develop life-threatening high-altitude pulmonary edema (HAPE) or high-altitude cerebral edema (HACE). The incidence of HAPE or HACE is altitude-dependent and is reportedly 0.1-8% (Sonna 2002; Basnyat and Murdoch 2003). Risk factors for the development of AMS include the rate of ascent, final altitude, exertion, and individual susceptibility factors, including age, presence of lung disease, and physical condition (Honigman et al. 1993; Basnyat and Murdoch 2003).

Experimental studies have confirmed the development of symptoms compatible with AMS in people exposed to hypobaric hypoxic conditions. For example, Shukitt-Hale et al. (1998) exposed 23 U.S. Army personnel (18-29 years of age) to an environment mimicking low altitude and two environments mimicking moderately high altitude, equivalent to 550, 4,200, and 4,700 m, respectively, for 4.5 h. The participants were evaluated using symptom, mood, and cognitive and motor performance measures. At 4,700 m, significant effects were exhibited in 7 of 9 symptom measures, 12 of 16 mood measures, and 7 of 10 cognitive and motor performance measures. Cognitive and motor performance was affected on relatively simple tasks, such as simple and choice reaction time and addition tests. At 4,200 m, significant effects were exhibited in 1 of 9 symptom measures, 4 of 16 mood measures, and 4 of 10 cognitive and motor performance measures. The authors concluded that exposure to a simulated altitude of 4,200 m was associated with fewer AMS symptoms compared with incidences seen

following exposures to the equivalent of 4,700 m, suggesting that even relatively small changes in altitude can have a dramatic effect on symptom reporting and performance. Shukitt and Banderet (1988) showed that acute human exposures to environmental conditions equivalent to an altitude of 1,600 m results in mood changes. Friendliness, clear thinking, dizziness, sleepiness, and unhappiness were increasingly reported at 4,300 m, whereas only sleepiness was over-reported at 1,600 m. At 4,300 m, the altered moods differed from baseline on the day of arrival (within 1-4 h), differed even more after 1 day (18-28 h), and returned to baseline by day 2 (42-52 h). Morning and evening values were similar at each altitude. Therefore, changes in mood states at higher altitudes have a distinct and measurable time course. Studies performed by Li et al. (2000a) in healthy young college students (17-18 years of age) showed that 1-h exposures to simulated high altitudes at or above 2,800 m were associated with decreased SaO_2 and adverse effects on mood state. Reduced performances on a visual four-choice reaction-time test were observed following a 1-h exposure to simulated high altitudes at or above 3,600 m (Li et al. 2000b).

The pathophysiology of high-altitude diseases has been reviewed (Roach and Hackett 2001; Basnyat and Murdoch 2003). It is thought that environmental hypoxia triggers several changes that precede AMS development, including impaired gas exchange, increased sympathetic activity, fluid retention and redistribution, increased cerebral blood flow, altered blood-brain barrier permeability, and ultimately cerebral edema (vasogenic edema) in the most severe cases. Development of HACE represents a severe end-stage manifestation of this vasogenic response. Rapid ascent to high altitude is also associated with the development of noncardiogenic pulmonary edema. HAPE is thought to be caused by the combination of hypoxia-induced pulmonary hypertension and increased permeability of the pulmonary capillary endothelium (Basnyat and Murdoch 2003). HAPE is further characterized by elevated pulmonary artery pressure, normal left atrial filling pressure, and normal ventricular function (Basnyat and Murdoch 2003).

Clinical manifestations of HAPE include a nonproductive cough, rales, dyspnea on exertion, fever, fatigue, weakness, resting tachycardia and tachypnea, and cyanosis. Left untreated, HAPE can be rapidly fatal. Risk factors for HAPE include moderate to severe exertion, exposure to cold temperatures, anxiety, young age, male gender, and possibly obesity. HAPE usually begins within the first 2-4 days after rapid ascent to high altitudes, and onset commonly occurs during the second night of sleep at high altitude. Most physicians have maintained that HAPE is unknown or rare at

elevations below 2,440 m (Raymond 2003). However, Gabry et al. (2003) described 52 lowlanders who developed HAPE after skiing at 1,400-2,400 m. Although this retrospective study could not rule out possible confounding etiologies for pulmonary edema, such as infection or illicit drug use, it provides intriguing evidence that some individuals might develop HAPE at lower altitudes than previously recognized (Raymond 2003). The barometric pressure at 1,500 m is 630 mmHg, and the PO_2 is about 132 mmHg. Those conditions are analogous to breathing air containing about 17.3% oxygen at sea level. A retrospective study by Cremona et al. (2002) further supports Gabry's observations that lung disease could occur in the absence of frank signs of HAPE. Cremona showed that at 4,559 m about 75% of 262 climbers who were not diagnosed with HAPE had an increase in closing volume suggestive of subclinical pulmonary edema. Forty (15%) of the participants also had radiologic or physical diagnostic evidence—rales or pulmonary edema on chest radiographs—indicating increased pulmonary extravascular lung volumes. Only one participant had clinical features consistent with full-blown HAPE.

Experimental Studies

Multiple human chamber studies have evaluated physiologic responses, clinical signs and symptom reporting, and effects on cardiovascular and cognitive performance in people exposed to low-oxygen environments. One issue that needs to be considered is whether responses to environmental hypoxia are influenced by barometric pressure. There is some evidence in sheep that pulmonary responses observed under normobaric hypoxic conditions and hypobaric hypoxic conditions are not equivalent; indeed, clinical effects are often more severe under hypobaric conditions (Levine et al. 1988). However, Sheedy et al. (1996) failed to demonstrate that normobaric hypoxic conditions and hypobaric hypoxic conditions resulted in different cardiopulmonary responses in rats. Roach et al. (1996) exposed nine healthy men (ages not specified) to a simulated high-altitude environment (equivalent to 4,564 m; barometric pressure = 432 mmHg; PiO_2 = 80 mmHg), a normobaric hypoxic environment (barometric pressure = 760 mmHg, PO_2 = 80 mmHg), or a normoxic hypobaric environment (barometric pressure = 432 mmHg; PiO_2 = 115 mmHg), which was achieved by adding supplemental oxygen to the chamber, for 9 h. Symptoms associated with AMS were more prevalent in the simulated high-

altitude environment (five of nine subjects developed AMS) than in the normobaric hypoxic environment (two of nine subjects developed AMS) despite the presence of similar SaO_2 values (about 83%) in the two treatment groups. A relative lack of AMS symptoms following exposures to normobaric hypoxic conditions was reported by Meehan (1986). It is unknown why combined environmental hypoxia and hypobaria exacerbates AMS symptoms in subjects. Tucker et al. (1983) reported greater pulmonary ventilation during normobaric hypoxic conditions (equivalent to 14% oxygen) than during a high-altitude (similar to 4,570 m) exposure in six resting subjects exposed for 2-h to the same PO_2. Studies by Loeppky et al. (1996, 1997) have largely substantiated those results. Collectively, these studies suggest that normobaric hypoxic conditions result in fewer adverse effects when compared with findings seen in people at equivalent hypoxic high-altitude (hypobaric) conditions. Because of the possible differences between the responses observed under normobaric and hypobaric conditions, the majority of the subcommittee's review focuses on experimental studies conducted under conditions that would be found on board submarines (that is, normobaric hypoxic atmospheres).

Exposure to environmental hypoxia under normobaric conditions can impair physical performance. For example, Taylor and Bronks (1996) reported that in healthy young male subjects (average age of 20.9 years; n = 14), exercise times were reduced about 25% when performing moderate exercise (30 to 60 watts) on a cycle ergometer and breathing an oxygen-deficient atmosphere (fraction of inspired oxygen [FiO_2] = 0.135) compared with exercise times recorded for the same subjects under normoxic conditions (FiO_2 = 0.2093). The SaO_2 values at exhaustion under hypoxic conditions were lower than those observed under normoxic conditions (69.9% vs 93.4%). Exercise under hypoxic conditions also was associated with increased heart rates, reduced maximal oxygen uptake, and increased plasma lactate and ammonia concentrations.

Piehl Aulin et al. (1998) examined whether normobaric hypoxic conditions affect erythropoiesis, blood pressure, physical performance, or mood. Healthy young male (n = 17) and female (n = 3) endurance athletes (20 to 32 years of age) were housed under normoxic, mild hypoxic (16.2% oxygen), or moderate hypoxic (14.9% oxygen) conditions intermittently (12 h hypoxic, 12 h normoxic) for 10 consecutive days followed by exposure to normoxic conditions for 7 days. PaO_2 and SaO_2 were significantly decreased during the 10-day intermittent hypoxic exposure. Living at normobaric hypoxia corresponding to an altitude of 2,000 m for 12 h per day was

associated with increased production of erythropoietin and stimulation of erythropoiesis within 2 to 5 days of initial exposure. Increased erythropoietin production and concomitant increases in red blood cell production also were observed by Rodriguez et al. (2000) in people exposed to hypobaric hypoxic conditions for 90 minutes (min) and by Berglund et al. (2002) in people (n = 7) exposed for 10 days to moderately hypoxic (reported PO_2 = 14 kilopascals, equivalent to 105 mmHg), normobaric conditions. Piehl Aulin et al. (1998) further report that submaximal and maximal oxygen uptakes, blood pressures at rest and during exercise, and profile of mood states (POMS) tests did not change during their study.

Hodkinson et al. (2003) examined whether normobaric hypoxia causes activation of coagulation and might therefore increase the risk of venous thromboembolism. These investigators exposed six healthy male volunteers to either dry air or a hypoxic gas mixture composed of 12.8% oxygen in nitrogen (equivalent to breathing air at 3,660 m) for 3 h. The volunteers were seated during exposure. Hodkinson et al. (2003) did not observe significant differences in hemostatic or endothelial markers between the control and hypoxic groups even though platelet and leukocyte counts were significantly higher in the hypoxic group. There were increases in fibrinogen and von Willebrand factor as well as rheological changes, but these changes were not significantly different from those exhibited in controls.

Altitudes as low as 1,500 m (PO_2 about 127.5 mmHg, equivalent to an atmosphere containing about 17.9% oxygen at sea level) have been associated with reduced cognitive and motor performance (Gustafsson et al. 1997). Chamber studies, including several experiments designed to replicate conditions found on board submarines, have shown that exposures to normobaric hypoxic conditions also might result in reduced cognitive performance. Karlin and Curtis (1945) demonstrated reduced physical performance and mental efficiency in submariners exposed to an atmosphere containing 17% oxygen and 3% carbon dioxide for 50 h. Shukitt et al. (1988) reported that short-term (15-day) exposure to normobaric atmospheres containing 13% oxygen can result in decreased cognitive function, altered mood states, moderate AMS symptoms, and impaired fine motor control. For 5 consecutive days, Cymerman et al. (2002) exposed seven submariners (31.8 ± 6.1 years of age) to environmental conditions that would be encountered on board a disabled submarine (environmental hypoxia, 16.75% oxygen; hypercapnia, 2.5% carbon dioxide; and cold temperatures, 4°C). Within 2 days, subjects reported cold stress and muscle discomfort that lasted throughout the rest of the study. Participants also developed decreased postural control after 66 h of exposure. Loss of bal-

ance was especially apparent when the testing was performed with the subject's eyes closed. The submariners did not report a significant increase in AMS symptoms despite the hypoxic conditions within the chamber. The study authors concluded that the observed effects would not impair submariners' abilities to perform their duties when participating in a rescue effort.

Gustafsson et al. (1997) and Linde et al. (1997) simulated normal work shifts on board Swedish and U.S. submarines under three normobaric hypoxic conditions. Young male volunteers (20-28 years of age, n = 22) participated in the experiments. The men were exposed to intermittent to nearly continuous hypoxic conditions and were tested using a battery of cognitive performance measures. Prolonged (10-day) continuous exposures to moderately hypoxic conditions (PO_2 = 105 mmHg) were associated with symptoms compatible with AMS. Symptoms of AMS were also exhibited during more intermittent exposures (for example, 24-h) at a PO_2 of 97.5 mmHg. Performance on one test of motor function (finger tapping) was adversely affected by intermittent exposures at a PO_2 of 97.5 mmHg or 10-day exposures at a PO_2 of 105 mmHg. Linde et al. (1997) reported that effects on cognitive performance were found to be small and were prevented when the PO_2 was maintained above 97.5 mmHg.

Physical exertion may further exacerbate the cognitive effects of normobaric hypoxic environmental conditions. Knight et al. (1990a) exposed 13 healthy male subjects (average age, 24 ± 6) to 13%, 17%, or 21% oxygen atmospheres for 15 days. Chamber carbon dioxide levels were raised to 0.9% (ambient air contains about 0.03%) to mimic conditions on board submarines. Subjects were tested at rest and under submaximal work rates designed to achieve 35% or 65% of the maximum rate of oxygen uptake. A significant reduction in SaO_2 (83-85% under hypoxic conditions vs 94% at ambient conditions) was observed during exercise in atmospheres containing 13% and 17% oxygen. Despite decreases in SaO_2, Knight et al. (1990a) did not demonstrate a significant decrement in the volunteers' abilities to solve computational problems during exposures to moderate hypoxic conditions even when subjects performed moderate exercise. Knight et al. (1990b) reported symptoms in 13 healthy male subjects (average age, 24 ± 6) exposed to both normoxic and hypoxic conditions for 15 days. Exposures were conducted under conditions that simulated atmospheres found on board submarines. The exposure scenarios were as follows: 21% oxygen for 3 days, 17% oxygen for 3 days, 21% oxygen for 3 days, 13% oxygen for 3 days, and 21% oxygen for 3 days. Because of the similarity in the subjects' physical measures (age, weight, height), the subcommittee presumes that the cohorts used in the two Knight studies

were identical. Significant changes in AMS scores were observed in volunteers when exposed to atmospheres containing 17% oxygen at hypobaric pressure (PO_2 = 98 mmHg) or when exposed to atmospheres containing 13% oxygen at normobaric pressure (PO_2 = 99 mmHg). Many of the subjects (42%) displayed symptoms compatible with AMS after 1 day of exposure to an atmosphere containing 13% oxygen. Fewer of the subjects (8%) exposed at 13% oxygen continued to report symptoms of AMS for the next 2 days. Symptoms compatible with AMS were observed in 3 of 11 of the subjects when they were exposed to an atmosphere containing 17% oxygen for 3 days and only when the barometric pressure in the chamber was reduced to 576 mmHg for the final 7 h of exposure (Knight et al. 1990a).

Cerebral hypoxia may produce slowing of the alpha rhythm, increases in the slow-wave components, and other changes in the human electroencephalogram (EEG). Van der Worp et al. (1991) used quantitative EEGs to investigate cerebral hypoxia in humans exposed to normobaric hypoxic conditions. Middle cerebral blood flow velocity and cortical EEGs were collected in a group of healthy young male subjects (20-27 years of age, n = 10) before and during exposures to environmental hypoxia when SaO_2 values were 80%, 70%, and 60%. Environmental hypoxia associated with an SaO_2 of 60% caused an increase in EEG slow-wave activity. Lesser degrees of environmental hypoxia caused only minimal EEG changes. Schellart and Reits (2001) demonstrated that acute exposures of healthy volunteers (18-52 years of age, n = 14) to normobaric hypoxic conditions (equivalent to an atmosphere containing about 10% oxygen) caused a rapid increase (within 20 min) in the amplitude of all bands. The volunteers also had significantly reduced SaO_2 (mean was 67.2 ± 6.0% after 20 min), and some individuals demonstrated sleepiness or reduced attentiveness when their SaO_2 dropped below 55%. Kraaier et al. (1988) showed that normobaric hypoxic conditions equivalent to conditions at 6,096 m caused increases in slow-wave activity and decreases in alpha-wave activity. Ozaki et al. (1995) observed similar EEG changes in people exposed to hypobaric hypoxic conditions equivalent to those at altitudes of 3,000-6,000 m. Changes in the alpha rhythm (10-11 hertz), the most sensitive EEG parameter examined, occurred at hypobaric hypoxic conditions equivalent to those found at 3,000 m. Increased environmental hypoxia further depressed that wave component. Changes in theta rhythm were also observed when conditions equivalent to those at an altitude of 5,000 m were reached.

An acute decrease in PaO_2 is a strong stimulus for increased cerebral blood flow. Van der Worp et al. (1991) showed that exposures to normo-

baric hypoxic conditions resulted in increased blood flow velocity. Huang et al. (1987) likewise showed that transient increases in cerebral blood flow occur in people following initial exposures to high-altitude conditions. Buck et al. (1998) used positron emission tomography (PET) with $[^{15}O]H_2O$ to assess cerebral blood flow in eight healthy volunteers (average age, 28 ± 5 years) exposed to normobaric hypoxic conditions that mimicked those at 3,000 or 4,500 m of elevation. PaO_2 values observed following exposures to simulated altitudes of either 3,000 or 4,500 m were 57.2% ± 6.5 and 40.9% ± 5.1, respectively. Increased brain blood flow was observed following exposures to hypoxic conditions associated with an altitude of 4,500 m. Changes were most pronounced in the hypothalamus (32.8% increase), thalamus (19.2% increase), and cerebellum (17.6% increase). Increases in other brain regions varied from 9.1% to 13.8%.

Occupational and Epidemiologic Studies

Few occupational studies have explored whether chronic hypoxia is associated with any adverse health effects. Basnyat and Litch (1997) assessed the incidence of illness among porters and trekkers in the Himalayan mountains. A cohort of 155 members of commercial trekking groups (102 Nepali porters, 31 Nepali trek staff, and 22 Western trekkers) were observed for 22 days as they ascended elevations ranging from 487 m to 5,100 m. Medical problems occurred in 45% of subjects. High-altitude pharyngitis and bronchitis (12%), AMS (8%), and gastroenteritis (6%) were the most common illnesses reported. West (1999) discussed how supplemental environmental oxygen can improve sleep quality, mental performance, productivity, and general well-being in high-altitude workers.

Airline personnel is another group that routinely works under hypoxic conditions. Commercial jets operate under reduced atmospheric pressure, low humidity, and mild hypoxic conditions. Nicholas et al. (1998) reported that U.S. pilots and navigators have exhibited significantly increased mortality due to cancer of the kidneys and renal pelvis, motor neuron diseases, and external causes. In addition, increased mortality due to prostate cancer, brain cancer, colon cancer, and cancers of the lip, buccal cavity, and pharynx was suggested. These results must be considered cautiously, because other environmental factors associated with high-altitude work, such as increased cosmic radiation, might also be implicated in these findings.

Effects in Animals

Because hypoxia has been well studied in humans, the subcommittee's review of the experimental animal literature focused on less-studied clinical end points, including histologic changes in organs following acute to chronic hypoxia. The other literature cited further describes the effects of severe hypoxia.

Acute Toxicity

Kleinsasser et al. (2003) reported that pigs can develop radiographic and clinical features compatible with early HAPE after 48-h exposures to normobaric hypoxic conditions (10% oxygen). There is some experimental evidence that prior respiratory-tract infection might increase the risk of HAPE. Carpenter et al. (1998) showed that weanling rats infected with Sendai virus that were exposed to normobaric hypoxic conditions (PO_2 = 76 mmHg) for 24 h exhibited the early stages of pulmonary edema.

Repeated Exposures, Subchronic Toxicity, and Chronic Toxicity

Subchronic (21-day) or chronic (>90-day) exposures to moderate to severe environmental hypoxia (for example, atmospheric oxygen <10-12%) in rats are associated with multiple pathologic changes, including reduced body weight and body-weight gain (Sheedy et al. 1996; Cervos Navarro et al. 1999; Lorente et al. 2002), adrenal gland and spleen atrophy (Wolman et al. 1993; Lorente et al. 2002), carotid body hypertrophy (Clarke et al. 2000), hypertension (Schweda et al. 2000), increased numbers of myocardial mitochondria (Cervos Navarro et al. 1999), right ventricular hypertrophy (Sheedy et al. 1996; Schweda et al. 2000), increased lung growth (Sekhon and Thurlbeck 1996), multi-organ lipidosis (Wolman et al. 1993), remodeling of the brain and lung microvasculature (LaManna et al. 1992; Aguirre et al. 2000), and hippocampal neuronal loss (Shukitt-Hale et al. 1996). Cervos-Navarro et al. (1991) found that cats exposed to decreasing amounts of oxygen (21%, 15%, 10%, 8%, 7%, and 5%) over a period of 320 days developed microvascular proliferation and decreased Purkinje cell numbers.

Reproductive Toxicity in Males

Hypoxia is hypothesized to reduce fertility (Vitzthum and Wiley 2003); however, few studies have been conducted to confirm that hypothesis. Saxena (1995) reported that rhesus monkeys exposed to a simulated altitude of 4,411 m (barometric pressure = 428.8 mmHg) for 6 h per day for 21 days developed decreased semen volumes, sperm counts, and sperm motility as well as elevations in pH and in fructose concentrations. Those changes were associated with degeneration of the germinal epithelium and spermatogenic arrest observed at the end of the exposure; monkeys had not recovered 3 weeks after the exposure. Gonzales et al. (1990) reported that rats exposed to conditions associated with an altitude of 4,340 m for 4 days developed pyknotic primary spermatocytes and spermatids, necrosis of numerous cells, and sloughing of primary spermatocytes. Gosney (1984) showed that young adult male Wistar albino rats exposed to hypobaric hypoxic conditions at a barometric pressure of 380 mmHg for 28 days had smaller testes compared with controls kept in ambient conditions. Histologic examinations of the testes revealed degeneration and sloughing of spermatogenic cells and changes in Leydig cell numbers in the hypoxic rats. Fahim et al. (1980) showed that chronic exposures to a simulated 6,000-m environment were associated with reduced plasma testosterone concentrations and vacuolization and pyknosis of spermatogenic tissues.

Immunotoxicity

There is very limited data available concerning immunologic effects associated with hypoxia. Klokker et al. (1993) exposed eight healthy male volunteers (22-28 years of age) to room air at a simulated altitude of 5,486 m for 20 min in a hypobaric chamber. Exposures to severe hypobaric hypoxic conditions resulted in transient increases in leukocyte concentrations and natural killer-cell numbers and activity.

Genotoxicity

No data could be found indicating that hypoxia results in genotoxicity.

Carcinogenicity

No data could be found indicating that hypoxia results in carcinogenicity. Airline pilots reportedly exhibit increased mortality due to cancer of the kidneys and renal pelvis (Nicholas et al. 1998). Those findings were not considered by the subcommittee, because airline personnel are exposed to increased cosmic radiation and other environmental factors that are absent on board submarines.

PHYSIOLOGIC, TOXICOKINETIC, AND MECHANISTIC CONSIDERATIONS

The mammalian alveolus is designed to absorb oxygen from the air and subsequently deliver it to the blood via a concentration gradient. The oxygen dissociation curve shows the percent saturation of hemoglobin at various PO_2 (Figure 11-1). The sigmoid shape of the oxygen dissociation

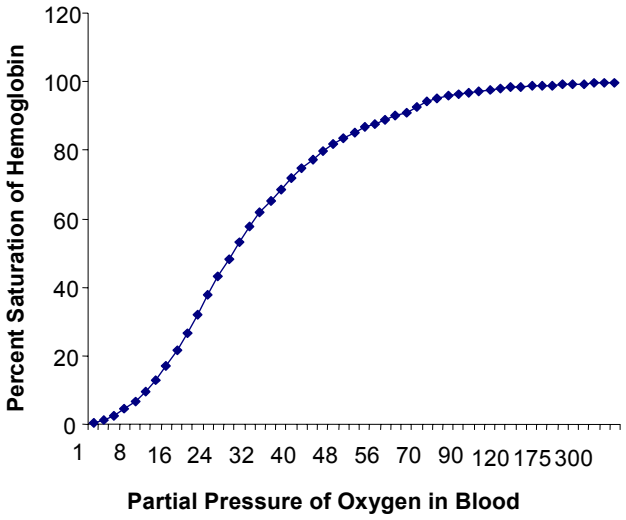

FIGURE 11-1 Hypothetical human blood oxygen dissociation curve at 37°C, pH = 7.4.

curve is a result of the cooperative binding of oxygen to the four polypeptide chains. Thus, hemoglobin is most attracted to oxygen when three of the four polypeptide chains are bound to oxygen. Increased body temperature, increased partial pressure of carbon dioxide, decreased blood pH, and increased 2,3-diphosphoglycerate favor the off-loading of oxygen from the hemoglobin molecule.

Under normal conditions, the plateau of the oxyhemoglobin dissociation curve occurs at a PO_2 of 70 mmHg. At high PO_2, usually in the lungs, hemoglobin binds to oxygen to form oxyhemoglobin. When the blood is fully saturated, all the erythrocytes are in the form of oxyhemoglobin. PO_2 below 60 mmHg have increasingly negative effects on the oxygen saturation of hemoglobin.

Oxygen circulates in the blood and diffuses to the tissues, via a concentration gradient, to be used in cellular metabolism. As the erythrocytes travel to tissues deprived of oxygen, the PaO_2 decreases. Consequently, oxyhemoglobin releases oxygen to form hemoglobin. The amount of oxygen available to tissues is dependent on the amount of oxygen entering the lungs, the efficiency of the pulmonary gas exchange, the blood flow to the tissues, and the ability of the blood to carry oxygen. Hypoxia is a relative deficiency of oxygen in the tissues and may be caused by a reduction in PO_2 , inadequate oxygen transport, or the inability of the tissues to use oxygen.

SUBCOMMITTEE RECOMMENDATIONS

The subcommittee's recommendation for EEGL and CEGL values for oxygen are summarized in Table 11-3. The current U.S. Navy values are provided for comparison.

1-Hour EEGL

Multiple chamber studies have exposed people to atmospheres containing ≤105 mmHg oxygen for several days (Gustafsson et al. 1997; Linde et al. 1997; Knight et al. 1990 a,b; Roach et al. 1996; Berglund et al. 2002). People exposed to those hypoxic conditions developed significant reductions in SaO_2 and clinical effects, including headaches and other symptoms of AMS, increased cerebral blood flow, reduced exercise endurance, increased heart rates, reduced maximal oxygen uptake, and

TABLE 11-3 Emergency and Continuous Exposure Guidance Levels for Oxygen (mmHg)

	Current U.S. Navy Values		NRC Recommended
Exposure Level	Maximum	Minimum	Minimum Values
EEGL			
1 h	220	130	105
24 h	160	130	127
CEGL			
90 days	160	130	140

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level, h, hour; mmHg, millimeters of mercury; NRC, National Research Council.

increased plasma lactate and ammonia concentrations (Knight et al. 1990a,b; Taylor and Bronks 1996; Buck et al. 1998). Despite the decreases in SaO_2, Knight et al. (1990b) did not demonstrate significant decrements in the volunteers' abilities to solve computational problems during moderate hypoxia, even while volunteers performed moderate exercise. The weight of evidence of the available chamber studies indicates that in an emergency, a submarine crew should be able to tolerate a 1-h exposure to air containing a PO_2 of 105 mmHg without developing adverse clinical effects.

24-Hour EEGL

Several studies have examined the responses of healthy men to hypoxic conditions mimicking those found on board U.S. submarines. In 1945, Naval researchers demonstrated reduced physical performance and mental efficiency among submariners exposed to 17% oxygen (129 mmHg) and 3% carbon dioxide for 50 h (Karlin and Curtis 1945). Cymerman et al. (2002) exposed seven submariners (31.8 ± 6.1 years of age) to environmental conditions that would be encountered on board a disabled submarine (127 mmHg oxygen, 2.5% carbon dioxide, 4°C) for 5 consecutive days. Within 2 days, subjects reported cold stress and muscle discomfort that lasted throughout the rest of the study. Participants also developed decreased postural control after 66 h of exposure. Loss of balance was especially apparent when the testing was performed with the subject's eyes closed. The submariners did not report significant increases in AMS symptoms despite the hypoxic conditions within the chamber. The authors of this

study concluded that the effects of the study conditions would not impair submariners' abilities to perform their duties when participating in a rescue effort. The weight of evidence indicates that in an emergency, a submarine crew should be able to tolerate a 24-h exposure to air containing 127 mmHg oxygen without developing adverse clinical effects. Commercial airplanes offer a useful comparison. They operate at cabin pressures equivalent to altitudes of 1,525-2,000 m (Hultgren 1997). The barometric pressure at 1,500 m is 630 mmHg, and the PO_2 is about 132 mmHg.

90-Day CEGL

Short-term (<2-3 days) experimental exposures to air with a PO_2 of about 132 mmHg have been associated with mood changes and reduced cognitive and motor performance (Shukitt and Banderet 1988). Those changes would not be acceptable during a 90-day deployment. The weight of evidence suggests that prolonged exposures to atmospheres containing about 140 mmHg would not cause adverse effects in healthy young adult males. The subcommittee anticipates that many crewmen will undergo physiologic adaptations in response to this mildly hypoxic environment, although others might experience drowsiness and other symptoms during the beginning of 90-day deployments. These effects are not expected to degrade performance of the crew or result in other adverse effects. Comparisons to naturally occurring conditions is noteworthy. The recommended 90-day CEGL for minimum levels of oxygen, 140 mmHg, is encountered at altitudes roughly equivalent to 1,000 m. Moreover, the U.S. Occupational Safety and Health Administration (OSHA) standard for workers in oxygen-deficient atmospheres requires air to be at least 19.5% oxygen. OSHA has classified air containing less than 19.5% oxygen to be immediately dangerous to life or health (IDLH) (29 CFR 1910.146(b).

DATA ADEQUACY AND RESEARCH NEEDS

Additional studies are needed to evaluate the appropriateness of the 90-day CEGL. The subcommittee could not find any studies examining the effect of subchronic exposure to mild hypoxia on mood state or cognitive performance. The subcommittee suggests the Navy perform prospective studies to evaluate submariners for complaints of headaches, fatigue, and

other symptoms that might be associated with the mild hypoxic environment often encountered on board submarines.

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Appendix A

Biographical Information on the Subcommittee on Emergency and Continuous Exposure Guidance Levels For Selected Submarine Contaminants

ERNEST McCONNELL (*Chair*) is president of ToxPath, Inc., a consulting firm in Raleigh, NC, that specializes in experimental toxicology and pathology. Before becoming a consultant, Dr. McConnell was director of the Division of Toxicological Research and Testing Program, National Toxicology Program at the National Institute of Environmental Health Sciences (NIEHS). He has served two terms as a member of the NRC Committee on Toxicology and on several NRC committees, including the Subcommittee on Manufactured Vitreous Fibers. He received his D.V.M. from Ohio State University and his M.S. in pathology from Michigan State University. He completed his residency in comparative pathology at the Armed Forces Institute of Pathology, Walter-Reed Army Medical Center.

RAKESH DIXIT is a study director–compound manager and biochemical toxicologist for Merck Research Laboratories, where he conducts safety assessment studies. His research interests include safety-toxicity biomarkers, safety assessment of pharmaceutical agents, biochemical mechanisms of toxicity, and toxicokinetics. He is the editor-in-chief of *Toxicology Mechanisms and Methods* and associate editor for *Toxicology Applied*

Pharmacology and *Journal of Toxicology and Environmental Health and Methods*. Dr. Dixit served on the NRC Subcommittee on Jet Propulsion Fuel 8. He received his Ph.D. in toxicology and biochemistry from Case Western Reserve University. He is board-certified in toxicology by the American Board of Toxicology.

DAVID DORMAN is director of the Division of Biological Sciences at CIIT Centers for Health Research. The primary objective of his research is to provide a refined understanding of chemically induced neurotoxicity in laboratory animals that will lead to improved assessments of potential neurotoxicity in humans. Dr. Dorman's research interests include evaluation of the effects of neurotoxic chemicals on potentially sensitive subpopulations; examination of chemical-induced effects on behavior and cognitive development; and the application of pharmacokinetic methods to the risk assessment of neurotoxicants. He received his D.V.M. from Colorado State University. He completed a combined Ph.D. and residency program in toxicology at the University of Illinois Champaign-Urbana and is a Diplomate of the American Board of Veterinary Toxicology and the American Board of Toxicology.

MAUREEN FEUSTON is senior director of toxicology at Sanofi--Synthelabo Research, a Division of Sanofi-Synthelabo Pharmaceuticals, Inc. At Sanofi-Synthelabo, she manages the toxicology program and serves as the company's primary source of information and guidance for developmental and reproductive toxicology issues. Prior to joining Sanofi-Synthelabo, she was responsible for general and reproductive toxicology at Mobil Oil Corporation's Environmental Health and Safety Laboratory. Dr. Feuston has held a number of elected positions in scientific societies, including president of the Middle Atlantic Reproductive and Teratology Association and council member of the Society of Toxicology's Reproductive and Developmental Specialty Section, and has served on numerous committees within the Teratology Society. She has also served on the NRC Subcommittee on Reproductive and Developmental Toxicants. She received her Ph.D. in developmental biology from the University of Cincinnati.

JACK HARKEMA is University Distinguished Professor in the College of Veterinary Medicine at Michigan State University (MSU). He is also the director of the Laboratory for Experimental and Toxicologic Pathology in the National Food Safety and Toxicology Center and the director of the Mobile Air Research Laboratory at MSU. Dr. Harkema's research is designed to understand the cellular and molecular mechanisms involved in the

pathogenesis of airway injury caused by the inhalation of airborne pollutants. Dr. Harkema received his D.V.M. from Michigan State University and his Ph.D. in comparative pathology from the University of California, Davis.

HOWARD KIPEN is a professor and director of the Clinical Research and Occupational Medicine Division in the Department of Environmental and Occupational Medicine at the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School. His research focuses on controlled-exposure studies of the effects of environmental agents, such as particulate air pollution, diesel exhaust, and solvents, and epidemiologic studies of medically unexplained symptoms. He has served as a member or chair of several IOM committees, including the Committee on the Persian Gulf Syndrome Comprehensive Clinical Evaluation Program. He received his M.D. from University of California at San Francisco and his M.P.H. from Columbia University School of Public Health. He is board-certified in internal medicine and occupational medicine.

LOREN KOLLER is an independent consultant and former professor and dean of the College of Veterinary Medicine at Oregon State University. His research interests include toxicologic, pathologic, and immunologic effects of toxic substances and the effect of environmental contaminants on tumor growth and immunity. He is a former member of the NRC Committee on Toxicology and participated on several of its subcommittees, including the Subcommittee on Immunotoxicity and the Subcommittee on Zinc Cadmium Sulfide. He is currently serving on the IOM Committee on the Assessment of Health Effects of Vietnam Veterans. He received his D.V.M. from Washington State University and his Ph.D. in pathology from the University of Wisconsin.

JOHN O'DONOGHUE is director of the Health and Environment Laboratories of Eastman Kodak Company. He also holds an appointment as adjunct associate professor of environmental medicine at the University of Rochester, School of Medicine and Dentistry. His research interests include neurotoxicology and toxicologic pathology. Dr. O'Donoghue has served on several NRC committees including the Committee on Toxicology and the Subcommittee on Toxicological Hazard and Risk Assessment. He received his V.M.D and Ph.D. from the University of Pennsylvania and is a Diplomate of the American Board of Toxicology.

JOYCE TSUJI is a principal scientist in the toxicology and health risk practice of Exponent, Inc. She is a board-certified toxicologist with experi-

ence in risk assessment and risk communication on projects in the United States and internationally. Her specific expertise includes exposure assessment, environmental health education, and biomonitoring for exposure to chemicals in the environment. She is currently serving on the NRC Subcommittee on Spacecraft Exposure Guidelines (SEGs) and served on the NRC Subcommittee on Submarine Escape Action Levels and the Subcommittee on Copper in Drinking Water. She received her Ph.D. in physiology and ecology from the Department of Zoology at the University of Washington.

ANNETTA WATSON is a senior research staff scientist in the Life Sciences Division of Oak Ridge National Laboratory (ORNL). She has been involved with the development of reference doses, acute exposure guideline levels (AEGLs), and other decision criteria for chemical warfare agents. Dr. Watson has also interpreted and applied toxicological information on hazardous materials to meet community emergency preparedness planning and training needs. She has served on numerous NRC committees, including the Committee on Toxicology, the Subcommittee on Toxicological Hazard and Risk Assessment, the Subcommittee on Guidelines for Military Field Drinking Water Quality, and the IOM Committee to Survey the Health Effects of Mustard Gas and Lewisite. She received a Ph.D. from the School of Agriculture at the University of Kentucky and an undergraduate degree in entomology from Purdue University.

CALVIN WILLHITE is a toxicologist employed by the California Department of Toxic Substances Control. Dr. Willhite was a member of the Chemical Substances Threshold Limit Values (TLV) Committee of the American Conference of Governmental Industrial Hygienists for ten years. Dr. Willhite currently serves on the editorial boards of the *Journal of Toxicology and Environmental Health, Part B, Critical Reviews*; *Toxicology*; *Reproductive Toxicology*; and *Toxicology and Applied Pharmacology*. He served on the NRC Committee on Toxicology and is a member of the NRC Subcommittee on Acute Exposure Guideline Levels, the NSF Health Advisory Board, and the National Toxicology Program Scientific Advisory Committee. He received his M.S. in toxicology from Utah State University and his Ph.D. in pharmacology from Dartmouth Medical School.

Glossary

Accommodation: The act or state of adjustment or adaptation.[1]

ACGIH (American Conference of Governmental Industrial Hygienists): ACGIH is a member-based organization and community of professionals that advances worker health and safety through education and the development and dissemination of scientific and technical knowledge. ACGIH publishes exposure guidance values called Threshold Limit Values (TLVs) and Biological Exposure Indices (BEIs). Exposures at or below TLVs or BEIs do not create an unreasonable risk of disease or injury. TLVs and BEIs are designed for use by industrial hygienists in making decisions regarding safe levels of exposure to various chemical substances and physical agents found in the workplace.[2]

Acute exposure: An exposure lasting 1 day or less.[3]

Acute exposure guideline levels (AEGLs): AEGLs "represent threshold exposure limits for the general public and are applicable to emergency exposures ranging from 10 min to 8 h. Three levels—AEGL-1, AEGL-2, and AEGL-3—are developed for each of five exposure periods (10 min, 30 min, and 1 h, 4 h, and 8 h) and are distinguished by varying degrees of severity of toxic effects. AEGL-1 is the airborne concentration 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 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 above which it is predicted that the general

population, including susceptible individuals, could experience life-threatening health effects or death."[4]

Adaptation: The acquisition of modifications that fit a plant or animal to life in a new environment or under new conditions.[1]

AEGL: *See* acute exposure guideline levels.

Aerosol: A suspension of liquid or solid particles in a gas.[5]

Alveolar macrophage: A mononuclear phagocytic cell arising from monocytic stem cells in bone marrow whose function is to ingest and digest foreign matter in the alveoli.[1]

Area under the curve (AUC): A measure of exposure that includes both duration and concentration. It is calculated from the curve that results when the concentrations of the test substance in some biologic tissue, typically blood, are plotted versus the exposure time.

ATA: *See* atmosphere absolute.

Atmosphere absolute (ATA): One atmosphere absolute (1-ATA) is the average atmospheric pressure exerted at sea level, 14.7 pounds per square inch (psi). Two-atmosphere absolute (2-ATA) is twice the atmospheric pressure exerted at sea level. If a physician prescribes 1 hour of hyperbaric oxygen treatment at 2-ATA, the patient breathes 100% oxygen at two times the atmospheric pressure at sea level for 1 hour.

ATSDR (Agency for Toxic Substances and Disease Registry): The ATSDR is an agency of the Department of Health and Human Services that was created by Congress under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), commonly known as the Superfund Act. Its mission is to serve the public by using the best science, taking responsive public health actions, and providing trusted health information to prevent harmful exposures and disease related to toxic substances. ATSDR defines minimal risk levels (MRLs).[6]

AUC: *See* area under the curve.

CAMS: *See* central atmosphere monitoring system.

CDC (Centers for Disease Control and Prevention): The CDC is an agency of the Department of Health and Human Services. The CDC promotes health and quality of life by preventing and controlling disease, injury, and disability. The National Institute of Occupational Safety and Health (NIOSH) is part of the CDC.[7]

CEGL: *See* continuous exposure guidance level.

Ceiling concentration: A concentration that shall not be exceeded during any part of a working exposure.[8]

Central atmosphere monitoring system (CAMS): CAMS monitors the submarine atmosphere by using "an infrared spectrometer to measure carbon monoxide and a mass spectrometer to measure oxygen, nitrogen, carbon dioxide, hydrogen, water vapor, and fluorocarbons 11, 12, and 114."[9]

Chronic exposure. An exposure lasting 6-24 months.[3]

Chronic obstructive pulmonary disease (COPD): COPD is "a disease characterized by chronic bronchitis or emphysema and airflow obstruction that is generally progressive, maybe accompanied by airway hyperreactivity, and may be partially reversible."[10]

Continuous exposure guidance level (CEGL): A CEGL is defined as a ceiling concentration designed to prevent any immediate or delayed adverse health effect or degradation in crew performance resulting from a continuous exposure lasting up to 90 days.

CO: Carbon monoxide.

CO_2: Carbon dioxide.

COPD: *See* chronic obstructive pulmonary disease.

Draeger tube: A monitoring device for acetone, ammonia, benzene, CO, CO_2, chlorine, hydrazine, hydrochloric acid, NO_2, ozone, sulfur dioxide, toluene, total hydrocarbons, methyl chloroform, and monoethanolamine.[11]

EEGL: See emergency exposure guidance level.

Electrostatic precipitator: A system to clear particles and aerosols from air.[12]

Emergency exposure guidance level (EEGL): An EEGL is defined as a ceiling concentration that will not cause irreversible harm or prevent performance of essential tasks, such as closing a hatch or using a fire extinguisher, during a rare emergency situation lasting 1-24 hours.

EPA: U.S. Environmental Protection Agency.

FEV_1: *See* forced expiratory volume.

Forced expiratory volume (FEV_1). FEV_1 is a standard test of lung function. It is the volume of air that can be forcibly exhaled in 1 second following a maximal inspiration.[11]

Forced vital capacity (FVC): FVC is a standard test of lung function. It is the maximal volume of air that can be exhaled as forcibly and rapidly as possible after a maximal inspiration.[11]

Fumes: Particulate, smoke-like emanations from the surface of heated metals.[6]

FVC: *See* forced vital capacity.

Gas: One of the three states of matter, characterized by very low density and viscosity (relative to liquids and solids); comparatively great expansion and contraction with changes in pressure and temperature; ability to diffuse readily into other gases; and ability to occupy with almost complete uniformity the whole of any container.[6]

H_2: Hydrogen.

Habituation: Decreased responsiveness to stimulation.[12]

IARC (International Agency for Research on Cancer): IARC is an agency of the World Health Organization. IARC's carcinogenicity classifications are as follows:[13]

Group 1. The agent (mixture) is carcinogenic to humans. The exposure circumstance entails exposures that are carcinogenic to humans.

Group 2A. The agent (mixture) is probably carcinogenic to humans. The exposure circumstance entails exposures that are probably carcinogenic to humans.

Group 2B. The agent (mixture) is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans.

Group 3. The agent (mixture, or exposure circumstance) is not classifiable as to carcinogenicity in humans.

Group 4. The agent (mixture, exposure circumstance) is probably not carcinogenic to humans.

Irritant: A toxicant that exerts its deleterious effects by causing inflammation of mucous membranes on contact. Irritants principally act on the respiratory system and can cause death from asphyxiation due to lung edema.[14]

Irreversible harm: Permanent damage or injury to health. Emergency exposure guidance levels (EEGLs) are designed to avoid or prevent irreversible harm.

LC_{01}: Lethal concentration in 1% of the sample population.

LC_{50}: Lethal concentration in 50% of the sample population.

LOAEL: *See* lowest-observed-adverse-effect level.

Lowest effect level: The lowest dose or exposure level in a study at which a statistically or biologically significant effect is observed in the exposed population compared with an appropriate unexposed control group.[15]

Lowest-observed-adverse-effect level (LOAEL): A LOAEL is the "lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group."[16]

MEA: Monoethanolamine.

Minimal risk level (MRL): ATSDR's "estimate of daily human exposure to a hazardous substance at or below which that substance is unlikely to pose a measurable risk of harmful (adverse), noncancerous effects. MRLs are calculated for a route of exposure (inhalation or oral) over a specified time period (acute, intermediate, or chronic)."[17]

MRL: *See* minimal risk level.

NAAQS: *See* national ambient air quality standards.

National ambient air quality standards (NAAQS): "The Clean Air Act, requires EPA to set National Ambient Air Quality Standards (NAAQS) for pollutants considered harmful to public health and the environment. The Clean Air Act established two types of national air quality standards. Primary standards set limits to protect public health, including the health of 'sensitive' populations such as asthmatics, children, and the elderly. Secondary standards set limits to protect public welfare, including protection against decreased visibility, damage to animals, crops, vegetation, and buildings. NAAQS have been set for six principal pollutants, which are called 'criteria' pollutants: carbon monoxide, nitrogen dioxide, ozone, lead, particulate matter, and sulfur dioxide."[18]

NH_3: Ammonia.

NIOSH: National Institute for Occupational Safety and Health.

NO: Nitric oxide.

NO_2: Nitrogen dioxide.

NOAEL: *See* no-observed-adverse-effect level.

NOEL. *See* no-observed-effect level.

No-observed-adverse-effect level (NOAEL): A NOAEL is "an exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered as adverse, nor precursors to specific adverse effects. In an experiment with several NOAELs, the regulatory focus is primarily on the highest one leading to the common usage of the term NOAEL as the highest exposure without adverse effect."[17]

No-observed-effect level (NOEL): A NOEL is the "greatest concentration or amount of a substance, found by experiment or observation, that causes no alterations of morphology, functional capacity, growth, development, or life span of target organisms distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure."[19]

OSHA (Occupational Safety and Health Administration): OSHA is an agency of the U.S. Department of Labor. It is authorized to set workplace health and safety standards for a wide variety of physical and chemical hazards and occupational situations. OSHA establishes permissible exposure limits (PELs) for a typical 8-hour workday within a 40-hour workweek and short-term exposure limits (STELs) applicable to a 15-min period within a workday.[20]

PEL: *See* permissible exposure limit.

PEL-TWA: *See* permissible exposure limit.

Permissible exposure limit (PEL): A PEL is the "maximum amount or concentration of a chemical that a worker may be exposed to under OSHA regulations."[21] According to OSHA regulations, the permissible exposure limit–time-weighted average (PEL-TWA) is a regulatory standard for a particular chemical expressed as "an average value of exposure over the course of an 8 hour work shift."[22]

RD_{50}: A statistically estimated concentration resulting in 50% reduction in respiratory rate.

Recommended exposure limit (REL): A REL is "an 8- or 10-h time-weighted average (TWA) or ceiling concentration recommended by NIOSH that is based on an evaluation of the health effects data."[22]

Reference concentration (RfC): EPA's estimate of "air exposure concentration to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime."[23]

REL: *See* recommended exposure limit.

Relative risk (RR): RR is an epidemiologic measure of association between an exposure or risk factor and disease incidence. It is expressed as a ratio of the incidence rate for exposed persons to the incidence rate for the unexposed.[24]

Reversible effect: An injury from which a target tissue or organ can recover or regenerate.

RfC: *See* reference concentration.

RR: *See* relative risk.

SEAL: *See* submarine escape action levels.

Short-term exposure limit (STEL): As defined by ACGIH, a STEL is a "15-minute TWA exposure for a regulated chemical that should not be exceeded at any time during a workday, even if the 8-hour TWA is within the TLV-TWA or PEL-TWA."[22]

Short-term public emergency guidance levels (SPEGLs): SPEGLs are "suitable concentrations for single, short-term, emergency exposures, of the general public."[25]

SMAC: *See* spacecraft maximum allowable concentrations.

Spacecraft maximum allowable concentrations (SMACs): SMACs are "concentrations of airborne substances (such as gas, vapor, or aerosol) that will not compromise the performance of specific tasks during emergency conditions. Exposure to 24-h SMACs will not cause serious or permanent effects but may cause reversible effects that do not impair judgment or interfere with proper responses to emergencies such as fires or accidental releases. Long-term SMACs (e.g., 7 day) are intended to avoid adverse health effects (either immediate or delayed) and to avoid degradation in crew performance with continuous exposure in a closed space-station environment. SMACs were developed for astronauts (healthy individuals)."[26]

SMR: *See* standardized mortality ratio.

SPEGL: *See* short-term public emergency guidance levels.

SSBN: Nuclear-powered ballistic missile submarines, Ohio class.

SSN: Nuclear-powered attack submarines. There are three SSN classes: Los Angeles, Seawolf, and Virginia.

Standardized mortality ratio (SMR): SMR is a measure of population health. It is calculated by taking the ratio of the number of deaths observed in the population of interest to the number of deaths expected on the basis of the mortality rates of a reference population.[24]

STEL: *See* short-term exposure limit.

Subchronic exposure: An exposure lasting 2-13 weeks or 10% of the test animal life-span.[3]

Submarine escape action levels (SEALs): At concentrations below a SEAL 1, respiratory and central nervous system function should not be impaired enough to significantly affect the ability to escape or to be rescued, and crew members can remain in the submarine without wearing eye and respiratory protection (EABs) for up to 10 days. At and above SEAL 1, but below SEAL 2, respiratory and central nervous system effects should not be severe enough to hamper ability to escape, and crew members would not be required to wear EABs but would plan to escape so that the last man leaves the submarine within 24 h. At and above a SEAL 2, unprotected exposure to the gas can result in impairment to respiratory and central nervous system function to an extent that the ability to escape would be compromised, and crew members should be required to wear EABs.[9]

Threshold Limit Value (TLV): A TLV is the "concentration in air of a substance to which it is believed that most workers can be exposed daily without adverse effect (the threshold between safe and dangerous concentrations). These values are established (and revised annually) by the American Conference of Governmental Industrial Hygienists and are time-weighted concentrations for a 7- or 8-hour workday and a 40-hour workweek."[20]

Time-weighted average (TWA): Under OSHA regulations, a TWA is the average concentration of a regulated chemical to which a worker may be repeatedly exposed during a conventional 8-h workday and a 40-h workweek without adverse effect.

TLV: *See* Threshold Limit Value.

TWA: *See* time-weighted average.

UF: *See* uncertainty factor.

Uncertainty factor (UF): A UF (e.g., 1, 2, 3, or 10) can be used when deriving human health risk reference values from experimental data to account for inter- or intraspecies differences, database gaps, extrapolations from high to low dose, or other adjustments required. Multiple UFs can be used in a calculation. A UF of 10 is considered to be a health-protective default value to be employed when little is known about a particular source of variability or uncertainty, such as intraspecies differences or lack of information on a relevant health effect. As additional research becomes available, UFs change as indicated by the new information.

Vent fog precipitator: A system used in the submarine engine room to clear the air of oil mists.

VOC*: See* volatile organic compounds.

Volatile organic compounds (VOCs): VOCs are organic chemicals that have high vapor pressure and easily form vapors at normal temperature and pressure.

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