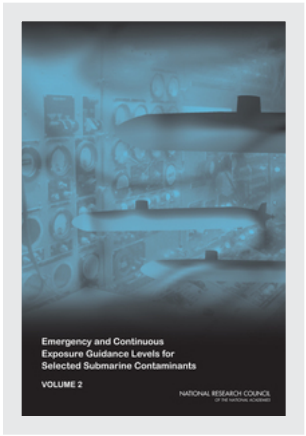

Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants: Volume 2 (2008)

DETAILS

332 pages | 6 x 9 | PAPERBACK

ISBN 978-0-309-11273-4 | DOI 10.17226/12032

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SUGGESTED CITATION

National Research Council. 2008. *Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants: Volume 2*. Washington, DC: The National Academies Press. https://doi.org/10.17226/12032.

Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants

VOLUME 2

Committee 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

NATIONAL RESEARCH COUNCIL
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Washington, D.C.
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This project was supported by Contract W81K04-06-D-0023 between the National Academy of Sciences and the U.S. Department of Defense. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the organizations or agencies that provided support for this project.

International Standard Book Number-13: 978-0-309-11273-4
International Standard Book Number-10: 0-309-11273-7

Additional copies of this report are available from

The National Academies Press
500 Fifth Street, NW
Box 285
Washington, DC 20055

800-624-6242
202-334-3313 (in the Washington metropolitan area)
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Preface

A submarine, of course, is an enclosed and isolated environment when submerged. Its crew works, eats, and sleeps in this environment and is exposed to air contaminants 24 h/day, unlike workers in a typical occupational environment, who have a respite from workplace exposures at the end of the workday or workweek. To protect the health of submariners, the U.S. Navy has developed 1-h and 24-h 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 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 Committee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants. This report, the second of two, provides the committee's rationale and recommendations regarding ammonia, benzene, 2,6-di-tert-butyl-4-nitrophenol, Freon 12, Freon 114, hydrogen, 2190 oil mist, ozone, surface lead, toluene, and xylene.

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 NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards 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 thank the following for their review of this report: Darol Dodd, The Hamner Institutes for Health Sciences; Terry Gordon, New York University; Rogene Henderson, Lovelace Respiratory Research Institute; Gary Krieger, NewFields, LLC; John Morris, University of Connecticut; Nathaniel Rothman, National Cancer Institute; George Rusch, Honeywell, Inc.

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 Richard Schlesinger, Pace University. Appointed by the National 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 committee and the institution.

We would like to acknowledge the assistance of Sylvia Talmage and Kowetha Davidson, of Oak Ridge National Laboratory, who provided information and input that aided in the development of the toluene and ammonia profiles, respectively.

The committee 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; Jean Hampton, senior fellow; Jennifer Saunders, associate program officer; Mirsada Karalic-Loncarevic, manager, Technical Information Center; Norman Grossblatt, senior editor; and John H. Brown, program associate.

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

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

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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 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 exposure 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 when necessary develop, EEGLs and CEGLs for a variety of substances. As a result of the Navy's request, the NRC convened the Committee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants in 2002.

STATEMENT OF TASK

Members of the committee were selected for their expertise in inhalation toxicology, neurotoxicology, immunotoxicology, reproductive and developmental toxicology, veterinary pathology, pharmacokinetics, epidemiology, and human-health risk assessment. The committee 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 the Navy's exposure levels should be changed on the basis of the committee's evaluation.
- For two submarine contaminants for which no guidance levels exist—surface lead and 2,6-di-tert-butyl-4-nitrophenol (DBNP)—determine whether sufficient data are available to develop EEGLs and CEGLs and, if so, 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 committee 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 reports. This is the committee's second report. It contains the EEGL and CEGL recommendations for the following chemicals of concern to the Navy: ammonia, benzene, DBNP, Freon 12, Freon 114, hydrogen, 2190 oil mist, ozone, surface lead, toluene, and xylene. All other chemicals were addressed in the committee's first report.

APPROACH TO STUDY

In conducting its evaluations, the committee 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

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

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). 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, 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 exposure guidance levels. The committee recognizes that statistical power of a study involving a small number of subjects needs to be considered. However, the committee did not develop criteria for assessing the adequacy of a study's statistical power, because individual studies, whether human or animal, were never used in isolation to derive an EEGL or CEGL value. When appropriate human data were not available, standard laboratory animal studies were used, and preference was given to nonhuman-primate studies. A weight-of-evidence approach was used to select key studies and ensure that the selected data were consistent with the overall scientific database.

For derivation of the EEGL and CEGL values, the committee 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 developing similar exposure levels provided in more recent NRC reports. The basis of the EEGLs was acute or short-term inhalation and ocular toxicity data, whereas the basis of the CEGLs was repeated inhalation exposure data, and the effects of cumulative exposures were considered for derivation of the CEGL. The most sensitive end points were emphasized for derivation of both exposure levels. The committee considered only health end points relevant to healthy young men on the assumption that no women are 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 susceptibility.

[2]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.

CONCLUSIONS AND RECOMMENDATIONS

In this report, the committee makes recommendations for 1-h and 24-h EEGLs and 90-day CEGLs for the following chemicals: ammonia, benzene, DBNP, Freon 12, Freon 114, hydrogen, 2190 oil mist, ozone, surface lead, toluene, and xylene. The recommendations are listed in Table S-1, and the Navy's current and proposed values are included in the table for comparative purposes. The bases of the committee's derivations are provided in the individual chemical profiles. Overall, the committee found substantial differences in the adequacy of the datasets used to derive the EEGL and CEGL values. Some chemicals, such as benzene and ozone, have robust datasets; others, such as DBNP, have a paucity of data available for determining the effects of inhalation exposure. Specific recommendations for research needed to improve the confidence of the derived exposure levels are provided in the individual chemical profiles.

The committee determined EEGL and CEGL values lower than the current exposure guidelines for hydrogen. The committee's values are consistent with the approach used by the National Aeronautics and Space Administration to derive its guideline levels and that used by the U.S. Environmental Protection Agency to set exposure standards for explosive gases. All other EEGL and CEGL values determined by the committee are similar to or slightly higher than the values proposed by the Navy.

For two chemicals (DBNP and surface lead), the committee could not recommend exposure guidance levels because data were insufficient. For DBNP, the animal toxicity database available includes only studies of single or repeat doses, primarily by the oral route, that assess a small number of end points. Moreover, many of the data points are conflicting. Thus, much uncertainty is associated with any attempt to estimate EEGL and CEGL values for this compound. The committee recommends that the data gaps be addressed with appropriately designed studies before any further consideration of potential EEGL and CEGL derivation.

For surface lead, no data were available to the committee on the physical nature, chemical identity, routes of exposure, or bioavailability of the lead-containing materials of concern. To conduct submarine-specific lead health risk analyses, data concerning generation, location, dispersion, and extent of onboard lead contamination, including the lead concentration in submarine drinking water, must be available. Available methods for site-specific human health risk assessment for lead-containing dust require rigorous estimates of the quantity of dust ingested daily. It appears unlikely that published estimates of house-dust lead exposure could be applied with confidence to a submarine. In addition, no data were available to the committee concerning submariners' urinary lead or blood lead. Thus, it is not clear whether a significant exposure of the crew to lead occurs. It is important to establish whether submariner blood lead concentrations differ from those of civilian adults and active military personnel not engaged in submarine operations who live in the United States. The committee

TABLE S-1 Comparison of U.S. Navy's Current and Proposed Exposure Guidance Levels with Those Recommended by the Committee

Chemical	Exposure Level	U.S. Navy Values, ppm		Committee Recommended Values, ppm
		Current	Proposed	
Ammonia	1-h EEGL	100	30	100
	24-h EEGL	100	20	50
	90-day CEGL	50	10	10
Benzene	1-h EEGL	50	10	40
	24-h EEGL	2	3	3
	90-day CEGL	1	0.1	0.2
DBNP	1-h EEGL 24-h EEGL 90-day CEGL	No current or proposed levels available		Insufficient data to derive guidance levels
Freon 12	1-h EEGL	2,000	2,000	4,000
	24-h EEGL	1,000	1,000	1,000
	90-day CEGL	100	100	300
Freon 114	1-h EEGL	2,000	2,000	2,000
	24-h EEGL	1,000	1,000	1,000
	90-day CEGL	100	100	125
Hydrogen	1-h EEGL	10,000	10,000	4,100
	24-h EEGL	10,000	10,000	4,100
	90-day CEGL	10,000	10,000	4,100
2190 oil mist[a]	1-h EEGL	—	10 mg/m^3 (forward)	20 mg/m^3
	24-h EEGL	—	2 mg/m^3 (forward)	2.5 mg/m^3
	90-day CEGL	—	0.3 mg/m^3 (forward)	0.3 mg/m^3
Ozone	1-h EEGL	1	0.3	0.5
	24-h EEGL	0.1	0.1	0.1
	90-day CEGL	0.02	0.02	0.02
Surface lead	1-h EEGL 24-h EEGL 90-day CEGL	No current or proposed levels available		Insufficient data to derive levels
Toluene	1-h EEGL	200	150	200
	24-h EEGL	100	50	100
	90-day CEGL	20	16	20
Xylene	1-h EEGL	200	100	200
	24-h EEGL	100	100	100
	90-day CEGL	50	50	50

[a]Navy values provided are for forward section of submarine. No current or proposed values were provided for aft section of submarine. The standards are defined as "respirable suspended particles" by the Navy.

Abbreviations: DBNP, 2,6-di-tert-butyl-4-nitrophenol.

highly recommends determining crew urinary lead or blood lead concentrations before submarine deployment and then identical measurements on completion of typical tours of duty. That approach would help to identify any submarine-associated lead exposure. If individual submariners are identified with increased blood lead, determination of the lead sources during deployment or as a result of onshore activity would be necessary.

RESEARCH RECOMMENDATIONS

The committee repeats and re-emphasizes here its research recommendations from its first report. The submarine atmosphere does not appear to be well characterized. For the chemicals reviewed in this report, only Freon 12, Freon 114, and hydrogen are regularly monitored in the submarine atmosphere. Exposure data, if available, on the other chemicals are limited to one-time sampling on a few submarines; whether the reported concentrations are representative of the submarine fleet is not known. Therefore, the committee agrees with the NRC report *Submarine Air Quality*[3] that the Navy should "survey various classes of submarines for trace contaminants and particulate matter" and monitor submarines to "provide [a] complete analysis of submarine air and data on exposure of personnel to contaminants." If the exposure assessments indicate that chemicals pose problems (that is, if concentrations are higher than 90-day CEGLs), relative source contributions of those chemicals should be determined. The committee notes that a few onboard sources, such as cigarette-smoking and some cooking methods, contribute to the formation of multiple compounds considered in this and its first report. Therefore, stricter management or elimination of those sources is likely to solve some exposure problems on submarines.

The committee did not address exposure to chemical mixtures. The committee recommends that empirical data that characterize mixtures found in submarine air be evaluated when they become available. The potential for antagonistic, additive, or synergistic interactions between contaminants in the submarine environment is subject to substantial uncertainty, remains largely unexamined, and needs to be studied.

As in the committee's first report, several of the chemicals evaluated in 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 such descriptors as "mild" or "mild to moderate," and the data on sensory-irritation thresholds can be highly variable. Research is needed to quantify the diverse methods and end points

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

used in sensory-irritation studies so that the data can be used in public-health 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-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 the 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) had 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 if necessary develop 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 Committee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants in 2002.

THE COMMITTEE'S CHARGE

Members of the committee were selected for their expertise in inhalation toxicology, neurotoxicology, immunotoxicology, reproductive and developmental toxicology, veterinary pathology, pharmacokinetics, epidemiology, and human-health risk assessment. The committee 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 acrolein, ammonia, benzene, carbon dioxide, carbon monoxide, formaldehyde, Freon 12, Freon 114, hydrazine, hydrogen, methanol, monoethanolamine, nitric oxide, nitrogen dioxide, 2190 oil mist, oxygen, ozone, toluene, and xylene.
- Determine whether the current or proposed guidance levels are consistent with the scientific data and whether the Navy's exposure levels should be changed on the basis of the committee's evaluation.
- For two submarine contaminants for which there are no guidance levels—surface lead and 2,6-di-tert-butyl-4-nitrophenol (DBNP)—determine whether sufficient data are available to develop EEGLs and CEGLs and, if so, 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 research as appropriate.

To accomplish its charge, the committee 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 reports. This is the committee's second report, and it contains evaluations of EEGLs and CEGLs for 11 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. Before entry into the submarine service, candidates receive a comprehensive physical and psychologic 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). Thus, the population that serves on U.S. submarines is, in general, an extremely healthy one.

Recent studies that have evaluated mortality patterns in U.S. submariners support the conclusion that submariners are extremely healthy. Charpentier et al. (1993) examined a cohort of 76,160 submariners who served on U.S. nuclear-powered submarines during the period 1969-1982. They compared mortality in the submariners with that in the general adult male population of the United States and found that the standardized mortality ratio (SMR) for total mortality was significantly less than 1.[1] The SMR was also significantly lower than that

[1]An SMR indicates whether mortality in a given population is greater (SMR > 1) or less (SMR < 1) than that in a comparison population.

expected in a military population. The SMRs for specific causes of mortality were also less than 1. SMRs approached 1 for only two causes: malignant neoplasms of the central nervous system (SMR, 1.03) and motor-vehicle accidents (SMR, 1.06). The results reported by the study authors were 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 just listed account for about 90% of the 2,044 medical events reported.

Although recent data indicate that U.S. submariners are a healthy population, some might be sensitive to particular air contaminants because of genetic predisposition or conditions arising during active duty. For example, Sims et al. (1999) reported that asthma led to the disqualification each year of 0.16% of the active-duty personnel serving in the Atlantic Fleet Submarine Force (the authors considered the asthma cases to be mild).

Tobacco smokers might be more or less sensitive to some air contaminants. Smoking is permitted only in restricted areas on U.S. submarines. The percentage of U.S. 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 on 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 collected from one submarine in 1997. 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, smoking policies on board submarines vary because they are determined by the commanding officer.

THE SUBMARINE ENVIRONMENT

The U.S. submarine fleet is composed mostly of two types of submarines (Thomas et al. 2000). Table 1-1 provides some distinguishing characteristics of the crews and patrols of the two submarine types.

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

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

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

[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.

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 by electrolyzing 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 by cigarette-smoking and hydrogen that is released in battery-charging are removed by a carbon monoxide–hydrogen burner that catalytically oxidizes the two components to carbon dioxide and water, respectively; hydrocarbons are also oxidized by this system. Activated-carbon filters help to 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 at sea.

When the submarine is submerged, air is recirculated in a closed-loop system. The system is composed of the forward-compartment air-circulation system and the engine-compartment air-circulation system (R. Hagar, Naval Sea Systems Command, personal commun., April 2, 2003). Figure 1-1 is a generalized schematic of a nuclear-powered attack submarine. The forward-compartment air-circulation system contains most of the air-purification equipment and oxy-

gen 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 serves as the mixing chamber. Stale air from the boat is exhausted to the fan room, and the fan room supplies treated air to the boat. The engine-compartment air-circulation system provides heating, cooling, and air distribution within the engine room and is designed to maintain its air temperature below 100°F. Electrostatic precipitators and other filters in this room treat its air. Air from the engine room is exhausted directly to the fan room, which supplies air directly to the engine room.

Special variations in the exhaust airflow path described above 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 also vent directly to the fan room, and air from the laundry dryers passes through lint screens and then to the fan room. About 50% of the air vented to the fan room passes through electrostatic precipitators, and air from the galley, scullery, pantry, and water closets goes through activated-charcoal filters before venting to the fan room. Cooking grease is removed from the range and fryer hoods by centrifugal force.

The central atmosphere monitoring system (CAMS) of the submarine 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 concentrations, although they are not used in submarines equipped with the newer version of CAMS. A portable oxygen detector verifies oxygen concentrations weekly. Colorimetric detector tubes are used weekly to measure concentrations of acetone, ammonia, benzene, carbon dioxide, carbon monoxide, chlorine, hydrazine, hydrochloric acid, methyl chloroform, monoethanolamine nitrogen dioxide, ozone, sulfur dioxide, toluene, and total hydrocarbons. During battery-charging, 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 of volatile organic compounds, ozone, acrolein, aldehydes, amines, and nitrosamines. Although some monitoring is conducted on submarines, several have reported that it is inadequate and provides few data on overall exposure (for example, see NRC 1988b).

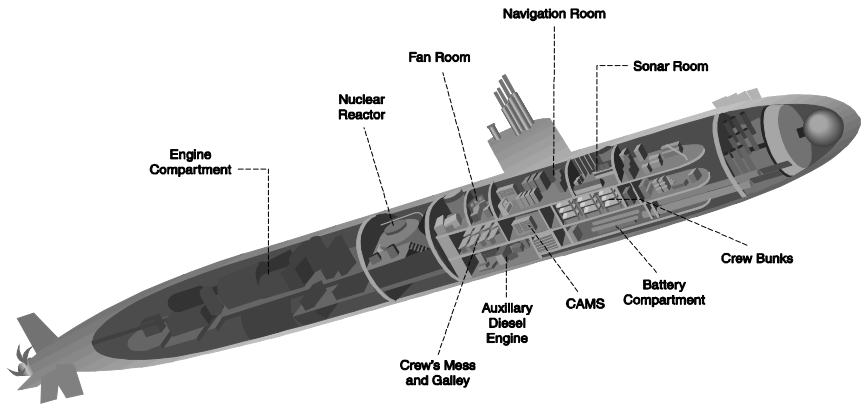

FIGURE 1-1 Generalized schematic of a nuclear-powered attack submarine. Source: Adapted from image courtesy of the Smithsonian/NMAH Transportation.

THE COMMITTEE'S APPROACH TO ITS CHARGE

The committee 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 committee's approach to data selection included the following elements:

- Whenever possible, primary references (published or unpublished study reports) were used to derive 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 for deriving exposure guidance levels. Evaluation of study quality required the professional expertise and judgment of the committee.
- Inhalation-exposure studies were used to derive exposure guidance levels. Data on other exposure routes were considered, especially when evaluating pharmacokinetics, metabolism, and mechanisms of toxicity.
- Human studies were preferred for developing the exposure guidance levels. The committee 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 the latter, because these were conducted in a controlled laboratory setting and allowed measurement of personal exposure and evaluation of end points relevant to derivation of exposure guidance levels. The committee recognizes that one potential problem with experimental studies is the statistical power of a study to detect an effect given the small number of subjects typically used. That design problem often exists in studies using humans or large animal species, such as nonhuman primates and dogs. However, the committee did not set a threshold for statistical power for two reasons. First, data presented in manuscripts or technical reports were often inadequate to allow the committee to perform independent calculations to determine the power of an experiment. Second, derivation of the EEGLs and CEGLs was never based solely on a single study; key studies were always supported by other human experimental studies, epidemiologic studies, or animal studies (see last bullet). To the best of the committee's knowledge, the data used were not obtained from uninformed subjects or by force or coercion.

- When high-quality human data were not available, standard laboratory animal studies were used to derive exposure guidance levels. The animal species used were those on which there were historical control data and those which were most relevant to humans. Nonhuman-primate studies were generally preferred but often were not available.
- A weight-of-evidence approach was used to select key studies that ensured 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.

The committee 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 reports (NRC 1992, 2001). The committee evaluated chemicals individually and did not address exposures to chemical mixtures. The committee recommends that empirical data that characterize mixtures found in submarine air be evaluated when they become available. The committee considered only health end points relevant to healthy young men on the assumption that women do not serve as permanent crew on board submarines. In deriving EEGLs and CEGLs, the committee assumed that maximal exercise is not achieved because of the confined conditions on a submarine. It also assumed that the submarine is operated at or near a pressure of 1 atm. The specific approaches adopted by the committee for developing EEGLs and CEGLs are outlined below.

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 that last 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, CEGLs would apply.

To develop 1-h and 24-h EEGLs, the committee reviewed relevant human and animal toxicity data and considered all health end points reported. The EEGLs were based on 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 committee used the available scientific literature or the guidance provided in *Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals* (NRC 2001).

In deriving EEGLs, the committee used uncertainty factors that ranged from 1 to 10. Those factors accounted for interspecies differences (extrapolation from animal to human populations, if applicable), intraspecies differences (possible variations in susceptibility that might be applicable to the healthy male population considered), extrapolations from a lowest observed-adverse-effect level to a no-observed-adverse-effect level; and weaknesses or critical gaps in the databases. The committee strove for consistency, but its overarching goal was a thorough case-by-case review of available data. Selection of uncertainty factors for each chemical reflects the committee's best judgment of the data on toxicity and mode of action. Because an uncertainty factor of 3 represents a logarithmic mean (3.16) of 10, the committee 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 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 committee used the basic approach outlined for developing EEGLs; relevant data were reviewed, sensitive end points evaluated, and appropriate uncertainty factors applied. The method differed only in that inhalation studies with repeated exposures, when available, were used as the primary basis of CEGL development. The effects of cumulative exposure over time were taken into account by 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 of greater than 1 in 10,000 exposed persons (NRC 1986b). For 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 committee evaluated the theoretical excess cancer risk resulting from exposure at the 90-day CEGLs. The committee considered deriving the cancer risk resulting from exposure at the 24-h EEGLs but concluded that such estimates would involve too much uncertainty. Additional information regarding cancer risk is provided in individual chapters as appropriate. The committee notes that COT typically has not proposed CEGLs for carcinogenic substances (NRC 1986b) but acknowledges that there is value in conducting such evaluations and has proposed 90-day CEGLs for compounds with known or suspected carcinogenic activity in humans.

Comparison with Other Regulatory Standards or Guidance Levels

The committee considered relevant inhalation exposure standards or guidance levels put forth by NRC and other agencies or organizations in its evaluations. However, it notes that the submarine 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-health standards are designed for repeated exposure throughout a working lifetime on the assumption that workers are exposed 8 h/day, 5 days/week for a working lifetime. Submariners can be exposed 24 h/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 4.5-5 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; such health effects might not be considered acceptable in setting conventional occupational-health or public-health exposure standards.

The committee 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 with EEGLs and CEGLs because SMACs are developed with similar criteria and address adverse

effects in 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 REPORT

This report contains the committee's rationale and recommendations for ammonia, benzene, DBNP, Freon 12, Freon 114, hydrogen, 2190 oil mist, ozone, surface lead, toluene, and xylene. Each chapter presents the relevant toxicologic and epidemiologic studies of a substance with selected chemical and physical properties, toxicokinetic and mechanistic data, and published regulatory and guidance levels for inhalation exposure. The committee's recommendations for exposure guidance levels and the research needed to define and support the recommendations are provided. The chemical profiles contained in this report are not comprehensive toxicologic profiles. Only data particularly relevant to the derivation of the EEGLs and CEGLs are discussed. References to recent authoritative reviews of the toxicology of some of the chemicals addressed in this report are provided.

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2

Ammonia

This chapter summarizes the relevant epidemiologic and toxicologic studies of ammonia. 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 committee considered all that information in its evaluation of the Navy's current and proposed 1-h, 24-h, and 90-day exposure guidance levels for ammonia. The committee's recommendations for ammonia exposure levels are provided at the end of this chapter with a discussion of the adequacy of the data for defining the levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

Ammonia is a corrosive alkaline gas at room temperature (Budavari et al. 1989). It is colorless and has a distinctive odor that has been described as sharp, pungent, and irritating (HSDB 2005). The odor threshold has been reported to range from 5 to 53 ppm (NRC 2002). Selected chemical and physical properties are listed in Table 2-1.

OCCURRENCE AND USE

Ammonia has several important industrial uses (Czuppon et al. 1992). It is a primary feedstock in the fertilizer industry, which is the largest consumer of ammonia. It is also used to synthesize explosives and products in the fibers and plastics industry. Ammonia also is a naturally occurring compound, an essential component of many biologic processes, and an intermediate in the global nitrogen cycle. Average global ammonia concentrations are estimated to range from 0.6 ppb to 3 ppb (ATSDR 2004).

TABLE 2-1 Physical and Chemical Properties of Ammonia

Synonyms	Anhydrous ammonia, ammonia gas
CAS registry number	7664-41-7
Molecular formula	NH_3
Molecular weight	17.03
Boiling point	–33.35°C
Melting point	–77.7°C
Flash point	NA
Explosive limits	NA
Specific gravity	0.639 at 0°C
Vapor pressure	8.5 atm at 20°C
Solubility	Solubility in water: 47% (0°C), 38% (15°C), 28% (30°C), 18% (50°C); soluble in chloroform and ether
Conversion factors	1 ppm = 0.7 mg/m^3; 1 mg/m^3 = 1.44 ppm

Abbreviations: NA, not available or not applicable.
Sources: Specific gravity from Czuppon et al. 1992; vapor pressure from Lewis 1993; all other data from Budavari et al. 1989.

Sources of ammonia on submarines include the sanitary system, decomposition of monoethanolamine (a chemical used in the carbon dioxide removal system), and decomposition of insulation blowing agents (Crawl 2003). NRC (1988) listed ammonia as a possible air contaminant on board submarines and reported a concentration of 2 ppm. No information was provided on sampling protocol, location, operations, or duration. No other exposure data were located.

SUMMARY OF TOXICITY

The database to characterize ammonia toxicity is sufficient and includes human and animal data suitable for derivation of exposure guidance levels. Multiple toxicologic reviews are available, including evaluations by the NRC (1966, 1987, 1994, 2002, 2007), the Agency for Toxic Substances and Disease Registry (ATSDR 2004), the American Conference of Governmental Industrial Hygienists (ACGIH 2001), and the National Institute for Occupational Safety and Health (NIOSH 1974). Information from those reviews is summarized in the following paragraphs.

Ammonia is a corrosive, alkaline, irritant gas that produces effects immediately on contact with moist mucous membranes of the eyes, mouth, and respiratory tract. It reacts with moist tissues to form ammonium hydroxide in an exothermic reaction; the thermal and chemical burns resulting from high-concentration exposures are a consequence of the heat of reaction and of the corrosive properties of the alkaline reaction product ammonium hydroxide.

Ammonia is a respiratory and ocular irritant; high concentrations can cause respiratory tissue injury and necrosis and penetrate the corneal epithelium. Because of its appreciable water solubility, ammonia is largely retained in the nasal mucosa, another common site of tissue injury after vapor exposure.

Because of its widespread commercial use and transport, accidental exposure to ammonia during industrial, farm, or transport accidents is not uncommon, and the toxicology literature contains numerous case studies and accident reports involving human exposures to high, but unknown, concentrations that have caused deaths or severe and long-lasting injuries. Although they contain useful background information, such reports provide little quantitative information regarding dose-response relationships, do not characterize exposure conditions expected on board a modern submarine, and hence will be given little consideration in the present analysis.

In addition to accident case reports, the ammonia database contains human experimental-exposure studies, epidemiologic studies, and laboratory animal experimental studies that characterize respiratory and ocular tissue injury, behavioral changes, reductions in respiration rates (such as RD_{50} values), potentially increased infectivity with pathogen challenge, or lethality.

As stated above, the human odor threshold for ammonia ranges from 5 to 53 ppm, and sensory fatigue ("adaptation" or "inurement") is documented. There is some subjective debate regarding the concentration at which respiratory and ocular irritation occurs, but there is a consensus that tissue is injured at vapor concentrations in excess of those at which ammonia can be detected by odor or ocular irritation; thus odor and ocular irritation have warning value for ammonia, although sensory fatigue often occurs after continuous or repetitive exposures.

Effects in Humans

Accidental Exposures

No reliable concentration data are available for characterizing human exposures sustained during the many transportation, industrial, and agricultural accidents in which injurious or lethal ammonia concentrations have been released. Most case reports contain no exposure estimates but demonstrate that high vapor concentrations have caused severe damage to the respiratory tract. Death was most likely to occur when exposures were high enough to cause pulmonary edema. Nonlethal, irreversible, or long-term effects occurred when damage progressed to the tracheobronchial region, as manifested in reduced performance on pulmonary-function tests, bronchitis, bronchiolitis, emphysema, and bronchiectasis. Nondisabling, reversible effects were manifested as irritation to the eyes, throat, and nasopharyngeal region. A few of the many accident case reports are summarized below. Additional case reports and details are presented

in NRC (2002; see Table 2-5, "Human Toxicity Data, Accidental Exposure to Ammonia," pp. 34-41) and ATSDR (2004).

Caplin (1941, as cited in NIOSH 1974) reported effects observed in 47 persons accidentally exposed to ammonia in an air-raid shelter when a transfer pipe containing ammonia was ruptured. Casualties were divided into three groups according to the degree to which they were affected: "mildly" (slight upper respiratory tract and eye irritation and hoarseness), "moderately" (productive cough and moist rales and more pronounced respiratory tract irritation), or "severely" (pulmonary edema with cyanosis, intense dyspnea, and persistent cough with frothy sputum). No deaths occurred among the nine "mildly" affected patients. Of the 27 "moderately" affected patients, three exhibited signs and symptoms similar to pulmonary edema and died within 36 h. Nine of the "moderately" affected patients developed bronchopneumonia within 2-3 days, and three died 2 days after the onset; mortality rate for the "moderately" affected patients was 22% (six of 27). The 11 "severely" affected patients developed pulmonary edema, and seven died within 48 h after exposure; mortality rate for the "severely" affected group was 64% (seven of 11).

Walton (1973) reported on the death of one of seven workers exposed to ammonia for an undefined duration in an industrial accident. The autopsy report noted marked laryngeal edema, acute congestion, pulmonary edema, and denudation of the bronchial epithelium. Survivors exhibited difficulty in breathing and burns of the eyes, mucous membranes, and skin; reduced pulmonary gas transfer and airway damage were apparent in survivors followed for 3 years after exposure.

A worker exposed to high concentrations of ammonia vapor estimated at 10,000 ppm for an undefined duration (perhaps a few minutes) experienced coughing, dyspnea, and vomiting soon after exposure (Mulder and Van der Zalm 1967). Three hours after initial exposure, the worker's face was "red and swollen," his mouth and throat were "red and raw," his tongue was swollen, speech was difficult, and conjunctivitis was present; he died of cardiac arrest 6 h after exposure. An autopsy revealed marked respiratory irritation, denudation of the tracheal epithelium, and pulmonary edema (Mulder and Van der Zalm 1967).

People acutely exposed to high concentrations of ammonia who survive immediate effects may die of complications weeks to months later. A 25-year-old man died 60 days after exposure to a high concentration of ammonia sustained in a farming accident (Sobonya 1977). The autopsy report noted damage to the bronchial epithelium, bronchiectasis, mucus plugging and mural thickening of the smallest bronchi and bronchioles, fibrous obliteration of small airways, and a purulent cavitary pneumonia characterized by large numbers of *Nocardia asteroides* (nocardial pneumonia). Three co-workers exposed in the same accident died immediately. Hoeffler et al. (1982) reported on a case of a 30-year-old woman who died 3 years after exposure to ammonia during an accident involving a tanker truck carrying anhydrous ammonia in Houston, Texas. Her injuries resulted in severe immediate respiratory effects, including pulmonary

edema. She required mechanically assisted respiration throughout her remaining life. Bronchiectasis was detected 2 years after exposure and confirmed at autopsy, which also showed bronchopneumonia and cor pulmonale.

Experimental Studies

Numerous clinical studies—summarized in NRC (1987, 2002, 2007), ATSDR (2004), and elsewhere—have been conducted in healthy human subjects—including allergic and nonallergic people, those with asthma, and smokers—exposed to monitored concentrations of ammonia for various durations in controlled settings. Clinical data on reversible and nondisabling effects include responses from resting and exercising subjects and address respiratory and cardiac function, airway resistance, granulocytes and monocytes in peripheral blood, cell concentrations in nasal lavage fluids, and various subjective measurements of ocular and respiratory irritancy and systemic effects. Although results of some of the earlier (such as 1940s) studies may be compromised by what is now considered limited analytic characterization of exposure concentrations, there are sufficient multiple and well-conducted clinical studies suitable for exposure-guideline estimation. The more quantitative studies are summarized in Tables 2-2 and 2-3 and the following text.

Verberk (1977) examined dose-response relationships of signs and symptoms after exposure to ammonia vapor at 50-140 ppm in an exposure chamber over increasing durations (30 min to 2 h). Respiratory function and subjective responses of two groups of adults were recorded. One group consisted of eight people familiar with the literature on ammonia effects but "not accustomed…by personal contact" to ammonia exposures (the informed group, 29-53 years old). The second group consisted of eight university students unfamiliar with the literature on ammonia effects and unfamiliar with experiments in laboratory situations (the naive group, 18-30 years old). All subjects had the opportunity to leave the exposure chamber at any time during the test. Histamine threshold challenge tests performed on each subject before ammonia exposure documented the absence of hypersusceptibility to nonspecific irritants. Four members of each group were smokers. Each group was exposed at 1-week intervals to ammonia at 50, 80, 110, or 140 ppm for 30 min, 1 h, or 2 h. Subjective responses (such as smell, eye irritation, throat irritation, and cough) were recorded every 15 min, and respiratory function—vital capacity (VC), forced expiratory volume at 1 sec (FEV_1), forced inspiratory volume at 1 sec (FIV_1)—was measured before and after each exposure. Chamber concentrations were monitored instantaneously with an infrared spectrometer. No subject inhaling any test concentration for any exposure duration exhibited more than a 10% decrease in VC, FEV_1, or FIV_1. Verberk (1977) considered that small percentage to be "no effect." The committee agrees that such small differences have minimal clinical significance. Furthermore, there was no group effect on those measures. Subjective-response scores did exhibit group effects and dose- and duration-response

TABLE 2-2 Subjective-Response Scores[a] of Informed[b] and Naive[b] Human Subjects Exposed to Ammonia Vapor at Various Concentrations

Perception and Exposure Duration	50 ppm (mean)		80 ppm (mean)		110 ppm (mean)		140 ppm[c] (mean)	
	Informed	Naive	Informed	Naive	Informed	Naive	Informed	Naive
Smell:								
½ h	2.0	2.5	2.0	3.0	2.2	3.0	2.0	3.8
1 h	2.0	2.5	2.0	3.0	2.1	3.0	2.0	4.0
2 h	2.0	3.0	1.5	3.0	2.1	3.0	2.0	WD
Eye irritation:								
½ h	1.5	0.8	1.6	1.6	2.7	2.6	3.0	3.0
1 h	1.5	0.8	1.7	1.6	2.7	2.5	2.9	3.2
2 h	1.0	1.3	1.5	2.0	2.2	2.5	2.3	WD
Throat irritation:								
½ h	0.5	0.5	0.8	1.1	1.4	2.0	1.0	3.8
1 h	0.5	0.7	1.0	1.5	1.4	2.6	1.3	4.5
2 h	0.7	1.6	0.8	2.0	1.0	3.0	1.2	WD
Urge to cough:								
½ h	0.2	0.2	0.4	0.7	0.8	1.7	0.6	2.3
1 h	0.2	0.1	0.5	1.0	0.7	2.0	0.9	1.8
2 h	0.3	0.7	0.6	1.5	0.5	2.0	0.6	WD
General discomfort:								
½ h	0.0	0.0	0.0	1.1	0.2	1.0	0.0	2.5
1 h	0.0	0.2	0.0	1.2	0.2	1.0	0.0	3.3
2 h	0.0	1.2	0.0	1.2	0.3	1.8	0.0	WD

[a]Based on a scale of 0-5: 0 = "no sensation," 1 = "just perceptible," 2 = "distinctly perceptible," 3 = "nuisance," 4 = "offensive," 5 = "unbearable."

[b]Informed subjects were academically familiar with the effects of ammonia but not accustomed to regular exposure to it; naive subjects were unfamiliar with literature documenting effects and had not experienced regular exposure. See Table 2-3 for additional details of experimental protocol.

[c]Only four of the naive subjects tolerated 140 ppm for 1 h; none tolerated 140 ppm for 2 h.

Abbreviations: WD, self-withdrawn from chamber.

Source: Adapted from NRC 2007; data from Verberk 1977.

relationships and are summarized in Table 2-2. In general, the informed group submitted response scores lower than the naive group.

Ihrig et al. (2006) evaluated the dose-response relationship of signs and symptoms (irritative, olfactory, and respiratory) during and after ammonia vapor exposures at 10-50 ppm in an exposure chamber according to the following exposure protocol: (a) 0 ppm for 4 h/day on day 1, (b) 10 ppm for 4 h/day on day 2, (c) 20 ppm for 4 h/day on day 3, (d) 20 ppm for 3 h/day with two 30-min peak exposures at 40 ppm (referred to hereafter as 20/40), and (e) 50 ppm for 4 h/day on day 5. The subjects were 43 male volunteers, 21-47 years old: 33 naive subjects unfamiliar with ammonia odor and 10 subjects who were regularly exposed to ammonia in the workplace; their smoking history was unreported. Subjective responses were elicited from them every hour of exposure at 10-40 ppm; at

TABLE 2-3 Summary of Experimentally Determined Human Nondisabling and Reversible Effects of Inhaled Ammonia

Concentration (ppm)	Time	Subjects and Effects	Reference
5	3-h exposure (1.5 h resting + 1.5 h exercising)	5 males and 7 females; healthy adults, 21-28 years old (mean, 25); smoking history unreported; n = 12 When compared with 0-ppm control, no inflammatory reaction in upper respiratory tract, no alteration in exhaled nitric oxide concentration, no alteration in bronchial response to methacholine; subjective reports of eye discomfort and smell ($p < 0.01$), headache, dizziness, and "feeling of intoxication" ($p < 0.05$) significantly greater than control; tendency toward sensory adaptation to subjective "solvent smell"	Sundblad et al. 2004
10	4 h	43 male volunteers (33 naive subjects, 10 ammonia workers); healthy adults, 21-47 years old; smoking history unreported; n = 43. Subjects examined by physician before and after exposure; tear-flow rates measured with paper strips; lung-function examinations included bronchial responsiveness; individual attention, reaction time, and powers of concentration tested at end of each exposure day; "no relevant effects of the ammonia exposure in these physical and neurophysiological examination could be found" Median rank of olfactory symptoms 0.2 (less than 1 = "hardly at all") in experienced subjects and 1.8 (2 = "somewhat") in naive subjects	Ihrig et al. 2006
20	4 h	43 male volunteers (33 naive subjects, 10 ammonia workers); healthy adults, 21-47 years old; smoking history unreported; n = 43 Subjects examined by physician before and after exposure; tear-flow rates measured with paper strips; lung-function examinations included bronchial responsiveness; individual attention, reaction time, and powers of concentration tested at end of each exposure day; "no relevant effects of the ammonia exposure in these physical and neurophysiological examination could be found" Median rank of olfactory symptoms 0.5 (less than 1 = "hardly at all") in experienced subjects and about 2.5 (2 = "somewhat") in naive subjects	Ihrig et al. 2006

20, 40	20 ppm for 3 h with two 30-min peaks at 40 ppm	43 male volunteers (33 naive subjects, 10 ammonia workers); healthy adults, 21-47 years old; smoking history unreported; n = 43 Subjects examined by physician before and after exposure; tear-flow rates measured with paper strips; lung-function examinations included bronchial responsiveness; individual attention, reaction time, and powers of concentration tested at end of each exposure day; "no relevant effects of the ammonia exposure in these physical and neurophysiological examination could be found" Median rank of olfactory symptoms 0.9 (less than 1 = "hardly at all") in experienced subjects and 3 ("rather much") in naive subjects	Ihrig et al. 2006
25	3-h exposure (1.5 h resting + 1.5 h exercising)	5 males and 7 females; healthy adults, 21-28 years old (mean, 25 years); smoking history unreported; n = 12 When compared with 0-ppm control, no inflammatory reaction in upper respiratory tract, no alteration in exhaled nitric oxide concentration, no alteration in bronchial response to methacholine; subjective reports of irritation in eye and upper airways increased over control in all categories ($p < 0.01$); headache, dizziness, "feeling of intoxication, etc." ($p < 0.01$ or $p < 0.05$); no tendency toward sensory adaptation to subjective reports of "solvent smell"	Sundblad et al. 2004
30	10 min	6 fit males, 23-44 years old (mean, 31 years) Odor moderately intense to highly penetrating; irritation faint or not detectable	MacEwen et al. 1970
32	5 min	10 healthy volunteers 1 had nasal dryness	Industrial Bio-Test Lab 1973 (as cited in ATSDR 2004 and WHO 1986)
50	5 min	10 healthy volunteers 2 had nasal dryness; NOAEL identified by ATSDR (2004)	Industrial Bio-Test Lab 1973 (as cited in ATSDR 2004 and WHO 1986)

(Continued)

TABLE 2-3 Continued

Concentration (ppm)	Time	Subjects and Effects	Reference
50	10 min	6 fit males, 23-44 years old (mean, 31 years) Highly penetrating odor; moderate irritation	MacEwen et al. 1970
50	30 min	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.0-2.5 (5 = "unbearable"); eye irritation ranked 0.8-1.5; throat irritation ranked 0.5; slight urge to cough; slight general discomfort; pre-exposure and postexposure measurements of FVC and FEV_1 exhibited no change from control; participants recorded subjective response every 15 min of exposure; in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977
50	1 h	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.0-2.5 (5 = "unbearable"); eye irritation ranked 0.8-1.5; throat irritation ranked 0.5-0.7; mild urge to cough; slight general discomfort; pre-expsoure and postexposure measurement of FVC and FEV_1 exhibited no change from control; in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977
50	2 h	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.0-3.0 (5 = "unbearable"); eye irritation ranked 1.0-1.3; throat irritation ranked 0.7-1.6; mild urge to cough; mild general discomfort; pre-exposure and postexposure measurement of FVC and FEV_1 exhibited no change from control; in general, naive subjects rated effects more severely than informed subjects, at all exposures	Verberk 1977

50	2-6 h/day, 6 weeks; workplace exposures, standard workplace physical activities	2 unacclimated subjects: 1 male, 1 female, 24-29 years old; 1 smoker No significant difference in respiratory rates, pulse, systolic and diastolic BP, FVC, and FEV_1; physician-observed mild eye, nose, and throat irritation not significantly different from control; no evidence of abnormal chest sounds, heart murmur, neurologic change, or weight change; no impairment	Ferguson et al. 1977
50	4 h	43 male volunteers (33 naive subjects, 10 ammonia workers); healthy adults, 21-47 years old; smoking history unreported; n = 43 Subjects examined by physician before and after exposure; tear-flow rates measured with paper strips; lung-function examinations included bronchial responsiveness; individual attention, reaction time, and powers of concentration tested at end of each exposure day; "no relevant effects of the ammonia exposure in these physical and neurophysiological examination could be found" 3 participants exhibited "slight conjunctival hyperemia"; irritative symptom median of 1 ("hardly at all") and largely unchanged over 4-h exposure; median rank of olfactory symptoms about 1.7 (2 = "somewhat") in experienced workers and about 3.2 (3 = "rather much") in naive subjects	Ihrig et al. 2006
72	5 min	10 healthy volunteers 3 had nasal, eye, and throat irritation; LOAEL identified by ATSDR (2004)	Industrial Bio-Test Lab 1973 (as cited in ATSDR 2004 and WHO 1986)
80	30 min	16 adults; 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.0-3.0 (5 = "unbearable"); eye irritation ranked 1.6; throat irritation ranked 0.8-1.1; mild urge to cough; moderate general discomfort; no measurable change from control in respiratory function (FVC, FEV_1); in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977

(Continued)

TABLE 2-3 Continued

Concentration (ppm)	Time	Subjects and Effects	Reference
80	1 h	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.0-3.0 (5 = "unbearable"); eye irritation ranked 1.6-1.7; throat irritation ranked 1.0-1.5; mild urge to cough; moderate general discomfort; no measurable change from control in respiratory function (FVC, FEV_1); in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977
80	2 h	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 1.5-3.0 (5 = "unbearable"); eye irritation ranked 1.5-2.0; throat irritation ranked 0.8-2.0; urge to cough; moderate general discomfort; no measurable change from control in respiratory function (FVC, FEV_1); in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977
25, 50, 100: ascending and descending sequentially weekly; 2 weeks at each concentration	2-6 h/day, 6 weeks; workplace exposures; normal workplace physical and mental tasks	4 unacclimated subjects: males, 26-46 years old; 2 smokers No adverse effects on respiratory function; no increase in frequency of eye, nose, throat irritation; only statistically significant increase was in FEV_1 ("improvement") with increasing ammonia concentration; no subjective reports of irritation; physician examinations indicate "mild" irritation of eyes, nose, and throat at 50, 100 ppm (0.11), not significantly different from control (0.09); after acclimation, continuous exposure at 100 ppm (with occasional excursions to 200 ppm) easily tolerated; exposure effects on workplace mental and physical tasks normally performed by chemical operator also evaluated (none)	Ferguson et al. 1977
100	5, 10, 15, 20, 30 sec	Individual, forced-air nostril delivery at 100 ppm (at 9 newtons/cm^2) for designated exposure periods separated by 15-min measurement of NAR; concentration-dependent increase in NAR but no significant differences between mean response in nonatopic and atopic (including those with allergic rhinitis) subjects	McLean et al. 1979

Mean, 102-336 (range, 71.5-492)	Apparently 95-120 min	18 healthy servicemen, mean 24.1 years old; exercising on cycle ergometer at 20 to 120 W; considered "submaximal" exercise "No material discomfort" but dryness of mouth and prickling sensation in nose; reversible ventilatory responses; ventilation minute volume significantly reduced at 151-336 ppm; no effect on respiratory minute volume at 102 ppm; exercise tidal volume significantly increased at 152 ppm; average reduction in ventilation minute volume of 6% (range, 3.5-10.0%) at all concentrations	Cole et al. 1977
110	30 min	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.2-3.0 (5 = "unbearable"); eye irritation ranked 2.6-2.7; throat irritation ranked 1.4-2.0 of 5; mild urge to cough; moderate general discomfort; no measurable change from control in respiratory function (FVC, FEV_1); in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977
110	1 h	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.1-3.0 (5 = "unbearable"); eye irritation ranked 2.5-2.7; throat irritation ranked 1.4-2.6; moderate urge to cough; moderate general discomfort; no measurable change from control in respiratory function (FVC, FEV_1); in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977
110	2 h	16 adults: 8 informed (7 males, 1 female, 29-53 years old), 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.1-3.0 (5 = "unbearable"); eye irritation ranked 2.2-2.5; throat irritation ranked 1.0-3.0; urge to cough; general discomfort; no measurable change from control in respiratory function (FVC, FEV_1); in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977

(Continued)

TABLE 2-3 Continued

Concentration (ppm)	Time	Subjects and Effects	Reference
140	30 min	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.0-3.8 (5 = "unbearable"); eye irritation ranked 3.0; throat irritation ranked 1.0-3.8; mild urge to cough; moderate general discomfort; no measurable change from control in respiratory function (FVC, FEV_1); in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977
140	1 h	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Odor perception ranked 2.0-4.0 (5 = "unbearable"); eye irritation ranked 2.9-3.2; throat irritation ranked 1.3-4.5; moderate urge to cough; moderate general discomfort; no measurable change from control in respiratory function (FVC, FEV_1); part of naive population withdrew; in general, naive subjects rated effects more severely than informed subjects at all exposures	Verberk 1977
140	2 h	16 adults: 8 informed (7 males, 1 female, 29-53 years old); 8 naive (6 males, 2 females, 18-30 years old) Naive group withdrew; odor perception ranked 2.0 by informed group; eye irritation ranked 2.3 by informed group; throat irritation ranked 1.2 by informed group; urge to cough; general discomfort; no measurable change from control in respiratory function (FVC, FEV_1).	Verberk 1977
143	5 min	10 healthy volunteers Nose, eye, throat, and chest irritation; lacrimation	Industrial Bio-Test Lab 1973 (as cited in ATSDR 2004 and WHO 1986)

500	15, 30 min (1 for 15 min, 6 for 30 min)	7 adult males at rest Increase in minute volume compared with control (+50-250%); nose and throat irritation (2) with nasal dryness and stuffiness; excessive lacrimation (2); immediate hyperventilation (3); change to mouth breathing (5); transient hypoesthesia of skin around nose and mouth (7); no significant change in blood or urinary urea, ammonia, blood-protein nitrogen; "slight" increase in pulse rate, BP in 1 of 2; nasopharyngeal irritation persisted for 24 h in 2; authors consider 500 ppm "physiologically undesirable"	Silverman et al. 1949
571 (mean NH_3TR; SEM, 41.5)	Single breath	14 subjects, 21-30 years old from cohort of 102 healthy, nonsmoking males, females Threshold for reflex glottis closure	Erskine et al. 1993
1,791 (mean NH_3TR; SEM, 52)	Single breath	14 subjects, 86-95 years old from cohort of 102 healthy, nonsmoking males, females Threshold for reflex glottis closure	Erskine et al. 1993

Note: See also Table 2-4 of NRC (2002) and Table 3-1 of ATSDR (2004) for additional detail. For full description of rankings used in Verberk (1977), see Table 2-2 of this chapter.
Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; BP, blood pressure; FEV_1, forced expiratory volume at 1 sec; FVC, forced vital capacity; LOAEL, lowest observed-adverse-effect level; NAR, nasal airway resistance; NOAEL, no-observed-adverse-effect level.
Source: Adapted from NRC 2007.

50 ppm, responses were elicited every 30 min. Subjective-response scores were to ammonia in the workplace; their smoking history was unreported. Subjective responses were elicited from them every hour of exposure at 10-40 ppm; at 50 ppm, responses were elicited every 30 min. Subjective-response scores were ranked as 0 = "not at all," 1 = "hardly at all," 3 = "somewhat," 4 = "considerably," and 5 = "very much." Medical examinations conducted before and after each exposure assessed physical response of the eyes and respiratory tract and cognitive skills (for example, lung-function test, including bronchial responsiveness; neuropsychologic examination for reaction time, attention, and concentration; and nasal resistance and lacrimation). Ihrig et al. (2006) report that, with the exception of three subjects in the 50-ppm exposure group who exhibited "slight conjunctival hyperemia," "no relevant effects of the ammonia exposure in these physical and neuropsychological examinations could be found." Furthermore, comparison of subjective symptoms reported by naive subjects vs ammonia workers indicated that habituation strongly influenced complaint reporting (for example, naive subjects reported more symptoms than experienced subjects at a given exposure).

McLean et al. (1979) examined the effect of ammonia on nasal airway resistance (NAR) in atopic and nonatopic human subjects and the potential inhibition of such effects by atropine or chlorpheniramine administration. Ammonia (100 ppm at 9 newtons/cm^2) was serially introduced into each nostril (successive exposure durations for each subject of 5, 10, 15, 20, and 30 sec/nostril followed by 15-min intervals). Exposures were followed by NAR measurement with a pneumotachograph attached to a facemask every minute for 5 min and then every 2 min for 10 min (total of 10 measurements over a 15-min period). Nonatopic subjects were screened on the basis of strict criteria that included a questionnaire, physical examination, spirometry, a nasal smear for eosinophils, and a battery of 19 prick and six intracutaneous allergen tests. Nonatopic subjects could possess no personal or immediate family history of atopic disease (allergic rhinitis, asthma, or atopic dermatitis), could have no more than 5% eosinophils in their nasal smears, and had to exhibit negative reactions on the battery of prick and intracutaneous allergen tests. Atopic subjects were screened on the basis of a characteristic history of allergic rhinitis and positive allergen-test reactions. Some subjects determined to be atopic had a history of asthma. All subjects had been symptom-free for several weeks before the study, and none was judged to be taking medications that would influence skin or mucosal tests. Individual baselines were obtained by collection of NAR measurements during a 15-min pre-exposure period. Additional tests included introduction of 0.1 mL of aerosolized phosphate-buffered saline, 0.1 mL of atropine sulfate, or 0.1 mL of chlorpheniramine maleate into the nostrils, each followed by exposure to 100-ppm ammonia vapor for 20 sec. NAR measurements after exposure increased significantly with increase in exposure duration from 5 to 20 sec. Negligible increases were noted in subjects exposed for 30 sec compared with 20 sec. There was no significant difference in NAR increase between atopic and nonatopic subjects exposed to ammonia, nor was there any significant difference between

the allergic-rhinitis subjects with and without a history of asthma. Atropine sulfate administration inhibited the NAR response to ammonia in atopic and nonatopic subjects by up to 89%, whereas administration of the antihistamine chlorpheniramine had no effect on ammonia-induced NAR. The study authors noted that the results of atropine and chlorpheniramine administration suggest that ammonia irritancy is mediated primarily by a parasympathetic reflex effect on the nasal vasculature and not by histamine release.

Industrial Bio-Test Laboratories, Inc. (1973, as cited in ATSDR 2004 and WHO 1986) determined the irritation threshold in 10 human volunteers exposed to ammonia vapor at 32, 50, 72, or 143 ppm for 5 min. Irritation was subjectively defined as annoyance to the nose, throat, eyes, mouth, or chest. Subjects demonstrated dose-related response of those subjective end points; effect severity was not noted.

MacEwen et al. (1970) studied six male workers, 23-44 years old (average, 31 years); all were considered "fit" in that each had passed either class II U.S. Air Force (USAF) or class II Federal Aviation Administration (FAA) physical examinations for flying. The test population included "nonsmokers, reformed smokers, and heavy smokers." Each subject underwent head-only exposure to ammonia at 30 or 50 ppm for 10 min and reported the degree of intensity and description of irritation of the nose and eyes on a subjective scale. Study results demonstrated a dose-related increase in the subjective response to ammonia at 30 and 50 ppm. At 30 ppm, irritation was reported by two of the six subjects as faint (grade 1) and by three as not detectable (grade 0), and one gave no response; for odor characteristics at 30 ppm, three subjects reported odor as strong or highly penetrating (grade 4), two reported odor as easily noticeable or moderate (grade 3), and one gave no response. At 50 ppm, irritation was reported by four subjects as moderate (grade 2), by one as faint or just perceptible (grade 1), and by one as not detectable (grade 0); odor was characterized as strong or highly penetrating (grade 4) for by all six subjects at 50 ppm.

Silverman et al. (1949) studied seven male subjects exposed to 500-ppm anhydrous ammonia through a nose and mouth mask for 30 min (six subjects) or 15 min (one subject). Respiratory rates, minute volumes, blood pressure, pulse rates, blood urea, serum nonprotein nitrogen, and urinary urea and ammonia were measured in each subject, and each subject provided subjective reactions. Results were as follows: increase (50-250%) of minute volume in all seven subjects; nose and throat irritation (two subjects) with nasal dryness and stuffiness; excessive lacrimation (two subjects); immediate hyperventilation (three subjects); change to mouth breathing (five subjects); transient hypoesthesia (decreased sensitivity) of skin around nose and mouth (seven subjects); no significant change in blood or urinary urea, ammonia, or blood-protein nitrogen; and "slight" increase in pulse rate and systolic and diastolic blood pressure in one of two measured subjects. Nasopharyngeal irritation persisted for 24 h in two subjects. Silverman et al. (1949) concluded from their results that exposure to 500-ppm ammonia was "physiologically undesirable."

Cole et al. (1977) studied the effects of exercise on 18 servicemen exposed

to ammonia vapor at 102, 152, 206, and 336 ppm in an exposure chamber while cycling under a load of 20 W increased to 180 W in 20-W increments for various durations that apparently were 95-120 min (based on assumptions of "zero time" and extrapolation from figures in the report). Subjects served as their own controls. Measurements of respiratory function (respiratory rate, minute volume, tidal volume, and oxygen uptake) and cardiac frequency were taken during each experimental period and compared with control values. Measured minute volume was decreased by 8%, 10%, and 6% at 152, 206, and 336 ppm, respectively. However, no clear dose-related trend was observed relative to the controls. Tidal volume was significantly decreased by 9% and 8% and respiratory frequency was increased by 10% and 8% at 206 and 336 ppm, respectively, compared with the control values. Those changes may indicate that vagal stimulation may have occurred; however, no clear dose-response relationship was observed. The small changes in tidal volume and respiratory frequency are unlikely to be clinically significant. During exposures, subjects noted "no material discomfort" but mouth dryness and prickling in the nose; these effects were reversible on cessation of exposure.

Sundblad et al. (2004) investigated the acute effects of repeated low-concentration ammonia exposures in chamber experiments, incorporating both rest and ergometric exercise. Seven females and five male healthy atopic adults, 21-28 years old (mean, 25 years), with no reported present or past symptoms of allergy or airway disease were exposed to ammonia in randomized order at 0, 5, and 25 ppm for 3 h on three occasions; the exposures were separated by at least 1 week in which subjects did not undergo experimental ammonia exposures. During each 3-h exposure, 1.5 h was spent at seated rest and 1.5 h in exercising on a bicycle ergometer at 50 W. Subjects ranked 10 subjective and transient symptoms selected to characterize irritation—such as eye, nose, and throat discomfort—and systemic response—such as headache, dizziness, nausea, and "feeling of intoxication." The latter symptoms were characterized by Sundblad and co-workers as "CNS [central nervous system] effects." Sundblad et al. (2004) offered no neurophysiologic measurements or other experimental data to support categorization of the latter symptoms as adverse CNS responses. The committee concludes that the systemic responses may be consequences of irritation or odor. Moreover, the exposure protocol meant that each subject was exposed to two ammonia concentrations, which may or may not have been separated by a sham exposure, depending on the randomized exposure sequence. Therefore, because of sensory fatigue, a subject's response to the second ammonia exposure may have been less vigorous than it would have been in previously unexposed subjects.

Sundblad et al. (2004) also evaluated changes resulting from exposure in the following parameters: lung function; bronchial responsiveness to methacholine; exhaled nitric oxide; cell composition in nasal lavage fluids; and peripheral blood profiles for leukocytes, monocytes, lymphocytes, basophilic granulocytes, eosinophils and complement factor C3b. Under the experimental conditions, ammonia exposures at 5 or 25 ppm did not induce detectable upper airway in-

flammation or increased bronchial response to methacholine according to any analytic measure used; an observed increase in number of blood granulocytes after exposure was considered an exercise effect by the investigators and has been noted by other investigators (Hansen et al. 1991).

Subjective-symptom rankings on a questionnaire exhibited a dose-response relationship. On the basis of questionnaire results, Sundblad et al. (2004) noted a tendency toward sensory adaptation to "solvent smell" in those exposed at 5 ppm but not those exposed at 25 ppm. Rankings of all 10 measured symptoms that characterized irritation and systemic response were significantly greater than in controls in the 25-ppm exposure group, but the 5-ppm exposure group exhibited higher rankings of only five symptoms. All symptomatic effects ranked were transient.

In the controlled workplace-exposure study of Ferguson et al. (1977), effects of ammonia vapor on three groups of two industrial workers exposed at 25, 50, or 100 ppm were evaluated after practice exposure at the same concentrations during a 1-week period. Subjects underwent exposure at a sodium bicarbonate plant in areas where ammonia concentrations of 25 and 50 ppm were achieved; controlled 100-ppm exposures took place in an exposure chamber. Exposure periods ranged from 2 to 6 h/day for 5 weeks. No adverse effects on respiratory function and no increase in frequency of eye, nose, and throat irritation were noted. The only statistically significant increase was an unexplained FEV_1 ("improvement") with increasing ammonia concentration. Participants contributed no subjective reports of irritation; physician examinations indicated that the resulting signs of "mild" eye, nose, and throat irritation at 50 and 100 ppm were not significantly different from control findings. After acclimation, exposure for up to 6 h at at least 100 ppm (averages of 103-140 ppm with occasional excursions to 200 ppm) was "easily tolerated" by subjects (Ferguson et al. 1977).

Erskine et al. (1993) used an inspiratory pneumotachograph to measure the concentration of ammonia required to elicit reflex glottis closure in 102 healthy nonsmoking subjects, 17-96 years old, after single intermittent breaths of ammonia vapor at about 500 to 2,000 ppm. The results demonstrated a strong positive correlation (coefficient, 0.85) between age and the ammonia concentration needed to trigger reflex glottis closure (NH_3TR). The mean NH_3TR for the group 86-95 years old was 1,791 ppm (SEM, 52; n = 14), and the mean NH_3TR for the group 21-30 years old was 571 ppm (SEM, 41.5; n = 14).

Occupational and Epidemiologic Studies

Most available occupational and epidemiologic studies were not designed to discriminate between ammonia and other workplace contaminants—such as endotoxins, fungi, bacteria, and respirable dusts—in their relative contributions to the development of observed adverse respiratory effects. Minor pulmonary-function deficits have been observed in swine workers exposed to ammonia in

combination with dust and endotoxin (Reynolds et al. 1996). Although ammonia concentrations as high as 200 ppm have been reported (Carlile 1984), mean exposure concentrations of 4-7 ppm were more typical for the swine workers examined (Reynolds et al. 1996; Donham et al. 1995). Other occupational cohort studies have examined farmers and farm workers employed in enclosed livestock buildings; substances measured include not only ammonia but also dusts, endotoxins, and various microorganisms and fungi (Cormier et al. 2000; Choudat et al. 1994; Donham et al. 1995, 2000; Ballal et al. 1998, all as cited in ATSDR 2004). The utility of the data from those studies is limited because of confounding by multiple exposures, fragmentary exposure-duration characterization, and lack of information on clinical signs and symptoms.

Holness et al. (1989) compared respiratory effects in a group of 58 workers (51 production and six maintenance workers at an industrial soda-ash production facility) chronically exposed to airborne ammonia vapor (mean ± standard deviation, 9.2 ± 1.4 ppm) with effects in a group of 31 plant workers with essentially no exposure to airborne ammonia (0.3 ± 0.1 ppm). During a 1-week period, the workers were assessed on the basis of a questionnaire (reporting of cutaneous or respiratory symptoms), sense of smell, baseline pulmonary function (FVC [forced vital capacity], FEV_1, FEF50 [forced expiratory flow at 50% FVC], and FEF75), or change in lung function over a workshift at the beginning and end of a workweek. There were no differences between the two groups in the characteristics studied and no relationship between concentration and duration of ammonia exposure and lung function. Holness and colleagues pointed out that study weaknesses included "lack of adequate exposure data" and the difficulties in accounting for both concentration and duration of exposure. In addition, there was no characterization of historical occupational ammonia exposures during years before the short period in which they measured workplace concentrations.

Effects in Animals

Thorough reviews of the results of controlled experimental exposures can be found in NRC (2002, 2007) and ATSDR (2004).

Acute Toxicity

Lethality values (LC_{50} and lowest experimental concentrations) for mice, rats, and cats receiving inhalation exposures are presented in Table 2-4; data on other exposure routes, such as rabbit intratracheal cannulation (Boyd et al. 1944), are not summarized here because of their limited application. Cats are the least characterized of the experimental species tested. The LC_{50} for rats ranged from 7,338 and 16,600 ppm for 60-min exposures to 40,300 ppm for a 10-min exposure (MacEwen and Vernot 1972; Appelman et al. 1982). The LC_{50} for mice ranged from 4,230 ppm for a 60-min exposure to 10,096 ppm for a 10-min

TABLE 2-4 Summary of Acute-Lethality Inhalation Data on Ammonia Exposure of Laboratory Animals

Species	Concentration (ppm)	Time	Effects	Reference
Rat	40,300	10 min	LC_{50}	Appelman et al. 1982
	33,433	10 min	10% mortality	
	28,595	20 min	LC_{50}	
	26,155	20 min	30% mortality	
	20,300	40 min	LC_{50}	
	18,047	40 min	20% mortality	
	16,600	60 min	LC_{50}	
	14,114	60 min	30% mortality	
Rat	7,338	60 min	LC_{50}	MacEwen and Vernot 1972
Mouse	10,152	10 min	LC_{50}	Silver and McGrath 1948
	8,723	10 min	25% mortality	
Mouse	4,837	60 min	LC_{50}	MacEwen and Vernot 1972
	4,550	60 min	30% mortality	
Mouse	4,230	60 min	LC_{50}	Kapeghian et al. 1982
	3,950	60 min	25% mortality	
	4,380	240 min	25% mortality[a]	
	1,350	240 min	100% survival	
Mouse	21,430	30 min	LC_{50}	Hilado et al. 1977, 1978
	19,048	30 min	25% mortality	
Cat	1,000	10 min	5% mortality	Dodd and Gross 1980

[a]No observation period after exposure.
Abbreviations: LC_{50}; statistically determined lethal concentration for 50% of sample population.
Source: Adapted from NRC 2007.

exposure (Silver and McGrath 1948; MacEwen and Vernot 1972; Kapeghian et al. 1982; Hilado et al. 1977, 1978).

Rats exposed to lethal ammonia concentrations exhibited signs of dyspnea and of irritation of the eyes and nose and lung hemorrhage on necropsy (Appelman et al. 1982). Mice exposed to lethal concentrations showed signs of irritation of the eyes and nose, labored breathing, and gasping with tremors, ataxia, convulsions, and seizures; pathologic lesions occurred in the alveoli (Silver and McGrath 1948; MacEwen and Vernot 1972; Kapeghian et al. 1982). Signs of toxicity in cats included death, poor general condition, severe dyspnea, anorexia, dehydration, bronchial breath sounds, sonorous and sibilant rhonchi, and coarse rales (Dodd and Gross 1980). Pulmonary-function tests provided evidence of airway damage throughout the experiment and of central lung damage on observation day 21. Gross lung examination showed congestion, hemorrhage, edema, and evidence of interstitial emphysema and collapse. Bronchopneumonia, which caused the death of one animal, was common after observation day 7.

Species comparisons of LC_{50} data by ten Berge et al. (1986) documented that mice are usually more sensitive to lethal concentrations of irritants in general, and ammonia in particular, than other experimental laboratory animals. ten Berge et al. also suggested that toxicity data on mice "do not provide an appropriate basis for predicting…the mortality response in humans" to many locally irritant gases, including ammonia. Data summarized in NRC (2007) indicate that the mouse is 2.7-4 times more susceptible than the rat to the toxic effects of ammonia inhalation exposure.

Acute sublethal toxicity after controlled experimental exposure to ammonia has been well summarized in the previous NRC (2002) report *Review of Submarine Escape Action Levels for Selected Chemicals* (Chapter 2, pp. 22-68, "Ammonia"; see Table 2-8, "Experimental Animal Toxicity Data…" pp. 44-56) and ATSDR report (2004; see Table 3-1, "Levels of Significant Exposure to Ammonia and Ammonium Compounds—Inhalation," pp. 27-40) and will not be presented at the same level of detail here. The database on acute sublethal exposure includes studies on mice, rats, and cats exposed at various concentrations (3-1,157 ppm) of ammonia for 10 min to 24 h.

In general, nondisabling, reversible effects in laboratory animals were mild after single exposure. Rats exhibited concentration-dependent cessation of tracheal ciliary activity during exposure at 3-90 ppm for 10 min (Dalhamn 1956 a,b): at 3, 6.5, and 90 ppm, ciliary activity ceased in 7-8 min, 150 sec, and 5 sec, respectively. Schaerdel et al. (1983) exposed rats to ammonia at 15-1,157 ppm for 24 h; no behavioral changes or irritation of the eyes or mucous membranes were exhibited, and no changes were noted in blood pCO_2 and pH. Small changes in pO2 occurred "within the normal range for rats." A 50% reduction in the respiration rate (RD_{50}) was noted in mice exposed at about 300 ppm for 30 min (Barrow et al. 1978).

There was no evidence of pulmonary lesions in mice or rats exposed at single nonlethal concentrations.

Repeated Exposure and Subchronic Toxicity

Effects in laboratory animals were mild and transient after repeated exposure (subchronic duration) to ammonia and are summarized in Table 2-5. Only minimal effects on respiratory epithelium of the upper respiratory tract were observed after continuous exposure at up to 714 ppm for several days (Schaerdel et al. 1983). Repeated exposure of Swiss-Webster mice to the experimental RD_{50} of 303 ppm for 6 h/day for 3 or 7 days was associated with reversible and minimal to moderate changes in respiratory epithelium that were not considered pathologic lesions (Buckley et al. 1984). Exposure of Swiss OF1 mice at 711 ppm, which is about 3 times the experimental RD_{50} of 257 ppm in this mouse breed, resulted in slight to moderate exfoliation, erosion, ulceration, and necrosis of the respiratory epithelium of the nasal cavity; no lower respiratory tract le-

sions were produced (Zissu 1995). Additional supportive studies (for example, Tepper et al. 1985; Manninen et al. 1988) are summarized in Table 2-5.

Except for nonspecific inflammation of the lungs at 1,101 ppm, repeated daily exposure of rats at 57 ppm for 114 days or at 222 or 1,101 ppm for 6 weeks (8 h/day) produced no effects (Coon et al. 1970). Almost all rats died after continuous exposure at 651 or 672 ppm for 65 days. Repeated exposure at 1,101 ppm for 6 weeks (8 h/day) produced transient dyspnea and lacrimation in dogs and rabbits, whereas continuous exposure at 672 ppm for 90 days resulted in signs of irritation of the eyes and nose and pathologic lesions in the lungs of dogs and rabbits and pneumonitis in several species (dog, rabbit, guinea pig, and monkey) (Coon et al. 1970).

Subchronic inhalation-exposure studies of male mice provide inconclusive evidence of nasal carcinoma (Gaafar et al. 1992), and gavage exposure of laboratory mice to ammonium ion at 42 mg/kg per day for 4 weeks provided no evidence of carcinogenicity (Uzvolgyi and Bojan 1980, as cited in ATSDR 2004).

The longest exposure in the available literature was 114 days of continuous exposure of male and female Sprague-Dawley and Long-Evans rats at 57 ppm (Coon et al. 1970); this protocol resulted in no clinical signs and no significant effects when compared with the control.

Done et al. (2005) evaluated continuous ammonia exposure (at 0.6, 10.0, 18.8, or 37.0 ppm) of weanling pigs (commercial herd) in combination with generated inspirable (artificial) dust (at 1.2, 2.7, 5.1, or 9.9 mg/m^3) for 5 weeks in a controlled-ventilation facility. The ammonia and dust concentrations evaluated were considered representative of commercial piggeries in the United Kingdom. Done and co-workers used a multifactorial design incorporating a total of 560 weanling pigs over 2 years. Daily clinical examination for respiratory, gastrointestinal, and ocular signs documented that the experimental exposures had no significant effects. Postmortem turbinate scores were low (for example, low clinical rhinitis), as were lung scores; neither turbinate nor lung scores were affected by exposure to ammonia or dust when compared with the controls. No significant differences in any monitored characteristic were noted when experimental pigs were compared with controls (Done et al. 2005).

Overall, studies of repeated exposure indicate that mice are more susceptible than other mammals tested repeatedly or for subchronic exposure durations.

Chronic Toxicity

No information characterizing chronic toxicity of ammonia exposure was located.

TABLE 2-5 Summary of Repeated and Subchronic Ammonia Exposure Studies in Laboratory Animals

Species	Concentration (ppm)	Exposure Duration	Effects	Reference
Rat (male, female Sprague-Dawley, Long-Evans)	222	8 h/day for 6 weeks	No deaths or clinical signs	Coon et al. 1970
	1,101	8 h/day for 6 weeks	Nonspecific inflammatory changes in lungs (colony infection); no deaths or clinical signs	
	57	Continuous, 114 days	No clinical signs; no significant effects when compared with controls	
	182	Continuous, 90 days	No clinical signs	
	375	Continuous, 90 days	"Mild" nasal discharge in 25% of test population	
	651	Continuous, 90 days	Day 25: mild dyspnea, nasal irritation, 63% mortality Day 65: 98% mortality	
	672	Continuous, 90 days	87% mortality	
Rabbit, guinea pig, dog, monkey	222	8 h/day for 6 weeks	Focal pneumonitis in one of three monkeys; no other signs or clinically significant effects	Coon et al. 1970
	1,101	8 h/day for 6 weeks	Dogs and rabbits exhibited transient lacrimation and dyspnea in week 1; nonspecific inflammatory changes in guinea pig lungs	
Rabbit, guinea pig, dog, monkey	57	Continuous, 90 days	No signs or clinically significant effects	Coon et al. 1970
	672	Continuous, 90 days	Dogs: heavy lacrimation, nasal discharge, hemorrhagic lung lesions in one of two Rabbits: erythema, discharge, corneal opacity, moderate lung congestion in two of three Guinea pigs: four of 15 died All (also in controls, but less severe): focal or diffuse interstitial pneumonitis; calcification in renal tubules and bronchial epithelium; cell proliferation in renal epithelium; myocardial fibrosis	

Rat	Serial exposure at 100, 300, 300, 100	6 h/day, with 2 days separating exposure concentrations	Measurements of running-wheel activity indicated decrease after more than 1 h at 100 ppm, decrease throughout 300-ppm exposures; authors attributed activity change to sensory irritation; activity depression transient; running-wheel activity increased after exposure cessation.	Tepper et al. 1985
Mouse	Serial exposure at 100, 300, 300, 100	6 h/day, with 2 days separating exposure concentrations	Similar to rat experience, as described above; at comparable concentrations, activity of mice decreased less than that of rats	Tepper et al. 1985
Weanling pig (commercial herd)	0.6, 10.0, 18.8, or 37.0 (in combination with artificial dust at 1.2, 2.7, 5.1 or 9.9 mg/m^3)	Continuous for 5 weeks	Multifactorial experiment performed on 560 weanling pigs over 2 years; daily inspections and clinical monitoring and postmortem examinations of 40 pigs in each of eight batches revealed "minimal gross pathology and …minor pathological changes of little significance"; low turbinate scores (for example, low clinical rhinitis) and low lung scores observed and unaffected by exposure to ammonia and dusts; no differences between control and exposed pigs in any respiratory, gastrointestinal, or ocular measure monitored, including nasal discharge and sneezing	Done et al. 2005
Rat	4, 24, 44, 165, 714	Continuous for 3 or 7 days	Minimal lesions in nasal-cavity respiratory epithelium at 7 days (undefined concentration); no change in trachea or lungs; no significant effects on blood gases or pH	Schaerdel et al. 1983
Rat (female)	25, 300	6 h/day for 5, 10, or 15 days	No treatment-related changes observed in lung, kidney, or liver; 300 ppm considered NOAEL	Manninen et al. 1988
Mouse (Swiss-Webster)	303 (RD_{50})	6 h/day for 5 days	Observed subjects exhibited no clinical signs; no lesions in tracheobronchial or pulmonary areas; nasal-cavity epithelium exhibited minimal exfoliation and other tissue changes, moderate squamous metaplasia and inflammatory changes	Buckley et al. 1984

(Continued)

TABLE 2-5 Continued

Species	Concentration (ppm)	Exposure Duration	Effects	Reference
Mouse (Swiss OF1)	78 (0.3 RD_{50}); 257 (RD_{50}), 711 (2.8 RD_{50})	6 h/day for 4, 9, or 14 days	Observed subjects exhibited no clinical signs; respiratory tract exhibited rhinitis with metaplasia and necrosis in nasal-cavity epithelium only at 3 times RD_{50}, with increasing severity at greater exposure duration (very severe on day 14); no lesions at RD_{50} for any exposure duration	Zissu 1995

Abbreviations: NOAEL, no-observed-adverse-effect level; RD_{50}; statistically estimated concentration resulting in 50% reduction in respiratory rate.

Reproductive Toxicity in Males

No information characterizing reproductive toxicity of inhalation, ocular, oral, or dermal exposure to ammonia of male humans or laboratory animals was located.

Immunotoxicity

Although secondary infections of respiratory lesions and skin burns can occur after exposure to concentrated ammonia vapors or aerosols (Caplin 1941; Sobonya 1977; Slot 1938; O'Kane 1983), there is no evidence that ammonia exposure impairs the human immune system (ATSDR 2004).

Laboratory animal studies of a variety of species indicate that repeated inhalation or whole-body exposure to some concentrations of ammonia is associated with a decrease in immune response—a decrease in "cell-mediated immune response" in guinea pigs challenged with tuberculin derivative after exposure to 90-ppm ammonia 24 h/day for 3 weeks (Targowski et al. 1984, as cited in NRC 2002) or a decrease in resistance when challenged with an infective bacterial dose. Examples of the latter include increased mortality in male mice exposed to the LD_{50} of *Pasteurella multocida* after exposure to 500-ppm ammonia 24 h/day for 7 days (Richard et al. 1978, as cited in ATSDR 2004), increased severity of clinical signs in rats inoculated with *Mycoplasma pulmonis* either before a 4-week exposure to 25-ppm ammonia 24 h/day (Broderson et al. 1976, as cited in ATSDR 2004) or before a 3- to 9-day exposure to 100-ppm ammonia 24 h/day (Pinson et al. 1986), and increased Newcastle disease infection rate in chickens exposed to the 48-h lowest observed-adverse-effect level (LOAEL) of 50 ppm and to the 72-h LOAEL of 20 ppm (Anderson et al. 1964, as cited in NRC 2002). Neumann et al. (1987, as cited in ATSDR 2004) noted a reduced gamma globulin concentration in pigs exposed to ammonia at 100 ppm 24 h/day for 31-45 days. It is thought that the experimental findings in laboratory animals represent the consequences of an effect secondary to tissue injury and inflammation resulting from the ammonia exposure.

Genotoxicity

ATSDR (2004) considers that the data on ammonia and ammonium ion exposures may indicate the presence of mutagenic and clastogenic properties.

A retrospective examination of fertilizer-factory workers with different occupational histories of ammonia exposure compared the frequencies of chromosomal aberrations and sister-chromatid exchanges (SCEs) and mitotic index; findings indicated increased chromosomal-aberration and SCE frequencies with "increasing length of exposure" (Yadav and Kaushik 1997, as cited in ATSDR 2004).

The frequency of mouse micronuclei increased after single intraperitoneal administration of ammonia at 12, 25, and 50 mg/kg in Swiss albino mice (Yadav and Kaushik 1997, as cited in ATSDR 2004).

Among the several cellular assays that were not lethal to the test system, positive results were observed in the following: chromosomal aberrations in chick fibroblasts exposed to buffered ammonia-ammonium chloride solutions, reduced cell division in mouse fibroblasts cultured in media with added ammonia and ammonium chloride, DNA-repair inhibition in mouse fibroblasts in media with added ammonium chloride, and decreased rate of DNA synthesis in mucosal cells of mouse ileum and colon in culture with added ammonium chloride (Rosenfeld 1932; Visek 1972; Capuco 1977; Zimber and Visek 1972, all as cited in ATSDR 2004).

Carcinogenicity

The Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) has not evaluated ammonia for evidence of human carcinogenic potential, because there are "no data," and so has made no formal determination regarding the carcinogenicity of ammonia (EPA 1991).

The International Agency for Research on Cancer (IARC) considered "aqueous ammonia" as one of several materials used for spot removal in its monograph on dry cleaning (IARC 1995). However, ammonia was not specifically examined for carcinogenic potential in the monograph, which focused on chlorinated and other industrial solvents (IARC 1995). IARC has not published an evaluation of the potential human carcinogenicity of ammonia.

ATSDR reported a case of epidermal carcinoma of the nasal septum in the survivor of a serious industrial accident in which the exposed person's upper lip and nose were accidentally splashed with a refrigeration mixture containing ammonia (Shimkin et al. 1954, as cited in ATSDR 2004). There was no evidence that the ammonia exposure was causal. ATSDR (2004) examined other cases of human inhalation exposure that followed ammonia spills but found no other case reports of carcinogenicity.

Adult male mice repeatedly exposed to ammonia vapor ("ammonia vapour of 12% ammonia solution," 15 min/day, 6 days/week, 8 weeks) exhibited "mitotic figures" and nasal carcinoma (one of 10) or nasal mucosal adenocarcinoma (one of 10) (Gaafar et al. 1992). There is no conclusive evidence that the ammonia exposures induced the observed carcinomas (ATSDR 2004).

Examination of mice exposed by gavage to ammonia dissolved in water at 42 mg of ammonium ion per kilogram per day for 4 weeks provided "no evidence of a carcinogenic effect" (Uzvolgyi and Bojan 1980, as cited in ATSDR 2004).

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

Toxicokinetics and mechanisms of action for ammonia have been thoroughly summarized in recent publications—such as NRC 2002, 2007, and ATSDR 2004—and will not be detailed here.

Metabolism and Toxicokinetics

Ammonia is a product of amino acid and dietary-protein metabolism and is found naturally in human blood and tissues. In the large intestine, bacterial degradation of urea can also form ammonia (Diamondstone 1982, as cited in NRC 1994). Synthesis of some amino acids (glutamine, asparagine, and arginine) requires the presence of ammonia (White et al. 1978, as cited in NRC 1994).

Depending on the route of exposure, ammonia can be metabolized to urea and glutamine in the liver (ingestion exposure; Visek 1972; Furst et al. 1969; Pitts 1971, as cited in NRC 2002) or metabolized to glutamine or protein in tissues (after subcutaneous or intraperitoneal exposures; Duda and Handler 1958; Furst et al. 1969, as cited in NRC 2002). Of more immediate interest to the present analysis is inhalation exposure, during which ammonia is largely retained and absorbed by tissues of the upper respiratory tract. For short-term exposure (less than 2 min), 83-92% of inhaled ammonia is retained in the nasal mucosa (Landahl and Hermann 1950), and only small amounts are absorbed in the systemic circulation. With longer and more concentrated inhalation exposure, retention in the nasal mucosa decreases until it attains equilibrium (4-30%) with the inhaled-air concentration after exposure of 10-27 min (Silverman et al. 1949). Within 3-8 min after exposure termination, ammonia in expired air decreases to pre-exposure concentrations. After inhalation exposure during an industrial accident to ammonia at concentrations sufficient to induce first- and second-degree burns, blood ammonia concentration in the exposed patient remained stable, and chest x-ray films were normal (Leduc et al. 1992); it is thought that this finding indicates either a lack of appreciable ammonia absorption from the respiratory tract or rapid detoxification of inhaled ammonia (NRC 2007).

It has been speculated that people with impaired liver function may have increased blood-ammonia concentrations after inhalation exposure (Swotinsky and Chase 1990).

Systemic ammonia is largely excreted by the kidney as urea and ammonium compounds and voided in urine. Systemic ammonia can also be excreted as urea in feces (Gay et al. 1969; Pitts 1971, both as cited in NRC 2002) and perspiration (Guyton 1981; Wands 1981, both as cited in NRC 2002). Silverman et al. (1949) reported no changes in blood or urinary ammonia, urea, or nonprotein nitrogen in seven human subjects exposed to ammonia vapor at 350-500 ppm for 30 min. Within the first 30 min, about 70-80% of inhaled ammonia was expired; Silverman and colleagues thought that this indicated ammonia satura-

tion of upper respiratory tract tissues. Depending on the subject, equilibrium was attained within 10-27 min after exposure at 350-400 ppm, when the concentration of ammonia in expired air remained stable. Silverman et al. (1949) calculated that if all retained ammonia had been absorbed into the blood, there would have been no significant change in blood or urinary urea, ammonia, or nonprotein nitrogen; this is consistent with the human experimental data.

The data of Silverman et al. (1949) also indicate that retention of ammonia in the human nasopharynx is concentration- and time-dependent.

Mechanism of Toxicity

Ammonia is a corrosive, alkaline, locally irritant gas that produces effects immediately on contact with moist mucous membranes of the eyes, mouth, and respiratory tract. It reacts with those and other moist tissues to form ammonium hydroxide in an exothermic reaction (Wong 1994); the thermal and chemical burns that result from high-concentration exposures are considered to be a consequence of the heat of reaction and of the corrosive properties of the alkaline reaction product ammonium hydroxide. Ammonia is a respiratory and ocular irritant; case reports of accidental industrial and agricultural releases of high (but unquantified) concentrations document the presence of respiratory tissue injury and necrosis and penetration of corneal epithelium with resulting corneal scarring (Leduc et al. 1992; Mulder and van der Zalm 1967; Sobonya 1977; Hatton et al. 1979. For additional cases, see ATSDR 2004; NRC 1987, 2007).

Although there is no evidence that CNS function is compromised by inhalation exposure to ammonia at about 100 ppm, persons with depressed liver function or liver failure (for example, hepatic encephalopathy or congenital or acquired hyperammonemia) accumulate excessive ammonia in the CNS (NRC 2002). Depending on concentration, low or high CNS ammonia accumulations can induce "stupor and coma" (consistent with hyperpolarization) or seizures (consistent with depolarization), respectively (NRC 2002). Ammonia intoxication in the CNS is associated with astrocyte swelling and morphologic change (for example, Alzheimer II astrocytes observed in cases of hyperammonemia) and adverse changes in astrocyte metabolism (Norenberg 1981; Albrecht 1996; Norenberg and Martinez-Hernandez 1979, as cited in NRC 2002).

The human odor threshold for ammonia is about 5-53 ppm (Pierce 1994), and sensory fatigue (adaptation) occurs with prolonged exposure. The study conducted by Ferguson et al. (1977) demonstrated adaptation to ammonia at up to 150 ppm, with excursions to 200 ppm, in people acclimated to ammonia at 25-100 ppm for 1 week. When subjects were exposed to mixed odors, the odor threshold for ammonia was 10-20 ppm (Ferguson et al. 1977). At a concentration of 30 ppm in a Rochester chamber (head-only exposures), fit and healthy male workers (who passed class II USAF or class II FAA flying physicals) described the odor as "easily noticeable, moderate intensity" (two of five subjects) or "strong, highly penetrating" (three of five); that indicated that the odor

threshold had been exceeded at 30 ppm (MacEwen et al. 1970). When groups of naive and informed subjects were exposed to ammonia for 30 min in a whole-body exposure chamber and mean results were compared, the naive group subjectively judged 50 ppm to be greater than "distinctly perceptible" but less than a "nuisance;" the judgement of informed subjects rated concentrations of 50 and 80 ppm as only "distinctly perceptible" after a 30-min exposure (Verberk 1977). After a 2-h exposure, the naive group judged 50, 80, and 110 ppm as a "nuisance" (Verberk 1977).

A more recent study of naive vs informed (workers familiar with workplace ammonia) subjects (Ihrig et al. 2006) evaluated the response of 43 male volunteers (21-47 years old) repeatedly exposed to ammonia at 10-50 ppm 4 h/day for 5 days in an exposure chamber. The median reported intensity of irritative symptoms and respiratory symptoms remained below 1 ("hardly at all") at all exposure concentrations (even at 50 ppm) for both naive and informed subjects. For olfactory symptoms, the median score of the naive group for the 20/40-ppm and 50-ppm exposure was between 3 and 4 (between "rather much" and "considerably"), whereas the median score of the informed group for the 20/40-ppm exposure was less than 1 (1 = "hardly at all") and for the 50-ppm exposure was less than 2 (2 = "somewhat") (Ihrig et al. 2006). Median annoyance rankings displayed by the naive group exceeded 4 ("rather much") at 50 ppm, whereas the median annoyance rankings by the informed group exposed at 50 ppm remained under 2. Habituation was evident in the informed group of subjects.

Although there are variable results and some debate regarding the concentrations at which respiratory and ocular irritation occurs, there is a consensus that tissue injury occurs at vapor concentrations higher than those at which ammonia can be detected by odor or irritation; thus, sensitivity to the odor of ammonia vapor imparts warning properties via odor and ocular irritation.

Susceptible Populations In tests of concentrations required to stimulate reflex glottis closure (NH_3TR) in healthy nonsmokers, Erskine et al. (1993) determined that the closure reflex of elderly people (86-95 years old) with a mean NH_3TR of 1,791ppm (SEM ,52 ppm) is less responsive to ammonia vapor than that of younger people (21-30 years old) with a mean NH_3TR of 571 ppm (SEM, 41.5 ppm). Erskine et al. (1993) point out that their earlier work (Erskine et al. 1992) indicated that "smokers have considerably more sensitive upper airway reflexes than non-smokers."

The human-subjects study of McLean et al. (1979) documented that nonatopic and atopic subjects, some of whom had a history of asthma, responded similarly on a NAR test to ammonia at 100 ppm introduced into each nostril under pressure for up to 30 sec. McLean and colleagues noted that experimental results suggest attenuation of NAR after ammonia exposure and that the attenuation is mediated primarily by parasympathetic reflex effects on the nasal vasculature, not by histamine release. Collectively, the results indicate that, at about 100 ppm, ammonia coming into contact with tracheobronchial or pulmonary regions would not be expected to induce a different effect on asthmatic people.

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

A number of organizations have established or proposed inhalation-exposure levels or guidelines for ammonia. Selected values are summarized in Table 2-6.

COMMITTEE RECOMMENDATIONS

The committee's recommendations for EEGL and CEGL values for ammonia are summarized in Table 2-7. The current and proposed U.S. Navy values are provided for comparison.

The literature contains human-exposure data derived from multiple clinical and monitoring studies that were carried out at ammonia concentrations and for exposure durations of interest and are thus suitable for direct estimation of exposure guidance levels. Over 100 adults were subjects of the clinical studies summarized in Tables 2-2 and 2-3, and 58 workers were monitored for the occupational epidemiologic study of Holness et al. (1989). Evaluated subjects were healthy people participating in a variety of activities (and thus uptake rates), including resting, working, and exercise. Groups represented were nonatopic and atopic people, including asthmatics (McLean et al. 1979; Sundblad et al. 2004); smokers and nonsmokers (MacEwen et al. 1970; Ferguson et al. 1977); and those with various thresholds for reflex glottis closure (Erskine et al. 1993). Given the breadth of data available on ammonia that include evaluation of a variety of groups involved in various activities, the committee concludes that the uncertainty surrounding the variability in susceptibility of the submarine crew to ammonia is most likely small.

1-Hour EEGL

On the basis of human data summarized in Table 2-3, the highest concentrations (102-140 ppm) in exposures of about 1 h resulted in minimal or no physiologic change in respiratory function (FVC, FEV_1, minute volume, and respiratory rate) or cardiac function (pulse rate and diastolic and systolic blood pressure) compared with control values (Verberk 1977; Ferguson et al. 1977; Cole et al. 1977). The protocol of Ferguson et al. (1977) incorporated serial daily exposures for 2 weeks. The exercise regimen incorporated into the study of Cole et al. (1977) and the workplace physical activity incorporated into the study of Ferguson et al. (1977) take into account the physical stress that may occur during an emergency situation onboard. The Ferguson et al. evaluation of potential exposure effects on a worker's ability to perform physical and mental tasks required in the course of daily duties of a chemical-plant operator is pertinent to tasks performed by submarine crew (for example, data-logging, computational tasks, and walking up and down flights of stairs).

TABLE 2-6 Selected Inhalation Exposure Levels for Ammonia from the NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	25	ACGIH 2001
	TLV-STEL	35	
NIOSH	REL-TWA	25	NIOSH 2005
	REL-STEL	35	
OSHA	PEL-TWA	50	29 CFR 1910.1000
Spacecraft			
NASA	SMAC		NRC 1994
	1-h	30	
	24-h	20	
	30-day	10	
	180-day	10	
Submarine			
NRC	EEGL		NRC 1987
	1-h	100	
	24-h	100	
	CEGL		
	90-day	50	
	SEAL 1 (10 days)	75	NRC 2002
	SEAL 2 (24 h)	125	
General Public			
ATSDR	Acute MRL	1.7	ATSDR 2004
	Chronic MRL	0.1	
NAC/NRC	AEGL-1 (1-h)	30	NRC 2007
	AEGL-2 (1-h)	160	
	AEGL-1 (8-h)	30	
	AEGL-2 (8-h)	110	

[a]The comparability of EEGLs and CEGLs with occupational-exposure and public-health standards or guidance levels is discussed in Chapter 1 ("Comparison with 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; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; 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; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

The preponderance of data from clinical studies indicates that even a multihour exposure to ammonia at about 100 ppm would result in few substantial

TABLE 2-7 Emergency and Continuous Exposure Guidance Levels for Ammonia

Exposure Level	U.S. Navy Values (ppm)		Committee Recommended Values (ppm)
	Current	Proposed	
EEGL			
1-h	100	30	100
24-h	100	20	50
CEGL			
90-day	50	10	10

Abbreviations: CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level.

physiologic effects. Among naive populations tested (Verberk 1977), exposure at 110 ppm for 1 h was judged to be at a "nuisance" level and to generate perceptible eye and throat irritation and perceptible urge to cough. Those effects are known to be reversible on cessation of exposure. Thus, a concentration of about 100 ppm is judged to be minimal and would not interfere with performance of critical tasks during an onboard emergency.

The weight of evidence from the strong human experimental data, including exercising subjects (Cole et al. 1977) and exercising smoking subjects (Ferguson et al. 1977) for the exposure duration of concern indicates a 1-h EEGL of 100 ppm for ammonia. The use of human data precludes the application of an interspecies uncertainty factor. Furthermore, that concentration does not induce significant differences in NAR when the response of atopic subjects, including asthmatics, is compared with that of nonatopic subjects in studies of direct (forced-air) ammonia-vapor contact with intranasal tissues (McLean et al. 1979). Therefore, no intraspecies uncertainty factor has been applied.

24-Hour EEGL

Pertinent, multihour, human exposure studies include those of Ferguson et al. (1977), Sundblad et al. (2004), Verberk (1977), and Ihrig et al. (2006). For ammonia exposure 2-6 h/day, 5 days/week for 2 weeks at 100 ppm, Ferguson et al. (1977) observed no eye, nose, or throat irritation significantly different from controls and no other effects except for FEV_1 improvement. Ferguson et al. (1977) observed that repeated exposure at 100 ppm as above, with excursion to 200 ppm, was "easily tolerated" by the human subjects. Sundblad et al. (2004) observed no detectable inflammation in upper airways (on the basis of multiple physiologic measurements) and no increased bronchial responsiveness to methacholine challenge in healthy and atopic subjects exposed to ammonia continuously for 3 h at 0, 5, or 25 ppm with exposures separated by an interval of at least a week; each subject exercised at 50 W for half of each exposure period. Sundblad et al. (2004) noted a dose-response relationship for multiple and transient symptoms of irritation and systemic response as reported on subject ques-

tionnaires. Verberk (1977) exposed informed and naive subjects to ammonia for 2 h at 110 or 140 ppm. At 110 ppm, informed and naive subjects reported marginally nuisance eye irritation and perceptible (informed) or nuisance (naive) odor; at 140 ppm, naive subjects withdrew from the exposure chamber before the passage of 2 h because of "offensive" concentrations, but no informed subjects withdrew (the informed group reported "perceptible" and "nuisance" odor and eye irritation at 140 ppm for 2 h) (see Table 2-3).

Ihrig et al. (2006) exposed naive and informed male volunteers (the latter "regularly exposed to ammonia in the workplace") to ammonia at 10-50 ppm 4 h/day for 5 days. Habituation was noted during the course of the study, and experienced subjects reported fewer symptoms than naive subjects. Medical examinations were conducted for tear-flow rates, lung function, bronchial responsiveness, cognitive function, and related end points after each exposure. Except for three subjects in the 50-ppm group who exhibited "slight conjunctival hyperemia," no relevant physical or neurophysiologic effects (such as reaction time, attention, and power of concentration) were observed (see Table 2-3).

The short-term (10-min) exposure studies of fit, male, military or military-contractor personnel (MacEwen et al. 1970) also provide valuable background regarding irritancy.

Pertinent animal studies were evaluated for valuable insight and to augment the human database. Animal studies include repeat-exposure and subchronic-toxicity estimates in rats, mice, rabbits, guinea pigs, and rabbits (Coon et al. 1970; Tepper et al. 1985; Manninen et al. 1988; Buckley et al. 1984; and Zissu 1995) (see Table 2-5). Preference is given to consideration of the rat and mouse data because these species are obligate nose-breathers; mice are considered unusually sensitive to the toxic effects of exposure to such respiratory irritants as ammonia (Ten Berge et al. 1986).

Coon et al. (1970) reported no deaths or clinical signs after exposure of rats at 222 ppm 8 h/day for 6 weeks and no deaths or attributable clinical signs at 1,101 ppm with the same exposure regimen. Tepper et al. (1985) observed transient changes in running-wheel activity in rats with exposure durations greater than 1 h at ammonia concentrations of 100 ppm and for all exposure durations at 300 ppm; mice undergoing the same protocol exhibited a similar but smaller activity-profile change; Tepper and colleagues attributed the changes to sensory irritation. The related mouse studies of Buckley et al. (1984) and Zissu (1995) examined the effects of ammonia exposure at various fractions or multiples of the RD_{50} (Swiss-Webster mouse RD_{50} of 303 ppm, Buckley et al.; Swiss OF1 mouse RD_{50} of 257 ppm, Zissu); subjects exhibited no clinical signs. At the RD_{50}, histopathologic examination identified minimal exfoliation and erosion, and moderate metaplasia and inflammation were exhibited in the nasal cavity epithelium. Rats continuously exposed at 182 ppm for 90 days exhibited no clinical signs and had normal hematologic, organ, and tissue values not different from control values for any measure examined (Coon et al. 1970; see Table 2-4).

The weight of evidence exhibited by the experimental data—which include those on exercising human subjects and smokers (Ferguson et al. 1977;

MacEwen et al. 1970; Sundblad et al. 2004), naive and informed human subjects (Verberk 1977; Ihrig et al. 2006), and mice and rats[1] exercising for multiple hours (Tepper et al. 1985; ten Berge et al. 1986)—provides the basis for estimating the 24-h EEGL for ammonia. It is important to note that close examination of data from MacEwen et al. (1970) and Verberk (1977) indicates that the human irritancy response (for example, eye and throat irritation) tends to "flatten" after exposure at 50 ppm for 30 min to 1 h even among naive subjects ("nuisance" concentrations of 50 ppm; Verberk 1977). The same naive subjects ranked exposure at 80-100 ppm as "offensive." Such irritancy effects are fully reversible on cessation of ammonia exposure. It is further noted that the recent and carefully collected human-exposure data of Ihrig et al. (2006) indicate threshold physiologic effects at 50 ppm (4 h/day for 5 days) by documenting transient conjunctival irritation (three subjects) and olfactory irritation rankings considered moderate ("somewhat" for experienced subjects and "rather much" for naive subjects); habituation was evident (Ihrig et al. 2006). Therefore, the human data led the committee to consider 50 ppm as a protective level of ammonia exposure for 24 h during emergency situations, given the present insufficiency of data for assessing human accommodation to 80-100 ppm for 24 h of continuous exposure.

The use of human data precludes application of an interspecies uncertainty factor. Furthermore, it is known that exposure at 100 ppm does not induce significant differences in NAR when the response of atopic subjects, including asthmatics, is compared with that of nonatopic subjects in studies of direct (forced-air) ammonia-vapor contact with intranasal tissues (McLean et al. 1979). Therefore, no intraspecies uncertainty factor has been applied. The committee's recommended 24-h EEGL is 50 ppm, which the committee judges sufficient to prevent a level of irritancy that could interfere with crew alertness and efficient work performance during an emergency.

90-Day CEGL

There are no reliable human experimental data on exposure durations greater than about 6 weeks (Ferguson et al. 1977; see Table 2-3). The committee considered subchronic experimental data on a susceptible laboratory species (rat) in which there are no documented clinical signs after continuous exposure at 57 ppm for 114 days or at 182 ppm for 90 days (Coon et al. 1970); the study's continuous-exposure protocol included "downtime" for animal feeding and chamber servicing equal to less than 2.2% of the experimental exposure duration. After continuous exposure at 375 ppm for 90 days, Coon et al. (1970) reported "mild" nasal discharge as the only noteworthy sign in rats (see Table 2-5). The committee did not use the latter finding regarding nasal tissue effects,

[1]Both rodents are obligate nose breathers, and mice are considered sensitive to ammonia.

because Coon and colleagues did not perform histopathologic evaluations of the nasal cavity to confirm the presence or absence of irritation response. More contemporary studies have shown that 5-week continuous exposure of weanling pigs at 37 ppm in combinations with dust at concentrations up to 9.9 mg/m^3 was associated with no significant changes in turbinate or lung tissue when compared with controls (Done et al. 2005). Furthermore, daily clinical monitoring for respiratory, gastrointestinal, and ocular signs demonstrated that the experimental exposures had no significant effect. Swine are increasingly considered a reasonable surrogate for human physiologic and tissue responses; thus, the study of Done et al. (2005) adds particular insight to human-exposure considerations.

It is reported that rodents exposed repeatedly to ammonia vapor at 711 ppm over a period of days develop lesions in the nasal respiratory epithelium (Zissu 1995).

Human data indicate that exposure to ammonia concentrations at up to 140 ppm over a period of hours or days is unlikely to cause irreversible systemic effects. Nevertheless, it appears that exposure at over about 110 ppm would be expected to generate eye, nose, throat, and chest irritation in naive or untrained human populations exposed for 90 days (Verberk 1977), even when sensory fatigue is accounted for. Although not yet experimentally characterized in long-term human studies, available dose-response data indicate that systemic toxicity at that concentration is not expected to be clinically significant (NRC 1987).

It is known that "most, if not all, individuals who are regularly exposed to ammonia develop a tolerance to its irritant effects" (Ferguson et al. 1977). Ferguson et al. (1977) evaluated skilled and experienced repair workers at a chemical manufacturing facility who underwent workplace exposure in areas where ammonia concentrations of 25 and 50 ppm were achieved; controlled 100-ppm exposure took place in an exposure chamber. Exposure periods ranged from 2 to 6 h/day for 5 weeks. No adverse effects on respiratory function and no increase in frequency of eye, nose, and throat irritation were noted by participants and examining physicians. After acclimation, up to 6 h of continuous exposure at at least 100 ppm (average, 103-140 ppm, with occasional excursions to 200 ppm) was "easily tolerated" (Ferguson et al. 1977). In the years before the Ferguson et al. study, facility workers did not voluntarily don respiratory protection until workplace ammonia reached 400-500 ppm. Persons who are naive with respect to ammonia do not exhibit such tolerance.

The human-exposure study of Ihrig et al. (2006) also compared the subjective and physiologic responses of naive vs experienced subjects (the latter commonly experienced workplace exposures to ammonia) to successive concentrations of 0, 10, 20, 20/40, or 50 ppm 4 h/day over 5 days. At all concentrations, the experienced subjects reported fewer symptoms than naive subjects. At 10 ppm, the median ranking of olfactory symptoms by naive subjects lay between the qualitative score of 1 ("hardly at all") and 2 ("somewhat"), whereas the median ranking by naive subjects at 20 ppm lay between 2 and 3 ("rather much"). The median ranking of olfactory symptoms by experienced subjects was less than 1 at 10, 20, and 20/40 ppm (Ihrig et al. 2006). Habituation was evident.

On the basis of response to questionnaires, the subjects of the Sundblad et al. (2004) study exposed at 25 ppm for 3 h (serial exposures) did not appear to exhibit sensory fatigue to "solvent smell"; sensory fatigue to odor was noted in subjects exposed at 5 ppm. Subjects exposed at 25 ppm ranked perceived discomfort for all 10 possible questionnaire symptoms significantly higher than during the sham exposure or when exposed at 5 ppm; however, no subjects are reported to have terminated the 25-ppm exposure prematurely. Nevertheless, the committee considers the Sundblad et al. (2004) study to be flawed in that it lacked control for odor perception and is thus confounded by the potential for irritancy as a consequence of generic odor perception rather than any sensory-irritancy response peculiar to ammonia. An ideal study protocol would have masked the odor of ammonia or used subjects who had no sense of smell. Reported irritation effects and breathing difficulties for the 5-ppm exposure group are recognized as small, odor-related, and generic.

The data of MacEwen et al. (1970) indicate that ammonia at 30 ppm was associated with "just perceptible" nasal and ocular effects in two of five naive volunteers exposed for 10 min. The data of Sundblad et al. (2004) indicate that ammonia at 25 ppm (3-h exposure) is associated with transient irritation of eyes, nose, and upper airways but no "detectable upper-airway inflammation or increased bronchial responsiveness to methacholine." When compared with sham exposures, ammonia at 25 ppm was also associated with increased reports of sensations of nausea, headache, and sensation of intoxication in some subjects. The symptomatology is consistent with an odor response, and the committee considers 25 ppm to be an odor-irritancy threshold in healthy, exercising populations of an appropriate age and thus comparable with the submarine-crew population of concern.

It is acknowledged that rigorous measurements of sensory fatigue have not been collected for continuous exposure approaching 90 days, so some degree of speculation is appropriate.

The human-subjects data of Sundblad et al. (2004), MacEwen et al. (1970), and Ihrig et al. (2006) are convergent in demonstrating irritancy in young adults in response to ammonia at about 20-30 ppm. The committee selects that range as the minimal LOAEL for irritancy due to odor and incorporates a factor of 3 to accommodate adjustment of the minimal LOAEL to a no-observed-adverse-effect level (NOAEL) for odor perception. The resulting estimate of 6.7-10 ppm is rounded to 10 ppm.

To minimize potential complaints regarding discomfort, annoyance, or ocular irritation among submariners confined for multiple weeks in ammonia atmospheres, the committee recommends a 90-day CEGL of 10 ppm. That CEGL should prevent potential degradation in submarine-crew performance resulting from sustained exposure to intense odor and nuisance concentrations and is below the human experimental concentrations associated with "moderate" irritation considered as an adverse effect. The Sundblad et al. (2004) data indicate that sensory fatigue for ammonia odor perception is likely to occur at some (undefined) ammonia concentration greater than 5 ppm but less than 25 ppm and

are indicative of the protective nature of a 10-ppm CEGL. Furthermore, the committee's recommended CEGL of 10 ppm is supported by the results of Sundblad et al. (2004), Verberk (1977), and Ihrig et al. (2006) indicating relatively static effects over time at 20-50 ppm; the results of Coon et al. (1970) documenting no signs or clinically significant effects in nonhuman primates continuously exposed for 90 days at 57 ppm; and the results of Done et al. (2005) showing no signs or clinically significant effects in weanling pigs continuously exposed at 37 ppm for 5 weeks.

As for the previous 1-h and 24-h EEGL estimates, there is little justification for application of an intraspecies uncertainty adjustment to accommodate asthmatics exposed to ammonia. Given the weight of evidence from workplace and clinical exposure studies, an ammonia concentration of 10 ppm as the CEGL is protective for submarine crews.

DATA ADEQUACY AND RESEARCH NEEDS

Quantitative exposure data are available on humans—including asthmatics, smokers, elderly people, and children—and laboratory animals, including such susceptible species as mice and rats. Most human studies suitable for quantitative assessment used short-term exposure (up to 2 h; one study incorporated exposure of 4 h and 6 h), which necessitate assumptions regarding the concentration-dependent nature of the toxic response to ammonia. Controlled human-exposure studies for extended exposure (especially 24-h continuous and multi-day exposure) are lacking in the database available for study. In addition, controlled experimental studies of humans are restricted to small numbers of subjects and exhibit incomplete protocols. Greater and more objective quantification of such subjective end points as irritation and nuisance is needed; however, evaluations using appropriate psychophysical methods also need to assess cognitive and emotional factors that affect subjective responses (Dalton 2002). Finally, there are few contemporary studies of long-term ammonia exposure of laboratory animals; the 90-day studies available for assessment were published in the early 1970s. Although they are sufficient for the current evaluation, corroborating evidence based on modern analytic and vapor-generation techniques would have been highly useful for application to the 90-day assessment.

The results of Verberk (1977; Table 2-2) and Ihrig et al. (2006) indicate that mere knowledge of and exposure experience with the irritant and odor properties of ammonia vapor can effectively reduce human avoidance behavior and increase tolerance to concentrations as great as 140 ppm for exposure as long as 2 h. That finding has operational significance for naval submarine command and warrants further serious consideration as a training opportunity for submarine crews. The committee echoes the previous recommendation of the Committee on Submarine Escape Action Levels regarding application of Verberk's (1977) findings to submarine-crew training curricula (NRC 2002) and recommends inclusion of the more recent Ihrig et al. (2006) human-exposure data.

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3

Benzene

This chapter summarizes the relevant epidemiologic and toxicologic studies of benzene. 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 committee considered all that information in its evaluation of the U.S. Navy's current and proposed 1-h, 24-h, and 90-day exposure guidance levels for benzene. The committee's recommendations for benzene exposure levels are provided at the end of this chapter with a discussion of the adequacy of the data for defining the levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

Benzene is a colorless flammable liquid at room temperature (Budavari et al. 1989). It has a distinctive odor with an odor threshold of 4.68 ppm (HSDB 2005). Selected physical and chemical properties are listed in Table 3-1.

OCCURRENCE AND USE

Benzene is widely used as a feedstock in the synthesis of many industrial intermediates—such as styrene, cyclohexane, aniline, and alkylbenzenes—that are used to produce plastics, resins, dyes, detergents, pharmaceuticals, and pesticides. It has limited use as a solvent because of its associated adverse health effects.

Benzene is a common contaminant of outdoor and indoor air. Sources of benzene exposure include tobacco smoke, gasoline vapors, exhaust from motor vehicles, and industrial emissions. In 1998, the average benzene concentration in

TABLE 3-1 Physical and Chemical Properties of Benzene

Synonyms	Cyclohexatriene, phenyl hydride
CAS registry number	71-43-2
Molecular formula	C_6H_6
Molecular weight	78.11
Boiling point	80.1°C
Melting point	5.5°C
Flash point	−11°C (closed cup)
Explosive limits	NA
Specific gravity	0.8787 at 15°C/4°C
Vapor pressure	94.8 mm Hg at 25°C
Solubility	Miscible with alcohol, chloroform, ether, carbon disulfide, carbon tetrachloride, glacial acetic acid, acetone, oils
Conversion factors	1 ppm = 3.19 mg/m^3; 1 mg/m^3 = 0.31 ppm

Abbreviations: mm Hg, millimeters of mercury; NA, not available or not applicable.
Sources: Budavari et al. 1989; HSDB 2005.

outdoor air in metropolitan areas was estimated to be 0.58 ppb (NCI 2003). However, overall average personal exposure has been estimated to be 4.7 ppb, probably because of passive exposure to cigarette smoke (Wallace 1989). Data have shown that homes with smokers have higher benzene concentrations (median, 3.3 ppb) than homes with no smokers (median, 2.2 ppb) (Wallace 1989). Air from a smoke-filled bar contained benzene at up to 11.3 ppb (Brunnemann et al. 1989). The mainstream-smoke benzene emission factor ranges from 5.9 to 73 µg per cigarette, and the sidestream-smoke factor ranges from 345 to 653 µg per cigarette (ATSDR 2005).

Sources of benzene on submarines include petroleum-derived fuels and lubricants, high-temperature paints, and smoking (Crawl 2003). A few measurements of benzene on submarines have been reported. Holdren et al. (1995) reported the results of air sampling at three locations over 6 h during the missions of two submarines. Depending on the sample-collection method, concentration ranges were 5.3-6.3 ppb and 5.5-6.5 ppb on one submarine and 5.8-10 ppb and 16-34 ppb on the other. Raymer et al. (1994) did not detect benzene in a similar sampling exercise on two other submarines. The committee notes that the results presented by Holdren et al. and Raymer et al. represent one-time sampling events on four submarines. Whether the reported concentrations are representative of the submarine fleet is not known, particularly because few details were provided about the conditions on the submarines when the samples were taken.

SUMMARY OF TOXICITY

Benzene is one of the most well-studied chemicals used today. It has been the subject of several comprehensive reviews and risk assessments (IARC 1982, 1987; NRC 1996; EPA 1998, 2002; IOM 2003; ATSDR 2005). Those reviews were used to guide the committee's evaluation. In addition, the committee obtained and reviewed the key primary literature that formed the basis of the earlier reviews and scientific literature published since those assessments were performed.

The earlier reviews concluded that benzene is associated with effects on the hematologic, immune, and nervous systems. Evidence of those effects is found in the occupational literature (Srbova et al. 1950; Yin et al. 1987a; Kraut et al. 1988; Rothman et al. 1996; Lan et al. 2004) and reports of controlled animal experiments (Gill et al. 1980; Rozen et al. 1984; Cronkite et al. 1985, 1989; Rosenthal and Snyder 1985; Molnar et al. 1986).

There is also agreement in previous reviews that benzene is a human carcinogen. The predominant cancer caused by benzene is acute myelogenous leukemia. Support for that conclusion comes from epidemiologic studies of workers exposed to benzene in rubber hydrochloride manufacturing plants (Rinsky et al. 1981, 1987) and in factories in China (Hayes et al. 1996, 1997; Yin et al. 1987b, 1989, 1996) and from inhalation studies of rodents that show that benzene causes cancer in multiple tissues, with strong evidence of lymphoid tumors in mice (Maltoni et al. 1989; Cronkite et al. 1984, 1985, 1989; Snyder et al. 1980, 1984, 1988; Farris et al. 1993).

Effects in Humans

Accidental Exposures

Deaths from acute inhalation of benzene in closed spaces, such as storage tanks, have been reported since the early 1900s (summarized in ATSDR 2005). Deaths occurred within hours or days and were attributed to asphyxiation, respiratory arrest, and central nervous system (CNS) depression or to pulmonary edema, hemorrhage, and cardiac collapse. At concentrations of 300-3,000 ppm, benzene causes drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. At lower exposures (estimated at over 60 ppm for 3 weeks), clinical evaluation has revealed symptoms of mucous membrane irritation and dyspnea (Midzenski et al. 1992).

Experimental Studies

A few studies of intentional exposure to benzene in humans were located in the older literature. In a kinetic and metabolic study, 23 students and laboratory assistants inhaled benzene at 47-110 ppm for 2-3 h (Srbova et al. 1950).

Benzene was measured in blood, urine, and exhaled breath. The investigators concluded that the durations of study were inadequate to achieve equilibrium between blood and air but that uptake was much more rapid (70-80%) in the first 5 minutes (min) of exposure than after 1 h (about 50%). The authors reported that "the volunteers had no subjective troubles." No further information is available to define the presence or absence of clinically evident responses.

Hunter and Blair (1972) exposed male volunteer laboratory staff to benzene at 19-125 ppm in inhalation chambers for up to 6-8 h to study metabolism and excretion, including phenol measurements. The lack of mention of health complaints or effects suggests that the exposures were tolerated with minimal symptomatic effects.

Occupational and Epidemiologic Studies

Prolonged exposure to benzene is well known to result in bone marrow hypoplasia, which leads to leukopenia, lymphopenia, anemia, or thrombocytopenia. Continued exposure can progress to pancytopenia and aplastic anemia. An increased risk of leukemia is well established (see ATSDR 2005). This section reviews some of the key occupational and epidemiologic studies on benzene.

Kellerova (1985) studied a group of workers chronically exposed to benzene at 45-155 ppm with peak exposures of 310 ppm. Compared with unexposed controls, there was a significantly higher percentage of abnormal and borderline electroencephalographic findings.

Inoue et al. (1986, 1988) conducted metabolic and biomonitoring studies of Chinese shoe-factory and printing workers chronically exposed to benzene. The geometric mean exposures in the 1986 study ranged from 1 to 76 ppm. The 1988 study, which involved only men, reported arithmetic mean exposures of 32 ± 25 ppm (time-weighted average [TWA]). That neither study described any adverse health symptoms or signs among the workers again suggests that symptomatic effects of benzene were minimal at most.

Neurotoxic effects were observed in sewage-treatment workers employed for a median of 5 years at a plant where benzene and toluene had been measured at up to 300 ppm and greater than 200 ppm, respectively. CNS symptoms included lightheadedness, fatigue, increased sleep requirement, and headache. A greater frequency of impairment on neurobehavioral tests was found with increased years of work in the plant, although the analyses were not adjusted for possible confounding factors. CNS symptoms resolved with a reduction in exposure, but neurobehavioral performance was not retested (Kraut et al. 1988).

Yin et al. (1987a) reported on the prevalence of subjective symptoms among male shoe and printing workers exposed chronically (average, 4.5 years) to benzene at an average concentration of 33 ppm (TWA). In a subgroup exposed at 1-40 ppm, dizziness and headache were significantly increased over those in controls and sore throat, dizziness, and headache were increased among

those exposed at 41-210 ppm. Although mean leukocyte counts in men exposed to benzene were similar to those in the control group, the prevalence of leukopenia was significantly greater in the benzene-exposed group. Lymphocyte counts were reduced in men exposed to a combination of benzene and toluene. The paper did not present exposure subgroup analyses of the hematologic data.

A cross-sectional study of hematologic measures—mean corpuscular volume, absolute lymphocyte count, white blood cell (WBC) count, red blood cell (RBC) count, hematocrit, and platelet count—was performed in 44 workers exposed to benzene at a median concentration of 31 ppm (TWA). When analyzed according to a median split, all six hematologic measures were reduced, but in the lowest-exposure group (median, 7.6 ppm, the TWA) only lymphocyte count was decreased; lymphopenia seemed to be the most sensitive end point in this study (Rothman et al. 1996).

Qu et al. (2002, 2003) evaluated the relationship between peripheral blood cell counts and personal benzene exposure in 130 Chinese workers and 51 controls in various industries. The median of the individual 4-week average benzene exposures was 3.8 ppm (range, 0.08-54.5). RBC, WBC, and neutrophil counts were significantly decreased with increased exposure; however, the values remained within the normal ranges. The authors reported results for a low-exposure group, but the supporting data were not presented, and the committee could not independently evaluate the authors' conclusions.

Lan et al. (2004) reported on hematotoxicity in 250 benzene-exposed Chinese shoe workers compared with 140 unexposed age- and sex-matched controls. Exposure characterization was based on repeated full-shift individual monitoring for up to 16 months before sampling. Total counts of WBCs, granulocytes, lymphocytes, B cells, and platelets were significantly lower in the exposed groups. In the 109 workers exposed to benzene at less than 1 ppm (arithmetic mean, 0.57 ppm), there was a significant reduction, but the dose-response data on those exposed at less than 1 ppm and those exposed at 1 to less than 10 ppm were relatively flat for WBC, granulocytes, and absolute lymphocyte counts. Consistent with the effect on multiple cell lines, progenitor cell colony formation (measured as colony-forming unit-granulocyte-macrophage [CFU-GM], burst-forming unit-erythroid [BFU-E], and colony-forming unit-granulocyte, erythroid, macrophage, megakaryocte [CFU-GEMM]) was significantly lower (all indexes) in the exposed groups (one exposed at less than 10 ppm and the other exposed at 10 ppm or more) than the control group. A greater proportional effect was observed in colony formation than in depression of mature cells. Genetic variants in two key metabolizing enzymes, myeloperoxidase and NAD(P)H quinone oxidoreductase, significantly influenced susceptibility to decreases in WBC counts. Lower WBC counts were observed in subjects with both genotypes (4,900 ± 1,240 cells/μL) than in subjects with either genotype (5,480 ± 1,120 cells/μL) or neither genotype (5,980 ± 1,420 cells/μL); this strongly suggests a gene-environment interaction. The authors state that dermal exposures did not contribute substantially to total benzene exposure, but supporting data for this statement are incomplete (Vermeulen et al. 2004). In a fol-

low-up analysis, Lan et al. (2005) reported that WBC counts were significantly decreased after control for multiple comparisons among workers with single nucleotide polymorphisms (SNPs) in five of 20 candidate genes; one SNP was associated with an increase in WBC counts. Those results also suggest genetic variability in the individual response to benzene exposure.

Shen et al. (2006) performed a follow-up study of the cohort used by Lan and co-workers (2004). Their study population included the same 250 workers who were exposed to benzene in two shoe-manufacturing factories and 140 unexposed controls in comparable populations who worked in three Chinese clothing-manufacturing factories. Controls were frequency-matched by sex and age to exposed workers. Benzene exposure characterization was based on repeated full-shift individual monitoring for up to 16 months before sampling. The exposed group had a mean (± SD) benzene air exposure in the month before phlebotomy of 5.4 ± 12.1 ppm. Total counts of WBCs, granulocytes, lymphocytes, B cells, and platelets were significantly lower in the exposed group. Shen et al. (2006) also examined the role of 24 SNPs in seven genes that survey the genome and participate in DNA double-strand break repair in peripheral WBC counts. They found that four SNPs in WRN (Ex4 –16 G > A, Ex6 +9 C > T, Ex20 –88 G > T, and Ex26 –12 T > G), one SNP in TP53 (Ex4 +119 C > G), and one SNP in BRCA2 (Ex11 +1487 A > G) were associated with decreased WBCs in benzene-exposed workers.

Hematologic surveillance of U.S. industrial workers exposed to benzene has found no increase in hematologic abnormalities (in WBCs, RBCs, or platelets) associated with chronic benzene exposure at less than 1.5 ppm (Tsai et al.1983, 2004; Collins et al. 1991, 1997).

Effects in Animals

Acute Toxicity

A 1940 review article examined the acute neurotoxicity of benzene in cats (von Oettingen 1940). Exposure was tolerated at 3,000 ppm for 30-60 min, was toxic at 7,500 ppm after 30-60 min, and was fatal at 20,000 ppm after 5-10 min.

Acute inhalation studies of benzene in rodents document lethality within minutes at 10,000-20,000 ppm (summarized in ATSDR 2005). Subchronic and chronic studies in animals consistently document bone marrow depression or immunotoxicity and largely confirm the human occupational experience. Mice exposed to benzene at 4,680 ppm for 8 h experienced a decrease in bone marrow colony-forming cells (Uyeki et al. 1977).

Statistically significant leukocytopenia was seen in mice treated with benzene at 100 ppm for 24 h. The authors concluded that it was a peripheral effect rather than a stem-cell effect, which developed with more prolonged exposure (Gill et al. 1980). In fact, animals that received unexposed transplanted stem cells and were later exposed to benzene at 100 ppm for 24 h showed no reduc-

tion in stem-cell survival, although by day 8 at this exposure stem-cell survival, measured as spleen colony formation, was zero. The study also demonstrated that intermittent exposure at up to 4,000 ppm (6 h/day, 5 days/week) could be tolerated much longer than continuous exposure at the same concentration.

Molnar et al. (1986) examined the neurotoxic effects of benzene in rats. They reported on a minimal narcotic concentration of 5,940 ppm for a 4-h exposure. Increased locomotor activity was observed in rats exposed to benzene at 2,000 ppm for 3-4 h but not in rats exposed for only 1-2 h.

Repeated Exposure and Subchronic Toxicity

Green et al. (1981) exposed CD-1 mice by inhalation to benzene at eight concentrations ranging from 1.1 to 4,862 ppm 6 h/day for 5 days. They reported a no-observed-adverse-effect level (NOAEL) of 10 ppm for reduction in WBCs, polymorphonuclear leukocytes, lymphocytes, and RBCs, whether examined in marrow or spleen, and a lowest observed-adverse-effect level (LOAEL) of 103 ppm.

Continuous exposure of mice to benzene at 21 ppm for 4-10 days led to significant decreases in nucleated cells and colony-forming granulopoietic stem cells in bone marrow; the effects of exposure for 8 h/day, 5 days/week for 2 weeks were similar (Toft et al. 1982).

Dempster et al. (1984) evaluated dose-effect and time-effect relationships between benzene exposure and hematologic and behavioral effects to determine whether such effects follow Haber's law (concentration [C] × exposure time [t] = response [k]) for extrapolating between short-term and long-term toxicity. Mice were exposed to benzene for 6 h/day until a minimal CT product of 3,000 ppm was achieved (3,000 ppm for 1 day, 1,000 for 3 days, 300 for 10 days, or 100 for 30 days). A CT relationship was observed for a reduction in lymphocyte counts, the most sensitive overall measure. Effects on RBCs varied. Counts declined significantly after exposure to benzene at 300 ppm for 3 days and at 100 ppm for 10 days but not at 1,000 ppm for 3 days or after 3,000 ppm for 1 day. The most sensitive behavioral index was increased milk-licking, which was observed in the 100-ppm group as early as the first exposure. Similarly, hindlimb grip strength was reduced significantly in the first session at 1,000 ppm but not at 300 ppm. A CT relationship was not observed for the neurobehavioral effects. Recovery from all changes was complete 60-70 days after cessation of exposure.

Sun et al. (1992) extended the Dempster et al. study to lower concentrations. They exposed Kunming mice to benzene at 0.78, 3.13, or 12.52 ppm 2 h/day, 6 days/week for 30 days. They reported increased forelimb grip strength at the two lowest concentrations but decreased strength at the highest concentration. At the highest concentration, brain, but not blood, acetylcholinesterase activity was significantly reduced (by about 10%). There were also decreases in the percentage of bone marrow precursors of RBCs in mice exposed with benzene at 3.13 ppm and in RBC and WBC precursors at 12.52 ppm.

Rozen et al. (1984) examined hematologic toxicity in mice after inhalation of benzene at 10-300 ppm 6 h/day for 6 days. At 10 ppm, there was decreased mitogen-induced blastogenesis of both B and T lymphocytes. The investigators also observed decreased femoral lipopolysaccharide-induced B-colony-forming ability but no change in the numbers of B lymphocytes. At 30 ppm and greater, depressed phytohemagglutinin-induced splenic blastogenesis was observed but no decrease in T lymphocytes. More significant changes were seen at 300 ppm.

Rosenthal and Snyder (1985) evaluated the effects of benzene on immune resistance to *Listeria monocytogenes* in mice. Mice were exposed to benzene at 10, 30, 100, and 300 ppm 6 h/day either 5 days before challenge or 5 days before and 7 days during challenge. The pre-exposure regimen produced evidence of infection only at 300 ppm, whereas exposures before and during challenge increased bacterial counts in mice exposed at 30 ppm and greater. B and T lymphocytes were depressed at all concentrations except 10 ppm.

Exposure of mice to benzene at 10 ppm 6 h/day, 5 days/week for 2 weeks produced no peripheral or bone marrow hematologic effects (Cronkite et al. 1989). A concentration of 25 ppm produced lymphopenia. At 100-400 ppm, there was a dose-related decrease in blood lymphocytes, bone marrow cellularity, and marrow content of colony-forming unit-spleen (CFU-S). Severe lymphopenia and decreased marrow CFU-S were observed in mice exposed at 300 ppm for 2-16 weeks, and recovery was complete within 2-4 weeks (Cronkite et al. 1985, 1989).

Ward et al. (1985) examined inhalation toxicity in CD-1 mice and Sprague-Dawley rats exposed to benzene at 1, 10, 30, and 300 ppm 6 h/day, 5 days/week for 13 weeks. Depression in all three peripheral blood lines (pancytopenia) was seen in mice exposed to benzene at 300 ppm, whereas similarly exposed rats showed decreased lymphocytes and increased neutrophil percentages. Histopathologic changes were seen in benzene-exposed mice in the thymus, bone marrow, lymph nodes, and spleen. The only lesion found in rats was a decrease in femoral marrow cellularity at 300 ppm.

Chronic Toxicity

Snyder et al. (1978, 1980, 1982, 1988) performed a series of lifetime exposure studies with rats and mice to evaluate the toxic effects of benzene. AKR/J mice developed severe lymphocytopenia, bone marrow hypoplasia, granulocytosis, and reticulocytosis from exposure to benzene at 300 ppm (6 h/day, 5 days/week). In similarly exposed Sprague-Dawley rats, lymphocytopenia and mild anemia were observed (Snyder et al 1978). At 100 ppm, AKR/J mice exhibited significant lymphycotopenia, reduced RBC counts, increased neutrophils, and bone marrow hypoplasia (Snyder et al. 1980). Studies of C57BL/6 mice (Snyder et al. 1980) and CD-1 mice (Snyder et al. 1982) exposed to benzene at 300 ppm 6 h/day, 5 days/week also reported lymphocytopenia, anemia, increased neutrophils, and bone marrow hyperplasia and hypoplasia. A

significant increase in hematopoietic neoplasms was observed in the C57BL/6 mice but not in CD-1 mice. C57BL/6 and CD-1 mice treated with a lifetime regimen of benzene at 300 ppm for 1 week followed by no exposure for 2 weeks exhibited lymphocytopenia, anemia, and significantly increased tumor incidences (Snyder et al. 1988).

Maltoni et al. (1982a,b, 1989) conducted a series of oral and inhalation carcinogenicity bioassays that demonstrated that benzene causes a variety of neoplasia in rodents. In the inhalation studies, Sprague-Dawley rats were exposed to benzene at 200-300 ppm 4-7 h/day, 5 days/week for a lifetime. There was an increased incidence of malignant tumors and carcinomas of the Zymbal glands and oral cavity and marginal increases in hepatomas and carcinomas of the nasal cavity and mammary glands.

Reproductive Toxicity in Males

Data on the effects of benzene on male reproductive end points are sparse. A single study of effects of paternal exposure to benzene on reproductive outcome (miscarriages) was negative (Strucker et al. 1994).

Ward et al. (1985) exposed groups of mice and rats to benzene at 1, 10, 30, and 300 ppm 6 h/day, 5 days/week for 13 weeks. Male mice exposed at 300 ppm exhibited testicular lesions, including minimal to moderately severe testicular atrophy or degeneration (seven of 40 mice), moderate to severe decreases in spermatozoa in the epididymal ducts (six of 40 mice), and a minimal to moderate increase in abnormal sperm forms (nine of 40 mice). The investigators reported that similar lesions of doubtful biologic significance were seen at lower concentrations.

Spano et al. (1989) evaluated the cytotoxic effects of benzene on mouse germ cells. Testicular monocellular suspensions were obtained from mice orally exposed to benzene at 1, 2, 4, 6, and 7 mL/kg, and flow cytometric analyses were performed periodically for up to 70 days after treatment. No effect on testicular weight occurred, but the relative percentages of some cell subpopulations (tetraploid and haploid cells) were different from those in control samples and indicated some cytotoxic damage of differentiating spermatogonia.

Immunotoxicity

There is substantial clinical and experimental evidence that benzene adversely affects human immune function; much of it was reviewed above in the occupational-epidemiology sections (see descriptions of Yin et al. 1987a; Lan et al. 2004) and with the hematology data in the animal section (see descriptions of Rozen et al. 1984; Cronkite et al. 1985, 1989; Rosenthal and Snyder 1985). Other studies that evaluated the immunotoxic effects of benzene are discussed below.

Depressed lymphocytes, increased susceptibility to infection, and altered immunoglobulin levels have been shown among painters exposed to benzene at 3.4-48 ppm (Marcus 1987; Lange et al. 1973).

Rozen and Snyder (1985) exposed mice to benzene at 300 ppm 6 h/day, 5 days/week for 23 weeks. They observed reduced mitogen-induced proliferation of bone marrow and splenic B and T lymphocytes and reduced counts of bone marrow and splenic B lymphocytes and thymic and splenic T lymphocytes.

Rosenthal and Snyder (1987) performed lymphocyte functional assays and tumor-challenge experiments in mice. Animals were exposed to benzene at 10, 30, and 300 ppm 5 days/week for 20 days in the functional assays and for 100 days in the tumor-challenge experiments. A delay in peak response of splenocytes to mitomycin-treated stimulator cells was observed at concentrations as low as 10 ppm. At 100 ppm, there was decreased tumor resistance in mice inoculated with viable PYB6 tumor cells. Tumor-lytic abilities of cytotoxic T lymphocytes were also decreased.

Genotoxicity

Benzene is not mutagenic in most in vitro test systems, and in vitro tests for chromosomal abnormalities have been mixed. However, in vivo animal and human studies have been positive for chromosomal aberrations and micronuclei and more mixed for sister-chromatid exchange (see reviews by Smith 1996; Whysner et al. 2004; ATSDR 2005).

Carcinogenicity

Many studies have examined the carcinogenic potential of benzene in animals and humans (for example, more than 40 epidemiologic studies). The evidence has been evaluated by a number of organizations and committees in setting regulatory standards and guidelines (IARC 1987; Paustenbach et al. 1993; EPA 1998, 2003; ACGIH 2004; WHO 2004; ATSDR 2005). The reviews agree that benzene is a known human carcinogen primarily on the basis that repeated exposure to relatively high doses in occupational settings is associated with acute nonlymphocytic leukemia. Linkage to other types of hematologic tumors—such as acute lymphocytic leukemia, non-Hodgkins lymphoma, multiple myeloma, and chronic lymphocytic leukemia—is still debated. Three large cohort studies of leukemia have been reported on.

A cohort of workers exposed to benzene in the manufacture of Pliofilm from rubber hydrochloride has been studied by the National Institute for Occupational Safety and Health (NIOSH) (Rinsky et al. 1981, 1987). The cohort consisted of over 1,000 workers employed at a facility in Ohio from the early 1940s to the 1970s. The standardized mortality ratio (SMR) for leukemia was 3.37 (95% confidence interval [CI], 1.54-6.41) for employees who were ever exposed. SMRs for cumulative doses were 1.09 (95% CI, 0.12-3.94) for 0-40 ppm-

years of exposure, 3.22 (95% CI, 0.36-11.65) for 40-200 ppm-years, 11.86 (95% CI, 1.33-42.85) for 200-400 ppm-years, and 66.37 (95% CI, 13.34-193.9) for more than 400 ppm-years (Rinsky et al. 1987). There is general agreement on mortality in the cohort but substantial uncertainty about the various exposure-estimating schemes used to derive each worker's cumulative exposure over the 40 years of operation, with particular uncertainty (due to sparse actual measurements) during the first 10 years of operation (1940-1950) (Kipen et al. 1989; Paustenbach et al. 1992). For risk-assessment purposes, the U.S. Environmental Protection Agency (EPA) has endorsed a modification of the NIOSH exposure measurements (summarized in Crump 1994).

Yin et al. (1996) reported on 74,828 Chinese men and women employed in 1972-1987 at 672 factories where benzene was used. They were followed for an average of 12 years. The relative risk of death from leukemia was 2.1 (95% CI, 1.0-5.3) in men and 2.8 in women (95% CI, 0.8-17.6). Overall significant increases in risk were found for the incidence of lymphohematopoietic malignancies, malignant lymphoma, all leukemia, acute myelogenous leukemia, myelodysplastic syndrome, and aplastic anemia. There was no adjustment for smoking or accounting for other industrial exposures.

A third study examined 17,525 Australian refinery workers and found a significant overall increase in total leukemia; more important, it included a nested case-control study that examined dose-response effects (Glass et al. 2003). Workers with over 8 ppm-years of cumulative exposure (13 cases) had a leukemia odds ratio of 11.3 (95% CI, 2.85-45.1). Duration of employment was not associated with leukemia risk. Recent updates of the cancer incidence in the cohort found no excess of all leukemias (Gun et al. 2004) or acute nonlymphocytic leukemia (Gun et al. 2006). However, it was noted that acute nonlymphocytic leukemia was found among workers classified as having medium or high exposure to hydrocarbons.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

About 30-50% of an inhaled dose of benzene is absorbed by the lungs over a number of hours; the absorption rate may approach 80% at the onset of exposure (Srbova et al. 1950). It is rapidly distributed to the tissues and accumulates in fatty tissues because it is lipophilic. About 30-50% is eliminated by the lungs, less than 1% is eliminated by the kidneys, and the remainder undergoes metabolic transformation (Srbova et al. 1950).

The major site of metabolism is the liver, but metabolism also occurs in the bone marrow, initially by mixed function oxidases. CYP2E1 catalyzes the initial step in benzene metabolism by oxidatively producing benzene oxide, which then rearranges to phenol and can be converted to hydroquinone by CYP2E1. Myeloperoxidase can convert phenol and hydroquinone to various toxic quinones or free radicals, which are thought to be myelotoxic and to con-

tribute to leukemogenicity. An important minor metabolite (in myelotoxicity and carcinogenesis) might be *trans,trans*-muconic acid.

Sabourin et al. (1989) exposed rats and mice for 6 h at a time to benzene at 5, 50, and 600 ppm, examining a CT constant of 300 ppm-h. Toxic metabolites (hydroquinone glucuronide and muconic acid) were greater at the low concentrations. Detoxification products (phenylglucuoronide and prephenyl mercapturic acid) increased at higher concentrations. Sabourin et al. (1987) noted that mice are significantly more metabolically active than rats and are a better model for human benzene toxicity than rats.

Dempster et al. (1984) explored the CT interaction of benzene inhalation in mice with concentrations of 100-3,000 ppm for 1-30 days for a constant of 3,000 ppm-days. Lymphocytes showed a constant decrease with all four exposure regimens. RBC declines, milk-licking, and grip strength failed to show a constant relationship (RBCs required long exposure, grip strength showed a threshold, and milk-licking showed an inverted U function). All hematologic and behavioral changes were reported to be completely reversible after cessation of exposure.

Kimura et al. (1971) examined variation in the oral LD_{50} of rats at three ages: 14 days, young adult, and older adult. LD_{50}s were 3.4, 9.8, and 5.6 mL/kg, respectively, indicating reduced toxicity in nonelderly adults vs older adults and younger animals.

A number of physiologically based pharmacokinetic (PBPK) models have been developed to simulate the disposition of benzene after inhalation exposure of animals (see, for example, Bois et al. 1991; Medinsky et al. 1989a,b,c; Sun et al. 1990; Cole et al. 2001) and humans (see, for example, Bois et al. 1996; Brown et al. 1998; Travis et al. 1990). Descriptions of the models are provided by the Agency for Toxic Substances and Disease Registry (ATSDR 2005). Most recently, a human PBPK model for benzene was developed by Yokley et al. (2006). It used Bayesian statistical methods to assess the variability that would arise from known or suspected variability in metabolic pathways involved in benzene metabolism. Pathways examined included those shown by Li and Yin (2006) to influence the hematotoxicity of benzene in humans.

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

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

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

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	0.5	ACGIH 2004
	TLV-STEL	2.5	
NIOSH	REL-TWA	0.1	NIOSH 2005
	REL-STEL	1	
OSHA	PEL-TWA	1	29 CFR 1910.1028
	PEL-STEL	5	
Spacecraft			
NASA	SMAC		NRC 1996
	1-h	10	
	24-h	3	
	30-day	0.1	
	180-day	0.07	
Submarine			
NRC	EEGL		NRC 1986
	1-h	50	
	24-h	2	
General Public			
ATSDR	Acute MRL	0.009	ATSDR 2005
	Intermediate MRL	0.006	
NAC/NRC	AEGL-1 (1-h)	52	EPA 2006
	AEGL-2 (1-h)	800	
	AEGL-1 (8-h)	9	
	AEGL-2 (8-h)	200	

[a]The comparability of EEGLs and CEGLs with occupational-exposure and public-health standards or guidance levels is discussed in Chapter 1 ("Comparison with 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; EEGL, emergency exposure guidance level; 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; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

COMMITTEE RECOMMENDATIONS

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

TABLE 3-3 Emergency and Continuous Exposure Guidance Levels for Benzene

Exposure Level	U.S. Navy Values (ppm)		Committee Recommended Values (ppm)
	Current	Proposed	
EEGL			
1-h	50	10	40
24-h	2	3	3
CEGL			
90-day	1	0.1	0.2

Abbreviations: CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level.

1-Hour EEGL

Immediate effects of benzene, examined most robustly for the CNS and the immune or hematologic system, are best ascribed to its direct effects, including those on peripheral blood cells, rather than to the metabolite effects that predominate after more prolonged exposure. The 1-h EEGL should be based on the acute, reversible symptoms of CNS depression rather than on immunotoxicity because it is intended to prevent conditions that would keep submariners from performing emergency responses. The study of Srbova et al. (1950) reported a NOAEL of 110 ppm for exposure of 2-3 h in humans. Although that study has limitations—such as reporting the absence of adverse effects in a single statement without data on individuals and passive collection of data on health end points without a questionnaire—it is a controlled human-exposure experiment. A number of other human studies, experimental and occupational, provide support that benzene at concentrations of around 100 ppm do not produce incapacitating symptoms. Examples include the study of laboratory staff exposed to benzene at 19-125 ppm for 6-8 h (Hunter and Blair 1972), studies of factory workers exposed chronically to benzene at up to 76 ppm (TWA) (Inoue et al. 1986, 1988), and reports of increased dizziness and headache after chronic exposure to benzene at up to 40 ppm and increased mucous membrane irritation at up to 210 ppm (Yin et al. 1987a). All the studies suggest no significant CNS impairment with benzene at up to at least 100 ppm, although they were not specifically designed to evaluate CNS effects. Supporting the human data is a study by Molnar et al. (1986), which examined neurotoxicity of benzene in rats. A minimal narcotic concentration of 5,940 ppm was reported for a 4-h exposure. Increased activity was the most sensitive end point for which they observed a NOAEL of 2,000 ppm. Thus, with a NOAEL of 110 ppm based on human data and a database uncertainty factor of 3, the 1-h EEGL is 37 ppm, which the committee rounded to 40 ppm. The uncertainty factor used for benzene recognizes that the human studies used to develop the exposure guideline predominantly evaluated clinical signs and symptoms and were not designed to examine more subtle neurobehavioral responses known to be important for structurally

related solvents, such as toluene. In recognition of this research need, the committee felt that a database uncertainty factor of 3 was appropriate. At concentrations that did not produce unconsciousness, there is no documentation in animals or humans of persistent sequelae of exposure of 1 h or less.

24-Hour EEGL

CNS and hematologic or immunologic effects associated with benzene were considered in setting the 24-h EEGL. Although human studies have documented benzene's ability to cause CNS depression and hematologic or immunologic effects over hours or days, there are no quantitative human exposure data on which to base a 24-h EEGL for either end point confidently. There are, however, reasonable data from animal studies that can be used because mice and humans are relatively similar in hematologic and immunologic effects of benzene. Numerous studies document the potential of benzene to reduce peripheral blood-cell counts over days to years of exposure. Gill et al. (1980) found a LOAEL of 100 ppm for reduction in circulating WBCs after a 24-h continuous exposure of mice, which was attributed to peripheral effects rather than metabolite-mediated marrow suppression. Reversibility itself was not documented for those effects, but the same study's report of no effect on bone marrow stem cells with the same 24-h exposure regimen suggests no impairment of bone marrow recovery capacity. However, the degree of immunosuppression that was shown to result from leukopenia indicates that concern about clinically meaningful, but temporary, immunosuppressive effects cannot be dismissed. The committee used those end points as the basis for a 24-h EEGL. An uncertainty factor of 3 was selected to account for the use of a LOAEL instead of a NOAEL because reversible peripheral WBC suppression is of limited toxicity. Other uncertainty factors applied were an interspecies uncertainty factor of 3 and an intraspecies factor of 3. The interspecies uncertainty factor reflects potential interspecies differences in pharmacodynamics rather than pharmacokinetics, because the end point is not driven by benzene metabolites, so pharmacokinetic variability is of little concern. The intraspecies uncertainty factor reflects the potential genetic susceptibilities reported by Lan et al. (2004, 2005) and Shen et al. (2006). Although the resulting calculation is 3.3 ppm (100/30), the committee recommends setting the 24-h EEGL at 3 ppm. That value will help to protect against acute CNS effects (see discussion of 1-h EEGL) and protect the key immunologic and myeloid target tissues. Other agencies have based their findings on the Dempster et al. (1984) study, but its exposure regimen of 6 h/day for multiple days was judged to be suboptimal for a submarine standard.

90-Day CEGL

The studies by Lan et al. (2004) and Shen et al. (2006) were deemed appropriate and were considered by the committee for derivation of the 90-day

CEGL value. Lan et al. (2004) showed statistically significant hematologic changes in multiple measures in the Chinese workers exposed to benzene for an average of 6 years at a mean concentration of 0.57 ppm. That report, however, has generated some debate, suggesting that the evidence of benzene-induced hematotoxicity is lacking for benzene exposures at less than 1 ppm. Criticisms raised by Lamm and Grünwald (2006) include the lack of a clear dose-response relationship for exposures at less than 1 ppm, the questionable toxicologic significance of the effects observed, and the fact that cell lines involved are distinct from those associated with myeloid leukemias. Moreover, the analysis by Lan et al. (2004) considers a much longer timeframe than what is typically relevant for a subchronic-exposure guideline. Shen et al. (2006) reported an association between total counts of WBCs, granulocytes, lymphocytes, B cells, and platelets and the cohort's benzene exposure that occurred in the preceding month (mean, about 5 ppm). The exposure assessment was considered relevant for derivation of the 90-day CEGL value. The value reported by Shen et al. (2006) is supported by the earlier Lan et al. study showing hematotoxicity following benzene exposure to at least 10 ppm. Therefore, the committee used 5 ppm as the point of departure for the 90-day CEGL.

The mean value reported by Shen et al. (2006) was used as a LOAEL for hematologic effects relevant for submariners. Because multiple cell lineages were affected, which provides some evidence of a bone marrow response, an uncertainty factor of 10 was applied for use of a LOAEL instead of a NOAEL. Interindividual variability in this cohort has been shown to be influenced by benzene metabolism (Lan et al. 2004) and repair genes (Shen et al. 2006), so an uncertainty factor for intraspecies variability of 3 was also applied. A full-scale uncertainty factor of 10 was not used, because the variability observed in the large cohort was minimal (about a 3-fold difference or less), and the cohort most likely included individuals with combinations of susceptible polymorphisms. Moreover, even the most susceptible individuals were not at markedly increased risk for a clinically adverse outcome over the long period under observation (Lan et al. 2004). Thus, the 90-day CEGL to prevent peripheral blood count depression is 0.2 ppm. This is supported by the NOAEL of 30 ppm in mice and rats for chronic hematologic toxicity (Ward et al. 1985). Cancer (leukemia) is ultimately the concern raised by these blood count depressions, and the carcinogenicity risk assessment below adds support to the CEGL derived by the committee.

CARCINOGENICITY ASSESSMENT

The committee relied on EPA's most recent cancer risk assessment (EPA 2003) to obtain a quantitative estimate of benzene carcinogenicity. EPA used leukemia mortality data from the study by Rinsky et al. (1981, 1987) and extrapolated with a low-dose linearity model using maximum likelihood estimates (Crump 1994). The estimated air concentrations associated with a lifetime (70-

year) risk of one in 10,000 (the U.S. Navy's acceptable cancer risk) range from 0.004 to 0.014 ppm. Adjusting for a career submariner's underwater exposure of about 5 years yields a range of 0.06-0.20 ppm. Uncertainty about inadequate exposure data and individual susceptibility is already accounted for in EPA's risk estimate, which is based on maximum likelihood estimates from a low-dose linear model.

DATA ADEQUACY AND RESEARCH NEEDS

The Lan et al. (2004) and Shen et al. (2006) studies go a long way toward providing a human dataset that can be used to explore low-level human exposures and chronic clinical outcomes. The human database on acute exposure to benzene is weak; however, it is unlikely to be improved, given the hazards of substantial benzene exposure. Mechanistic information on the metabolites that produce specific effects (bone marrow depression vs leukemia) might allow more specific standard-setting, but at present the data are considered adequate. The database on reproductive outcomes is sparse and would benefit from new bioassays.

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4

2,6-Di-tert-butyl-4-nitrophenol

This chapter summarizes the relevant epidemiologic and toxicologic studies on 2,6-di-tert-butyl-4-nitrophenol (DBNP). Selected chemical and physical properties and toxicokinetic and mechanistic data are also presented. Because of the lack of available data, the committee was unable to recommend exposure guidance levels for DBNP. A discussion of the research needed to allow derivation of exposure guidance levels is provided at the end of this chapter.

PHYSICAL AND CHEMICAL PROPERTIES

DBNP is a yellow crystalline powder that is relatively soluble in organic solvents but insoluble in water (Alexander et al. 2001). Selected chemical and physical properties are listed in Table 4-1.

OCCURRENCE AND USE

A yellow substance was observed in the U.S. submarine fleet, especially in newer boats, in builders' yards and during sea trials (MacMahon et al. 1999). Investigations during 1992-1993 by the U.S. Navy and the Electric Boat Division of General Dynamics Inc. determined that the yellow substance was DBNP, which is derived from an antioxidant, 2,6,-di-tert-butylphenol (DBP), in TEP 2190 steam-turbine lubricating oil (a synthetic turbine oil MILSPEC-L-17331). Analysis of samples of TEP 2190 lubricating oil found that it contained DBP at less than 10,000 ppm (Alexander et al. 2001). Release of small amounts of lubricating oil during operation of the steam turbine allows DBP to come into contact with electrostatic precipitators in air-handling systems that can nitrate DBP and thereby generate DBNP (Alexander et al. 2001). In 1993, the specification for TEP 2190 lubricating oil was changed by the Naval Sea Systems Command

TABLE 4-1 Physical and Chemical Properties of 2,6-Di-tert-butyl-4-nitrophenol

Synonyms	2,6-Di-*t*-butyl-4-nitrophenol; dibutyl-4-nitrophenol; di-tert-butyl-4-nitrophenol; di-tertiary-butyl-4-nitrophenol; dibutyl-*p*-nitrophenol; dibutyl-*p*-nitrophenol; di-*t*-butyl-*p*-nitrophenol
CAS registry number	728-40-5
Molecular formula	$C_{14}H_{21}NO_3$
Molecular weight	251
Boiling point	NA
Melting point	157.0ºC
Flash point	NA
Explosive limits	NA
Specific gravity	NA
Vapor pressure	NA
Solubility	NA
Conversion factors	1 ppm = 10.27 mg/m^3; 1 mg/m^3 = 0.09741 ppm

Abbreviations: NA, not available or not applicable.
Source: Data on molecular weight and melting point from Alexander et al. 2001.

to limit the amount of DBP in TEP 2190 oil to no more than 10 ppm (Still et al. 2005). However, the remaining stockpile of DBP-containing TEP 2190 lubricating oil in inventory is being used to supply the fleet for a number of years (MacMahon et al. 1999). Still et al. (2005) indicate that exposure to DBNP may also occur in many submarines because of the mixing of "old" and "new" TEP 2190 oil in submarine turbine systems and storage tanks. Exposures to DBNP are otherwise limited. DBNP was proposed as a miticide for treatment of resistant mite infections in mammals (Vesselinovitch et al. 1961), but there is no indication that it was ever used for this purpose.

SUMMARY OF TOXICITY

Toxicity information on DBNP is limited to animal studies in as much as no case reports, experimental-exposure studies, or epidemiologic studies resulting from human exposure to it are available. The toxicologic database on DBNP primarily includes acute-exposure studies and a few repeated-dose studies. No adequate data from inhalation-toxicity, subchronic toxicity, mutagenicity, carcinogenicity, or reproductive-toxicity studies are available.

After single or repeated oral or intraperitoneal (ip) doses, rats show clinical signs (prostration, rapid breathing, hyperthermia, and rapid induction of rigor mortis after death) that are consistent with inhibition of mitochondrial oxidative metabolism (Alexander et al. 2001; Carpenter et al. 1997). A single oral dose of 40 mg/kg in dimethyl sulfoxide (DMSO) and canola oil is sufficient to induce

hyperthermia and death in male rats (Still et al. 2005). However, the toxicity of DBNP appears to be significantly affected by the solvent used as the vehicle in toxicity studies (Vesselinovitch et al. 1961; Alexander et al. 2001; Still et al. 2005). Animals that survive acute exposure to DBNP are generally reported to appear clinically normal 24 h after exposure, although decreased weight gain may persist after recovery from near-lethal exposure (Alexander et al. 2001). Histopathologic changes, typically cellular degeneration, have been reported in smooth muscle, skeletal muscle, cardiac muscle, liver, kidneys, and spleen after exposure to DBNP by gavage or ip injection (Vesselinovitch et al. 1961; MacMahon et al. 1999; Alexander et al. 2001). Repeated ip doses have been reported to cause enlargement of the liver and changes in cellular enzyme levels (Carpenter et al. 1997). Skin contact with DBNP does not result in visible signs of dermal irritation or injury even at high doses, and absorption of DBNP through the skin is minimal (Vesselinovitch et al. 1961; Alexander et al. 2001; Inman et al. 2003; Pershing et al. 2006). However, when DBNP at 2 g/kg was held in contact with the skin for 24 h, two of five rabbits showed changes in weight gain but no other clinical signs of toxicity (Alexander et al. 2001). After gavage, DBNP is poorly absorbed and slowly excreted in the urine as a glucuronide conjugate (Holder et al. 1971; Carpenter et al. 1997). Enterohepatic recirculation may play a role in the low clearance rate of DBNP and its glucuronide metabolite (Holder et al. 1971; Carpenter et al. 1997). The slow elimination of DBNP is reported to result in evidence of cumulative toxicity after repeated oral and ip dosing (Holder et al. 1971; Vesselinovitch et al. 1961). In vitro studies with isolated mitochondria have identified deficits in stage 3 and stage 4 oxidative metabolism after exposure to DBNP (Carpenter et al. 1997). The in vitro findings are consistent with the clinical signs associated with DBNP exposure and suggest that DBNP is an inhibitor of mitochondrial respiration. The toxicity profile of DBNP is similar to that of 2,4-dinitrophenol (2,4-DNP), a well-recognized inhibitor of mitochondrial respiration (ATSDR 1995).

Effects in Humans

Accidental Exposures

No information on accidental exposures was identified.

Experimental Studies

No information on experimental studies was identified.

Occupational and Epidemiologic Studies

In 1992-1993, as noted above, U.S. Navy submarine crews noticed a yellowing of bulkheads and other structures during underwater periods and sea tri-

als. The yellowing was determined to be due to the presence of DBNP (Alexander et al. 2001). Crew members also reported that their skin turned yellow when they came into contact with contaminated surfaces. Deposition of the yellow material was visible on structural elements of the submarine interior and most pronounced in the engineering compartment; Alexander et al. (2001) indicated that DBNP was most likely present on clothing, bedding, eating utensils, and other surfaces in submarines because DBNP was distributed throughout the submarine by the ventilation system. Analysis of data collected by a Navy contractor indicated that airborne DBNP concentrations in submarines using the "old" TEP oil were less than 3.0 to 13 ppb 24-h/day for 90-day operation periods (Alexander et al. 2001). Laboratory simulation of the submarine operational environment using the "old" TEP oil reported DBNP up to 122 ppb (Alexander et al. 2001). No data have been reported on whether DBNP exposure affects the health of exposed crew members. The highest DBNP surface concentration documented in submarines using the "old" TEP oil is 0.2 $\mu g/cm^2$ (J. McDougal, personal communication cited in Inman et al. 2003).

Although there are no reports of human toxicity associated with DBNP exposure, DBNP has toxicologic similarities in animal studies to 2,4-DNP, a dinitrophenol on which there are human data. A 1919 study and a 1946 study reported respiratory difficulty and deaths after inhalation exposure to 2,4-DNP in the occupational environment (Perkins 1919; Gisclard and Woodward 1946, both as cited in ATSDR 1995). However, no quantitative exposure data were associated with those incidents, and there was also dermal, and possibly oral, exposure to 2,4-DNP in the working environment where the cases occurred (ATSDR 1995). There are no other reports of human toxicity associated with inhalation of 2,4-DNP (ATSDR 1995). The primary database on human effects associated with 2,4-DNP stems from the oral administration of 2,4-DNP as a weight-loss medication in the 1930s. Ingestion of 2,4-DNP has been associated with agranulocytosis, cataracts, peripheral neuropathy, and serious dermatologic effects in humans (ATSDR 1995). On the basis of those human exposures, the U.S. Environmental Protection Agency (EPA) established an oral reference dose of 0.002 mg/kg per day for 2,4-DNP by the application of a safety factor of 1,000 to a lowest observed-adverse-effect level (LOAEL) of 2 mg/kg per day, a dose that caused cataracts in humans (EPA 2005). Humans appear to be more susceptible to the development of cataracts than other mammals exposed to 2,4-DNP; among other animals cataracts have been induced only in birds after 2,4-DNP administration (ATSDR 1995). No inhalation reference concentration has been calculated for inhalation exposure to 2,4-DNP because of the lack of adequate data (EPA 2005).

Effects in Animals

The oral toxicity of DBNP appears to be significantly affected by the type of vehicle used to create the solutions administered. DBNP is not soluble in wa-

ter but is soluble in common organic solvents (Vesselinovitch et al. 1961). As described below, the toxicity of DBNP in aqueous vehicles is less than that reported in studies conducted with organic solvents. The purity of the DBNP used in toxicity studies may be another confounding factor in interpreting the data from them. The studies reported by Vesselinovitch et al. (1961) did not identify the source, method of manufacture, or purity of the DBNP they used. The studies undertaken by Carpenter et al. (1997), MacMahon et al. (1999), Alexander et al. (2001), Inman et al. (2003), and Still et al. (2005) have used material made with a method described by Rivera-Nevares et al. (1995), and the purity of the DBNP used in these studies is reported to be 97-99.5%, the primary impurity being DBP at concentrations of 1-3%.

Acute Toxicity

The oral LD_{50} of DBNP in male and female Sprague-Dawley rats was reported to be 500 and 450 mg/kg, respectively (Vesselinovitch et al. 1961) when it was given by gavage in a 0.2% aqueous carboxymethylcellulose solution. At lethal doses, rats showed general depression of activity starting 3 h after dosing; deaths occurred 4 h to 3 days after dosing. The oral LD_{50} value in male guinea pigs was reported to be 800 mg/kg (Vesselinovitch et al. 1961).

MacMahon et al. (1998, as cited in MacMahon et al. 1999) reported that the oral LD_{50} of DBNP given to male F-344 rats in a corn-oil vehicle was 82 mg/kg, and the no-observed-adverse-effect level (NOAEL) was less than 50 mg/kg. Effects were hyperthermia (as determined by measurement in the ear canal) and mild histopathologic degenerative changes in multiple organs (skeletal-, cardiac-, and smooth-muscle degeneration and minor hepatic- and renal-cell degeneration).

In a study of Sprague-Dawley rats given DBNP in corn oil, the oral LD_{50} was 93 mg/kg (MacMahon et al. 1999). Survivors at each dose were all five at 0 mg/kg, all five at 62.5 mg/kg, four of five at 78 mg/kg, two of five at 98 mg/kg, none of five at 250 mg/kg. Clinical abnormalities at all doses included prostration, hyperthermia, and labored respiration. Survivors recovered clinically (normothermic) within 24 h after dosing, although rats dosed at 98 mg/kg initially lost body weight and grew at a lower rate than animals in the other groups. Histopathologic changes in animals that died after exposure at 250 mg/kg included minimal degeneration of skeletal-, cardiac- and smooth-muscle fibers with occasional contraction bands and minimal degenerative changes in individual hepatocytes and renal tubule epithelial cells. The NOAEL was less than 62.5 mg/kg.

The difference in LD_{50} values between the study of Vesselinovitch et al. (1961) and the studies of MacMahon et al. (1998, as cited in MacMahon et al. 1999) and MacMahon et al. (1999) were hypothesized to be due to differences in rat strain and the vehicle used to administer the test substances (Alexander et al. 2001).

Alexander et al. (2001) conducted a series of exposures to address the differences observed in the Vesselinovitch et al. and MacMahon et al. studies. Those studies involved groups of five male or female F-344 rats given single oral doses of DBNP in a corn-oil vehicle (500, 275, 100, 50, or 0 mg/kg in males and 450, 275, 100, 50, or 0 mg/kg in females) and male Sprague-Dawley rats given single oral doses of DBNP in corn oil (250, 98, 78, 62.5, or 0 mg/kg) or in a 2% aqueous carboxymethylcellulose solution (DBNP at 98 mg/kg). The maximal observation period for all exposure groups was 14 days. Mortality was 100% in groups given doses of at least 250 mg/kg. Mortality was 20-80% in groups given 78-100 mg/kg. Mortality was zero in groups given 50 or 62.5 mg/kg. Maximal time to death was 4 h, 41 min for F-344 rats and 22 h, 25 min for Sprague-Dawley rats; death occurred sooner at the higher doses. Mortality was 60% in Sprague-Dawley rats given DBNP at 98 mg/kg in corn oil and 20% in rats given DBNP at 98 mg/kg in carboxymethylcellulose. Prostration, rapid breathing, increased body temperature, and muscle rigor were observed in all DBNP-exposed groups whether the animals died or survived. At 500 mg/kg, male F-344 rats convulsed before death. On histologic examination, mild, multifocal myofiber degeneration was observed in skeletal and cardiac muscle from F-344 rats given DBNP at 100-500 mg/kg. "Less significant changes" were observed in "muscle and other cell types" from F-344 rats given 50 mg/kg. Among Sprague-Dawley rats given DBNP at 275 mg/kg in corn oil, hepatic and pulmonary congestion were common findings. Histologic examination of these Sprague-Dawley animals showed degenerative and necrotic changes in individual hepatocytes and renal tubule epithelial cells. Weight loss was greater in DBNP-dosed animals than in controls. Although control animals returned to normal weight gain after gavage dosing, the DBNP animals did not return to normal weight gain during the 14-day observation period. The oral LD_{50}s calculated separately in the male F-344 rats and Sprague-Dawley rats were 80 mg/kg. The oral LD_{50} in the female F-344 rats was 50-100 mg/kg, or close to 80 mg/kg.

Still et al. (2005) reported giving male Sprague-Dawley rats single oral doses of DBNP at 15 or 40 mg/kg, which had been prepared by first dissolving it in DMSO and then adding it to canola oil. A small amount of ^{14}C-labeled DBNP was included in the mixture. Six of 16 of the rats dosed at 40 mg/kg died within 24 h of dosing. Necropsy examination of animals that died after DBNP dosing revealed edema or congestion of the thoracic cavity and lung hemorrhages. Animals that survived 40 mg/kg showed prostration, no auditory-startle response, reduced locomotor activity, and muscular rigidity for up to 8 days after dosing. After dosing at 15 mg/kg, rats exhibited lethargy and reduced startle response during the first 24-48 h. They were indistinguishable from controls 7-8 days after dosing.

The ip LD_{50}s in guinea pigs and mice were reported to be 580 mg/kg in males and 700 and 850 mg/kg for males and females, respectively (Vesselinovitch et al. 1961). The ip LD_{50}s in male and female Sprague-Dawley rats were reported to be 270 and 260 mg/kg, respectively (Vesselinovitch et al. 1961). Vesselinovitch et al (1961) also reported that rats given single lethal ip doses of

DBNP (300, 400, or 600 mg/kg) had histologic lesions in the heart (patchy, waxy degeneration of muscle fibers), liver (mild to moderate fatty change), spleen (lymphorrhexis, abortive mitosis, and other cellular changes), and kidneys (zonal necrosis of renal tubule epithelium). In rats given 130 mg/kg ip, renal lesions were accompanied by increased blood urea nitrogen, decreased urinary concentrating capacity, decreased urinary calcium, and decreased blood pressure (Vesselinovitch et al. 1961).

Intravenous (iv) administration of DBNP to rodents (species unstated) at 3.3 mg/kg in DMSO caused a marked increase in body temperature and death within 1 h, followed by the rapid onset of rigor mortis (Rivera-Nevares et al. 1995). The authors of the iv study considered the signs observed after DBNP administration to be consistent with depletion of ATP stores due to disruption of mitochondrial function.

Application of 1,000 mg/kg to the shaved backs of rats resulted in no systemic toxicity and no evidence of dermal irritation (Vesselinovitch et al. 1961). Five New Zealand white rabbits had DBNP applied directly to their shaved backs at 2,000 mg/kg; a control group was available for comparison (Alexander et al. 2001). The DBNP was held in place with a bandage for 24 h, and the animals were observed for abnormalities for 14 days. The rabbits showed no adverse clinical signs resulting from the exposure. On necropsy, no treatment-related lesions were reported, and histologic analysis of the DBNP-contact skin was not affected by contact with DBNP. Two of five DBNP rabbits showed statistically significant changes in weight gain compared with the control group. DBNP was below the limit of detection in "the blood and the organs" of exposed rabbits.

Repeated Exposure and Subchronic Toxicity

Vesselinovitch et al. (1961) fed groups of six male and six female Sprague-Dawley rats diets containing 0, 0.05%, 0.1%, 0.2%, or 0.4% DBNP for up to 16 weeks. Deaths were zero, zero, zero, six, and 12, respectively. Feed consumption and body weight were reduced in the surviving animals in the 0.2% group, but not in animals at the lower doses.

Vesselinovitch et al. (1961) reported studies in which groups of five female Sprague-Dawley rats were dosed ip with DBNP at 0, 10, 25, 50, or 100 mg/kg in 0.2% carboxymethylcellulose daily for up to 60 days. Deaths were zero, zero, two, five, and five, respectively and occurred on days 5-30 in the 25- and 50-mg/kg groups and on days 0-5 in the 100-mg/kg group. At 10 mg/kg, animals grew normally. At 25 mg/kg, the animals maintained their starting body weight but did not gain weight.

Carpenter et al. (1997) dosed groups of four male F-344 rats ip with DBNP at 0 or 10 mg/kg in DMSO for 10 days. Rats were monitored for body-weight gain, water consumption, urinary and fecal output, and behavior. Growth rate and urinary production were reported to be unaffected by DBNP. Presuma-

bly, water consumption and behavior were unaffected as well, although they were not mentioned.

Repeated ip injections of DBNP at 25 mg/kg for at least 58 days resulted in no significant inhibition of body-weight gain, but increased liver weight, increased the liver-to-body weight ratio, decreased the concentration of hepatic fatty acid binding protein (54% when measured as mg per gram liver or 17% when measured as mg per 100 g body weight), increased the concentration of bile acid sulfotransferase (BST) (71% when measured as mg per 100 g body weight, but no increase when measured as mg per g liver), and increased the concentration of dopamine sulfotransferase (31% when measured as mg per g liver or 28% when measured as mg per 100 g body weight) (Carpenter et al. 1997). Chromatographic analysis of hepatic BST revealed most of the BST in the DBNP rats was BST I, a typical pattern for female rats, whereas control rats showed the male profile for BST, which is three isofunctional BSTs. The change in BST suggests that DBNP exposure resulted in an altered expression of this endocrine-modulated enzyme.

Chronic Toxicity

No chronic-toxicity information was identified.

Reproductive Toxicity in Males

No reproductive-toxicity information was identified. However, the EPA Endocrine Disruptor Research Initiative includes a project to evaluate DBNP for endocrine effects, including testicular function (Still 2006). Still et al. (2005) found no ^{14}C-DBNP or ^{14}C-labeled metabolites in the testes of male rats after oral dosing with DBNP at 15 or 40 mg/kg. Still et al. (2005) suggested that the blood-testis barrier may prevent exposure of the testes to DBNP and its metabolites at the doses tested.

Immunotoxicity

No information on immunotoxicity was identified.

Genotoxicity

No genotoxicity studies of DBNP were identified. In general, activation of aromatic nitro-containing compounds requires metabolism to an aromatic hydroxylamine intermediate and eventual formation of highly reactive intermediates, such as the nitrenium ion, that can interact with DNA (Takahashi et al. 1978). Intestinal reduction by gut microflora is involved in forming reactive intermediates from some nitro-containing compounds. Neither of the metabolic

studies reported to date identified formation of DNA-reactive intermediates from DBNP (Holder et al. 1971; Carpenter et al. 1997).

Carcinogenicity

No documentation of any U.S. federal or federal advisory group review of DBNP for carcinogenicity was identified. The above discussion on genotoxicity is pertinent to consideration of DBNP for carcinogenicity.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

Holder et al. (1971) found that 28.1% of a 20-mg oral dose of ^{14}C-DBNP (randomly labeled in tert-butyl groups and given in carboxymethylcellulose) was excreted unchanged in a methanol extract of feces collected from Wistar rats (body weight 250-390 g) over a 5-day period. Maximal excretion was observed 48 h after dosing and ceased by 72 h. A dose of 1.0 mg ^{14}C-DBNP orally resulted in excretion of about 33% and 20% of the radiolabel in the urine and feces, respectively, after 5 days. When rats were pretreated with neomycin to reduce microflora in the gut, excretion rates were altered to 23% and 34% in the urine and feces, respectively. Bile contained only small amounts of radiolabel (1.4%) whether or not rats received neomycin. After ip administration, about 60% of a dose (0.192 mg or 1.0 mg) of ^{14}C-DBNP was eliminated in the urine; and biliary excretion amounted to about 30% of the dose after ip (0.192 mg) or iv (0.096 mg) administration in an aqueous ethanol solution. Overall, the data indicate that DBNP is slowly absorbed from the gastrointestinal tract with a large amount of it unchanged in the feces after oral dosing. Absorption through the gut after oral administration was about 50% of the dose. Metabolism of DBNP by the gut microflora appears to aid absorption: excretion of unabsorbed ^{14}C-DBNP in the feces increased after neomycin treatment. Failure to detect unchanged DBNP in the urine or bile indicates that once DBNP is absorbed, it is excreted only after metabolism.

Still et al. (2005) found that a single dose of DBNP at 15 mg/kg containing 1.5 μCi of ^{14}C-DBNP in DMSO and canola oil resulted in a significant increase in radioactivity counts after 24 h in the fat > liver > kidneys > heart > lungs > brain > striated muscle > spleen. Six days after dosing, radioactivity remained increased in liver > brain > fat > kidneys > heart; at 10 days after dosing only the liver continued to show a significant increase in radioactivity. ^{14}C in the blood peaked 7.5 h after dosing and was cleared from the blood by 24 h after dosing. ^{14}C in the urine peaked at 96 h and decreased incrementally thereafter. Some 23% of the oral dose was excreted in the feces within the first 24 h, and 54% was excreted in 96 h. An additional 3% of the radiolabel was excreted over the next 72 h. The authors suggested that DBNP or its metabolites may be able to accumulate in the body after continuous or repeated exposures.

Carpenter et al. (1997) studied several biochemical measures associated with exposure of F-344 rats to DBNP. Ring-labeled ^{14}C-DBNP reached peak blood concentrations in 5-10 min when given to male F-344 rats by ip injection (91.2 μg). Oral administration of a similar dose resulted in peak blood ^{14}C-DBNP in about 1 h. The rapid phase of clearance that occurs in the first hour after ip dosing is followed by a pseudosteady state that continues for a week and results in the removal of 4-6% of the radiolabel from the blood. The rapid phase is due to distribution of DBNP to body tissues and elimination in the urine and feces. Within 24 h after ip dosing with DBNP at 0.4 mg/kg, 20% and 12% of the dose is excreted in the urine and feces, respectively, and tissue distribution includes the liver (14-16%), spleen (3-5%), kidneys (8-10%), heart (2-5%), brain (0.8-1.2%), muscle (0.5-1%), fat (11-13%), and blood (6-8%). DBNP is slowly excreted from the body: 82-90% is excreted in the urine and feces within 10 days after a single ip dose of 0.4 mg/kg. Urinary and fecal excretion is 18-20% and 12-15%, respectively, during the first 2 days after dosing and decreases considerably thereafter. A single metabolite isolated from the urine, bile, and feces was a glucuronide conjugate of DBNP; no parent DBNP was isolated from these media, and no metabolite with a reduced nitro group was found. Those results are in agreement with those of Holder et al. (1971) and indicate that, once absorbed, DBNP is excreted after phase II metabolism as a glucuronide conjugate. Enterohepatic circulation of DBNP glucuronide is likely to contribute to the low rate of elimination of DBNP.

As described above, five New Zealand white rabbits had DBNP at 2,000 mg/kg applied directly to their shaved backs for 24 h. DBNP was below the limit of detection in "the blood and the organs" of exposed rabbits (Alexander et al. 2001).

In vitro dermal absorption studies using rat skin also found very little absorption of DBNP (J. McDougal, personal communication cited in Inman et al. 2003). Using isolated perfused porcine skin flaps exposed to ^{14}C-DBNP, Inman et al. (2003) found that among several exposure scenarios (40.0 μg/cm^2 in 100% ethanol, 40.0 μg/cm^2 in 85% ethanol and 15% water, 4.0 μg/cm^2 in 100% ethanol, and 4.0 μg/cm^2 in 85% ethanol and 15% water) the highest absorption measured was 1.08%. The highest mass of ^{14}C-DBNP absorbed was 0.5 μg. That most of the ^{14}C-DBNP applied was found on the surface of the skin where it was applied indicates very low skin absorption of DBNP.

Disposition and uptake-elimination profiles of a single dose of DBNP were quantified by using human skin grafted onto athymic nude mice (Pershing et al. 2006). DBNP was measured in samples of stratum corneum, epidermis, and dermis after exposure for 0.5, 1, 2, 4, 8, and 24 h. The C_{max} (maximal concentration) of DBNP in the stratum corneum was 1,663 ± 602 μg/cm^3. The C_{max} in the epidermis and dermis was about 0.03 and 0.003 times that, respectively. T_{max} (time to maximal concentration) occurred at 0.5-1.0 h in the three compartments. Over a 24-h interval, the greatest amount of DBNP remained in the stratum corneum, and elimination half-lives were 9-11 h in the three layers.

DBNP was quickly absorbed into the stratum corneum, but overall absorption across the skin graft was minimal.

DBNP-dosed animals have a clinical appearance (hyperthermia, rigor of skeletal and cardiac muscle, convulsions, and weight loss) that is shared by animals dosed with dinitrophenols, such as 2,4-DNP, and other agents that act as uncouplers of oxidative metabolism (Alexander et al. 2001). On the basis of quantum-mechanics calculations, Rivera-Nevares et al. (1995) suggested that DBNP might be second only to 3,5-di-tert-butyl-4-hydroxybenzylidene malonitrile (the most potent known uncoupler of oxidative phosphorylation) as an uncoupler of ATP. However, in vitro data reported by Carpenter et al. (1997) found DBNP to be one-third less potent than 2,4-DNP as an inhibitor of mitochondrial respiration.

Other in vitro studies have explored relative effects of exposure to DBNP on protein synthesis, mitochondrial respiration, and cell toxicity and species differences in these end points. When liver slices were used, human tissue was less sensitive than rat tissue to DBNP (Carpenter et al. 1997). End-point susceptibility in both species was ATP content > protein synthesis > LDH release > K+ leakage. At 50 μM DBNP, the ATP content of rat liver slices was reduced by 70% compared with a 30% reduction in human liver slices. At the same concentration, protein synthesis was reduced by 60% in rat liver slices and 30% in human tissue. When hepatocytes (WP-344 cells) were cultured in the presence of DBNP, loss of cellular viability was 8, 12, or 100 % after a 24-h exposure at 0.1, 0.2, or 2 μg/mL. Isolated rat liver mitochondria showed reduction in both stage 3 (at 200 or 300 nmol) and stage 4 (at 1, 2, or 5 nmol) respiration.

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

The occurrence of DBNP in the submarine environment is due to the unintended release of DBP from submarine steam-turbine systems that use TEP 2190 as a lubricating oil. The nitration of DBP by the electrostatic precipitator in the submarine air-handling system results in the formation of DBNP. DBNP has no known industrial or commercial use and is not reported to have natural sources. There are no published recommendations for inhalation exposure to DBNP from other national or international bodies.

COMMITTEE RECOMMENDATIONS

Contact with DBNP in the submarine environment potentially involves oral, dermal, and inhalation exposure. Significant dermal absorption of DBNP appears to be unlikely: studies reported by Vesselinovitch et al. (1961), Alexander et al. (2001), Inman et al. (2003), and Pershing et al. (2006) indicate that DBNP is absorbed to only a minor extent, if at all, through the intact skin. Although Alexander et al. (2001) reported reduced weight gain in a proportion of

the animals exposed at 2,000 mg/kg under occlusion for 24 h, it is unlikely that crew members would encounter such high exposure, because the maximal surface concentration determined in a submarine environment is reported to be 0.2 μg/cm^2 (J. McDougal, personal communication cited in Inman et al. 2003).

Oral exposure to DBNP is considered possible, but the degree of exposure by this route will depend to some extent on personal and environmental hygiene in the submarine. With the small amount of information available, it is not possible to estimate DBNP exposure by the oral route reliably. Because the DBP in TEP 2190 oil has been reduced from no more than 10,000 ppm to no more than 10 ppm (Alexander et al. 2001; Still et al. 2005), the surface contamination levels in submarines that have converted to using TEP 2190 oil manufactured with reduced DBP levels are likely to be significantly lower than the highest determined before the current (post-1993) TEP oil MILSPEC went into effect, 0.2 μg/cm^2.

There are no reports of the study of inhalation exposure to DBNP. Airborne DBNP in submarines using "old" TEP oil has been measured at less than 3.0 to 13 ppb 24 h/day for 90-day operation periods (Alexander et al. 2001). Laboratory simulation of the submarine operational environment reported DBNP concentrations from "old" TEP oil as high as 122 ppb (Alexander et al. 2001).

The routes of exposure used in animal studies that could be a basis for calculation of EEGL and CEGL values are oral, ip, and iv administration. The adverse clinical signs in rats associated with ip, iv, and oral administration of DBNP are similar. None of the iv studies include sufficient detail for deriving EEGL and CEGL values. Available data (and their limitations) from oral and ip studies considered in deriving EEGL and CEGL values are discussed in the following paragraphs.

Exposure to DBNP at acutely toxic doses results in prostration, rapid breathing, hyperthermia, and rapid induction of rigor mortis after death—signs that are consistent with inhibition of mitochondrial oxidative metabolism (Alexander et al. 2001; Carpenter et al. 1997). The lowest lethal single dose of DBNP reported is 40 mg/kg given by oral gavage. The oral LD_{50}s in rats given DBNP vary widely. Much of the variability may be attributable to the use of aqueous vs organic solvent vehicles, inasmuch as some studies with aqueous vehicles report larger LD_{50}s. However, rat strain and DBNP purity may also be sources of variability in the determination of LD_{50}s. Histopathologic changes, primarily cell degeneration, have been described in skeletal muscle, cardiac muscle, smooth muscle, liver, kidneys, spleen, and lungs of rats that died after lethal exposures to DBNP. Residual signs in animals that survive the acute phase of toxicity at near lethal doses are typically reported to include reduction in body weight gain.

In vitro studies of DBNP have demonstrated that the compound has the ability to uncouple oxidative phosphorylation; this is a property shared with 2,4-DNP (ATSDR 1995). Clinical signs and histopathologic lesions associated with DBNP and 2,4-DNP are very similar; the male rat oral LD_{50}s of DBNP (50 mg/kg) and 2,4-DNP (38 mg/kg) are also similar (ATSDR 1995). Significant

differences between the two chemicals are the induction of cataracts and peripheral neuropathy by 2,4-DNP but not by DBNP. However, those difference may be due to inadequacies in the testing of DBNP or the use of test species (rats, mice, guinea pigs, and rabbits) insensitive to the development of chemically induced cataracts. None of the studies conducted with DBNP have been of sufficient duration to rule out the possibility that DBNP may induce peripheral neuropathy similar to that induced by 2,4-DNP.

No studies have demonstrated a convincing NOAEL or LOAEL of acute oral exposure to DBNP. The lowest LOAEL of a single dose of DBNP is 15 mg/kg given to male rats in a study reported by Still et al. (2005). After dosing with DBNP, rats exhibited lethargy and reduced startle response during the first 24-48 h; recovery appeared complete within 7-8 days, when test animals were regarded as indistinguishable from controls. The presence of central nervous system (CNS) effects in rats given DBNP at 15 mg/kg correlates with the presence of ^{14}C label derived from DBNP in the brain for the first 24 h after dosing (Still et al. 2005). The reduction of acoustic-startle response in the rats given 15 mg/kg is an indicator that CNS performance may be seriously impaired at this exposure.

Most of the reports identify ip DBNP at 25 mg/kg as toxic in rats (Vesselinovitch et al. 1961; Carpenter et al. 1997). Repeated ip doses of 10 mg/kg are reported to be without effect on mortality and body weight for 60 days of exposure. Rats have also been fed DBNP at doses at about 25 and 50 mg/kg per day for 16 weeks without effect on mortality or body weight (Vesselinovitch et al. 1961). However, none of the data were considered to be adequate for use in recommending EEGL or CEGL values.

The Navy's Bureau of Medicine and Surgery (BUMED) has attempted to set allowable exposure limits for DBNP in the submarine environment (Alexander et al. 2001). However, inadequacies of the DBNP database were so great as to preclude the development of an inhalation reference value. Recognizing the deficiencies, Alexander et al. (2001) calculated a range of maximal allowable DBNP exposure levels for submarine crews by applying safety factors to the rat oral LD_{50} of 80 mg/kg and LOAEL of 50 mg/kg. On the basis of the application of safety factors of 1,000 and 100,000 to the rat LD_{50}, they calculated a maximal allowable atmospheric concentration of 27.3-0.273 ppb. Application of the same safety factors to the LOAEL resulted in the calculation of a maximal allowable atmospheric concentrations of 17.53-0.1753 ppb. Alexander et al. (2001) compared the data with the measured and simulated concentrations of DBNP in the submarine atmosphere, which were less than 3.0 ppb to 122 ppb, and concluded that more research was necessary to clarify the risk associated with DBNP. If the maximal allowable atmospheric concentrations by Alexander et al. (2001) were recalculated with the more recent data reported by Still et al. (2005) in which effects were seen at 15 mg/kg, the recalculated maximal allowable concentrations would be lower than those identified in Alexander et al. (2001).

Because of the inadequacy of the DBNP database, the committee agrees with Alexander et al. (2001) regarding the need for further information and is unable to recommend EEGL and CEGL values for DBNP exposure.

DATA ADEQUACY AND RESEARCH NEEDS

The U.S. Navy is reported to have reduced the DBP in TEP 2190 lubrication oil to less than 10 ppm. Presumably, the reduction has led to a substantial reduction in the potential exposure of DBNP in the submarine environment. However, no document substantiating that presumption was made available to the committee. The animal-toxicity database available for assessment of hazard and risk includes only single or repeat-dose studies, primarily via the oral route, with a small number of end points assessed. The committee considered deriving exposure guidance levels on the basis of noninhalation exposure routes, but there were insufficient data to support the route-to-route extrapolation. Furthermore, the overall database available for determining EEGL and CEGL values is small, and many of the data points are conflicting. Thus, much uncertainty is associated with any attempt to estimate exposure guidance levels for this compound. The committee recommends that, at a minimum, a short-term inhalation study be conducted that looks at a comprehensive set of end points before an EEGL or CEGL value is established.

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5

Freon 12

This chapter summarizes the relevant epidemiologic and toxicologic studies on Freon 12, or dichlorodifluoromethane. 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 committee considered all that information in its evaluation of the Navy's current and proposed 1-h, 24-h, and 90-day exposure guidance levels for Freon 12. The committee's recommendations for Freon 12 exposure levels are provided at the end of this chapter 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

Freon 12 is a nonflammable, colorless gas at room temperature. Like Freon 114, it has a faint ether-like odor at high concentrations (Budavari et al. 1996). Selected chemical and physical properties are listed in Table 5-1.

OCCURRENCE AND USE

In industrial settings, Freon 12 has been used as an aerosol propellant, a foam-blowing agent, and a refrigerant (Garcia 2000; WHO 1990). The primary source of Freon 12 in the submarine environment is through the air-conditioning and refrigerant plants (Garcia 2000; Crawl 2003). Several measurements of Freon 12 on submarines have been reported. Data collected on nine nuclear-powered ballistic missile submarines indicate an average Freon 12 concentration of 11 ppm (range, 0-61 ppm) and data collected on 10 nuclear-powered attack submarines indicate an average Freon 12 concentration of 13 ppm (range, 0-1,033 ppm) (Hagar 2003). Holdren et al. (1995) reported the results of air sam-

TABLE 5-1 Physical and Chemical Properties of Freon 12

Synonyms and trade names	FC 12, CFC-12, difluorodichloromethane, fluorocarbon-12, R 12
CAS registry number	75-71-8
Molecular formula	CCl_2F_2
Molecular weight	120.92
Boiling point	−29.8ºC at 760 mm Hg
Melting point	−158ºC
Flash point	NA
Explosive limits	NA
Specific gravity	1.1834 g/mL (57°C)
Vapor pressure	5.7 atm (20°C)
Solubility	Insoluble in water (0.028 g/100 g at 25°C); soluble in alcohol, ether
Conversion factors	1 ppm = 4.95 mg/m^3; 1 mg/m^3 = 0.202 ppm

Abbreviations: NA, not available or not applicable.
Sources: Flash point and explosive limits from HSDB 2005; all other data from ACGIH 2001.

pling at three locations conducted over 6 h during the missions of two submarines. Sampling indicated concentrations of 2.072-5.476 ppm and of 2.740-3.035 ppm, depending on the collection method, on one submarine, and concentrations of 0.452-3.015 ppm and of 2.092-2.938 ppm, depending on the collection method, on the other submarine. Raymer et al. (1994) reported the results of a similar sampling exercise (two submarines, three locations, and sampling duration of 6 h). Freon 12 concentrations were reported at 4.0 and 1.4 ppm in the fan rooms, 2.0 ppm in the galleys, and 1.8 and 4.0 ppm in the engine rooms.

SUMMARY OF TOXICITY

The toxicity of Freon 12 has been studied in a number of species exposed acutely and repeatedly. Most of those studies, however, were conducted in the 1970s and earlier and lack complete documentation. Some studies, although frequently cited in review articles, are unpublished or were published in foreign journals and were available only in published reviews. The information evaluated indicates that Freon 12 has relatively low acute toxicity by inhalation (for example, LC_{50}s over 500,000 ppm in animals) with weak narcotic and moderate cardiac-sensitizing effects. The EC_{50}s (the concentrations at which a specified effect is observed in 50% of a test population) for cardiac sensitization, the most serious toxic effect, have been reported to be at least 50,000 ppm in dogs and other animals given intravenous epinephrine and over 100,000 ppm in animals

with endogenous epinephrine. Respiratory and circulatory system effects, such as bronchoconstriction and changes in heart rate, have also been observed in animals acutely exposed at those concentrations. In general, the few data in humans are consistent with data in animals in the types of effects and effect levels. Because administered doses of epinephrine that caused cardiac arrhythmia with Freon 12 exposure were considerably higher than would occur endogenously, under normal conditions, neurologic or other effects—such as pulmonary effects—are more likely than cardiac effects at lower Freon 12 concentrations. Freon 12 concentrations associated with no or minimal effects in animals and a small number of healthy human subjects are about 1,000 ppm.

Chlorofluorocarbon mixtures appear to have greater toxicity than individual compounds alone at the same concentration as the mixture, although reports are based on higher concentrations (for example, over 10,000 ppm), and no information is available on lower concentrations.

Effects of repeated or longer-term exposures are generally similar to those of acute exposures. Thus, Haber's law (concentration [C] × exposure time [t] = response [k]) for extrapolating toxicity between short-term and long-term exposures does not appear to apply for Freon 12. That observation is consistent with the pharmacokinetics of Freon 12, which is rapidly absorbed and eliminated almost entirely by inhalation with little metabolism. Equilibrium blood concentrations and appearance in cerebral spinal fluid occur within minutes of exposure, and elimination is complete within 20-50 min after exposure ceases.

Freon 12 has not been reported to be genotoxic, and long-term studies in animals exposed orally or via inhalation and available epidemiologic data do not show evidence of carcinogenicity. No evidence of male reproductive toxicity was found. No studies were available to evaluate immunotoxicity via inhalation.

Effects in Humans

Accidental Exposures

No published reports of deaths or other effects of humans resulting from accidental exposures to airborne Freon 12 were located. Freon 12 and other chlorofluorocarbons have been involved in cases of intentional inhalation that resulted in death that was most likely related to effects on the heart (for example, cardiac arrhythmia, possibly aggravated by increased catecholamine from stress or moderate hypercapnia) (NRC 1984; WHO 1990). Chlorofluorocarbons, including Freon 12, have been evaluated in connection with deaths of people who had asthma and who used inhalers containing chlorofluorocarbons. Chlorofluorocarbon toxicity in those cases has been discounted because of the small amount of chlorofluorocarbon exposure compared with known toxic levels from experimental studies and cases of deliberate abuse (WHO 1990), although Aviado (1994) believes that chlorofluorocarbons may have contributed to the deaths of those who had asthma.

Experimental Studies

A few studies have been conducted in small numbers of healthy human volunteers. Most of them involved short-term exposure, such as 2.5 h or less (see Table 5-2). Exposures generally at tens of thousands of parts per million and above are associated with electroencephalographic (EEG) changes and decrements in neurobehavioral performance (Kehoe 1943, as cited by NRC 1984; Azar et al. 1972). Reduced ventilatory capacity and decreased heart rate were reported at a concentration as low as 10,000 ppm (Valić et al. 1982), but not by Azar et al. (1972), and cardiac arrhythmia and amnesia were noted in a subject at 100,000 ppm (Kehoe 1943, as cited in NRC 1984). No irritation or effects on the heart, central nervous system (CNS), or a variety of clinical measures were reported at 1,000 ppm (Azar et al. 1972; Emmen et al. 2000; Stewart et al. 1978). The longest exposure period examined in those studies was 8 h/day, 5 days/week for 2-4 weeks. The details of this study are discussed below.

Some of the highest exposures in human studies were reported in an unpublished study involving two subjects (Kehoe 1943, as cited in NRC 1984, WHO 1990, Garcia 2000). One subject tolerated Freon 12 at up to 60,000 ppm for 80 min but showed cardiac arrhythmia followed by amnesia within 10 min when exposed at 110,000 ppm. The other, exposed at 40,000 ppm for 14 min and at 20,000 ppm for 66 min, reported a tingling sensation and humming in the ears and showed EEG changes, slurred speech, and decreased psychologic test scores but no cardiac effects.

Valić et al. (1977) exposed 10 subjects to Freon 12 and several other chlorofluorocarbons, including mixtures of the compounds. Ventilatory capacity was measured after 15-sec and 45-sec exposures. Electrocardiographic (EKG) changes were measured at various intervals after the start of a 15-sec exposure period and continuously during a 60-sec exposure period. Significant reduction (although "not clinically alarming") in ventilatory lung capacity (maximum expiratory flow at 50% [MEF50] or 75% [MEF75] of vital capacity), bradycardia, and increased variability in heart rate were reported for the individual compounds, and mixtures had stronger respiratory and cardiac effects. Exposure to Freon 12 at 27,000 ppm for 45 sec was reported to cause reductions in MEF50 and MEF75 of 3.4% and 5.6%, respectively. Smaller reductions were reported after a 15-sec exposure. In one subject, exposure to a 90%:10% mixture of Freon 12:Freon 11 (8,3000:1,800 mg/m^3 for 15 sec and 8,900:1,600 mg/m^3 for 60 sec) but not to the individual chlorofluorocarbons or other mixtures was associated with tachycardia and inversion of the T wave. Valić et al. (1982) exposed 11 subjects for 35 or 130 min to Freon 12 and reported acute reduction in ventilatory lung capacity at 10,000 ppm (significant decrease in MEF50 and MEF25 for a 35-min but not 130-min exposure) and 17,500 ppm (significant decrease in MEF50, MEF25, forced expiratory volume at 1 sec [FEV_1], and forced vital capacity [FVC] for both exposure periods) but not at 90 ppm. Significant concentration-dependent decreases in heart rate at 10,000 ppm and 17,700 ppm were also reported.

TABLE 5-2 Summary of Human Toxicity of Freon 12

Exposure Period	No. Subjects	End Point	NOAEL (ppm)	Adverse-Effect Level (ppm)	Reference
15 sec, 45 sec (pulmonary tests) 15 sec, 60 sec (EKG tests)	10	None of the subjects had a history of cardiovascular or pulmonary disease; pulmonary effects—3.4% decrease in MEF50, 5.6% decrease in MEF75; cardiac effects—reduced heart rate and respiratory sinus arrhythmia for all compounds tested; tachycardia and negative T wave in one subject exposed to 10%:90% mixture of Freon 11:Freon 12	—	27,000	Valić et al. 1977
10 min	1	Amnesia and cardiac arrhythmia	—	110,000	Kehoe 1943, as cited in Garcia 2000 and NRC 1984
14 min, then 66 min	2	EEG changes, slurred speech, and decreased psychologic test scores in one subject; other subject tolerated 60,000 ppm for 80 min without such effects	40,000 then 20,000	—	Kehoe 1943, as cited in NRC 1984
35 min, 130 min	11	Reduction in MEF50 and MEF25 (at 10,000 ppm, 35-min exposure but not 130-min exposure); reduction in FEV_1 and FVC at 17,500 ppm only; decrease in heart rate at both concentrations	90	10,000; 17,500	Valić et al. 1982, as cited in WHO 1990
1 h; two exposures, one per week	4 males, 4 females, 20-24 years old	No clinically significant changes in laboratory measures (of blood and urine), blood pressure, pulse, EKG or lung function	1,000 or 4,000	—	Emmen et al. 2000
2.5 h; once a week, for 2 weeks	2	Healthy men 28 and 34 years old; no effects on clinical observations, laboratory tests, subjective impressions, EKG, equilibrium; 7% reduction in psychomotor scores	1,000	10,000	Azar et al. 1972
8 h/day, 5 days/week for 2-4 weeks	8	No effect on cognitive or motor function tests, clinical measures of blood and urine, spirometry, EKG, EEG, or irritation symptoms	1,000	—	Stewart et al. 1978

Abbreviations: EKG, electrocardiography; EEG, electroencephalography; FVC, forced vital capacity; MEF25, maximum expiratory flow at 25% of vital capacity; MEF50, maximum expiratory flow at 50% of vital capacity; NOAEL, no-observed-adverse-effect level.

Azar et al. (1972) reported a small reduction (7%) in performance on psychomotor tests (clerical tasks, sorting, manual dexterity, and mental arithmetic) in two healthy men after 150 min at 10,000 ppm but no effects at 1,000 ppm. Subjects were tested in the chamber for 2.5 h/day, 5 days/week for 2 weeks separated by a week of no chamber exposures but 2 days of practice tests. During the weeks with testing in the chamber, control-air exposure was used on Mondays, Wednesdays, and Fridays. Exposure at 1,000 ppm and 10,000 ppm occurred on Tuesday and Thursday, respectively, in the first week, and the opposite order was used on these days in the second week of chamber testing. Scores at each concentration for each week were compared with the average of scores from the 3 days of control-air exposure. Other observations included clinical observations, blood tests, subjective impressions, and continuous EKG monitoring. The authors concluded that single exposures of 2.5 h or less at 10,000 ppm could be tolerated without permanently affecting health.

In a more recent experimental study, Emmen et al. (2000) exposed four healthy men and four healthy women (20-24 years old) to Freon 12 at 1,000 ppm and 4,000 ppm for 1 h. Subjects were exposed twice in different weeks. No clinically significant changes from reactions in clean air were measured in blood and urine (hematology and clinical chemistry) 24 h after exposure; in blood pressure, pulse, and EKG before, during, or after exposure; or in lung function (peak expiratory flow) 45 min before and 75 min after exposure.

Stewart et al. (1978) exposed healthy volunteers for the longest period. Up to four healthy men and four healthy women were exposed to Freon 12 at 250, 500, and 1,000 ppm for 1 min to 8 h to assess absorption, excretion, and physiologic effects. When those exposures caused no health effects in any subjects, the subjects were exposed at 1,000 ppm 8 h/day, 5 days/week for 2-4 weeks. Physical examinations, subjective symptom surveys, blood and urinary analysis for clinical measures, spirometry, EKG, EEG, adrenal gland function, motor or cognitive tests, or health monitoring over a year after exposure showed no treatment-related effects. In contrast, eight men exposed repeatedly to Freon 11 (considered to be more toxic than Freon 12) at 1,000 ppm showed minor decrements in some cognitive tests (Stewart et al. 1978).

Occupational and Epidemiologic Studies

Exposure to Freon 12 has been widespread because of its use as a refrigerant and as an aerosol propellant in consumer products and medicinal inhalers (Marier et al. 1973; Ritchie et al. 2001). Only a few occupational or epidemiologic studies that involved Freon 12 were located. Those studies also involve exposure to other chlorofluorocarbons. Edling and colleagues (Edling and Olson 1988 in Swedish, as cited in WHO 1990; Edling et al. 1990) examined 89 refrigeration workers exposed mainly to Freon 12 (56% of cases), although several other chlorofluorocarbons were involved. Chlorofluorocarbon concentrations measured by personal monitors exceeded 750 ppm at least once (as 1-min mean

values) for 60 of the 89 workers. The highest concentration recorded was 14,000 ppm, and the highest time-weighted average concentration for 8 h was 280 ppm. No significant differences in EKG measurements between nonexposed and exposed periods were found, nor was a concentration-related trend found when subjects were grouped by exposure, although effects in subjects in the medium-exposure group were borderline significant (Wilcoxon's test, $p = 0.05$, one-tailed). No differences were found in simple reaction-time measurements before and after exposure.

Mortality in 539 refrigeration construction and repair workers (employed more than 6 months) was not increased (18 deaths vs 26 expected) (Szmidt et al. 1981 in Swedish, as cited in WHO 1990 and Rusch 2000). Freon 12 was among several chlorofluorocarbons used by the workers. No significant increases in total tumor deaths, lung cancer deaths, or cardiovascular deaths were reported. Restricting the analysis to those employed more than 3 or 10 years did not change the findings. Exposure of six refrigeration workers to Freon 12 and hydrochlorofluorocarbon (HCFC) 22 at concentrations that occasionally reached 1,300 to 10,000 ppm was not associated with cardiac problems compared with plumbers who had no such exposure (Antti-Poika et al. 1990, as cited in Rusch 2000).

Effects in Animals

Toxicity of Freon 12 has been examined in several animal species, including rats, mice, guinea pigs, dogs, cats, and monkeys. Dogs in particular have been studied for the cardiac-sensitizing effects of Freon 12 and other chlorofluorocarbons. Studies in dogs have generally been conducted in conscious animals, whereas those in other species have used anesthetized animals. General anesthesia makes the heart less responsive to epinephrine, and this confounding factor needs to be taken into account in interpreting the animal data. Results of studies of Freon 12 in animals are generally consistent with those of experimental studies in humans (see Table 5-3). In general, at equivalent air concentrations of Freon 12, effects noted after brief exposure (for example, 5 min) appear to be similar to those reported after longer exposure (for example, an hour) or repeated exposure.

Acute Toxicity

Cardiac sensitivity in exercising dogs appears to occur at Freon 12 concentrations of about 100,000 ppm (Mullin et al. 1972). At those concentrations, CNS-depressant effects also occur (see Table 5-3). Dogs exposed to Freon 12 and intravenous epinephrine show signs of cardiac arrhythmia beginning around 50,000 ppm (Reinhardt et al. 1971), and the concentration of Freon 12 associated with arrhythmia increases with decreasing epinephrine dose (Rusch 2000).

TABLE 5-3 Summary of Animal Toxicity of Freon 12

Species (no.)	Exposure Period	End Point	NOAEL (ppm)	Adverse-Effect Level (ppm)	Reference
Dog (12)	30 sec	No cardiac arrhythmia with fright	80,000	—	Reinhardt et al. 1971
Rat (5)	2 min	Anesthetized; reduced pulmonary compliance and tidal volume	—	50,000	Watanabe and Aviado 1975
Rat (5, 5, 4)	5 min	Unanesthetized; no arrhythmias; acceleration of heart rate (10% at 400,000), although not statistically significant	—	100,000, 200,000, 400,000 and 20% O_2	Watanabe and Aviado 1975
Mouse (3)	4 min	Anesthetized; 8% increase in pulmonary resistance, 6% decrease in pulmonary compliance (tests of significance not reported)	—	20,000	Brody et al. 1974
Mouse (4)	4 min	Anesthetized before exposure; no cardiac arrhythmia with or without epinephrine; increase in height of QRS complex (13.9%) and decrease in heart rate (9.3%); slowing of heart rate reduced when epinephrine was also administered	—	400,000	Brody et al. 1974
Dog (4)	5 min	EC_{50} for cardiac arrhythmia with epinephrine.	20,000, 40,000	80,000	Clark and Tinston 1972
Dog (12)	5 min	Cardiac arrhythmia in 5 dogs with epinephrine	—	50,000	Reinhardt et al. 1971
Monkey (3)	5 min	Anesthetized before exposure; 9% decrease in aortic blood pressure; changes in other measures not significant	—	50,000	Aviado and Smith 1975

Monkey (4)		15% increase in pulmonary resistance and 10% increase in aortic heart rate; changes in pulmonary compliance (-11%), respiration minute volume (-1%), and aortic blood pressure (-15%) not significant.	—	100,000	Aviado and Smith 1975
Monkey (3)	5 min	Anesthetized before exposure; no change in heart rate	50,000, 100,000	—	Belej et al. 1974
		Nonsignificant decrease (10%) in myocardial force	50,000	—	
		Significant decrease (20%) in myocardial force	—	100,000	
Dog (3)	5 min	Anesthetized before exposure; increased pulmonary resistance and heart rate at both concentrations; reduced minute volume at 200,000 ppm; no significant effect on aortic blood pressure	—	100,000, 200,000	Belej and Aviado 1975
Mouse (8 at 200,000 ppm, 6 at 400,000 ppm)	6 min	No arrhythmia with or without epinephrine in anesthetized mice; supplemental oxygen administered	200,000, 400,000	—	Aviado and Belej 1974
Rabbit, dog	10 min	No cardiac arrhythmia; administered to anesthetized animals with tracheal cannula	200,000, 500,000 and 20% O_2	—	Paulet et al. 1975a
Rat (4)	15 min once a week, exposed 4 times at each concentration	Decrease in operant performance at 140,000 ppm; however, operant behavior measured pre-exposure and postexposure over the course of the study did not significantly differ (no lasting effect)	40,000, 60,000, 80,000, 100,000	140,000	Richie et al. 2001

(Continued)

TABLE 5-3 Continued

Species (no.)	Exposure Period	End Point	NOAEL (ppm)	Adverse-Effect Level (ppm)	Reference
Dog (8)	16 min	Cardiac arrhythmia with exercise; anesthetic effects in 6 animals	—	≥100,000	Mullin et al. 1972
Rat (20)	<17 min	Deficits in motor function; effect levels for Freon 12 with and without supplemental oxygen were similar	—	170,000-460,000	Ritchie et al. 2001
Rabbit, dog	20 min	Anesthetized; no significant effect on blood measures (electrolytes, pH, glucose, urea, protein, cholesterol)	200,000 and 20% O_2	—	Paulet et al. 1975b
Dog (6)	1 h	No cardiac arrhythmia with epinephrine	25,000	—	Reinhardt et al. 1971
Rat (10), rabbit (5)	Two 1-h exposures per day for 15 days	No significant effect on basal metabolism, thirst or diuresis, plasma electrolytes	50,000 and 20% O_2	—	Paulet et al. 1975b
Rat, guinea pig	2 h	Deaths in rats but not in guinea pigs	—	600,000[a]	Schloz 1962, as cited in ACGIH 2001
Rat, guinea pig, cat	Several hours	No deaths	300,000-800,000[a]	—	Studies reviewed by ACGIH 2001
Mouse	24 h	Histologic evidence of increased leukocyte infiltration of alveolar wall relative to controls; exudate in bronchioles	—	10,000	Quevauviller et al. 1963

Cat (2), rat (5), guinea pig (3), dog (2)	3.5 h/day, 5 days/week for 4 weeks	No adverse effects	100,000	—	Scholtz 1962, as cited in WHO 1990
Rat (90 of each sex), mouse (60 of each sex)	4 h/day, 5 days/week; rat: 104 weeks; mouse: 78 weeks	No evidence of carcinogenicity or effects on body weight	1,000, 5,000	—	Maltoni et al. 1988
Dog (6)	6 h/day, 7 days/week for 90 days	No adverse effects on behavior and appearance, food or water consumption, body weight, clinical measures, heart rate, EKG, blood pressure, sight, hearing, dentition, organ weights, or histologic examinations	5,000	—	Leuschner et al. 1983
Rat (40)	6 h/day, 7 days/week for 90 days	No adverse effects on behavior and appearance, food or water consumption, body weight, clinical measures, sight, hearing, dentition, organ weights, or histologic examinations	10,000	—	Leuschner et al. 1983
Dog, monkey, guinea pig	7-8 h/day for 3.5-56 days	Some deaths, CNS reactions.	—	200,000	Summarized by Clayton 1967

(Continued)

TABLE 5-3 Continued

Species (no.)	Exposure Period	End Point	NOAEL (ppm)	Adverse-Effect Level (ppm)	Reference
Rat (15), guinea pig (15), monkey (3), rabbit (3), dog (2)	8 h/day, 5days/week for 6 weeks	1 rat died but no visible signs of toxicity in survivors; focal necrosis in livers of guinea pigs; one monkey had heavy pigment deposits in liver, spleen, and kidney; all species but dogs had lung congestion, other organs appeared normal; histopathologic examination showed nonspecific interstitial inflammatory changes in lungs of experimental and control animals (same control group as for 90-day continuous-exposure study)	836	836 (guinea pig)	Prendergast et al. 1967
Rat (15), guinea pig (15), monkey (3), rabbit (3), dog (2)	Continuously for 90 days	2 rats, 1 guinea pig died but no visible signs of toxicity in survivors; focal or submassive necrosis in livers of guinea pigs; no pathologic changes reported in tissues of other species; nonspecific inflammatory changes in lungs of all species and in controls	807	807 (guinea pig)	Prendergast et al. 1967
Mouse (30 female); dog (3 of each sex)	Mice: 5 days/week for 23 months	Exposed to mixtures of CFCs, including about 50% Freon 12; no sign of toxicity or lung tumors	970 mg/kg per day (686 ppm)[b]	—	Smith and Case 1973
	Dogs: 7 days/week for 12 months	No sign of toxicity or irritation in lung tissue sections; no changes in hematology, blood chemistry, EKG, heart histology, urinalysis	2,240 mg/kg per day (1,584 ppm)[b]	—	Smith and Case 1973

[a]No information on whether supplemental oxygen was used.
[b]Reported as a dose; equivalent air concentration for 24-h exposure for a human in parentheses.
Abbreviations: CFC, chlorofluorocarbon; CNS, central nervous system; EC_{50}, the concentrations at which a specified effect is observed in 50% of a test population; EKG, electrocardiography; EEG, electroencephalography; MEF50 and MEF25-forced expiratory flow at 50% or 25% of vital capacity; FEV_1, forced expiratory volume at 1 sec; FVC, forced vital capacity; NOAEL, no-observed-adverse-effect level.

Epinephrine doses used in these studies are reported to be higher than (for example, 10 times as high as) endogenous concentrations (Reinhardt et al. 1971). The epinephrine dose, 5 μg/kg, used by Clark and Tinston (1972) was the highest that could be administered without causing serious cardiac arrhythmia by itself. Sensitization of the heart to cardiac arrhythmia is reported to be temporary. Injection of epinephrine 10 min after 0.5-min exposure at a sensitizing concentration (80,000 ppm) of Freon 12 had no effect on cardiac rhythm (Clark and Tinston 1972).

Effects observed with acute exposure to Freon 12 at 100,000 ppm and above include bronchoconstriction in monkeys (Aviado and Smith 1975) and dogs (Belej and Aviado 1975) and increased heart rate and decreased myocardial force (Aviado and Smith 1975) in monkeys. Rats were reported to show pulmonary resistance and other lung-function effects at 50,000 ppm (Watanabe and Aviado 1975); except for a decrease in aortic blood pressure (which was not significant at 100,000 ppm), such changes at 50,000 ppm were not significant in monkeys (Aviado and Smith 1975). Mice exposed for 24 h at 10,000 ppm showed lung changes on histopathologic examination, including greater leukocyte infiltration of alveolar walls relative to controls and an exudate in the bronchioles (Quevauviller et al. 1963). Exposure to a mixture of Freon 12 and Freon 11 or Freon 114 was associated with no clinical signs but had microscopic effects, such as alveolar hemorrhage (Quevauviller et al. 1963). However, the old report lacked sufficient details to evaluate whether the effects were entirely treatment-related. Control animals showed similar effects (focal congestion of the alveolar walls with leukocyte infiltration) to a lesser degree. The order in which mice from the various groups were euthanized and the method of euthanasia were not specified. Some of the lesions observed could be caused by the method of euthanasia. Background pulmonary inflammatory disease may also have contributed to the lesions observed.

Rats showed no significant differences in operant performance measures (such as number of food rewards and number of errors) during 15-min exposures at 40,000, 60,000, 80,000, or 100,000 ppm compared with effects of exposure to clean air; however, exposure at 140,000 ppm resulted in a significant reduction in the number of food rewards and an increase in the ratio of errors to rewards (Richie et al. 2001).

Repeated Exposure and Subchronic Toxicity

Animal studies provide useful information for assessing effects of repeated or longer-term inhalation exposure because of the few human studies, although the original papers on several of the studies could not be obtained for critical evaluation (see Table 5-3). One of the useful studies for evaluating CEGLs for submariners is the 90-day exposure study of Prendergast et al. (1967), which used exposure for 24 h/day, 7 days/week (continuously) for 90 days and repeated exposure more similar to occupational settings (8 h/day, 5

days/week for 6 weeks). They exposed 15 rats, 15 guinea pigs, three rabbits, two dogs, and three monkeys to Freon 12 at about 800 ppm under each of the two exposure regimens. Hematologic characteristics were measured before and after exposure, body weights were measured monthly, and visible signs of toxicity (behavior, physical appearance, respiration pattern, locomotor activity, and prostration) were monitored during the exposure period. Animals were necropsied at the end of the study, and histopathologic evaluations of the heart, lungs, liver, spleen, and kidney were conducted. The same control group of animals was used for both repeated and continuous experiments.

A few deaths were observed in rats (one after repeated and two after continuous exposure) and guinea pigs (one after continuous exposure). No visible signs of toxicity were noted in the surviving exposed animals. Death rates observed in control animals were seven of 304 rats, two of 314 guinea pigs, none of 34 dogs, one of 57 monkeys, and two of 48 rabbits. After continuous exposure, body-weight gain in exposed rabbits and guinea pigs was decreased, although the starting weight of rabbits was greater than that of controls; and other species had similar (rats) or greater (dogs and monkeys) weight gain relative to controls. A high incidence of lung congestion was reported in all species but dogs, and nonspecific inflammatory changes were observed in the lungs of all species, including control animals. Fatty infiltration of the liver was observed in all guinea pig liver sections examined; several sections displayed focal or submassive necrosis.

Repeated exposure to Freon 12 for 6 weeks was also associated with no visible signs of toxicity (Prendergast et al. 1967). Other effects reported were similar to those observed after continuous exposure, although the liver changes (focal necrosis) in guinea pigs were considered less severe than those observed after continuous exposure. Weight loss occurred in dogs and monkeys. Lung congestion (except in dogs) and nonspecific interstitial inflammatory changes (in all species) were also noted in lung tissue; however, such changes were also noted in the lungs of control animals. Heavy pigment deposits in the liver, spleen, and kidney were reported in one monkey. Although the authors attributed the focal liver necrosis to the treatment (repeated exposure), they expressed some uncertainty in concluding that the effect was caused by exposure to Freon 12. Regarding the submassive necrosis of the liver after continuous exposure, the authors stated that the effect may have been due to the continuous nature of the exposure or the greater susceptibility of guinea pigs. In a later study (Prendergast et al. 1967), they did not attribute lung congestion to the treatment.

Four rats that received 20 acute exposures (15 min once a week) to Freon 12 at 40,000, 60,000, 80,000, 100,000, and 140,000 ppm showed a significant change in operant performance (measured by number of food rewards for and errors in completing learned tasks) only at 140,000 ppm (Richie et al. 2001). Operant performance was measured before, during, and after Freon 12 exposure. The rats were exposed to each concentration four times successively from the lowest to the highest concentration and received one exposure per week for a total of 20 weeks (four exposures at each of five concentrations). Performance

measured pre-exposure and postexposure showed no differences over the course of the study.

Forty rats exposed at 10,000 ppm and six dogs exposed at 5,000 ppm 6 h/day, 7 days/week for 90 days showed no adverse effects in behavior, food or water consumption, feces, sight, dentition, hearing, or hematologic, urinary, or other clinical measures (Leuschner et al. 1983). No changes from controls were found in internal organ weights measured in 11 rats and 12 dogs or in histologic examinations of 27 organs of 20 exposed rats examined and all dogs.

Chronic Toxicity

Chronic studies of inhalation or oral exposure to Freon 12 have reported few effects. Smith and Case (1973) exposed 30 mice and three adult dogs of each sex to chlorofluorocarbon mixtures 49-50% Freon 12 and about 25% CFC-11, 25% CFC-114, and small amounts of CFC-113. The authors reported daily doses rather than air concentrations: 970 mg/kg per day for mice and 2,240 mg/kg per day for dogs. Assuming an inhalation rate of 20 m^3/day and 70-kg body weight, those doses would be equivalent to 24-h air concentrations for a human of 686 ppm and 1,584 ppm. Mice exposed 5 min/day at the specified dose 5 days/week for 23 months showed no signs of toxicity or lung tumors. Dogs exposed once a day by face mask (exposure period not specified but probably much less than 24 h) 7 days/week for 1 year showed CNS-depressant effects immediately after dosing for a few minutes but no indication of toxicity or irritation when lung tissue sections were examined. The immediate CNS depression observed would probably not be expected if dogs were exposed at the same daily dose by inhalation of a lower Freon 12 concentration over 24 h. No changes were observed in hematology, EKG, heart histology, blood chemistry, or urinalysis. No effects on body weight were reported in an inhalation-carcinogenicity study in rats and mice by Maltoni et al. (1988), described below under "Carcinogenicity."

Reproductive Toxicity in Males

Few reports mention male reproductive toxicity of Freon 12 (NRC 1984; WHO 1990; ACGIH 2001). A three-generation oral-gavage study administered Freon 12 in corn oil to rats at average doses of 15 and 150 mg/kg per day (Sherman 1974, as cited in WHO 1990). No adverse effects on fertility, percentage of live births, or viability of offspring were reported. Freon 113 was examined in a one-generation reproduction study in which male and female rats were exposed by inhalation at 5,000 ppm or 12,500 ppm 6 h/day, 5 days/week for 10 weeks (males) or 3 weeks (females). Males were then paired with two females and exposed 6 h/day for 14 days. Mated females were exposed 6 h/day until day 20 of gestation, when they were allowed to give birth, and the offspring were

observed for up to 4 weeks. No adverse effects on standard reproductive indexes were reported (EPA 1983, as cited in WHO 1990).

Immunotoxicity

Information was insufficient to assess the immunotoxicity of Freon 12.

Genotoxicity

The mutagenic potential of chlorofluorocarbons, including Freon 12, has been examined in a number of in vitro tests and in vivo in rats (dominant lethal mutation study of Freon 12 administered orally at 15 and 150 mg/kg per day). All studies reviewed by WHO (1990) and ACGIH (2001) were negative.

Carcinogenicity

As noted above, Smith and Case (1973, as cited in WHO 1990) reported no evidence of lung tumors in mice and dogs after 23 months of inhalation exposure at 970 mg/kg per day and 12 months at 2,240 mg/kg per day, respectively, to a mixture of chlorofluorocarbons containing about 50% Freon 12 (25% Freon 11, 25% Freon 114, and 0.5-1% Freon 113). Maltoni et al. (1988) examined the carcinogenicity of Freon 12 and Freon 11 in 180 rats and 120 mice of both sexes exposed at 1,000 or 5,000 ppm 4 h/day, 5 days/week for 104 weeks. No evidence of carcinogenicity related to Freon 12 or Freon 11 was reported. Chronic oral-carcinogenicity studies of Freon 12 in rats and dogs have also been negative (Sherman, 1974; reviewed by WHO 1990 and EPA 1995 IRIS RECORD—1995 is the last revised date).

Szmidt et al. (1981 in Swedish, as cited in WHO 1990) found no significant increases in total tumor deaths or lung-cancer deaths in refrigerator construction and repair workers. Restricting the analysis to those employed more than 3 or 10 years did not change the findings.

Freon 12 is not listed in the National Toxicology Program 11th *Report on Carcinogens*.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

The most serious and potentially life-threatening toxic effect of inhalation of chlorofluorocarbons, such as Freon 12, is cardiac toxicity, which has been demonstrated in multiple animal species. According to Aviado (1994), three situations increase the sensitivity of the heart to the effects of chlorofluorocarbons: the injection of epinephrine, coronary ischemia or cardiac necrosis, and experimental bronchitis or pulmonary thrombosis. A common feature of those situations is a direct or indirect increase in cardiac irritability caused by epineph-

rine. General anesthesia, however, reduces cardiac sensitivity to the effects of chlorofluorocarbons (Aviado 1994), so studies in which animals were anesthetized are not representative of exposures associated with cardiac effects in unanesthetized animals. Anesthesia in mice and rats has also been shown to block the accelerating effects of chlorofluorocarbons on heart rate and instead to result in bradycardia.

According to Aviado (1994), the mechanism of chlorofluorocarbon toxicity originates in irritation of the respiratory tract, which by a simple reflex response influences the heart rate before absorption of the compound. That is followed by depression of cardiac function after chlorofluorocarbon absorption and by sensitization of the heart to sympathomimetic amines (Aviado 1994). Jiao et al. (2006) investigated potential mechanisms of cardiac-sensitization arrhythmia induced by simultaneous exposure to halocarbons and epinephrine. They used rat cardiomyocytes and found that the combination of the halocarbon CF_3Br and epinephrine had a unique effect on the electrophysiology of cardiomyocytes, specifically, reduction in conduction velocity associated with phosphorylation of gap-junction channel proteins. Jiao et al. (2006) note that effects on other ion channels may contribute to the risk of arrhythmia during cardiac sensitization and that their cardiomyocyte recording system cannot directly demonstrate actual arrhythmic effects.

Among the species studied, the guinea pig is the most resistant to cardiovascular effects (Aviado 1994). The rat and mouse are intermediate in susceptibility, and the dog and monkey are more sensitive species. Experimental data indicate that the dog may be more sensitive than the monkey to cardiac effects. Aviado cautions against assuming that responses in the monkey would be more similar to those in humans, because of the lack of studies that would allow such a definitive comparison and because some studies had indicated that monkeys have no response or a response opposite that in dogs and other species.

In addition to the reflex-induced bronchospasm, chlorofluorocarbons are postulated to reduce pulmonary compliance by reducing pulmonary surfactants (Aviado 1994). On the basis of the effect of atropine pretreatment in blocking pulmonary resistance caused by chlorofluorocarbons in the anesthetized mouse, bronchoconstriction in the mouse appears to result from vagal innervation of the lungs. Depression of respiratory movements at high exposure is related to the anesthetic properties of these compounds. In general, the dog appears to be less sensitive to respiratory toxicity than the mouse or rat.

The rapid onset and reversibility of symptoms (such as cardiac and CNS effects within seconds to minutes) and the little adherence to Haber's Law are consistent with the rapid appearance of inhaled Freon 12 in the blood and its rapid elimination (Blake and Mergner 1974; Paulet et al. 1975a). Inhaled Freon 12 (500,000 ppm for 10 min) in dogs and rabbits diffused rapidly into the bloodstream, cerebral spinal fluid (evaluated in dogs only), urine, and bile and reached equilibrium in blood within 2 min in rabbits and 5 min in dogs (Paulet et al. 1975a). Emmen et al. (2000) reported nearly maximal blood concentrations in eight human subjects (exposure at 1,000 ppm and 4,000 ppm) in 15 min. Af-

ter 10-min exposure at 200,000 or 500,000 ppm, elimination of Freon 12 from the blood was complete within 85 sec in the rabbit and within 20-30 min in dogs (Paulet et al. 1975a).

Although inhalation of high concentrations of Freon 12 results in its rapid appearance in the blood because of its low blood:air partition coefficient, little is absorbed from the lungs. Single breath studies in human volunteers indicated that much of what is inhaled is exhaled unabsorbed (Morgan et al. 1972). The amount that reached the bloodstream from such a brief exposure was rapidly cleared: only a small fraction remained 5 min after exposure (Morgan et al. 1972). Adir et al. (1975) reported that inhalation of an administered dose of 3,225-4,506 mg over 12-20 min to three dogs or a dose of 777 mg over 16.75 min to a human volunteer resulted in near maximal blood concentration within the first 5 min. Consistent with their finding of a blood:air partition coefficient for Freon 11 that is 5-6 times higher than that for Freon 12, Adir et al. (1975) reported 77% of the administered dose of Freon 11 over the exposure period was absorbed in dogs compared with 55% of Freon 12. Freon 12 was also eliminated more rapidly from the blood within about 50 min for the dogs and the human subject compared with longer than 100 min for Freon 11. A review by WHO (1990) reported that studies in rats and monkeys indicated that Freon 12 is slightly more readily absorbed than Freon 114.

Emmen et al. (2000) reported biphasic elimination from blood in most of the eight human subjects that was independent of concentration (1-h exposure at 1,000 ppm or 4,000 ppm)—a mean half-life of 7 min for the first phase of elimination and a mean half-life of 36 min for the second phase. Elimination occurs largely by the lungs with little metabolism (less than 1%), as shown in dogs (6-20 min of ventilation at 8,000-12,000 ppm [Blake and Mergner 1974] and humans (less than 0.2%; 7-17 min of inhalation at 1,000 ppm [Mergner et al. 1975]). In the study of anesthetized dogs (Blake and Mergner 1974), essentially all the radiolabeled Freon 12 was exhaled within an hour, and only traces of radioactivity appeared in the urine or with exhaled carbon dioxide. That longer exposures (50-90 min) or pretreatment with phenobarbital did not change the results indicates little biotransformation. Adir et al. (1975) developed a pharmacokinetic model for predicting blood and tissue concentrations of Freon 12 in dogs and humans and concluded that continuous 8-h exposure at 1,000 ppm would result in a venous blood concentration that was well below concentrations reported to sensitize a dog's heart to intravenously injected epinephrine (Azar et al. 1973).

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

A number of organizations have established inhalation exposure levels or guidelines for Freon 12. Selected values are summarized in Table 5-4.

TABLE 5-4 Selected Inhalation Exposure Levels for Freon 12 from the NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	1,000	ACGIH 2001
NIOSH	REL-TWA	1,000	NIOSH 2004
OSHA	PEL-TWA	1,000	29 CFR 1910.1000
Spacecraft			
NASA	SMAC		Garcia 2000
	1-h	540	
	24-h	95	
	30-day	95	
	180-day	95	
Submarine			
NRC	EEGL		NRC 1984
	1-h	10,000	
	24-h	1,000	

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

Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; 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; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

COMMITTEE RECOMMENDATIONS

The committee's recommendations for EEGL and CEGL values for Freon 12 are summarized in Table 5-5. The current and proposed U.S. Navy values are provided for comparison.

TABLE 5-5 Emergency and Continuous Exposure Guidance Levels for Freon 12

Exposure Level	U.S. Navy Values (ppm)		Committee Recommended Values (ppm)
	Current	Proposed	
EEGL			
1-h	2,000	2,000	4,000
24-h	1,000	1,000	1,000
CEGL			
90-day	100	100	300

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level.

1-Hour EEGL

Exposure limits for Freon 12 should be set to prevent narcosis and other CNS effects, cardiac arrhythmia, tachycardia or bradycardia, and bronchoconstriction. Liver effects (fatty liver and necrosis) were noted in guinea pigs after continuous 90-day or repeated subchronic exposure, but no other species have shown these effects even in chronic studies. Data from human experimental studies can be used to set EEGLs with support from animal studies.

NRC (1984) recommended a 1-h EEGL of 10,000 ppm on the basis of a small decrease in psychomotor performance in two humans and no effects after exposure at 1,000 ppm for 2.5 h (Azar et al. 1972). NRC (1984) does not mention effects on ventilatory capacity and reductions in heart rate reported in a study of 11 subjects exposed to Freon 12 at 10,000 ppm and 17,200 ppm for 35 or 130 min (Valić et al. 1982). Effects at 10,000 ppm appeared to be mild with no significant change in FEV_1 or FVC and a significant reduction in MEF50 and MEF25 after the 35-min exposure (5.8% and 12% decrease, respectively) but not the 130-min exposure. Such effects would not be considered clinically significant (Pellegrino et al. 2005) or detrimental to submariners' performance in an emergency situation. Few studies by other research groups are available to evaluate pulmonary and heart-rate effects in humans at concentrations of about 10,000 ppm. Azar et al. (1972) reported no pulmonary or EKG effects but a 7% reduction in psychomotor scores in two subjects exposed for 2.5 h at 10,000 ppm but not at 1,000 ppm. Some studies have not found such effects at higher concentrations in animals (Table 5-3). Studies in humans and animals at around 1,000 ppm have generally reported few effects even after repeated exposure Emmen et al. (2000) exposed eight people and reported no pulmonary or EKG effects at air concentrations as high as 4,000 ppm for 1 h. This study is considered the most relevant for deriving a 1-h EEGL, with supporting information from the other short-term human studies involving a total of 21 people. No interspecies factor is necessary, because human data are used. No intraspecies factor was used, because of the consistency in responses from human subjects around 4,000 ppm. Specifically, effects in humans are reported to begin at about 10,000 ppm, and no effects were noted at concentrations as high as 4,000 ppm. Thirteen people exposed at 10,000 ppm for a little over 2 h (Valic et al. 1982; Azar et al. 1972) showed relatively mild effects that indicate that any possible effects at lower concentrations in more sensitive people would be acceptable for a 1-h exposure in an emergency situation. Eight people showed no significant effects related to chlorofluorocarbon toxicity at 4,000 ppm or 1,000 ppm for 1 h, and 10 additional subjects tested at 1,000 ppm for longer periods than 1 h (Azar et al. 1972; Stewart et al. 1978) showed a similar lack of effects in measures related to chlorofluorocarbon toxicity. Thus, the committee recommends a 1-h EEGL of 4,000 ppm.

24-Hour EEGL

No 24-h exposure studies in humans are available to evaluate an EEGL for this period. Mice exposed at 10,000 ppm for 24 h showed no clinical effects but had microscopic lung changes, such as increased leukocyte infiltration of alveolar walls relative to controls, which showed similar effects to a smaller degree (Quevauviller et al. 1963). However, sufficient detail was not provided to ascertain whether the effects were treatment-related, and pulmonary inflammation differs from the respiratory effects (bronchospasm) of chlorofluorocarbons in other studies. Animals exposed continuously at around 800 ppm for 90 days showed few treatment-related effects (Prendergast et al. 1967; see Table 5-3); given the nature of the effects (liver effects in guinea pigs), development of them would be of less concern after a 24-h exposure. NRC (1984) recommended a 24-h EEGL of 1,000 ppm on the basis of the lack of effects on psychomotor performance in two human subjects after 2.5 h of exposure at 1,000 ppm (Azar et al. 1972) and rapid elimination of Freon 12 in expired air. A key study for setting an EEGL is that by Stewart et al. (1978). This relatively well-conducted study examined a number of end points and reported that exposure of eight healthy human subjects at 1,000 ppm 8 h/day, 5 days/week for 2-4 weeks resulted in no effects on cognitive or motor function, changes in clinical measures, spirometry, EKG, EEG, or irritation symptoms (Stewart et al. 1978). Although the literature does not indicate much difference in effect level between 1-h and multihour exposures, potential mild effects on respiration, the CNS, or heart rate might not be as tolerable for a 24-h period, so use of a more protective EEGL for a 24-h than for a 1-h exposure period is justified. Thus, on the basis of the weight of evidence, a 24-h EEGL of 1,000 ppm appears to be appropriate inasmuch as there was little or no effect in human and animal studies. Given the concurrence of a number of studies in animals and humans on that concentration and the acceptability of mild effects in an emergency situation, no additional uncertainty factors are warranted.

90-Day CEGL

Longer-term and repeated studies of Freon 12 are generally without effect at about 1,000 ppm and in some cases higher (rats exposed at 10,000 ppm and dogs at 5,000 ppm 6 h/day for 90 days; Leuschner et al. 1983). Rats, guinea pigs, rabbits, monkeys, and dogs exposed at 807 ppm continuously for 90 days or at 836 ppm 8 h/day, 5 days/week for 6 weeks showed liver effects only in guinea pigs, which may be more sensitive to this effect than other species (Prendergast et al. 1967). Exposure to other chlorofluorocarbons has also been noted to be associated with fatty liver in guinea pigs (Aviado 1994). Studies in other species at higher concentrations and for longer exposure have not reported liver effects attributable to Freon 12 exposure.

Observations of lung congestion and nonspecific inflammatory changes in lung tissue were not noted to be treatment-related, as were the liver changes in guinea pigs reported by Prendergast et al. (1967). Although effects on the lungs have been associated with Freon 12 exposure at higher air concentrations, the lung effects also were noted in control animals and do not appear to be related to bronchoconstriction associated with Freon 12. Stewart et al. (1978) reported no effect on cognitive or motor function, clinical measures of blood and urine, spirometry, EKG, EEG, or irritation symptoms in eight healthy volunteers exposed at 1,000 ppm 8 h/day, 5 days/week for 2-4 weeks. A Freon 12 concentration of 1,000 ppm from Stewart et al. (1978) was thus used as the initial basis of a NOAEL, with support from the animal data of Prendergast et al. (1967). Application of an uncertainty factor of 3 to account for database uncertainties associated with the lack of longer-term continuous studies in humans resulted in a CEGL of 300 ppm. An uncertainty factor of 3 is supported by Freon 12 toxicokinetics, which indicate that cumulative effects would not occur with time, particularly at the concentrations tested, because Freon 12 is rapidly eliminated with little metabolism. The little metabolism also obviates the need to consider metabolic differences among humans. Thus, the mechanistic data, other studies in humans, and the longer-term animal studies are supportive of the use of an uncertainty factor of 3 applied to the NOAEL of Stewart et al. (1978).

DATA ADEQUACY AND RESEARCH NEEDS

Although several studies of various species are available, including controlled studies in a small number of human subjects, most of them are not recent (that is, within the last 20 years), and in many cases the full nature of effects and study methods could not be evaluated, because of limitations in reporting or because the studies were unpublished or otherwise not readily available for review. Information on the effects of chronic inhalation exposure, carcinogenicity, or male reproductive or immune system effects is generally less adequate. The available evidence, however, indicates that Freon 12 is rapidly absorbed and eliminated with little metabolism and that neither cancer nor most other toxic effects would be expected at the proposed EEGLs and CEGL. Additional studies to define the nature of effects at 1,000-10,000 ppm and the effects of chronic exposure would increase confidence in that prediction. Evidence from the literature also indicates that mixtures of chlorofluorocarbons may result in a lower effect level than predicted from the effect levels of individual chlorofluorocarbons alone. Thus, if mixtures of chlorofluorocarbons could be present in submarines, effect levels for the mixtures should be evaluated.

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6

Freon 114

This chapter summarizes the relevant epidemiologic and toxicologic studies on Freon 114, or 1,1,2,2-tetrafluoro-1,2,-dichloroethane. 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 committee considered all that information in its evaluation of the Navy's current and proposed 1-h, 24-h, and 90-day exposure guidance levels for Freon 114. The committee's recommendations for Freon 114 exposure levels are provided at the conclusion of this chapter 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

Freon 114 is a noncorrosive, nonflammable, colorless gas. At high concentrations, it has been found to have an ether-like odor (Budavari et al. 1996). Selected chemical and physical properties are listed in Table 6-1.

OCCURRENCE AND USE

Freon 114 has been used historically as a refrigerant and aerosol propellant in industrial settings, consumer products, and medical devices (NRC 1984; WHO 1990). The primary source of Freon 114 in the submarine environment is the air-conditioning system and refrigerant plants (NRC 1984; Crawl 2003). Several measurements of Freon 114 on submarines have been reported. Data collected on nine nuclear-powered ballistic missile submarines indicate an average Freon 114 concentration of 8 ppm (range, 0-146 ppm) and data collected on 10 nuclear-powered attack submarines indicate an average concentration of

TABLE 6-1 Physical and Chemical Properties of Freon 114

Synonyms and trade names	FC-114, cryofluorane, fluorocarbon 114, dichlorotetrafluoroethane, 1,2-dichlorotetrafluoroethane, halocarbon 114, tetrafluorodichloroethane, 1,1,2,2-tetrafluoro-1,2-dichloroethane, 1,2-dichloro-1,1,2,2-tetrafluoroethane
CAS registry number	76-14-2
Molecular formula	$C_2Cl_2F_4$
Molecular weight	170.93
Boiling point	3.8°C
Melting point	–94°C
Flash point	Nonflammable
Explosive limits	NA
Specific gravity of liquid	1.5312 at 0°C
Vapor pressure	1,444 mm Hg at 20°C
Solubility	Soluble in ether, alcohol, water (0.01%)
Conversion factors	1 ppm = 7 mg/m^3; 1 mg/m^3 = 0.14 ppm

Abbreviations: NA, not available or not applicable.
Sources: Flash point from OSHA 1999; all other data from Budavari et al. 1989, 1996; NRC 1984.

8 ppm (range, 0-99 ppm) (Hagar 2003). Holdren et al. (1995) reported the results of air sampling at three locations conducted over 6 h during the missions of two submarines. Sampling indicated concentrations of 1.225-1.540 ppm and 0.822-0.914 ppm, depending on the collection method, on one submarine and 1.608-2.072 ppm and 1.256-1.490 ppm, depending on the collection method, on the other submarine. Raymer et al. (1994) report the results of a similar sampling exercise (two submarines, three locations, and sampling duration of 6 h). Freon 114 concentrations were reported as 4.2 and 7.0 ppm in the fan rooms, 2.8 and 7.0 ppm in the galleys, and 5.6 ppm in the engine rooms.

SUMMARY OF TOXICITY

The toxicity of Freon 114 has been studied in a number of mammalian species for acute effects, particularly those involving cardiopulmonary function. Few repeat-exposure studies have been conducted with Freon 114. Most of the studies with Freon 114 were conducted before 1975, and only a few toxicity end points were included. Freon 114, like other chlorofluorocarbons (CFCs), has a relatively low degree of acute toxicity by inhalation (for example, the 2-h inhalation LC_{50} in rats is over 600,000 ppm); central nervous system (CNS) and pulmonary-depressant and cardiac-sensitizing effects occur at relatively high exposure concentrations. Estimated EC_{50} values in dogs for cardiac sensitization

are less than 50,000 ppm when the intravenous (iv) epinephrine dose is 8 μg/kg, 100,000 ppm when it is 5 μg/kg, and over 100,000 ppm in dogs exercised to induce endogenous epinephrine release. Respiratory and circulatory effects, such as bronchospasm and changes in heart rate, have also been observed in animals with acute, high-concentration exposure to Freon 114. The few human data are consistent with the animal data in terms of mild respiratory and cardiac effects at 21,000 ppm. Because the cardiac arrhythmias seen after Freon 114 exposure of animals require high exposure to Freon 114 and epinephrine nearly simultaneously, CNS-depressant effects are more likely than cardiac effects to occur at lower concentrations. Effects of repeated exposure to Freon 114 are generally similar to those of acute exposure. Freon 114 is rapidly absorbed and eliminated almost entirely by exhalation with little metabolism. It produced no mutations in an abbreviated Ames/Salmonella test. Data for assessing reproductive, immune system, and carcinogenic effects of exposure to Freon 114 were not available for review.

Effects in Humans

Accidental Exposures

Reinhardt et al. (1971) reported that from 1967 to 1971 65 deaths were associated with the practice of "sniffing," or intentionally inhaling, aerosol products, including those with fluorocarbon propellants. Freon 114 was widely used in consumer aerosol products at the time, but no deaths were specifically attributed to it. The CFCs commonly used as aerosol propellants, including Freon 114, have been evaluated as potential causal agents in the deaths of young asthmatics using bronchodilator inhalers. However, none of the reports identifies Freon 114 as a cause of death.

Experimental Studies

Valić et al. (1977) demonstrated in humans that CFCs, including Freon 114, are able to induce bronchospasm, a biphasic change in ventilatory capacity (maximum expiratory flow at 50% [MEF50] and 75% [MEF75] of vital capacity), bradycardia, and inversion of the T wave with a single acute inhalation. Valić and co-workers exposed 10 men (20-24 years old) to Freon 114 at 21,000 ppm for 15 or 60 sec or to a 30:70 mixture of Freon 12 and 114 (about 7,000:14,000 ppm for 15 sec or about 8,300:15,680 ppm for 60 sec). After exposure for 45 sec, significant biphasic reduction in ventilatory lung capacity (MEF50), bradycardia, and increased variability in heart rate were reported for Freon 114, and the mixture had a more pronounced effect. Exposure to Freon 114 and to the Freon 12 and 114 mixture for 15 sec caused similar reductions in MEF50. Cardiac effects included inversion of the T wave in a few cases and atrioventricular block in one case with a 15-sec exposure to Freon 114 but no

life-threatening cardiac arrhythmia. No data were reported on effects on MEF75 after exposure to Freon 114. No threshold for cardiopulmonary effects was demonstrated in this study. However, Aviado (1994) concluded from the work of Stewart et al. (1978) with Freon 11 that human exposure at 1,000 ppm 8 h/day, 5 days/week for 18 exposures was without evidence of electrocardiographic changes, pulmonary-function changes, or subjective effects. Because Freon 11 is considered more cardiotoxic than Freon 114 (Aviado 1994), the no-observed-adverse-effect level (NOAEL) of Freon 114 would be expected to be higher than 1,000 ppm.

Occupational and Epidemiologic Studies

No occupational or epidemiologic studies of Freon 114 were identified. Deaths related to CFC exposure have been reported in the occupational environment associated with solvent use, foam-blowing, and refrigeration leaks; however, none of the reports is linked to Freon 114 exposure (see Aviado [1994] for a review of this topic).

Effects in Animals

The toxicity of Freon 114 has been studied in several animal species, including rats, mice, guinea pigs, dogs, cats, and monkeys. The primary effects of exposure to Freon 114 via inhalation are cardiopulmonary effects that can be induced in mice, rats, dogs, and rhesus monkeys. The majority of cardiopulmonary testing has been conducted in dogs with protocols that involve either iv injection of epinephrine or increases in endogenous epinephrine through exercise or induction of fright. Studies in dogs have generally been conducted in conscious animals; those in other species have used anesthetized animals. Because general anesthesia makes the heart less responsive to epinephrine, it is a confounding factor that needs to be considered in interpreting the animal data. In guinea pigs, fatty infiltration of the liver has been reported after exposure to Freon 114; however, this effect has not been reported in other species. In general, the effects observed in acute-exposure animal studies are consistent with the few human data available on Freon 114, and the effects observed in repeat-dose animal studies are consistent with those observed in acute-exposure animal studies.

Acute Toxicity

Acute-toxicity data on Freon 114 are summarized in Table 6-2. Reviews of Freon 114 have been conducted by Aviado (1994), ACGIH (1986, 2001), WHO (1990), and NRC (1984).

TABLE 6-2 Summary of Toxicity of Freon 114 in Animals

Species (no.)	Exposure Period	End Point	NOAEL (ppm)	Adverse-Effect Level (ppm)	Reference
Dog (12)	30 sec	Mild effect on heart rhythm in 1 dog, marked effect on heart rhythm in 1 dog, convulsions in 5 dogs	—	800,000	Reinhardt et al. 1971
Dog (7)	1.5-16 min	1 dog had marked cardiac effects while running at 300 fpm to induce epinephrine release; response was replicated in follow-up exposure; exposure cut short because dogs could not tolerate it	—	40,630	Mullin et al. 1972
Dog (7)	1.5-16 min	1 dog had marked cardiac response after 1.5 min of exposure; exposure cut short because dogs could not tolerate it	—	60,600	Mullin et al. 1972
Dog (12)	5 min	Cardiac arrhythmia in 1 dog with epinephrine	—	25,000	Reinhardt et al. 1971
Dog (12)	5 min	Marked cardiac response in 7 dogs; EC_{50}, <50,000 ppm	—	50,000	Reinhardt et al. 1971
Dog (4)	5 min	Cardiac sensitization in dogs given epinephrine at 5 μg/kg; EC_{50}, 100,000 ppm	25,000, 50,000	100,000	Clark and Tinston 1972
Dog (3)	5 min	Pulmonary resistance, pulmonary compliance, respiratory minute volume, heart rate, and aortic blood pressure measured in anesthetized dogs, supplemental oxygen administered	25,000	50,000, 100,000, 200,000	Belej and Aviado 1975
Rhesus monkey (3)	5 min	Cardiac arrhythmia and other cardiopulmonary effects in anesthetized open-chest preparations	—	50,000, 100,000, 200,000	Belej et al. 1974; Aviado and Smith 1975

(Continued)

TABLE 6-2 Continued

Species (n)	Exposure Period	End Point	NOAEL (ppm)	Adverse-Effect Level (ppm)	Reference
Mouse (3 or 4 males)	6 min	No cardiac changes in anesthetized animals, supplemental oxygen administered	400,000	—	Aviado and Belej 1974
Mouse (3 or 4 males)	6 min	Cardiac changes in anesthetized animals given epinephrine, second-degree block observed	100,000	200,000, 400,000	Aviado and Belej 1974
Dog (6)	16 min	No cardiac arrhythmia while running for 25 min at 300 fpm to induce epinephrine release	25,300	—	Mullin et al. 1972
Rat (8)	<30 min	Cardiac and pulmonary function at constantly rising concentration of Freon 114 in anesthetized animals	50,000	100,000	Friedman et al. 1973
Mouse (10/group)	30 min	Five of 10 died at 700,000 ppm, eight of 10 at 800,000 ppm	500,000	700,000, 800,000	Paulet and Desbrousses 1969
Mouse	30 min	No deaths	~700,000	—	Paulet 1969, as cited in WHO 1990
Rat	30 min	No deaths	~750,000	—	Paulet 1969, as cited in WHO 1990
Rabbit	30 min	No deaths	~750,000	—	Paulet 1969, as cited in WHO 1990
Guinea pig	2 h	Irregular breathing but “no toxic action”	—	8,000-47,000	Nuckolls 1933, as cited in ACGIH 1986
Rat	2 h	Incoordination	—	~300,000	Schloz 1961, as cited in WHO 1990
Rat	2 h	Deep narcosis	—	~600,000	Schloz 1961, as cited in WHO 1990

Rat, guinea pig	2 h	Disturbed equilibrium in rats and guinea pigs	—	300,000-400,000	Schloz 1962, as cited in WHO 1990
Dog (1)	8 h	No death	200,000	—	Yant et al. 1932, as cited in WHO 1990
Dog (1)	16 h	Death	—	200,000	Yant et al. 1932, as cited in WHO 1990
Guinea pig	24 h	Incoordination	—	~400,000	Schloz 1961, as cited in WHO 1990
Mouse (3)	24 h	No clinical effects but microscopic evidence of hemorrhage in lungs	—	10,000	Quevauviller et al. 1963
Dog (5)	8 h/day, 3-4 days	All five died	—	200,000	Yant et al. 1932, as cited in WHO 1990
Guinea pig (6)	8 h/day, 4 days	Fatty degeneration of liver	—	200,000	Yant et al. 1932, as cited in WHO 1990
Dog (3)	8 h/day, 21 days	Incoordination, tremors, convulsions during initial 3-5 days of exposure; tolerance developed	—	141,000	Yant et al. 1932, as cited in WHO 1990
Rat (10)	2.5 h/day, 5 days/week, 2 weeks	Decreased body-weight gain and blood polymorphonuclear leukocytes, increased blood lymphocytes, vascular congestion, and exudates in lungs	—	200,000	Paulet and Desbrousses 1969
Mouse (10)	2.5 h/day, 5 days/week, 2 weeks	Decrease of 9% in body weight	—	200,000	Paulet and Desbrousses 1969

(Continued)

TABLE 6-2 Continued

Species (n)	Exposure Period	End Point	NOAEL (ppm)	Adverse-Effect Level (ppm)	Reference
Rat (20 adult, 10 immature), mouse (20 adult)	2.5 h/day, 5 days/week, 2 months	No significant effect on mortality, body-weight gain, blood-cell counts, pulmonary pathology, or electrolytes	10,000	—	Paulet and Desbrousses 1969
Cat, rat, guinea pig, dog	3.5 h/day, 5 days/week, 20 exposures over 4 weeks	No adverse effects	100,000	—	Schloz 1962, as cited in WHO 1990
Guinea pig (8)	8 h/day, 21 days	Fatty degeneration of liver	—	141,000	Yant et al. 1932, as cited in WHO 1990
Dog (3/sex per group)	6 h/day, 7 days/week, 90 days	No significant effect on mortality, body weight, clinical examinations, EKG, blood pressure, hematology, clinical chemistry, urinalysis, necropsy, organ weights, or histopathology	5,000	—	Leuschner et al. 1983
Rat (20/sex per group)	6 h/day, 7 days/week, 90 days	No significant effect on mortality, body weight, clinical examinations, hematology, clinical chemistry, urinalysis, necropsy, organ weights, or histopathology	10,000	—	Leuschner et al. 1983
Rat, rabbit	2 h/day, 5 days/week, 8 months (rats), 9 months (rabbit)	No significant changes in hematology, histopathology, or EEG	10,000	—	Desoille et al. 1973

Mouse (30), rat (8/sex per group), dog (1-3/sex per group)	Mice, rats, puppies: 5 min twice a day; dogs: twice a day to discharge of propellant	No significant effect on body weight, hematology, clinical chemistry, or histopathology	164-2,240 mg/kg per day; mixture containing 25-50% Freon 114	—	Smith and Case 1973

Abbreviations: CNS, central nervous system; EKG, electrocardiography; EEG, electroencephalography; fpm, feet per minute; MEF50, MEF75-maximum expiratory flow at 50%, 75% of vital capacity.

Paulet and Desbrousses (1969) reported that mortality in mice exposed to Freon 114 for 30 min was none of 10 at 500,000 ppm, five of 10 at 700,000 ppm, and eight of 10 at 800,000 ppm. Exposure of a dog to Freon 114 for 16 h at about 200,000 ppm was lethal, but exposure for 8 h was not lethal (Yant et al. 1932, as cited in WHO 1990).

No deaths were reported in mice, rats, or rabbits exposed to Freon 114 for 30 min at about 700,000, about 720,000, or about 750,000 ppm, respectively (Paulet 1969, as cited in WHO 1990). Nuckolls (1933, as cited in ACGIH 1986) reported irregular breathing but no toxic action in guinea pigs exposed at 8,000-47,000 ppm for 2 h. Scholz (1961, as cited in WHO 1990) reported incoordination in guinea pigs exposed at about 400,000 ppm for 24 h. Scholz (1961, as cited in WHO 1990) reported incoordination (at about 300,000 ppm) and deep narcosis (at about 600,000 ppm) in rats exposed to Freon 114 for 2 h. Quevauviller et al. (1963) exposed three mice to Freon 114 at 10,000 ppm for 24 h without observing clinical effects, but hemorrhage was observed in the lungs on microscopic examination.

Cardiac sensitization (an increase in the reactivity of the heart to epinephrine) was demonstrated in one of 12 beagles exposed to Freon 114 at 25,000 ppm for 5 min (Reinhardt et al. 1971). Seven of the 12 dogs experienced a marked cardiac response at 50,000 ppm for 5 min. The marked response included ventricular fibrillation and cardiac arrest in two dogs. The authors reported that at high concentrations of Freon 12, an exposure time of only 30 sec was sufficient to induce cardiac sensitization and that hypoxia made the heart more sensitive to induction of sensitization. In an experiment designed to induce the release of endogenous epinephrine, they exposed 12 beagles to an atmosphere of Freon 114 at 800,000 ppm and oxygen at 200,000 ppm while surprising the dogs with loud noises. That exposure paradigm resulted in mild effects in one dog, a marked effect in one dog (bigeminal rhythm with areas suggestive of multiple ventricular beats), and convulsions in five dogs (mild seizures characterized by spasticity of the extremities). Similar effects in dogs were demonstrated by Mullin et al. (1972) at a Freon 114 concentration of 40,630 ppm but not 25,000 ppm in dogs exercising on a treadmill to induce release of endogenous epinephrine. Clark and Tinston (1972) reported an EC_{50} for cardiac sensitization of 100,000 ppm in beagles that were pretreated with epinephrine at 5 μg/kg and exposed to Freon 114 for 5 min. Exposure at 25,000 and 50,000 ppm did not cause cardiac abnormalities in epinephrine-pretreated dogs. Anesthetized mice exposed to Freon 114 at 100,000-400,000 ppm did not develop cardiac arrhythmias, but pretreatment with epinephrine induced second-degree block in one or two mice exposed at 200,000 or 400,000 ppm, respectively (Aviado and Belej 1974).

The effects of Freon 114 on cardiac and pulmonary function were measured in anesthetized dogs that were given Freon 114 vapor at 25,000, 50,000, 100,000, or 200,000 ppm via the endotracheal route for 5 min with 15-min washout periods between exposures (Belej and Aviado 1975). At 25,000 ppm, no effects on measures of pulmonary resistance, pulmonary compliance, respira-

tory minute volume, heart rate, and aortic blood pressure were observed. At 50,000 ppm, there was a significant reduction in pulmonary compliance but not the other measures. At 100,000 and 200,000 ppm, pulmonary resistance increased, pulmonary compliance was reduced, and heart rate increased significantly; a reduction in aortic blood pressure occurred only at 200,000 ppm. Cardiac and pulmonary effects have also been studied after administration of Freon 114 as an aerosol directly into the upper airway or into the trachea of dogs. Dose measurement was by cylinder actuations (five, 10, or 20 actuations); each actuation resulted in the release of 120 mL of Freon 114 under pressure. Aerosol application into the upper airway resulted in an irritation reflex that induced bradycardia and bronchoconstriction. Administration of 15-20 actuations into the trachea induced tachycardia. Bronchodilation was induced by one to 15 actuations, and 20 actuations induced bronchoconstriction and exaggerated epinephrine-induced tachycardia.

Cardiac and pulmonary function was studied in anesthetized rats exposed to Freon 114 at a constantly rising concentration (Friedman et al. 1973). No effect on function was observed at 50,000 ppm, but at 100,000 ppm, heart rate and tidal volume decreased, and pulmonary resistance increased. At 150,000 ppm, respiratory rate decreased, and pulmonary resistance no longer increased.

Cardiac and pulmonary function was also studied in anesthetized rhesus monkeys with an open-chest model (Belej et al. 1974; Aviado and Smith 1975). Groups of three monkeys were exposed to Freon 114 at 50,000-200,000 for 5 min. Concentrations of 100,000-200,000 ppm induced tachycardia, hypotension, respiratory depression, and an increase in respiratory stimulation. The majority of the findings were statistically significant only at 200,000 ppm. The authors reported cardiac arrhythmia at 50,000-100,000 ppm, but no data were presented on this end point.

After a series of studies in different species, Aviado (1975a,b) classified Freon 114 as a low-pressure propellant of intermediate toxicity on the basis of respiratory and cardiac effects in multiple species (monkeys, dogs, rats, and mice) at exposures of 50,000-200,000 ppm.

Repeated Exposure and Subchronic Toxicity

No adverse effects were reported in cats, rats, dogs or guinea pigs exposed to Freon 114 at 100,000 ppm 3.5 h/day for 20 exposures (Scholz 1961, as cited in WHO 1990).

Groups of 30 rats (20 adult and 10 immature) and 20 mice (adult) were exposed to Freon 114 at 10,000 ppm 2.5 h/day, 5 days/week for 2 months with no deaths or other adverse effects on body-weight gain, blood-cell counts, and pulmonary pathology (Paulet and Desbrousses 1969). Exposure of rats at 200,000 ppm 2.5 h/day, 5 days/week for 2 weeks produced a decrease in body-weight gain, a decrease in blood polymorphonuclear leukocytes, and an increase in blood lymphocytes (Paulet and Desbrousses 1969). Vascular congestion and

exudates were observed microscopically in the pulmonary alveoli and bronchioles. Mice exposed with the same experimental protocol lost about 9% of their body weight.

Exposure of dogs to Freon 114 at 200,000 ppm 8 h/day was lethal to all five dogs in 3-4 days (Yant et al. 1932, as cited in WHO 1990). At 141,000 ppm, dogs developed incoordination, tremor, and convulsions during the first exposures, but tolerance developed after 3-5 days of the 21-day study (Yant et al. 1932, as cited in WHO 1990).

In the guinea pig, slight liver effects (fatty degeneration) were observed at 200,000 ppm (8 h/day for 4 days) and 141,000 (8 h/day for 21 days) (Yant et al. 1932, as cited in WHO 1990).

Leuschner et al. (1983) found no adverse effects in dogs (three per sex per group) exposed to Freon 114 at 5,000 ppm 6 h/day, 7 days/week for 90 days. Similarly, groups of 20 rats per sex exposed at 10,000 ppm 6 h/day, 7 days/week for 90 days showed no adverse effects (Leuschner et al. 1983). Exposure to Freon 114 had no toxic effects on mortality, body weight, clinical examinations, hematology, clinical chemistry, urinalysis, necropsy, organ weights, and histopathology in both rats and dogs. The dogs were also examined for electrocardiographic effects and effects on blood pressure (before and after norepinephrine administration), and no adverse effects were found.

Repeated brief inhalation exposure of mice, rats, and dogs (5 min or the time it took to discharge an aerosol container) resulted in doses of a propellant containing 25-50% Freon 114 of 164-2,240 mg/kg per day for 2 weeks to 23 months. Other than ataxia and slight sedation in dogs that lasted only a few minutes, no effect on body weight and no hematologic, clinical-chemistry, or histopathologic effects were observed (Smith and Case 1973).

Repeated application of a 40% solution of Freon 114 in sesame oil had no effect on rabbit skin (Schloz 1962, as cited in WHO 1990). Repeated application of a Freon 114 spray resulted in local irritation of rat skin and the mucous membranes of rabbit eyes (Quevauviller 1965 and Quevauviller et al. 1964, as cited in ACGIH 2001). No injury to the eye was observed microscopically.

Chronic Toxicity

Desoille et al. (1973) exposed male rats and rabbits to Freon 114 at 10,000 ppm 2 h/day, 5 days/week for 8 months (rats) or 9 months (rabbits). No significant changes in clinical end points, hematology, histopathology, or electroencephalography were identified.

Reproductive Toxicity in Males

No relevant information on male reproductive toxicity after exposure to Freon 114 was located. Although some hydrogenated CFCs have been reported

to disrupt spermatogenesis, no CFCs have been reported to cause similar effects (Aviado 1994).

Immunotoxicity

No relevant information on immunotoxicity after exposure to Freon 114 was located. No other CFC has been reported to cause immunotoxicity (Aviado 1994).

Genotoxicity

Freon 114 was negative for reverse mutation in *Salmonella typhimurium* strains TA 100 and TA1535 in the presence of a rat liver, Aroclor 1254-induced S9 mixture (Longstaff et al. 1984).

Carcinogenicity

A group of 30 female mice was exposed via inhalation to a propellant mixture that contained 25% Freon 114 for 23 months without evidence of lung tumors (Smith and Case 1973). The dose of Freon 114 that was inhaled was calculated to be 970 mg/kg per day based on the respiratory volume of mice (Smith and Case 1973). No other animal cancer bioassay data were identified on Freon 114. Freon 11, a prototypical CFC, did not show carcinogenicity in a National Cancer Institute (NCI) rodent bioassay (Aviado 1994). That result is important because it differentiates CFCs from carbon tetrachloride, which did produce evidence of carcinogenicity in a similar NCI bioassay (Aviado 1994).

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

The most serious effect of Freon 114 after inhalation is cardiac toxicity, which has been demonstrated in four animal species. According to Aviado (1994), three situations increase the sensitivity of the heart to effects of CFCs—injection of epinephrine, coronary ischemia or cardiac necrosis, and bronchitis or pulmonary thrombosis. A common feature of those situations is a direct or indirect increase in cardiac irritability caused by epinephrine. In contrast, general anesthesia reduces cardiac sensitivity to the effects of CFCs (Aviado 1994). The mechanism of CFC toxicity originates in irritation of the respiratory tract, which by a simple reflex response influences the heart rate before absorption of the CFC (Aviado 1994). That is followed by depression of cardiac function after absorption of the CFC and sensitization of the heart to sympathomimetic amines (Aviado 1994). Of the species studied after exposure to CFCs, the guinea pig is the most resistant to cardiovascular effects. The rat and the mouse are intermediate in susceptibility, and the dog and the monkey are more sensitive. Data de-

veloped with Freon 114 indicate that the dog may be more sensitive than the monkey to cardiac effects. According to Aviado, there is no reason to consider the monkey the more appropriate species for extrapolation of CFC effects to humans. In addition to the reflex-induced bronchospasm caused by CFCs, CFCs are postulated to reduce pulmonary compliance by reducing pulmonary surfactants (Aviado 1994).

Freon 114 can be absorbed through the skin and gastrointestinal tract, but inhalation is the most common route of exposure, and exhalation the most important route of elimination. Peak blood concentrations of Freon 114 are reached immediately at the end of a short-term aerosol or vapor exposure (Dollery et al. 1970; Morgan et al. 1972). Using ^{38}Cl-labeled Freon 114, Morgan et al. (1972) found that although Freon 114 appears rapidly in the blood after inhalation, it is poorly absorbed and relatively rapidly exhaled because of its poor lipid solubility. Pulmonary elimination of ^{38}Cl-labeled Freon 114 is rapid after cessation of exposure: more than 50% is eliminated within the first minute after exposure. It is assumed that Freon 114, like Freon 12, diffuses rapidly into the cerebral spinal fluid and is excreted in the urine and bile (Paulet et al. 1975). Retention of ^{38}Cl-labeled Freon 114 in the human body was 12.3% ± 4.1% (SD) 30 min after inhalation of a single breath (Morgan et al. 1972). After 30 min, the amount of exhaled Freon 114 is low, and further excretion is slow. Similar results were obtained in dogs exposed to an aerosol of Freon 114 by Shargel and Koss (1972). When dogs were exposed to Freon 114 vapor at 50,000 ppm via a face mask, blood concentrations of Freon 114 peaked at about 15 min; after exposure at 100,000 ppm, a plateau was reached at about 10 min (Clark and Tinston 1972). The threshold for cardiac sensitization induced by a dose of epinephrine at 5 μg/kg was 50,000 ppm (a concentration at which none of four dogs was sensitized) to 100,000 ppm (a concentration at which two of four dogs were sensitized) (Clark and Tinston 1972). Cardiac sensitization associated with Freon 114 is a temporary effect that requires maintenance of critical concentrations of Freon 114 and epinephrine to persist in the dog (Clark and Tinston 1972). Reduction of blood Freon 114 concentrations below a critical concentration eliminates sensitization of the heart.

Adir et al. (1975) developed a pharmacokinetic model for predicting blood and tissue concentrations of Freon 12 in dogs and humans. On the basis of the model, they concluded that continuous 8-h exposure at 1,000 ppm would result in a venous blood concentration that was well below concentrations reported to sensitize the dog heart to intravenously injected epinephrine (Azar et al. 1973). Because the EC_{50}s for cardiac sensitization of Freon 12 and Freon 114 are similar (80,000 ppm and 100,000 ppm, respectively), their blood concentrations at the EC_{50}s are similar (35 μg/mL and 34 μ/mL, respectively), and their uptake and elimination curves are similar (Clark and Tinston 1972), the safety level predicted for Freon 12 by Adir et al. (1975) may also apply to Freon 114.

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

A number of organizations have established or proposed inhalation exposure levels or guidelines for Freon 114. Selected values are summarized in Table 6-3.

COMMITTEE RECOMMENDATIONS

The committee's recommendations for EEGL and CEGL values for Freon 114 are summarized in Table 6-4. The current and proposed U.S. Navy values are provided for comparison.

EEGL and CEGL values for Freon 114 should prevent significant CNS depression; changes in cardiac rhythm, including cardiac arrhythmia; and pulmonary changes, including bronchospasm and reduction in pulmonary compliance. The scientific literature has not identified other potential human health effects of exposure to Freon 114 or other prototypical CFCs. The accumulation of fat in the liver has been reported in guinea pigs exposed to Freon 114; however, this effect has not been observed in other animal species. In a series of experiments with mice, rats, monkeys, and dogs exposed to Freon 114 at high concentrations for brief periods (5 min), Aviado (1973) identified the dog as the most sensitive species for induction of cardiac and pulmonary effects and 50,000 ppm as the lowest effective concentration of Freon 114. In other studies with dogs, 50,000 ppm is a NOAEL for cardiac effects in dogs given epinephrine at 5 μg/kg, 25,000 ppm is a NOAEL for induction of cardiac effects in exercising dogs, and 25,000 ppm is a concentration at which one of 12 dogs receiving epinephrine at 8 μg/kg had cardiac effects (Mullin et al. 1972; Reinhardt et al. 1971; Clark and Tinston 1972). Valić et al. (1977) demonstrated bronchospasm and mild cardiac effects in young men exposed to Freon 114 at 21,000 ppm for 15, 45, or 60 sec. Studies demonstrating CNS-depressant effects have been conducted only at high exposures in laboratory animal species.

On the basis of a review of the cardiac and pulmonary toxicity of a series of CFCs, including Freon 114 and Freon 12, Aviado (1975a,b, 1994) was able to classify the CFCs as of high, intermediate, and low toxicity. Cardiac and pulmonary test results from studies on multiple species resulted in classification of Freon 114 and Freon 12 as having intermediate toxicity and significantly less toxic than Freon 11. Freon 114 and Freon 12 are similar in cardiac and pulmonary toxicity; hypotension occurs in dogs exposed to Freon 114 but not in dogs exposed to Freon 12. There are some differences in respiratory response between Freon 114 and Freon 12, but they tend to be related to the species tested and do not indicate a clear difference between Freon 114 and Freon 12. Although Freon 12 and Freon 114 appear to behave similarly, the 1-h EEGL and

TABLE 6-3 Selected Inhalation Exposure Levels for Freon 114 from the NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	1,000	ACGIH 2001
NIOSH	REL-TWA	1,000	NIOSH 2005
OSHA	PEL-TWA	1,000	29 CFR 1910.1000
Submarine			
NRC	EEGL		NRC 1984
	1-h	10,000	
	24-h	1,000	
	CEGL		
	90-day	100	

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

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

TABLE 6-4 Emergency and Continuous Exposure Guidance Levels for Freon 114

Exposure Level	U.S. Navy Values (ppm)		Committee Recommended Values (ppm)
	Current	Proposed	
EEGL			
1-h	2,000	2,000	2,000
24-h	1,000	1,000	1,000
CEGL			
90-day	100	100	125

Abbreviations: CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level.

90-day CEGL differ for the two compounds (see sections that follow), because the database on Freon 114 is not as robust as that on Freon 12, and a more conservative approach was required for Freon 114.

Intraspecies uncertainty factors were not applied to the derivation of the EEGL and CEGL values, because there is little evidence of metabolic or pharmacokinetic differences underlying the toxicity of Freon 114. Furthermore, data on epinephrine-sensitized animals were included in the assessment of Freon

114, and this provides an additional level of safety for potentially sensitive individuals.

1-Hour EEGL

As indicated above (see Table 6-2), 25,000 ppm is the lowest observed-adverse-effect level (LOAEL) for cardiac effects in the epinephrine-sensitized dog and the NOAEL for cardiac effects in exercising dogs exposed for 16 min to Freon 114. Valić et al. (1977) was able to demonstrate mild cardiac and pulmonary effects in young men exposed to Freon 114 at 21,000 ppm for less than 60 sec. Using 21,000 ppm as the LOAEL for exposure of humans to Freon 114, the application of an uncertainty factor of 10 to convert a LOAEL to a NOAEL results in a proposed EEGL of about 2,000 ppm based on cardiac and pulmonary effects. No time adjustment has been made in this proposal, because exposure at 21,000 ppm appears to result in a blood Freon 114 concentration that is approaching, but below, the critical level for induction of significant cardiac effects in humans, whereas exposure at 2,000 ppm should result in a blood concentration that is well below the critical level for cardiac effects. Exercising dogs, which are considered a sensitive test model, exposed at a similar concentration (25,000 ppm) did not show cardiac effects after a 16-min exposure to Freon 114. Exposure at 2,000 ppm would not be expected to result in a blood Freon 114 concentration that would approach the critical level for cardiac effects.

A proposed EEGL can also be based on CNS depression; however, the database on CNS depression is not as robust as that on cardiac and pulmonary end points. In rats, mice and rabbits, exposure at 700,000-750,000 ppm for 30 min produced no deaths (Paulet 1969). Paulet and Desbrousses (1969) reported no effect in mice exposed at 500,000 ppm, but 700,000 ppm was an LC_{50}. Incoordination, an indication of CNS depression, was observed in rats during or shortly after exposure to Freon 114 at about 300,000 for 2 h (Scholz 1961, as cited in WHO 1990). If 300,000 ppm is used as the LOAEL for CNS depression, an uncertainty factor of 3 can be applied to account for interspecies differences in sensitivity to Freon 114, an uncertainty factor of 10 can be applied to estimate a NOAEL from a LOAEL, and a database uncertainty factor of 3 can be applied to account for the quality of the data. Application of those three uncertainty factors results in a proposed 1-h EEGL of about 3,000 ppm.

Additional evidence that exposure at 2,000-3,000 ppm is unlikely to result in adverse effects is provided by the absence of effects in dogs examined after repeated exposure at 5,000 ppm 6 h/day, 7 days/week for 90 days, in rats after exposure at 10,000 ppm 2-6 h/day for as long as 8 months, and in rabbits after exposure at 10,000 ppm 2 h/day for 9 months (Paulet and Desbrousses 1969; Desoille et al. 1973; Leuschner et al. 1983).

On the basis of the overall weight-of-evidence approach, an EEGL value of 2,000 ppm is proposed to protect against CNS, cardiac, and pulmonary effects associated with exposure to Freon 114.

24-Hour EEGL

Two studies with 24-h exposure durations have been published. Guinea pigs exposed to Freon 114 at about 400,000 ppm for 24 h of were reported to show incoordination (Scholz 1961, as cited in WHO 1990). Mice exposed to Freon 114 at 10,000 ppm for 24 h showed no clinical signs of toxicity, but hemorrhage was visible in the lungs on histopathologic examination (Quevauviller et al. 1963, as cited in ACGIH 1986). Studies by Paulet and Desbrousses (1969), Desoille et al. (1973), and Leuschner et al. (1983) did not find similar evidence of pulmonary hemorrhage in multiple species of experimental animals even after multiple exposures to Freon 114. Pulmonary hemorrhage has not been identified as an effect associated with exposure to any of the CFCs (Aviado 1994). Pulmonary hemorrhage was not considered a significant effect of exposure of humans to Freon 114, because of the lack of reproducible data demonstrating pulmonary hemorrhage after exposure and because it commonly occurs during euthanasia of experimental animals. Using 10,000 ppm as a NOAEL, the committee applied an uncertainty factor of 3 to account for interspecies differences in sensitivity and an uncertainty factor of 3 to account for inadequacies in the database. No time adjustment was used to derive the EEGL, because the length of the exposure period in the animal studies was 24 h. Application of the two uncertainty factors results in a proposed 24-h EEGL of 1,000 ppm.

90-Day CEGL

A number of repeat-exposure studies of Freon 114 have been conducted at high concentrations for short periods, typically 2-3 h, each day. A small number of longer-duration studies have been conducted with Freon 114. In studies of dogs and guinea pigs exposed to Freon 114 at 141,000 ppm 8 h/day for 21 days, acute CNS signs were initially seen in dogs, but tolerance developed, and fatty livers were observed in guinea pigs (Yant et al. 1932, as cited in WHO 1990). Leuschner et al. (1983) reported that dogs exposed at 5,000 ppm and rats exposed at 10,000 ppm showed no adverse effects after exposures that lasted 6 h/day, 7 days/week for 90 days. Because dogs are generally considered more sensitive to the effects of Freon 114 than rodents, the 5,000-ppm dog exposure reported by Leuschner et al. (1983) was used as a NOAEL for Freon 114. In contrast with the database on Freon 12, there are no 90-day continuous-exposure studies or human repeated-exposure studies on which to base the CEGL value. Therefore, unlike the situation with Freon 12, a time-adjustment factor of 4 is applied to the NOAEL from the dog study, yielding a value of 1,250 ppm. In the absence of more robust data from animal and human exposure studies, that ap-

proach will result in a more conservative CEGL value. Application of two uncertainty factors of 3 to account for interspecies variability and the quality of the database results in a proposed CEGL value of 125 ppm.

DATA ADEQUACY AND RESEARCH NEEDS

Most of the studies in the database on Freon 114 were conducted before 1975 and the publication of standardized protocols and good-laboratory-practice guidelines for the assessment of potentially toxic substances. Therefore, they evaluated few toxicity end points, and effects on many organ systems and functions were not assessed. The database on Freon 114 includes few repeat-dose animal studies and no comprehensive long-term or continuous-exposure animal studies. In contrast with the database on some other CFCs, few data are available on human exposure to Freon 114. It is recommended that inhalation studies designed to specifically address a broad array of organ systems under the continuous-exposure conditions typical in the submarine environment be conducted.

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7

Hydrogen

This chapter summarizes relevant information on hydrogen gas, referred to as hydrogen in this profile. Selected chemical and physical properties are presented. The committee considered all those data in its evaluation of 1-h, 24-h, and 90-day guidance levels for hydrogen. The committee's recommendations for hydrogen concentrations considered the toxicity and explosivity of the gas in the reduced-oxygen environment present onboard submarines. The committee's recommendations for maximum hydrogen concentrations are provided at the conclusion of this chapter with a brief summary of the adequacy of the data used for defining them.

PHYSICAL AND CHEMICAL PROPERTIES

Hydrogen is a colorless, odorless, and tasteless gas (Budavari et al. 1989). It is the lightest gas and is explosive in air at concentrations greater than about 4% (Lewis 1996). In contact with chlorine, oxygen, or other oxidizers, hydrogen is flammable and explosive and burns with a nearly invisible flame (Budavari et al. 1989). Selected chemical and physical properties are listed in Table 7-1.

OCCURRENCE, USES, AND SOURCES OF EXPOSURE

Hydrogen is the most abundant element and is present in Earth's atmosphere at about 0.5 ppm (Windholz et al. 1976). It is formed during electrolysis of water as a byproduct of oxygen generation or by passing water vapor over heated iron. It is produced naturally by gut bacterial degradation of oligosaccharides (Hopfer 1982). Humans produce hydrogen at about 50 mg/day (Olcott 1972). Hydrogen is found in aircraft (NRC 2002) and space-shuttle air at about 100 ppm (NRC 1992). Charging shipboard batteries produces hydrogen. Thus, the hydrogen found onboard a submarine can reflect the low ambient concentrations found in the air, biologic sources,

TABLE 7-1 Physical and Chemical Properties of Hydrogen Gas

Synonyms	Protium
CAS registry number	1333-74-0
Molecular formula	H_2
Molecular weight	2.00
Boiling point	–252.77°C
Melting point	–259.2°C at 54 mm Hg
Flash point	NA
Explosive limit	4.1% by volume in air (lower limit)
Density	0.00008987 g/cm^3 at 20°C
Vapor pressure	NA
Solubility	NA
Conversion factors	1 ppm = 0.082 mg/m^3; 1 mg/m^3 = 12.2 ppm

Abbreviations: NA, not applicable or not available.
Sources: Explosive limit from Lewis 1996; density from Dean 1979; other data from Budavari et al. 1989.

and its release from marine batteries as a byproduct. Several measurements of hydrogen on submarines have been reported. Data collected on nine nuclear-powered ballistic missile submarines indicate an average hydrogen concentration of 0.03% (range, 0-0.63%) and data collected on 10 nuclear-powered attack submarines indicate an average hydrogen concentration of 0.02% (range, 0-0.75%) (Hagar 2003). Carbon monoxide and hydrogen in submarine air are oxidized to carbon dioxide and water in a specialized burner (U.S. Naval Systems Command 1979).

SUMMARY OF TOXICITY

At very high concentrations in air, hydrogen is a simple asphyxiant gas because of its ability to displace oxygen and cause hypoxia (ACGIH 1991). Hydrogen has no other known toxic activity. This profile considers only hydrogen gas and excludes health effects associated with other isotopic forms (deuterium or tritium) and hydrogen-containing chemicals (Windholz et al. 1976). Hydrogen-induced asphyxiation may occur at lower hydrogen concentrations when oxygen concentrations are also reduced as onboard a submarine. However, hydrogen concentrations needed to induce hypoxia even in a low-oxygen environment would far exceed the explosive limit of the gas. Thus, occupational exposure standards are set on the basis of the explosivity of hydrogen rather than its toxicity.

Hydrogen As an Asphyxiant Gas

Hydrogen can displace oxygen and result in asphyxiation and hypoxia. Air onboard a submarine is maintained at lower oxygen concentrations (about 19.5%) than in the natural environment to reduce the risk of fires. Hagar (2003) reported that the routine average partial pressure of oxygen (PO_2) on nuclear-powered attack submarines is 118-180 mm Hg (mean, 149 mm Hg); similar values were reported for nuclear-powered ballistic missile submarines. Minimum values recommended by NRC (2007) for the oxygen 1-h EEGL, 24-h EEGL, and 90-day CEGL are 105, 127, and 140 mm Hg, respectively. Assuming reasonably high humidity, an atmosphere with 28.2% hydrogen (282,000 ppm) is required to reduce the submarine mean PO_2 of 148 mm Hg (19.5%) to the 1-h oxygen EEGL of 105 mm Hg (14%); that is,

$$H_2 \text{ concentration} = [(19.5\% - 14\%)/19.5\%] \times 100 = 28.2\%.$$

Accordingly, hydrogen concentrations of 14.3% and 5.6% are required to reduce normal mean submarine oxygen concentrations to the 24-h EEGL and 90-day CEGL values, respectively.

Health effects associated with hydrogen mimic other forms of hypoxia. As alveolar partial pressure of oxygen is reduced, visual acuity in dim light declines as a reduction in arterial blood oxygen depresses the function of retinal rod cells. Hypoxia will lead to stimulation of medullary chemoreceptors and then to a compensatory increase in pulmonary ventilation. Important consequences of mild hypoxia include impaired judgment, reduction in ability to perform discrete motor movements, short-term memory loss, mental fatigue, headache, occasional nausea, and increase in reaction times. Rapid asphyxiation is characterized by tachypnea, cyanosis, sweating, cardiac arrhythmia, depression of the central respiratory center followed by loss of consciousness, and coma (reviewed in NRC 2007).

Hydrogen as an Explosive Gas

Hydrogen is an explosive gas. The U.S. Environmental Protection Agency (EPA 1988) recommends evacuation of personnel when the concentration of an explosive gas reaches 10% of the lower explosive limit. Ten percent of the lower explosive limit, or 4,100 ppm, of hydrogen is less than the hydrogen concentration required to reduce oxygen in submarine air to the 1-h or 24-h EEGL or the 90-day CEGL. Therefore, the hydrogen EEGL and CEGL values are based on hydrogen explosivity rather than adverse health effects arising from asphyxiation. Explosive limits of hydrogen in a lowered-oxygen atmosphere as would be found aboard a submarine are unknown.

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

Inhalation exposure levels for hydrogen have been established by the National Aeronautics and Space Administration (NASA) and are shown in Table 7-2. The American Conference of Governmental Industrial Hygienists (ACGIH 2004) classifies hydrogen as a simple asphyxiant, and no exposure limit has been assigned. ACGIH (1991) notes that the major hazard posed by hydrogen is due to its flammable and explosive properties.

COMMITTEE RECOMMENDATIONS

A health-based exposure standard would consider hydrogen-induced asphyxiation to be the critical effect. As noted earlier, clinical signs associated with hydrogen-induced hypoxia would occur if the oxygen concentration were reduced to below the 1-h EEGL (105 mm Hg), the 24-h EEGL (127 mm Hg), or the 90-day CEGL (140 mm Hg) (NRC 2007). However, the lower explosive limit for hydrogen in air is 41,000 ppm, and 10% of this concentration is 4,100 ppm. That value is appreciably lower than hydrogen concentrations required to produce hypoxia. Therefore, the EEGL and CEGL values for hydrogen (see Table 7-3) are based on explosivity rather than toxicity arising from its asphyxiant properties. Because of the seriousness of an onboard explosion, a safety factor of 10 was used in deriving the EEGL and CEGL values (to represent 10% of the lower explosive limit). Application of the safety factor agrees with the approaches used by NASA to derive the spacecraft maximum allowable concentration (Wong 1994) and that used by EPA (1988) to set exposure standards for explosive gases.

TABLE 7-2 Selected Inhalation Exposure Levels for Hydrogen

Organization	Type of Level	Exposure Level (ppm)	Reference
Spacecraft			
NASA	SMAC		Wong 1994
	1-h	4,100	
	24-h	4,100	
	30-day	4,100	
	180-day	4,100	

Abbreviations: NASA, National Aeronautics and Space Administration; SMAC, spacecraft maximum allowable concentration.

TABLE 7-3 Emergency and Continuous Exposure Guidance Levels for Hydrogen

Exposure Level	U.S. Navy Values (ppm)		Committee Recommended Maximum Values (ppm)
	Current	Proposed	
EEGL			
1-h	10,000	10,000	4,100
24-h	10,000	10,000	4,100
CEGL			
90-day	10,000	10,000	4,100

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level.

DATA ADEQUACY AND RESEARCH NEEDS

Control of submarine air concentration of hydrogen is required to eliminate the explosive threat posed by this gas. Enacting suitable control measures essentially eliminates concern about adverse health effects associated with acute or chronic exposure to hydrogen at concentrations associated with an explosive hazard. However, the present discussion presumes that hydrogen is biologically inert and acts as a simple asphyxiant. No acute-exposure or repeated-exposure studies of hydrogen are available. Likewise, pharmacokinetic and metabolic information on hydrogen is unavailable (Wong 1994).

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8

2190 Oil Mist

The U.S. Navy requested that the committee review and recommend inhalation exposure guidance levels for oil mist, specifically turbine oil with military nomenclature 2190 TEP. However, no relevant health-effects data specific to 2190 TEP were located in the public literature. Therefore, to determine exposure guidance levels, the committee had to define a petroleum distillate that it could use as a surrogate for evaluating health effects. In the absence of relevant data on 2190 TEP, the committee reviewed and evaluated literature on highly and severely refined distillate base stocks—a broad category of petroleum distillates that includes the base stock used in 2190 TEP (The Petroleum High Production Volume Testing Group 2003; CONCAWE 1986)—that were also insoluble in water. In general, lubricating base oils fit that characterization, and some information on the lubricating oil base stock of 2190 TEP (CAS no. 64742-54-7) was available. Other literature sources were evaluated when deemed appropriate.

This chapter summarizes relevant epidemiologic and toxicologic studies of the selected petroleum distillates mentioned above. Chemical and physical properties, toxicokinetic and mechanistic data, and inhalation exposure levels from other agencies are also presented. The committee considered all that information in its evaluation of the Navy's proposed 1-h, 24-h, and 90-day exposure guidance levels for oil mist. The committee's recommendations for oil mist exposure levels are provided at the conclusion of the chapter with a discussion of the adequacy of the data for defining the levels and the research needed to fill remaining data gaps. The effects of specific additives possibly present in the final petroleum products, such as sulfur or phosphate additives, were considered to be outside the scope of this assessment. Additives are usually proprietary materials and are used to improve the physical properties of products (Mackerer 1989). However, one additive, 2,6-di-tert-butyl-4-nitrophenol, is discussed in Chapter 4 of this report.

PHYSICAL AND CHEMICAL PROPERTIES

2190 TEP is a hydrotreated heavy paraffinic distillate that may have been further refined by severe solvent extraction, severe hydrocracking, or severe hydrotreating (Chevron 2001). It is described as a clear colorless to pale yellow liquid. Few physical and chemical property data are available; however, Table 8-1 provides information from material-safety data sheets provided by Navy suppliers.

OCCURRENCE AND USE

Mineral oil of inhalable particle size is called oil mist. The size of the particles depends on the process by which they are generated. Oil mists can potentially be generated in a variety of applications, which include metalworking, textile machinery, mist lubrication, and machining processes (ACGIH 2003; CONCAWE 1986). In submarines, generation of oil mist occurs primarily in the engine room. Inhalation and dermal contact are two possible exposure routes. The focus of this review is inhalation because adverse health effects resulting from dermal exposure are considered minimal provided that adequate personal-hygiene measures, such as wearing protective clothing and washing hands, are followed. Dermal toxicity of highly refined oils in humans is briefly summarized in CONCAWE (1986) and consists primarily of dermatitis and acne induced by oil.

TABLE 8-1 Physical and Chemical Data on Turbine Oil (Symbol 2190 TEP)

Synonyms and trade names	Lubricating oil[a]
CAS registry number	64742-54-7
Molecular formula	—
Molecular weight	—
Boiling point	<315°C
Melting point	NA
Flash point	NA
Explosive limits	NA
Specific gravity	0.86-0.87 at 15.6°C
Vapor pressure	<0.01 mm Hg at 38°C
Solubility	Soluble in hydrocarbons; insoluble in water
Conversion factors	—

Note: The Navy provided material-safety data sheets from two other suppliers (Equilon and Imperial). The little information on chemical and physical properties from those other sources was consistent with the data provided by Chevron (2001).
[a]The Petroleum HPV Testing Group (2003).
Abbreviations: NA, not applicable or not available.
Source: Data from Chevron 2001.

SUMMARY OF TOXICITY

A summary of the literature on occupational exposure to lubricating oil base stocks with and without additives is presented in Table 8-2. Animal data are summarized in Table 8-3. In addition, six articles (CONCAWE 1986; Mackerer 1989; Kenny et al. 1997; NRC 1997; NIOSH 1998; The Petroleum HPV Testing Group 2003) have summarized the available literature.

No relevant information on accidental exposures or experimental studies in humans was identified. However, occupational exposure to petroleum oil mists was associated primarily with effects on the respiratory system. Symptoms observed in automobile workers included coughing, wheezing, and phlegm, as reported by Kriebel et al. (1997), Greaves et al. (1997), and Ameille et al. (1995) at exposures (geometric means) of 0.19 mg/m^3, 0.43 mg/m^3, and 2.2 mg/m^3, respectively. Effects on respiratory function—as measured by reductions in cross-shift response in forced expiratory volume in 1 sec (FEV_1)—were demonstrated by Kriebel et al. (1997) and Kennedy et al. (1989) at the exposures defined previously. Marine engineers exposed to oil mists demonstrated similar effects on the respiratory system at a time-weighted average (TWA) of 0.45 mg/m^3 (Svendsen and Hilt 1997, 1999). A synergistic effect on respiratory function between inhaled tobacco smoke and oil mist has been suggested (Ameille et al. 1995).

Results of pulmonary exposure of laboratory animals to lubricating oils are similar to those observed in humans in occupational settings except that the animals were generally exposed at much higher concentrations. The target organ in animals was the respiratory tract. The most prevalent effect was the occurrence of foamy macrophages in the lungs. In general, the rat and the dog were the most sensitive species compared with rabbits, mice, and hamsters (Wagner et al. 1964). Acute exposures to metalworking fluids have been shown to be sensory and pulmonary irritants in mice (Schaper and Detwiler 1991). Straight oils caused sensory irritation that decreased within 1 h; pulmonary irritation was not observed until 2 h of exposure. The highest concentration tested was 2,816 mg/m^3. That is consistent with the low toxicity (primarily mucous membrane irritation of the upper respiratory tract) observed after acute exposure to oil mists with profile characteristics outside those defined in the present review (CONCAWE 1986; Dalbey and Biles 2003). Four- and 13-week exposures to oil mists with the same CAS number as 2190 TEP resulted in lung pathology at concentrations of 50 mg/m^3 and greater; pulmonary function was not affected at concentrations as great as 1,000 mg/m^3 (Dalbey et al. 1991; Dalbey 2001). Dogs and rats exposed to oil mist for up to 26 months at 5.5-105.8 mg/m^3 did not have an increase in tumor incidence (Stula and Kwon 1978; Wagner et al. 1964).

TABLE 8-2 Effects of Inhalation of Mist Oil on Humans

Oil Type, Characteristic[a]	Exposure Concentration	Exposure Duration	Subjects and Effects	Reference
Group S: cutting oil, straight (CO) Group E: mineral oil, soluble (MO) Group D: CO + MO Group C: control, unexposed assembly workers	Arithmetic mean: 2.6 + 1.8 mg/m^3 Geometric mean: 2.2 + 1.9 mg/m^3	Chronic for at least 1 year	Subjects from automobile industry in France: Group S, 40 males; Group E, 51 males; Group D, 139 males; Group C, 78 males. Effects evaluated: respiratory symptoms (by questionnaire), pulmonary function (FEV_1 and FVC), ventilatory impairment, bronchial reactivity There was no difference in prevalence of respiratory symptoms among groups; however, Groups S and D combined had significantly higher prevalence of cough or phlegm than Groups C and E; prevalence of cough and phlegm increased in straight-oil-exposed groups when adjusted for duration of exposure and smoking; interaction between ventilatory impairment and smoking was observed in straight-oil-exposed groups; bronchial reactivity was not affected by exposure to mineral oil; the committee found that no significant adverse effects were noted in Group S alone, and there was apparent interaction between cutting oil and smoking	Ameille et al. 1995
Cutting oil mist, not defined	Heavy, moderate, minimal	>5 years, workers in oil-mist-exposed jobs 1938-1967	2,485 male subjects who worked as machinists Mortality from various cancers evaluated No effect on incidence of respiratory cancer relative to expected; increase in cancer of large intestine and stomach was observed	Decoufle 1978
Metalworking fluid Straight, paraffinic or naphthenic, with or without sulfur or chlorine	Cross-sectional survey: selection of 2-year exposure window was based on report in which symptoms of cough, wheeze, and phlegm were	2 years	Subjects from automobile industry (UAW-General Motors). 1,676 male subjects (20.0% exposed to straight metalworking fluids, 24.9% exposed to soluble metalworking fluids, 12.6% exposed to synthetic metalworking fluids, 42.5% worked on assembly or were off	Eisen et al. 1997 (Study appears to be reanalysis of

Soluble Synthetic Assembly (controls)	found to predate diagnosis of asthma by about 2 years		work) Standard respiratory survey was used, and pulmonary-function tests were conducted; Cox proportional-hazards model was used Slight increase in RR for straight oil depending on whether year of hire was before or after 1970 (pre-1970: RR, 1.8; post-1970: RR, 2.0); increase was greater for synthetic oils; results provided possible evidence that exposure to straight oils may cause occupational asthma; primary objective of reanalysis study was to evaluate bias by selecting asthmatics out of work environment	Greaves et al. [1997])
Metalworking fluid Straight, paraffinic or naphthenic, with or without sulfur or chlorine Soluble Synthetic Assembly (controls)	Extrathoracic particle size: >9.8 µm Thoracic particle size: <9.8 µm Respirable particle size: <3.8 µm >0-0.1 mg/m^3; >0.1-0.5 mg/m^3; >0.5 mg/m^3 Note: Exposures are estimates; results are expressed as mg/m^3-years = quantitative estimate of past metalworking fluid exposure	1917-1985 Subjects had worked for at least 3 years; average duration of employment was 20 years	108 male subjects from automobile industry; 538 males in control group; subjects were from three plants (I, II, III) Case-control study to evaluate larynx cancer (squamous-cell carcinoma) Results suggested about 2-fold excess in larynx-cancer risk in workers exposed to straight metalworking fluid (combined plants I, II, III); OR for cancer increased with increasing exposure: >0.5 mg/m^3-years, OR, 2.23 (95% CI, 1.25-3.980); exposure, 0.5 mg/m^3; separate analysis of plants demonstrated increase in OR for metalworking fluid exposure in Plant I only Committee notes that confounding factors, such as sulfur content, also showed association with increased OR; association with sulfur may be associated in increased PAH content; authors did not attribute finding to smoking or alcohol intake, because there was no increase in lung cancer or cirrhosis	Eisen et al. 1994

(Continued)

TABLE 8-2 Continued

Oil Type, Characteristic[a]	Exposure Concentration	Exposure Duration	Subjects and Effects	Reference
Mineral oil mist Components of mist not defined	Mortality-study exposure concentrations: 0.07 mg/m^3 (minimum), 1.5 mg/m^3 (median), 3.7 mg/m^3 (mean), and 110 mg/m^3 (maximum) Prevalence-study exposure concentrations: 0.07 mg/m^3 (minimum), 1.0 mg/m^3 (median), 5.2 mg/m^3 (mean), and 110 mg/m^3(maximum)	≥5 years Mortality during 1942-1961	Subjects worked in machine shops (Kodak); mortality study had 3,122 in control group and 343 in "mist oil" group; over 1,700 were in prevalence study In mortality study, causes of death were compared; in prevalence study, authors evaluated FVC and FEV_1 and used questionnaire to assess cough, phlegm, dyspnea, wheezing, smoking status, and age In mortality study, no effects of oil mist on mortality were observed; in prevalence study, no evidence of adverse association between respiratory effects and mist oil was noted	Ely et al. 1970
Metalworking fluid: Straight mineral oil Soluble oil emulsions Water-based synthetic	0.43 ± 0.26 mg/m^3 (straight mineral oil) 0.55 ± 0.17 mg/m^3 (soluble oils) 0.41 ± 0.08 mg/m^3 (synthetics) Size-selective cut points were 9.8 µm (thoracic aerosol fraction) and 3.5 µm (respirable aerosol fraction) with geometric standard deviation of 1.2 for each	≥2 years Current employees	Subjects worked at General Motors facilities; 1,811 male machinists exposed to variety of mineral oils (364 to straight mineral oil, 452 to soluble oil emulsions, 226 to water-based synthetic oils); 769 males in internal reference group Effects evaluated with respiratory questionnaire: cough, phlegm, dyspnea, wheezing, chest tightness, self reported asthma and bronchitis Exposure-response relationships suggested association of respiratory symptoms (cough, phlegm, wheezing) with exposure to straight and synthetic fluids; synthetic oils had highest prevalence of symptoms, followed by straight oils and soluble oils (least)	Greaves et al. 1997

Turning Dept. – acid-refined mineral oils in 1926-1976 plus sulfur Grinding Dept. – "complex"	Average exposure concentration was estimated to be 5 mg/m^3 or more before 1965 Turning Dept. – 2.0 mg/m^3 (median; range, 0.3-3.4 mg/m^3) Grinding Dept. – 2.6 mg/m^3 (range, 1.0 – 7.3 mg/m^3) Also sodium nitrite and chromium	>5 years Subjects employed 1950-1966; had to be alive on 1/1/58 to participate	Subjects (788) working in metal industry Study evaluated cancer morbidity pattern of employees based on employee register Exclusive of cancer of scrotum, 39 cases of cancer were observed compared with 154.3 expected; cancer of scrotum was observed in 4 turners; committee notes that oils used now are more highly refined than oil used during 1950-1967	Jarvholm et al. 1981
Aerosols of cutting oils and cooling lubricants: Straight mineral oil Oil emulsions Synthetic fluids	Total aerosol concentration: Assembly workers, 0.07-0.44 mg/m^3 Machinists, 0.16-2.03 mg/m^3 Low: <0.20 mg/m^3 Medium: 0.20-0.55 mg/m^3 High: > 0.55 mg/m^3 End points measured Monday and Friday, before and after shift of workweek to demonstrate acute pulmonary response Particle size distribution was similar across oil types	≥ 6 months	Subjects were automobile workers and included 89 machine operators and 42 unexposed male assembly workers End points evaluated included acute pulmonary responses (FEV_1, FVC, PEF, MMEF [measured by spirometry] as measure of cross-shift lung-function changes) Machine operators exposed to aerosols of coolants and mineral oils had significant drop in cross-shift FEV_1 response relative to assembly workers; response was associated with inhalable aerosol >0.20 mg/m^3; there was no difference from Monday to Friday in FEV_1 response	Kennedy et al. 1989

(Continued)

TABLE 8-2 Continued

Oil Type, Characteristic[a]	Exposure Concentration	Exposure Duration	Subjects and Effects	Reference
Metalworking fluid: Straight and soluble	Straight: Mean: 0.243 mg/m^3 (SD, 0.265) Geometric mean: 0.193 (GSD, 1.79; range, 0.079-2.023). Airborne concentrations of inhalable particles, culturable bacteria, and endotoxins were measured Personal full-shift inhalable mass particle sample was collected with seven-hole sampler	≥ 1 month	Subjects were automobile workers (170 nonmachinists, 216 machinists); number of samples, 74 (straight metalworking fluid) and 139 (soluble metalworking fluid) Pulmonary-function tests were conducted with FEV_1 and FVC; respiratory symptoms were assessed with a questionnaire; end points were measured on single day There was evidence that chronic and acute respiratory symptoms were more prevalent in machinists than in nonmachinists; effects were also observed in nonmachinists; it should be noted, however, that many "nonmachinists" were at one time machinists in same plant; results were consistent with Kennedy et al. (1989) Present study tried to determine causal agent (such as endotoxins, fungal contaminants, various oil components) within oils responsible for toxicity Authors stated that "the ability of this study to quantify the acute irritant effects of MWF [metalworking fluids] accurately, and to identify the MWF constituents or exposure conditions amenable to environmental control was limited by the relatively low exposures in the plant selected for study and by the smaller than anticipated number of workers with exposure to straight or soluble MWF"	Kriebel et al. 1997
Mists and vapors of mineral oils and kerosene Medium to heavy naphthenic, acid-treated, hydrotreated	0.15-0.30 mg/m^3; spike, 2,000-4,000 mg/m^3	5-35 years of exposure	Subjects included 25 cable plant workers; 25 in control group Effects evaluated included pulmonary fibrosis with radiography, FEV_1, FVC; respiratory function was evaluated with questionnaire; McNemar's test for statistical analysis Fibrosis was observed in seven of 25 exposed workers and one of 25 controls; prevalence of respiratory symptoms did not differ	Skyberg et al. 1986

Medium to heavy paraffin, solvent-refined, severely hydrotreated			Committee notes that composition of mineral oil is unclear, because it is defined as kerosene and may contain aromatic hydrocarbons	
Mists and vapors of mineral oils and kerosene Medium to heavy naphthenic, acid-treated, hydrotreated Medium to heavy paraffin, solvent-refined, severely hydrotreated	Mineral oil vapor: 50-100 mg/m^3 Mineral oil mist: 0.5-1.5 mg/m^3	At least 3 years; 1963-1983 (followed up in 1990)	Subjects included 37 cable plant workers and 25 controls (radiographic analysis) Effects evaluated included pulmonary fibrosis (radiography) and lung function Fibrosis was observed in 10 of 25 cable workers and one of 25 controls; carbon monoxide transfer factor was decreased in exposed group Committee notes that composition of mineral oil is unclear, because it is defined as kerosene and may contain aromatic hydrocarbons	Skyberg et al. 1992
Mineral oil, composition undefined	Undefined	Undefined	288 subjects with scrotal cancer Study evaluated second primary tumors after detection of scrotal tumors Significant excess in second primary tumors of larynx, bronchus, and lip observed with mineral oil exposure	Waldron 1975

(Continued)

TABLE 8-2 Continued

Oil Type, Characteristic[a]	Exposure Concentration	Exposure Duration	Subjects and Effects	Reference
Mist oil: Lubricating oil, bp 300-700°C Fuel oil, bp 175-300°C	Mean concentration in engine room: 0.20 mg/m^3; mean concentration during tasks: 1.3 mg/m^3; two tasks with highest concentration were pressure testing of valves (2 mg/m^3) and maintenance of propeller shaft (1.5 mg/m^3) TWAC: 0.45 mg/m^3 for 5-h day at mean value and 2-h day at task (range, 0.12-0.74 mg/m^3); lowest TWAC: 0.12 mg/m^3; highest TWAC: 0.74 mg/m^3	>5 years 152 engineers Ferry trips take 10-20 min; 20-70 departures/day; 2 weeks onboard followed by 2 weeks off	Subjects were marine seamen (169 current marine engineers, 28 former marine engineers); 295 controls Effects evaluated with questionnaire (respiratory symptoms, MMI, cough, wheezing, dyspnea, chronic bronchitis). Significant increase (0.05 level) in MMI and dyspnea observed in marine engineers Confounding factors: engineers also had history of exposure to oil mist, asbestos (1950-1970), welding fumes, and other irritating gases	Svendsen and Hilt 1997
Mist oil: Lubricating oil, bp 300-700°C Fuel oil, bp 175-300°C	TWAC: 0.45 mg/m^3 for 5-h day at mean value and 2-h day at task (range, 0.12- 0.74 mg/m^3)	>5 years 152 engineers Ferry trips take 10-20 min; 20-70 departures/day; 2 weeks onboard followed by 2 weeks off	Subjects included marine seamen (68 engineers with chest x-rays films classified according to ILO system), 101 controls; spirometry was evaluated in 44 engineers and 71 controls Effects evaluated included respiratory function Borderline statistical significance (0.08) for emphysema based on ILO; FEV% was significantly decreased in marine engineers; reduced FEV% in absence of decreased FEV_1 can be interpreted as sign of emphysema Authors interpreted findings as possibly indicating that mist oil can impair respiratory function and increase abnormal findings in lungs; however, they concluded that findings were weak and further investigation was warranted	Svendsen and Hilt 1999

[a]Elemental sulfur is added to metalworking fluid to retard oil breakdown and improve lubricating properties under extreme temperature and pressure conditions.
Abbreviations: bp, boiling point; FEV_1, forced expiratory volume at 1 sec; FVC, forced vital capacity; MMEF, maximum midexpiratory flow; MMI, mucus membrane irritation; MWF, metalworking fluid; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; PEF, peak expiratory flow; RR, rate ratio; SD, standard deviation; TWAC, time-weighted average concentration; UAW, United Automobile Workers.

TABLE 8-3 Effects in Animals: Inhalation of Mist Oil

Species (no.)	Oil Type, Characteristic	Exposure Concentration	Exposure Duration	Effects	Reference
Mice, Swiss-Webster (4 mice per experiment per group)	Metalworking fluids (aerosolized) from 3 General Motors plants: 10 fluids, one of which was unused (new, neat) straight oil (100% sulfonized mineral oil, sample F) and another was used straight oil (sample F'; no additional chemical analysis available) Soluble and synthetic oils also tested but not considered relevant for this review	F: about 200-2,492 mg/m^3 F': about 400-2,816 mg/m^3 RD_{50} values obtained from concentration-response relationships; for samples F and F', RD_{50} had to be extrapolated because at concentrations tested RD_{50} was not achieved RD_{50}/mMAD/GSD F: 325,000 mg/m^3 (extrapolated; highest concentration tested, 2,492 mg/m^3)/2.7 µm/2.1 F': 110,100 mg/m^3 (extrapolated; highest concentration tested, 2,816 mg/m^3)/2.6 µm/2.0	Single 3-h exposures	Effects evaluated were changes in animal respiration; sensory and pulmonary irritation response at 1, 2, and 3 h; lung histopathology immediately after exposure, 24 h after exposure, 14 days after exposure. Effects evaluated included sensory irritation, defined as stimulation of trigeminal nerve ending in nasal mucosa resulting in lengthening of expiratory phase of each breath, and pulmonary irritation, defined as stimulation of vagal nerve endings resulting in pause between breaths With exposure to straight oils (samples F and F'), sensory irritation was observed immediately on exposure but decreased within 1 h or sooner; pulmonary irritation was observed after about 2 h of exposure and became more pronounced by end of 3-h exposure Respiratory frequency decreased rapidly on exposure reaching plateau at about 2 h; recovery was immediate at lower concentrations, slower at higher concentration 24 h after exposure, mild interstitial pneumonitis was seen in mice exposed to Samples F and F'; little difference was seen relative to controls immediately after exposure and 14 days after exposure Straight oils were least potent of oils tested; authors concluded that additives are important in determining potency of oils; samples F and F' had fewest additives of oils tested; straight oils were not considered irritating	Schaper and Detwiler 1991

(Continued)

TABLE 8-3 Continued

Species (no.)	Oil Type, Characteristic	Exposure Concentration	Exposure Duration	Effects	Reference
Wistar rats (8 males per group; 6 males in control group)	Mineral oil mist: mildly refined and derived from naphthenic crude oil. Mineral oil A: low viscosity used as impregnation fluid; 29 wt% aromatic hydrocarbons, 70 wt% saturated hydrocarbons. Mineral oil B/C (1:1): used in cable splicing; B, 31 wt% aromatics, 68 wt% saturated; C, 45 wt% aromatics, 54 wt% saturated. Also tested 3 synthetic crude oils: C15-C20 alkylbenzenes and polybutene; these groups are not discussed in this review	Total aerosol/vapor: Mineral Oil A: 126-770 mg/m^3 Mineral Oil B/C: 75-748 mg/m^3	7 h/day, 5 days/week for 2 weeks	Clinical observations included body weight, histopathology, oil deposition in fat tissue and brain. No deaths occurred in mineral-oil-exposed groups; all groups gained weight; however, mineral oil A (high concentration) had significantly lower mean body weight at necropsy; lung weights were increased at both concentrations of mineral oil A, but response was not dose-related; liver weights were increased in mineral oil A and B/C groups but only at highest concentration; statistical significance was not achieved. No macroscopic changes were seen in any of exposed groups; mineral oil B/C induced statistically significant increases in number of alveolar macrophages (748 mg/m^3) and degree of vacuolization ($\geq$75 mg/m^3); damage to bronchial mucosa was also seen in mineral oil B/C groups ($p<0.01$, 748 mg/m^3). Slight fatty liver degeneration was seen in high-dose mineral oil A group; sinusoidal dilatation was also observed in animals exposed to mineral oil B/C. Mineral oil A (only oil evaluated) was detected in fatty tissues and was retained 2 weeks after completion of exposure. Data excluded from assessment; chemical and physical data on mineral oil tested are different from 2190 TEP; materials tested are not representative of lubricating oils but are more representative of kerosenes	Skyberg et al. 1990 Follow-up to cable worker studies

Rats 15 males and 15 females per group Additional 10 male rats for specialized testing of pulmonary function	Generic cutting oil (GCO): CAS no. 64742-65-0 (85%) plus additives Gear oil (GO): CAS no. 64742-54-7 and CAS no. 64742-57-0 (combined 97%) plus small amount of additives Commercial engine oil (CEO): CAS no. 64762-65-0 (100 and 300 SUS) (94%) plus additives	GCO: 50, 150, 500 mg/m^3 GO: 60, 150, 520 mg/m^3 CEO: 50, 150, 400 mg/m^3	6 h/day, 5 days/week for 13 weeks	Effects evaluated included hematology, clinical chemistry, organ weights, histopathology, and pulmonary function (GCO: quaisistatic deflation pressure-volume curves, pulmonary hydroxyproline; GO: same as GCO plus lung volume; CEO: same as GO but no pulmonary hydroxyproline measured) Effects of three formulations were similar; the lung was target organ GCO: alveolar macrophages with addition of minor hyperplasia of alveolar epithelial cells at 50 mg/m^3 increasing in number and severity with increasing dose; thickening of alveolar walls at 500 mg/m^3; granulopoiesis in sternum at 500 mg/m^3; increase in lung weight at middle and high doses; shift in WBC differential at high dose (increase in circulating neutrophils and decrease in lymphocytes) GO: changes were similar to those with GCO with addition of minor hyperplasia of alveolar epithelial cells; increase in lung weight at middle and high concentrations; shift in WBC differential at middle and high concentrations. CEO: similar to changes with two previous formulations without hyperplasia Pulmonary function was generally unaffected.	Dalbey 2001

(Continued)

TABLE 8-3 Continued

Species (no.)	Oil Type, Characteristic	Exposure Concentration	Exposure Duration	Effects	Reference
Rats	Hydrotreated base oil (HBO): CAS no. 64742-54-7 Solvent-refined oil (SRO): CAS no. 64742-70-7 White oil (WTO): CAS no. 8042-47-5	50, 210, 1,000 mg/m^3	4 weeks	Effects evaluated included hematology, clinical chemistry, organ weights, and histopathology Only lung and associated lymph node changes were observed; the main histologic changes observed included accumulation of foamy macrophages in alveoli, infiltration of neutophils and lymphocytes associated with foamy macrophages and slight thickening of alveolar wall	Dalbey et al. 1991
Dogs, rabbits, mice, rats, hamsters (males) mongrel dogs, Dutch rabbits, Golden hamsters, Holtzman SD rats, CF No. 1 mice, CAF1/Jax mice (pulmonary-tumor-susceptible strain)	Light mineral oil: naphthene base saturated hydrocarbons (95% naphthenes: 27% noncondensed, 68% 2- to 6- ring naphthenes; 5% paraffins)	Low dose: mean, 5.2 mg/m^3; range, 3.8-6.6 mg/m^3 High dose: mean, 93.1 mg/m^3; range, 83.0- 104.2 mg/m^3	12-26 months Interim sacrifices were performed at 3 and 6 months and terminally after 1 year of exposure at 5 mg/m^3; at 100 mg/m^3, interim sacrifices occurred at 3, 6, 12, and 18 months and at 26-month termination; no exposed or control dogs at either concentration were included in 3-month sacrifice or 100-mg/m^3	Effects evaluated included body weight, hematology, and respiratory function (including histopathology, enzyme activities [serum and lung: BAP, MgAP], respirometry [rabbits only]). Body weights: All species: no significant differences were observed between exposed and control groups Hematology: All species: no significant changes were observed between exposed and control groups (NOAEL, 93.1 mg/m^3) Respiratory function: Rabbits: no significant differences were observed between exposed and control groups based on minute ventilation or oxygen consumption (NOAEL, 93.1 mg/m^3) Biochemistry: Dogs: no significant differences were observed between exposed and control groups at 5 mg/m^3 for 12 months; at 100 mg/m^3 (dogs, serum) BAP and MgAP were significantly increased at 12 months; BAP and MgAP were increased at 18 months, but only BAP reached	Wagner et al. 1964

sacrifice CAF1/Jax mice were exposed at 100 mg/m^3	significance Rabbits: no significant differences were observed between exposed and control groups (serum, lung tissue) Rats: no significant differences were observed between exposed and control groups at 5 mg/m^3 for 12 months (serum, tissue); BAP and MgAP were significantly increased at 100 mg/m^3 during the 6-month and 1-year evaluations Hamsters: significant differences were observed at 100 mg/m^3 (lung tissue); NOAEL, 93.1 mg/m^3 Pathology: Dogs: at 6-month sacrifices, concentrations demonstrated differing degrees of reaction to inhaled oil (foamy macrophages, clear droplets); this response was also evident at 5-mg/m^3 terminal sacrifice at 1 year; significant pulmonary alveolar and hilar lymph node oil deposition and/or lipid granuloma formation after about 12 months at 100 mg/m^3. Rabbits: essentially no response to inhaled oil mist Rats: prominent presence of macrophages with oil-containing cytoplasm; there was some evidence of interstitial pneumonia; pulmonary tissue alterations were of significance only at 100 mg/m^3 Mouse: no major pathologic response was evident (NOAEL, 5.2 mg/m^3)

(Continued)

TABLE 8-3 Continued

Species (no.)	Oil Type, Characteristic	Exposure Concentration	Exposure Duration	Effects	Reference
Dogs (4) Rats (5-20) Gerbils (3-9) CD mice (12-19) JAX mice (17-27)	Mineral oils, complex (70% paraffinic) with acetone and finish adjuvants, textile fiber finishes	5.5 ± 1.2 mg/m^3 105.8 ± 17.8 mg/m^3 Each concentration also contained 1,000 ppm acetone.	Chronic, 6 h/day, 5 days/week Dogs: 24 months Rats: 12 months with recovery at 1, 2, 6, 10 months; 12-24 months without recovery Gerbils: 12 months with recovery at 0.25, 0.5, 1, 2 months. Mice (CD): 10 months without recovery Mice (JAX): 12 months without recovery	Effects evaluated included pulmonary pathology Study not considered relevant, because complex mixture contained acetone and multiple additives; intent of study was to compare results with those of Wagner et al. (1964); results were comparable Data support absence of carcinogenicity in variety of animals exposed to mineral oils	Stula and Kwon 1978

Abbreviations: BAP, basic alkaline phosphatase; CEO, commercial engine oil; GCO, generic cutting oil; GO, gear oil; GSD, geometric standard deviation; MgAP, magnesium activated alkaline phosphatase; MMAD, mass median aerodynamic diameter; NOAEL, no-observed-adverse-effect level; RD_{50}; a statistically estimated concentration resulting in 50% reduction in respiratory rate.

Effects in Humans

Accidental Exposures

No relevant information was identified.

Experimental Studies

No relevant information was identified.

Occupational and Epidemiologic Studies

Fifteen studies of the general health effects of occupational exposure to lubricating oil mists were reviewed (see Table 8-2). In all cases, exposure was chronic, from at least 1 month to 35 years. No relevant studies of acute exposure to oil mists were identified. However, two studies (Kennedy et al. 1989; Kriebel et al. 1997) measured "acute pulmonary responses" (health effects measured on a single day) to metalworking fluid but after an exposure period of at least 1 month. Automobile workers (machine operators) exposed to aerosols of cutting oils and coolant fluids for at least 6 months demonstrated a significant drop in FEV_1 relative to assembly (control) workers (Kennedy et al. 1989); the response was associated with exposures greater than 0.20 mg/m^3. In a study by Kriebel et al. (1997), workers exposed to mineral oil at 0.24 mg/m^3 for at least 1 month, with respiratory effects being measured on a single day, provided some evidence of acute and chronic pulmonary respiratory symptoms. The results of Kriebel et al. (1997) are considered equivocal at best because nonmachinists (control population) demonstrated similar respiratory effects.

Most of the reviewed literature addressed chronic respiratory effects elicited after exposure of more than 1 year. Of the studies reviewed, only five (Ameille et al. 1995; Greaves et al. 1997; Eisen et al. 1997; Svendsen and Hilt 1997, 1999) were considered relevant for EEGL and CEGL development; of the five studies, three were specific to automobile workers, and two to marine engineers. In a study conducted by Ameille et al. (1995), automobile workers did not demonstrate an increased prevalence of respiratory symptoms when exposed to straight cutting oils at a geometric mean concentration of 2.2 mg/m^3 for a duration of at least 1 year. However, a combined analysis of workers exposed to straight cutting oil or a mixture of straight cutting oil and soluble cutting oil did exhibit an increased prevalence in cough or phlegm. Respiratory function and pulmonary function were also impaired in straight-cutting-oil-exposed workers who smoked.

Results of Greaves et al. (1997) suggested an association of increased reporting of respiratory effects (cough, phlegm, and wheezing) with exposure to metalworking fluids (synthetic oils > straight oils > soluble oils) after exposure of at least 2 years. The average exposure concentration was 0.43 mg/m^3. Eisen et al. (1997) reanalyzed those data to evaluate bias in the selection of asthmatics out of the work-

place. Their analysis demonstrated a slight increase in the rate ratio for asthma in workers exposed to straight metalworking fluid. As was observed in the Greaves et al. study (1997), the effects were greatest in the workers exposed to synthetic fluids.

A significant increase in mucous membrane irritation and dyspnea (Svendsen and Hilt 1997) and a decrease in respiratory function (Svendsen and Hilt 1999) were observed in marine engineers exposed to mist oil at a TWA of 0.45 mg/m^3 for more than 5 years. The engineers also had previous potential exposure to asbestos, welding fumes, and other irritating gases. The authors concluded that the findings of the respiratory-function evaluation were weak and that additional investigational work was needed.

Effects in Animals

Acute Toxicity

Schaper and Detwiler (1991) exposed Swiss-Webster mice to different aerosolized metalworking fluids obtained from three General Motors plants. Mice were exposed to straight (new or "neat" and used), soluble, or synthetic oils; only the results with straight oil are discussed here. Exposure concentrations ranged from about 200 to 2,492 mg/m^3 and from about 400 to 2,816 mg/m^3 for the neat and used metalworking fluid, respectively. For both neat and used oils, six concentrations were tested at 3-h exposure periods with four mice per exposure. Sensory irritation, as defined in Table 8-3, was observed immediately on exposure to both oils at all concentrations tested, but the irritation decreased within 1 h or less. Pulmonary irritation was apparent at 2 h on exposure to all oil mists. Mild interstitial pneumonia was observed after exposure to both the neat and used straight oils at the highest concentration tested.

Repeated Exposure and Subchronic Toxicity

As a follow-up to occupational-exposure studies of cable-plant workers exposed to mists and vapors of mineral oil and kerosene (Skyberg et al. 1986), Skyberg and co-workers (1990) exposed Wistar rats to two mineral oil mists derived from a mildly refined naphthenic crude oil for 7 h/day, 5 days/week for 2 weeks. One of the oils was representative of an oil used as an impregnation fluid (mineral oil A), and the other was used in cable splicing (mineral oil B/C). Exposure concentrations ranged from 126 to 770 mg/m^3 for mineral oil A and 75 to 748 mg/m^3 for mineral oil B/C. A significantly reduced necropsy body weight was observed in the high-dose group exposed to mineral oil A. Increased liver and lung weights were observed, but statistical significance was not achieved. Macroscopic changes were not observed in the lungs of any of the exposed groups. Mineral oil B/C induced significant increases in the number of alveolar macrophages (at 748 mg/m^3) and the degree of vacuolization (at greater than 75 mg/m^3). Damage to the bronchial mucosa (including ciliary loss), increased number of goblet cells, and cellular disorien-

tation were observed after exposure to all oils, except mineral oil A, at 748 mg/m^3. Liver pathology was noted as slight fatty liver degeneration (mineral oil A, 770 mg/m^3) and sinusoidal dilatation (mineral oil B/C, ≥75 mg/m^3). Mineral oil A was detected in the fat tissue of exposed animals and was retained 2 weeks after the completion of exposure.

Several inhalation studies of oil mists, with the characteristic profile outlined by the committee, were conducted by Dalbey et al. (1991) and Dalbey (2001). Two of the petroleum distillates tested, including one hydrotreated base stock and one gear oil, had the same lubricating base stock as 2190 TEP (CAS no. 64742-54-7). In a standard 4-week toxicity study, whole-body exposure to hydrotreated base oil at 50, 210, and 1,000 mg/m^3 for 6 h/day, 5 days/week, only lung and associated lymph node changes were observed (Dalbey et al. 1991). The main histologic changes observed at 210 and 1,000 mg/m^3 were accumulation of foamy macrophages in alveoli, infiltration of neutrophils and lymphocytes associated with the foamy macrophages, and a slight thickening of the alveolar wall; concentration dependence was demonstrated. Similar pathology of the lung and slight hyperplasia of alveolar epithelial cells were observed on exposure to gear oil (CAS no. 64742-54-7 and 64742-57-0) for 13 weeks at 60, 150, and 520 mg/m^3 (Dalbey 2001). Additional changes included an increase in lung weight and a shift in the white-blood-cell (WBC) differential (≥150 mg/m^3). Pulmonary function was not affected in any treatment group.

Chronic Toxicity

Wagner et al. (1964) exposed five species—dogs, rabbits, mice (CF No. 1 strain and CAF1/Jax strain), rats, and hamsters—to two concentrations of light mineral oil (naphthenic base) for 1 year (5 mg/m^3) to 26 months (100 mg/m^3). CF No. 1 mice were used to determine responses to exposures both histologically and physiologically and were used to assess longevity. CAF1 mice, which were used as a model to evaluate tumorigenic potential, were exposed only at 100 mg/m^3. No significant changes in body weight or hematologic characteristics were observed in any of the test species. Respiratory function was not affected in the rabbits, the only species tested this way. Basic alkaline phosphatase (BAP) and magnesium-activated alkaline phosphatase (MgAP) were monitored in all species. No significant differences from control animals were observed in rabbits (5 and 100 mg/m^3) or in dogs, rats, and hamsters (5 mg/m^3). In general, BAP and MgAP were increased in dogs, rats, and hamsters at 100 mg/m^3 as early as 6 months of exposure. No pathologic response was evident in the lung tissue of mineral-oil-exposed rabbits and mice. Pathologic responses (as evidenced by foamy macrophages and oil-droplet formation) were observed in dogs and rats. Those effects were apparent at 12 months of exposure in dogs (5 mg/m^3). Significant pulmonary alveolar and hilar lymph node oil deposition and granuloma formation were also observed at 12 months but only at 100 mg/m^3. In rats, pulmonary tissue alterations were of significance only at 100 mg/m^3.

Stula and Kwon (1978) evaluated the chronic toxicity of a complex mineral oil containing adjuvants and acetone in dogs, rats, mice, and gerbils. The primary objective was to determine whether the toxicity profile observed with pure mineral oil mist (Wagner et al. 1964) would be altered by the addition of adjuvants and acetone. The animals were exposed to the complex mixture for 12-24 months 6 h/day, 5 days/week at 5 and 100 mg/m^3 in combination with 1,000-ppm acetone. Relative to the results of Wagner et al. (1964), inhalation toxicity was not significantly altered by the addition of adjuvants and acetone. Oil mist was detectable in lung macrophages of all species tested at both concentrations. Oil microgranulomas were observed in rats and dogs only at the higher concentration. The data were not considered relevant to the present analysis, because of the composition of the test material. However, the data do confirm the results of Wagner et al. (1964).

Reproductive Toxicity in Males

Sperm morphology and counts were not adversely affected in male rats exposed to hydrotreated base oil at 1,000 mg/m^3 6 h/day for 4 weeks (Dalbey et al. 1991).

Immunotoxicity

No relevant information was identified.

Genotoxicity

Solvent-refined hydrotreated heavy paraffinic distillate was negative in the modified salmonella mutagenicity assay (Blackburn et al. 1986).

Carcinogenicity

Four studies of the carcinogenic potential of mineral oil in humans were reviewed (Waldron 1975; Decoufle 1978; Jarvholm et al. 1981; Eisen et al. 1994). Results are detailed in Table 8-2. Although it has been reported that workers exposed to mist oils have an increased risk of cancer, contamination of the mineral oils with polycyclic aromatic hydrocarbons (PAH, some known to be carcinogenic) confounds interpretation of the observed results. Metalworking fluids, such as 2190 TEP, that are highly or severely refined have low concentrations of PAHs when "unused" and are classified as A4 (not classifiable as a human carcinogen).

Severe processing can significantly reduce or eliminate the carcinogenic potential of crude oils, as has been demonstrated in mouse-skin painting studies (Kane et al. 1984). On the basis of the results of a modified salmonella mutagenicity assay (Blackburn et al. 1986), solvent-refined hydrotreated heavy paraffinic distillate was not predicted to be carcinogenic.

As discussed above, Wagner et al. (1964) exposed dogs, rabbits, mice (CF No. 1 strain and CAF1/Jax strain), rats, and hamsters to two concentrations of light mineral oil (naphthenic base) daily for 1 year (5 mg/m^3) to 26 months (100 mg/m^3). Significant pulmonary alveolar and hilar lymph node oil deposition or lipid granuloma formation were observed after 12 months in the dog. Although Stula and Kwon (1978) evaluated whether the toxicity profile observed with pure mineral oil mist would be altered by the addition of adjuvants and acetone, their results discussed above support those of Wagner et al. (1964).

Wagner et al. (1964) evaluated the tumorigenic potential of light mineral oil in a lung-tumor-sensitive strain of mice (CAF1/Jax). Mice were exposed to light mineral oil at 100 mg/m^3 6 h/day, 5 days/week. Animals were sacrificed monthly from 7 months to 13 months of exposures, and the lungs were processed for histologic evaluation. The collective results of the studies were equivocal. Percentage differences in tumor incidences of 20% and 15% were observed in oil-exposed mice compared with control mice at 10 and 11 months, respectively. However, at 12 and 13 months, the percentage difference was 10% and 13%, respectively; and control mice exhibited more tumors than the oil-exposed mice.

The negative results of the mutagenicity study and rodent carcinogenicity studies support the view that there is no carcinogenic potential in animals. The material-safety data sheet provided by the supplier states that 2190 TEP is not classified as a carcinogen by the National Toxicology Program or by the International Agency for Research on Cancer (Chevron 2001).

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

Although there is recent concern about the cardiac and pulmonary toxicity of respirable particulate matter from ambient air pollution, mechanistic understanding is insufficient to implicate oil mist particles as a health hazard for otherwise healthy adults. On the basis of animal studies, 2190 TEP would be expected initially to cause an inflammatory reaction if inhaled into the alveolar (deep) region of the lung. Deposition in the deep lung will depend to some extent on the size of the droplets; that is, if smaller than 3-5 μm, they can be expected to reach this area. Furthermore, "fine" oils, such as 2190 TEP, can spread over the surface of the airways and alveoli, depending on the dose. Oil deposited in the airways can be expected to be removed from the lung within a few days by normal physiologic mechanisms, such as the mucoescalator apparatus. However, oil deposited in the alveolar region cannot be removed from the lung to any substantial extent. In that region, the oil will first induce an inflammatory reaction whose extent will be directly dose-dependent. Initially, as demonstrated in animal studies (Skyberg et al. 1990), the oil is taken up (phagocytized) by alveolar macrophages. After a period of weeks, the oil can be found in macrophages of the draining lymph nodes, where it is essentially inert (it causes little reaction at this site). If the dose is large enough in the lung, the macrophages can coalesce and form foreign-body giant cells (Dalbey 2001). If the lung reaction is severe enough, the chronic inflammation can result in

interstitial pneumonitis and fibrosis (Wagner et al. 1964). Because the oil cannot be metabolized what reaches the deep lung can be expected to remain there or in the draining lymph nodes for long periods. However, there is no evidence that the lesions are progressive or that they will result in cancer in either the lung or lymph nodes.

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

There are no inhalation exposure levels for 2190 TEP oil mist. However, there are a few occupational standards for mineral oil mist, and they are listed in Table 8-4.

COMMITTEE RECOMMENDATIONS

The committee's recommendations for EEGL and CEGL values for oil mist are summarized in Table 8-5. The proposed U.S. Navy values are provided for comparison.

1-Hour EEGL

Because of the lack of human data on the health effects of short-term exposure to oil mist, data from animal studies were used. The point of departure for estimating the 1-h EEGL was 200 mg/m^3, which is the lowest observed-adverse-effect level from Schaper and Detwiler (1991). The committee concluded that that concentration would not affect task completion by a submariner. At that concentration for 3 h, aerosolized metalworking fluid produced sensory irritation

TABLE 8-4 Inhalation Exposure Levels for Mineral Oil Mist

Organization	Type of Level	Exposure Level	Reference
Occupational			
ACGIH	TLV-TWA	0.2 mg/m^3, inhalable particulate mass (draft)	ACGIH 2003
NIOSH	REL-STEL	10 mg/m^3	NIOSH 1997
	REL-TWA	5 mg/m^3	
OSHA	PEL-TWA	5 mg/m^3	29 CFR 1915.1000

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; REL, recommended exposure limit; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

TABLE 8-5 Emergency and Continuous Exposure Guidance Levels for Oil Mist

Exposure Level	U.S. Navy Proposed Values (mg/m^3)[a]	Committee Recommended Values (mg/m^3)
EEGL		
1-h	10 (values forward)	20
24-h	2 (values forward)	2.5
CEGL		
90-day	0.3 (values forward)	0.3

[a]U.S. Navy values are for forward section of submarine. No current or proposed values were provided for aft section of submarine.

Abbreviations: CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level.

in mice that decreased in 1 h or less and became more pronounced at 3 h. Interstitial pneumonitis was observed at 24 h but not 14 days after exposure. The effect was reversible after exposure ended. Pulmonary irritation was not observed until 2 h of exposure. An uncertainty factor of 3 to account for interspecies differences was applied because animal species and humans respond similarly regarding pulmonary effects. A database uncertainty factor of 3 was applied to account for the lack of data specific to 2190 oil mist and the need to use data on surrogate oils to derive an exposure guidance level. No intraspecies uncertainty factor was applied, because the submariner population would be expected to react similarly to the pulmonary effects. Application of the interspecies and database uncertainty factors results in a 1-h EEGL of 20 mg/m^3. That estimate is considered to be protective because it is based on a response after a 3-h exposure.

24-Hour EEGL

No relevant human information was available for determining the 24-h EEGL value. The committee considered the publication by Skyberg et al. (1986) but decided that the composition of the petroleum distillate was too dissimilar from 2190 TEP and lubricating oils. Therefore, the point of departure for estimating the 24-h EEGL was the recommended 1-h EEGL, 20 mg/m^3. Because the animals were exposed to the test metalworking fluid for 3 h, a time-duration adjustment factor of 8—(24 h)/(3 h) = 8—was applied to the 1-h EEGL, resulting in a 24-h EEGL of 2.5 mg/m^3.

90-Day CEGL

To determine the 90-day CEGL, the committee considered two studies of petroleum distillates with the same lubricating base stock as 2190 TEP (CAS no.

64742-54-7) tested on rats (Dalbey 2001; Dalbey et al. 1991). In a standard 4-week inhalation-toxicity study, whole-body exposure to hydrotreated base oil at 50, 210, and 1,000 mg/m^3 resulted in lung and associated lymph node changes (Dalbey et al. 1991). The no-observed-adverse-effect level (NOAEL) was 50 mg/m^3. The main histologic changes observed were accumulation of foamy macrophages in alveoli, infiltration of neutophils and lymphocytes associated with the foamy macrophages, and a slight thickening of the alveolar wall. Those effects were considered minimal. Similar pathology of the lung and slight hyperplasia of alveolar epithelial cells were observed on inhalation exposure of rats to gear oil (combination of CAS no. 64742-54-7 and 64742-57-0) for 13 weeks at 60, 150, and 520 mg/m^3 (Dalbey 2001). Additional changes included an increase in lung weight and a shift in the WBC differential ($\geq$150 mg/m^3). Pulmonary function was not affected. Because the latter study combined two lubricating base stocks, the committee used the first study (NOAEL, 50 mg/m^3) as the initial point of departure. In a study conducted by Ameille et al. (1995), automobile workers did not demonstrate an increased prevalence of respiratory symptoms when exposed to straight cutting oils at a geometric mean concentration of 2.2 mg/m^3 for at least 1 year. However, a combined analysis of workers exposed to straight cutting oil or a mixture of straight cutting oil and soluble cutting oil did exhibit an increased prevalence in cough or phlegm. Respiratory function and pulmonary function were also impaired in straight-cutting-oil-exposed workers who smoked. In humans, exposures to metalworking fluids at 0.243 mg/m^3 for at least 1 month (Kriebel et al. 1997) and 0.20 mg/m^3 for at least 6 months (Kennedy et al. 1989) resulted in similar respiratory responses (significant drop in FEV_1 response). Because exposure data in humans are not as well controlled as in the animal studies and the oils were different from 2190 TEP, the rat studies were considered more appropriate for setting the 90-day CEGL. Starting with 50 mg/m^3 as the initial point of departure, an uncertainty factor of 3 was applied to account for interspecies differences because animals and humans demonstrate similar respiratory symptoms on exposure to oil mist. A duration adjustment factor of 16.8—(7/5 [days])(24/6 [h])(3/1 [months])—was applied. A database uncertainty factor of 3 was also applied to account for the lack of data specific to 2190 oil mist and the need to use data on surrogate oils to derive an exposure guidance level. No intraspecies uncertainty factor was applied, because the submariner population would be expected to react similarly to the pulmonary effects. Application of the uncertainty and duration-adjustment factors results in a 90-day CEGL of 0.3 mg/m^3.

DATA ADEQUACY AND RESEARCH NEEDS

The committee recommends analysis of the oil mist to which the submariners are exposed. That mist oil should then be evaluated in animals for potential adverse health effects. If the Navy does not agree with the approach taken by the committee to estimate exposure guidance levels in this profile, acute and 90-day animal studies should be conducted with 2190 TEP. The committee recommends that used 2190

TEP (used in the same manner as in a submarine) be characterized to determine the aromatic components present.

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9

Ozone

This chapter summarizes relevant epidemiologic and toxicologic studies of ozone. 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 committee considered all that information in its evaluation of the Navy's current and proposed 1-h, 24-h, and 90-day exposure guidance levels for ozone. The committee's recommendations for ozone exposure guidance levels are provided at the conclusion of the chapter with a discussion of the adequacy of the data for defining them and the research needed to fill remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

Ozone is a highly reactive atmospheric gas whose molecule consists of three atoms of oxygen. At ambient temperatures, it is a pale blue gas that is a powerful oxidizer (Wojtowicz 1996). It is very reactive, and all phases (gas, liquid, and solid) are combustible and explosive. Some describe ozone as having a pungent odor that is detectable at 0.01 ppm (Wojtowicz 1996). Others describe it as having a "pleasant, characteristic" odor at concentrations below 0.2 ppm but as "irritating" at concentrations above 0.2 ppm (Budavari et al. 1989). Selected physical and chemical properties are summarized in Table 9-1.

OCCURRENCE AND USE

Ozone is widely used in water treatment because of its ability to disinfect; to eliminate taste, odor, and color; to lower turbidity; to remove iron and manganese; and to degrade a variety of organics, including detergents, pesticides,

TABLE 9-1 Physical and Chemical Data on Ozone

Synonyms	Triatomic oxygen
CAS registry number	7782-44-7
Molecular formula	O_3
Molecular weight	48.00
Boiling point	−111.9˚C
Melting point	−193˚C
Flash point	NA
Explosive limits	NA
Specific gravity	2.144 g/L at 0˚C
Vapor pressure	NA
Solubility	49 mL/100 mL water at 0˚C; soluble in alkaline solvents and oils
Conversion factors	1 ppm = 1.96 mg/m^3; 1 mg/m^3 = 0.51 ppm

Abbreviations: NA, not available or not applicable.
Sources: Solubility data from HSDB 2005; all other data from Budavari et al. 1989.

and proteins (Wojtowicz 1996). It is used to treat drinking water, industrial process streams, and municipal wastewater effluents and to treat water in cooling towers, swimming pools, and spas. It is also used for pulp delignification and bleaching and in the production of specialty organic chemicals and intermediates.

Ozone occurs naturally in the stratosphere at concentrations of 1-10 ppm and shields Earth from biologically damaging ultraviolet (UV) radiation (Wojtowicz 1996). In the stratosphere, short-wave UV radiation directly splits molecular oxygen (O_2) into atomic oxygen ($O\cdot$) that rapidly combines with O_2 to form ozone. In the troposphere, "ground-level" ozone is generated predominantly by a series of complex reactions involving nitrogen oxides, oxygen, and sunlight. Nitrogen dioxide (NO_2) absorbs longer-wavelength UV radiation, and this results in the generation of $O\cdot$ and nitric oxide (NO). $O\cdot$ then combines with O_2 to form ground-level ozone. NO_2 is regenerated by the reaction of NO with the newly formed ozone. In the absence of volatile organic compounds (VOCs), that reaction would approach a steady state with no buildup of ozone. However, atmospheric VOCs react with $O\cdot$ to produce oxidized compounds and free radicals that react with NO to form more NO_2. Consequently, the NO scavenging of ozone is upset, and this results in increased ozone concentrations.

In urban areas—such as Los Angeles, California—with high motor-vehicle traffic that emits large amounts of VOC-containing exhaust and with intense midday sunlight, complex atmospheric reactions are common place and result in what is termed photochemical smog. Ozone, the principal oxidant pollutant in photochemical smog, is considered both an environmental and a public-

health concern and is classified by the U.S. Environmental Protection Agency (EPA) as a criteria pollutant. EPA has established an 8-h national ambient air quality standard (NAAQS) concentration of 0.08 ppm for ozone (EPA 1996). In 1999, an estimated 90 million residents of the United States lived in areas where ambient ozone concentrations exceeded the NAAQS. Average background concentrations in the United States, in the absence of local anthropogenic emissions, are estimated to range from 0.02 to 0.04 ppm in the afternoon and are highest during spring (Fiore et al. 2003).

Ozone concentrations in airliner cabins on some flights may exceed the Federal Airline Administration and EPA NAAQS. Increased concentrations of ozone are expected primarily on aircraft without ozone converters or with malfunctioning converters that fly at high altitudes (NRC 2002). According to federal airline regulations, ozone in the cabin may not exceed 0.25 ppm at any time during a flight and may not exceed an average of 0.1 ppm during a 3-h flight above 27,000 feet. Mean ozone concentrations on aircraft have been reported to range from 0.022 ppm (Nagda et al. 1989) to 0.20 ppm (Waters 2001).

Potential sources of ozone in a submarine include motors, vent-fog precipitators, copying machines, and laser printers (Crawl 2003). No measurements of ozone concentrations onboard submarines have been reported in the literature.

SUMMARY OF TOXICITY

The toxicity of inhaled ozone has been extensively reviewed (EPA 1996). Numerous studies of controlled acute exposure have been conducted in human and laboratory animals. Study results have demonstrated that ozone is a potent irritant to the upper and lower airways that, when inhaled, results in impairments in pulmonary function and increased airway hyperresponsiveness with concurrent airway tissue injury and inflammation. The following is a brief review of important toxicologic studies in the scientific literature that were relevant to the committee's discussion and determination of appropriate guidance levels for ozone.

Effects in Humans

Accidental and Occupational Exposure

In an occupational setting, pulmonary congestion was reported in welders who used an inert-gas shielded-arc process that generated ozone at concentrations as high as 9 ppm (Kleinfeld and Giel 1956). Similar effects have been reported in welders exposed to ozone concentrations below 2 ppm (Challen et al. 1958). The effects were not observed when exposure concentrations were near 0.2 ppm. An accidental human exposure for 2 h to a high concentration of ozone (1.5 ppm) caused a 20% reduction in timed vital capacity of the lung and other effects (Chambers et al. 1957).

Experimental Studies

The harmful effects of inhaled ozone have been studied extensively in healthy and high-risk human subjects and in laboratory animals (EPA 1996); however, only the studies that are most relevant to the safety of submarine crew members (healthy men) are discussed in this report. Several well-designed studies have been conducted to investigate the pulmonary responses of healthy, nonsmoking human subjects acutely exposed to near ambient concentrations of ozone in environmentally controlled inhalation chambers. Those acute ozone exposures have resulted in pulmonary-function alterations, such as a decrease in inspiratory capacity; mild bronchoconstriction; rapid, shallow breathing during exercise; and symptoms of cough or pain on inspiration. Ozone exposure has been shown to result in airway hyperresponsiveness (as demonstrated by increased physiologic response to a nonspecific bronchoconstrictor, such as methacholine) and airway injury and inflammation (as assessed with bronchoalveolar lavage [BAL] or bronchial biopsy). An ozone-induced decrease in inspiratory capacity results in a decrease in forced vital capacity (FVC) and total lung capacity (TLC) and, in combination with mild bronchoconstriction, contributes to a decrease in the forced expiratory volume in 1 sec (FEV_1). The response of healthy adults to inhalation of ozone occurs in three phases: a delay phase in which no response to ozone is detected, an onset phase during which breathing frequency begins to increase, and a response phase during which breathing frequency stabilizes at a new higher level (Schelegle et al. 2007). Table 9-2 provides a summary of controlled ozone-exposure studies in humans that are discussed further below.

DeLucia and Adams (1977) exposed subjects to ozone at 0, 0.15, and 0.30 ppm for 1 h, while they were at rest and exercising continuously at three workloads, from light to heavy, with minute ventilation (VE) of 28-66 L/min. Significant time-dependent increases in breathing frequency and decreases in FEV_1 and forced midexpiratory flow (FEF25-75%) were observed in subjects after exposure at 0.30 ppm but only during heavy exercise. In another study, Folinsbee et al. (1978) exposed four groups of subjects (10 per group) to ozone at 0, 0.3, and 0.5 ppm for 2 h. One group was exposed at rest, and the other groups were exposed during intermittent exercise at levels requiring VE of 30, 50, or 70 L/min. They found that there were decrements in pulmonary function, such as FEV_1, even in resting subjects at 0.5 ppm and at 0.3 ppm with exercise. Horvath et al. (1979) also examined changes in pulmonary function during resting exposure to ozone at 0, 0.25, 0.50, and 0.75 ppm. In this study, resting 2-h exposure at 0.75 and 0.50 ppm caused significant mean decrements in FVC of 10% and 5%, respectively. However, ozone at 0 and 0.25 ppm induced no pulmonary decrements. On the basis of the studies of Folinsbee et al. (1978) and Horvath et al. (1979), which investigated the effects of ozone exposure on sedentary, healthy, young adults, the lowest concentration of ozone causing significant

TABLE 9-2 Controlled Exposure of Healthy Human Subjects to Ozone and Observed Effects on Pulmonary Function

Concentration (ppm)	Exposure Duration and Activity	Subjects and Effects	Reference
0.15, 0.30	1 h at rest and light to heavy workloads	6 men, 22-42 years old Mean FEV_1 decrements of 14% and 6.1% at 0.30 ppm with moderate and heavy exercise, respectively	DeLucia and Adams 1977
0.5	2 h at rest	40 men, 18-28 years old Decrease in mean FEV_1 (7%) and FVC (6%)	Folinsbee et al. 1978
0.25, 0.50, 0.75	2 h at rest	8 men and 5 women, 21-22 years old Mean FVC decrements of 5% and 10% at 0.50 and 0.75 ppm, respectively	Horvath et al. 1979
0.20, 0.30, 0.40	30-80 min with light to heavy exercise	8 men, 22-46 years old Decrease in FEF with heavy exercise with an effective dose of 0.2-0.3	Adams et al. 1981
0.12, 0.18, 0.24, 0.30, 0.40	2.5 h, IE	20-29 men per group, 18-30 years old Decrease in FVC, FEV_1 and FEF at 0.12 ppm	McDonnell et al. 1983
0.10, 0.15, 0.20, 0.25	2 h, IE	20 men, 21-29 years old Decrease in FEV_1 (>5%) and specific airway conductance (>15%) at 0.15 ppm	Kulle et al. 1985
0.12, 0.18, 0.24	1 h, heavy workload (competitive exercise)	10 men, 19-29 years old Decrease in FVC and FEV_1 at 0.18 ppm	Schelegle and Adams 1986
0.12	6.6 h, IE	10 men, 18-33 years old Mean FEV_1 decrements of 13% after 6.6 h and FVC of 8.3%; cough and discomfort increased with exposure; airway responsiveness to methacholine doubled after ozone exposure	Folinsbee et al. 1988
0.08, 0.10	6.6 h, IE	38 men, mean age 25 years Mean FEV_1 decrements of 8.4% at 0.08 ppm and 11.4% at 0.10 ppm; cough and discomfort increased with exposure	McDonnell et al. 1991

0.08, 0.10, 0.12	6.6 h, IE	22 men, 18-33 years old Decreased FVC and FEV_1 throughout exposure; mean FEV_1 decrements of 7.0%, 7.0%, and 12.3%, respectively	Horstman et al 1990
0.12	6.6 h/day, IE 5 consecutive days	17 men, mean age 25.4 years Mean FEV_1 decrements of 12.8%, 8.7%, 2.5%, 0.6%, and improvement of 0.2% on days 1-5, respectively; methacholine airway responsiveness increased by 100% on all exposure days; symptoms increased on first ozone day but were absent on last 3 exposure days	Folinsbee et al. 1994
0.30	1 h, CE	12 men, 18-34 years old Mean decrements of FEV_1 17.0-17.9%	McKittrick and Adams 1995
0.25	1 h, CE	32 men and 28 women, 22 ± 0.6 years old Mean FEV_1 decrements of 15.9% in men and 9.4% in women; FEV_1 decrements -0.4 to 56%	Ultman et al. 2004
0.1, 0.4	1 h, IE	12 men and 3 women, healthy, nonsmoking adults Neutrophils increased in BAL 6 h after exposure at 0.4 ppm.	Morrison et al. 2006
0.04, 0.06, 0.08	6.6 h, IE	15 men and 15 women, 22.8 ± 1.2 and 23.5 ± 3.0 years old, respectively Exposures included square-wave and triangular concentration profiles; at 0.08 ppm average, responses were observed earlier with the triangular profile (when ozone concentration was 0.15 ppm) than with the square-wave profile; no significant effects at 0.04 or 0.06 ppm	Adams 2006

Abbreviations: BAL, bronchoalveolar lavage; CE, continuous exercise; FEF, forced expiratory flow; FEV_1, forced expiratory volume at 1 sec; FVC, forced vital capacity; IE, intermittent exercise.

pulmonary function decrements has been determined to be 0.5 ppm for 2 h with average decrements of about 4% and 7% in FVC and FEV_1, respectively (EPA 1986).

Adams et al. (1981) exposed subjects to ozone at 0, 0.2, 0.3, or 0.4 ppm during continuous exercise at one of two workloads for 30-80 min. Eight trained male subjects (22-46 years old) completed 18 protocols, including exposure via mouthpiece to filtered air and to ozone at three concentrations, while exercising continuously for 30-80 min. The ozone effective dose was significantly related to pulmonary-function impairment and exercise ventilatory-pattern alteration. Multiple regression analysis, however, substantiated the predominant importance of ozone concentration, with the threshold for ozone toxicity during exercise at a moderately heavy workload—about 65% maximal O_2 uptake ($VO_{2\,max}$)—shown to be between 0.20 and 0.30 ppm.

McKittrick and Adams (1995) conducted a study designed to determine further what effect exercise pattern has on ozone-induced pulmonary responses when the total inhaled dose of ozone at a given concentration is kept the same. They exposed 12 aerobically trained men to ozone at 0.3 ppm for 1 h during continuous exercise and 2 h during intermittent exercise with equivalent estimated total doses of ozone. The two exposure regimens led to similar pulmonary-function alterations, but symptoms were slightly less during the last rest period of the intermittent-exercise exposure than at the end of the continuous exposure.

After brief exposure to ozone at concentrations over a few tenths of a part per million, exposed people have reported discomfort in the form of headache and dryness of the throat, nasal passages, and eyes. McDonnell et al. (1983) conducted a study designed to determine the lowest ozone concentration at which group mean decrements in pulmonary function occur in heavily exercising healthy young men. Subjects (20-29 per group) were exposed at 0, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm at a VE of 67 L/min for 2.5 h (15-min rest, 15-min exercise). Significant decrements in FVC, FEV_1, and FEF25-75% and an increase in cough were observed at 0.12 ppm, and there were concentration-dependent responses in all variables measured at concentrations greater than 0.24 ppm. Similar studies have also demonstrated significant decrements in pulmonary function with ozone exposures as low as 0.12 ppm (Kulle et al. 1985; Seal et al. 1993).

In a more recent study, Ultman et al. (2004) reported pulmonary responses in 60 healthy nonsmoking adults (32 men, 28 women) exposed to ozone at 0.24 ppm for 1 h with controlled exercise at a target VE of 30 L/min. They found considerable intersubject variability in FEV_1, with responses ranging from a 4% improvement to a 56% decrement. One-third of the subjects had decrements of more than 40%.

In a study directed at investigating possible mechanisms of pulmonary epithelial damage, Morrison et al. (2006) exposed six healthy nonsmoking adults to ozone at 0.1 ppm and seven similar subjects at 0.4 ppm with ^{99m}technetium-

labeled diethylene-triamine-penta-acetate (^{99m}Tc-DTPA) and performed BAL 1 or 6 h after exposure on different occasions. Five control subjects were exposed to filtered air. All study participants were exposed during intermittent exercise. Decreases in FEV_1 were observed immediately and at 1 h after exposure at 0.4 ppm. Ozone exposure did not affect ^{99m}Tc-DTPA lung clearance, but neutrophils increased in BAL fluid 6 h after exposure at 0.4 ppm. Superoxide anion release from BAL leukocytes decreased after 1 h of exposure at 0.1 ppm and after 6 h of exposure at 0.4 ppm. At 0.4 ppm, products of lipid peroxidation in BAL fluid decreased at 1 and 6 h. There was no change in antioxidant capacity of the lung epithelium or glutathione concentrations as measured after BAL at either concentration of ozone.

Controlled environmental exposure-chamber studies of longer duration have been reported (Folinsbee et al. 1988; Horstman et al. 1990; McDonnell et al. 1991). Adult volunteers were exposed for 6.6 h to ozone at 0.08, 0.10, or 0.12 ppm in whole-body chambers. Moderate exercise was performed for 50 min each hour for 3 h in the morning and afternoon. Folisbee and co-workers found that pulmonary-function decrements became greater after each hour of exposure at 0.12 ppm, with FVC declining by 8.3% and FEV_1 declining by 13% at the end of the sixth hour of exposure. Ozone exposure also caused increasing symptoms of cough and chest discomfort and increases in airway responsiveness to methacholine challenge. Similar studies were conducted to investigate the effects of ozone at 0.08 ppm on pulmonary function in exercising people (Horstman et al. 1990; McDonnell et al. 1991). Both studies found significant changes in spirometric measurements and significant increases in airway reactivity, specific airway resistance, and respiratory symptoms. At exposure concentrations of 0.08 ppm and 0.1 ppm, Horstman et al. (1990) found mean FEV_1 decreases of 7% and 8%, respectively. Likewise, McDonnell et al. (1991) found FEV_1 decreases at 0.08 and 0.1 ppm ozone of 8.4% and 11.4%, respectively. The FEV_1 response data in that study were best fitted to a three-parameter logistic model, suggesting that the ozone pulmonary-function response relationship has a sigmoid shape. That suggests that the induced response has a plateau, which indicates that at the given ozone concentration, workload, and length of exposure, no further increase in response is predicted with increasing exposure duration.

Folinsbee et al. (1994) extended their controlled-exposure studies by exposing healthy, nonsmoking men subjects to ozone at 0.12 ppm for 6.6 h while they exercised for 50 min of every hour at a ventilation rate of 39 L/min (moderate exercise) each day for 5 consecutive days. Although spirometric performance decreased with ozone exposure on the first day, the decrease was less on the second day and returned to control values on the third day. However, airway responsiveness to methacholine challenge (a measure of airway reactivity) increased progressively from day 1 through day 5.

In reviewing data from the literature, McDonnell et al. (1997) found that acute ozone exposure-response models of changes in lung function in humans should be consistent with the following observations: (1) for exposures of less

than 8 h, the response increases with increasing concentration (C), VE, and duration of exposure (T); (2) the response is nonlinear in each of the three exposure variables, and the exposure-response curve is concave upward at low values of the three variables; (3) with increasing T, the response reaches a plateau whose magnitude is a function of the rate of exposure; (4) with increasing C, the response appears to approach a plateau; (5) people vary in their response to ozone, and this variability becomes more pronounced at higher concentrations; and (6) older adults tend to be less responsive than younger adults. Using previously published data on 485 healthy young adult men exposed for 2 h to ozone at one of six concentrations while exercising at one of three levels, McDonnell et al. (1997) identified a sigmoid model that was consistent with previous observations of ozone pulmonary-response characteristics and was found to predict the mean response accurately with independent data. They did not find that the response was more sensitive to changes in C than in VE. They found that the response to ozone decreases with age.

Adams (2006) found that chamber exposure to ozone at an average of 0.08 ppm that more closely simulated the summertime ambient pollution exposure profile, which has a triangular shape, compared with the typical chamber exposure, which is a square wave, resulted in significantly greater FEV_1 response and total symptom severity response at 4.6 h, whereas responses at 6.6 h were not significantly different.

Controlled ozone-exposure studies of subjects with mild to moderate asthma suggest that they are at least as sensitive as nonasthmatic subjects. There was a tendency toward increased ozone-induced pulmonary-function decrements in asthmatic subjects relative to nonasthmatic subjects exposed to ozone at up to 0.2 ppm for 4-8 h (Scannell et al. 1996). Similarly, Alexis et al. (2000) reported that statistically significant ozone-induced decreases in FEV_1 in mildly atopic asthmatics tended to be greater than those in healthy subjects when both were exposed at 0.4 ppm for 2 h. Horstman et al. (1995) found that people with mild to moderate asthma exposed at 0.16 ppm for a longer duration (7.6 h) had reductions in FEV_1 that were significantly greater those in healthy subjects (19% vs 10%, respectively). Information derived from ozone exposure of tobacco-smokers is more limited. The general trend is that smokers are less responsive to ozone under controlled exposure conditions (Framptom et al. 1997; Torres et al. 1997).

Lippman (1993) reviewed the relevant literature that addresses pulmonary inflammatory responses to ozone in humans under controlled exposure conditions. He reported that ozone-induced pulmonary inflammation is detectable at concentrations as low as 0.1 ppm. He did not find an apparent threshold for ozone-induced pulmonary inflammation as measured with BAL. Devlin et al. (1991) exposed nonsmoking men randomly to filtered air (no ozone) and air with ozone at 0.10 or 0.08 ppm for 6.6 h with moderate exercise (VE, about 40 L/min). BAL was performed 18 h after each exposure, and cells and fluid were

analyzed. The BAL fluid of volunteers exposed to ozone at 0.10 ppm had significantly more neutrophils (PMNs), protein, prostaglandin E2 (PGE2), fibronectin, interleukin-6 (IL-6), and lactate dehydrogenase (LDH) than BAL fluid from the same volunteers exposed to filtered air. Moreoever, there was a decrease in the ability of alveolar macrophages to phagocytize yeast via the complement receptor; this suggested an ozone-induced impairment of lung defense mechanisms. Exposure at 0.08 ppm while exercising also resulted in significant increases in PMNs, PGE2, LDH, IL-6, alpha 1-antitrypsin and decreased phagocytosis via the complement receptor. The investigators concluded that exposure of humans to ozone at a concentration as low as 0.08 ppm for 6.6 h is sufficient to initiate an inflammatory reaction in the lung.

Epidemiologic Studies

There have been no reported epidemiologic studies of health effects in submariners exposed to onboard ozone. Numerous epidemiologic studies have examined the relationship of high ambient outdoor ozone concentrations to hospital admissions and daily morbidity and mortality. Some studies have examined the effects of sensitive populations, such as asthmatic children and the elderly; however, these groups are not relevant to the healthy male submariner population and are not further considered here.

EPA (1996) has thoroughly reviewed the epidemiologic dataset. Several studies have reported associations of adverse human health effects with exposure to increased ambient ozone (EPA 1996; Medina-Ramon et al. 2006). In one study, healthy adults had significant decrements in lung function when exercising outdoors and exposed to ambient ozone at 0.021-0.124 ppm (Spektor et al. 1988b). Similarly, healthy children attending a summer camp and exposed to ozone at the ambient concentration of 0.12 ppm had significant decrements in average FVC, FEV_1, peak expiratory flow rate, and FEF (Spektor et al. 1988a). A study of Taiwanese mail carriers indicated a reduction in peak expiratory flow rates that occurred sometime after exposure to ambient ozone at 0.006-0.096 ppm (Chan and Wu 2005). A study of adult hikers exposed to ambient ozone at 0.028-0.079 ppm while undergoing moderate exercise did not identify significant effects on lung function (Giradot et al. 2006). Several recent hospital admission and emergency-department visit studies in the United States (Peel et al. 2005), Canada (Burnett et al. 1997), and England (Anderson et al. 1998) have reported associations between an increase in ozone and an increase in risk of emergency-department visits and hospital admissions. In France, a short-term (1-2 days) increase in ozone exposure has been correlated with acute coronary events in middle-aged adults without heart disease (Ruidavets et al. 2005). Statistical modeling of exposure-response curves for ozone concentration and mortality indicate that even low concentrations of ozone, in the range of 0.01-0.25 ppm, are associated with an increased risk of premature death in the general U.S. population (Bell et al. 2006).

Effects in Animals

Acute Toxicity

Numerous toxicologic studies of inhaled ozone have demonstrated that the respiratory tract is the principal target for toxicity in laboratory animals. Acute exposures (3-4 h) to ozone at high concentrations (greater than 2 ppm) have been shown to cause death in laboratory rodents because of severe lung injury that results in alveolar edema, congestion, and hemorrhage. Four-hour exposures of rats, mice, and hamsters resulted in LC_{50}s of 2.1-9.9 ppm for rats, 2.1-9.9 ppm for mice, and 15.8 ppm for hamsters (Saltzman and Svirbely 1957).

Acute exposures of laboratory animals to ozone at much lower, nonlethal concentrations (less than 1 ppm), some of which are near ambient concentrations commonly in urban atmospheres with photochemical smog ($\leq$ 0.5 ppm), have been reported to cause airway epithelial injury particularly in the nasal passages and the distal conducting airways, especially in the centriacinar regions of the lung where terminal conducting airways have interfaces with the most proximal gas-exhange regions of the lung (the alveolar parenchyma). The more distal pulmonary alveoli in the deep lung of laboratory animals, including nonhuman primates, do not appear to be adversely affected by acute or chronic exposures to ozone at the low concentrations. Most of the reported morphologic studies of ozone-induced injury in laboratory animals exposed at near ambient concentrations have focused on the airway lesions in the pulmonary centriacinus. Fewer studies have been specifically designed to examine ozone-induced lesions in the upper respiratory tract, such as in the nose.

In general, the character of the airway epithelial changes induced by ozone is similar among laboratory animal species, including rodents and nonhuman primates. Some cell types in the surface epithelium lining affected airway sites are particularly susceptible to acute exposures at low concentrations and may undergo cellular degeneration or cell death. The epithelial cells most sensitive to ozone injury are ciliated cells and nonciliated cuboidal cells in the surface epithelium lining the proximal nasal airways, ciliated cells in the distal bronchiolar airways, and the alveolar type II cells lining the alveoli in the walls of respiratory bronchioles and proximal alveolar ducts. Loss of those sensitive epithelial cells due to death and exfoliation is quickly followed by reparative cellular proliferation and an abnormal increase in the numbers (hyperplasia) or size (hypertrophy) of more resistant nonciliated cells that include mucous goblet cells in the nasal passages, Clara cells in the terminal and respiratory bronchioles, and alveolar type II cells in the proximal alveolar ducts.

Several studies have investigated the time course of pulmonary inflammation after acute ozone exposure in laboratory rodents and rabbits. Maximal increases in total protein, albumin, and the number of PMNs in BAL fluid occur 8-18 h after the end of an acute exposure. Ozone-induced increases in total protein and albumin (indicators of increased permeability) and PMNs (cellular indicators of acute inflammation) depend on several factors, including species, strain,

concentration, exposure duration, and exercise during exposure. Hatch et al. (1986) investigated the acute inflammatory responses of five species (mice, guinea pigs, rats, hamsters, and rabbits) exposed to ozone at several concentrations, ranging from 0.2 to 2.0 ppm for 4 h. They found that guinea pigs were the most responsive (increased BAL fluid protein at 0.2 ppm or higher), rabbits were the least responsive (affected only at 2.0 ppm), and rats and mice were intermediate in their measured responses (effects only at 1.0 ppm or higher). Bhalla and Hoffman (1997) reported that rats exposed for 3 h at 0.5 ppm, but not 0.3 or 0.15 ppm, had increased permeability and inflammation in the lung. Dye et al. (1999) investigated strain-related differences in rats acutely exposed at 0.5 ppm and found that Wistar rats had significantly greater lung injury and inflammation than Sprague Dawley or F344 rats. The rat strain least sensitive to acute ozone injury was the F344 rat. Several studies have indicated that as ozone exposures continue for 3-7 days, the increases in BAL fluid PMNs and protein peak in the first few days and then attenuate, returning to near pre-exposure numbers. Van Bree et al. (2002) and colleagues have shown that rats exposed to ozone for 5 consecutive days had lower levels of protein, fibronectin, IL-6, and inflammatory cells than rats exposed for 1 day.

Exercise-induced enhancement of ozone-induced lung injury has been demonstrated in rats acutely exposed at 0.3 ppm (Mautz et al. 1985). The abundance and severity of pulmonary lesions increased as exercise and exposure duration were increased. Preliminary results also indicate that bacterial endotoxin, a common contaminant of indoor air, can enhance ozone-induced metaplasia in the nonciliated epithelium of the proximal nasal airway of the rat (Harkema and Wagner 2005).

Repeated Exposure and Subchronic Toxicity

Toxicologic studies of the nasal airways in laboratory rodents and nonhuman primates exposed to ozone has been reviewed recently (Nikasinovic et al. 2003). Macaques exposed to ozone at 0.15 ppm for 6 days (8 h/day) had acute neutrophilic rhinitis with alterations to the nasal transitional and respiratory epithelium in the anterior regions of the nasal passages. The nasal epithelial lesions in the exposed monkeys consisted of ciliated-cell necrosis, degeneration of ciliated cells with few or shortened cilia, and mucous-cell hyperplasia or metaplasia (Harkema et al. 1987). Exposures of laboratory rats at 0.8 ppm, but not 0.12 ppm, for 3 or 7 days (8 h/day) caused nasal epithelial injury with increased cellular proliferation, which resulted in epithelial hyperplasia and mucous-cell metaplasia in the nasal transitional (nonciliated cuboidal) epithelium lining the proximal nasal airways (Harkema et al. 1989; Johnson et al. 1990). Those data and data from several later toxicity studies in rats suggest that the rat nasal epithelium is less sensitive to ozone injury than that of nonhuman primates (Hyde et al. 1994).

Dungworth et al. (1975) reported that macaque monkeys exposed to ozone at 0.2-0.8 ppm 8 h/day for 7 days had hyperplasia and hypertrophy of the epithelium lining respiratory bronchioles in the centriacinar regions of the lung. The ozone-induced epithelial alterations were accompanied by accumulations of cellular debris and numerous alveolar macrophages in the affected airway lumina. The investigators stated that the threshold for the histologically detectable changes in the monkeys was below 0.2 ppm and most likely closer to 0.1 ppm. However, the ozone-induced alterations during the first 3-4 days of exposure did not increase in severity after 7 days of exposure, and they suggested cellular adaptation and apparent resistance to any further ozone-induced injury.

In later studies in the same laboratory (Harkema et al. 1987), macaques were exposed to ozone at 0.15 or 0.30 ppm for 90 days (8 h/day). After 90 days of exposure, there was ciliated cell necrosis, degenerated ciliated cells with few or attenuated cilia, and mucous-cell hyperplasia in the surface epithelium lining the proximal nasal airways. A neutrophilic inflammatory cell influx (acute rhinitis) was also present at 6 days, but not 90 days. The same ozone-exposed monkeys had moderate to marked hyperplasia of bronchiolar epithelium in the pulmonary centriacinar regions with increases in luminal macrophages (Harkema et al. 1993). There were no morphometrically determined differences in the severity of the bronchiolar epithelial lesions among the different ozone-exposed groups. The airway epithelial alterations did not appear to be concentration- or time-dependent. In contrast with the acute response to ozone in the nose, there was no evidence of epithelial-cell necrosis or inflammatory-cell influx (other than an increase in macrophages) accompanying the epithelial hyperplasia in the respiratory bronchioles of monkeys exposed for 6 or 90 days.

A small amount of work has been completed in studying the effects of ozone on the central nervous system. Groups of 10 male Wistar rats were exposed to air or ozone at 0.25 ppm 4 h/day for 15 or 30 days (Pereyra-Munoz et al. 2006). Motor activity measured over a 5-min period for both ozone-exposed groups was significantly decreased, to comparable degrees, after 15 and 30 days of exposure. Lipid peroxidation measured in the striatum of six rats per group was increased in a time-dependent manner in both ozone-exposed groups. The remaining four rats per group were used in histochemical preparations and for morphologic study. Increases were observed in the expression of dopamine and adenosine 3',5'-monophosphate-regulated phosphoprotein of 32 KD in the striatum after 30 days of exposure and in the expression of inducible nitric oxide synthase and copper-zinc superoxide dismutase in both the striatum and substantia nigra after 15 and 30 days of exposure. The number of neurons in the substantia nigra (stained with the Klüver-Barrera technique or histochemically for tyrosine hydroxylase) was reduced in a time-dependent manner in the ozone-exposed groups. There are no reports in the scientific literature demonstrating that exposure of humans to ozone results in neurotoxicity.

Chronic Toxicity

Long-term exposures (more than 90 days) to ozone have been conducted in laboratory rodents and macaques (Catalano et al. 1995; Chang et al. 1992; Tyler et al. 1988). Chang et al. (1992) exposed rats to a background concentration of 0.06 ppm 13 h/day, 7 days/week for 1, 3, 13, and 78 weeks with a sole daily 9-h spike (5 days/week) that rose to 0.25 ppm. The integrated concentration of the daily exposure with the spike was 0.19 ppm. The investigators found that in the terminal bronchioles, cilia were lost (at 78 weeks) and the surface area of Clara cells was decreased (at 1, 3, 13 and 78 weeks). There was also a progressive increase in epithelial hyperplasia, fibroblast proliferation, and thickening of the interstitial matrix from 13 to 78 weeks. There was a general postexposure recovery from the pulmonary lesions except the fibrotic interstitial changes, which were still apparent 17 weeks after the end of the chronic exposure. Pulmonary-function alterations consistent with restrictive lung disease and fibrotic lesions were also found in similarly exposed rats (Costa et al. 1995).

Tyler et al. (1988) exposed young monkeys (7 months) and rats to ozone at 0.25 ppm 8 h/day, 5 days/week over an 18-month period. Some animals were exposed throughout the entire 18-month period, and others were exposed only during alternate months (total of 9 months of exposure). At the end of the exposure, monkeys in both groups had developed respiratory bronchiolitis, increased volume density of respiratory bronchioles, and alterations in lung growth. The monkeys that received ozone exposures only during alternate months had for the most part pulmonary alterations equivalent to those in the group receiving ozone exposures throughout the entire 18-month period and in some cases greater alterations, such as greater collagen deposition. In the rat study, there were no significant differences between the two exposure groups; both groups had more bronchiole-alveolar duct junctions as determined by morphometric analyses.

A comprehensive study of F344 rats exposed at 0.12, 0.5, or 1.0 ppm 6 h/day, 5 days/week for 3 or 20 months has been summarized by Catalano et al. (1995). Detailed morphometric examinations of the noses and lungs of the animals were conducted by a team of investigators in several institutions (Chang et al. 1995; Harkema et al. 1994; Pinkerton et al. 1995; Stockstill et al. 1995). Adverse effects were found in the nasal, tracheobronchial, and pulmonary centriacinar airways. Rats chronically exposed at 0.5 and 1.0 ppm, but not 0.12 ppm, had marked alterations in the nasal airways consisting of chronic rhinitis, turbinate atrophy, epithelial hyperplasia, and mucous-cell metaplasia or hyperplasia. Chronic exposure to ozone at all concentrations caused epithelial alterations in the centriacinar regions of the lung. Similar nasal and pulmonary lesions have been reported in mice exposed at 0.5 or 1.0 ppm for 2 years (Herbert et al. 1996). The lesions were shown to persist with an additional 6 months of exposure.

Although it is well documented that the nasal and pulmonary alterations in all laboratory animals are similar, the concentrations at which ozone-induced

lesions are observed differ among rodents and nonhuman primates. After reviewing comparable acute and chronic ozone-exposure studies in rodents (rats and mice) and in macaques, Hyde et al. (1994) estimated that monkeys are about 10 times more sensitive to the development of ozone-induced nasal and pulmonary lesions.

In a more recent study, infant monkeys (30 days old) were episodically exposed to ozone at 0.5 ppm alone or with house-dust mite allergen (HDMA) 8 h/day, 5 days/week every 14 days for a total of 11 ozone episodes (Schelegle et al. 2003). The 6-month episodic exposure to ozone alone or with HDMA caused profound remodeling of the distal airways and centriacinar region and loss of bronchiolar airways.

Reproductive Toxicity in Males

There are few reports in the scientific literature on the effects of ozone on the male reproductive system. Exposure of male and female mice to ozone at 0.05-0.09 ppm before breeding did not affect pregnancy rate, the weight of live fetuses, or skeletal or soft-tissue malformations in offspring (Zhou et al. 2006). In a second study, male rats were exposed to ozone at 0.5 ppm or control air 5 h/day for 50 days (Jedlinska-Krakowska et al. 2006). The number of successful matings and the survival of pups were equivalent in the two groups. The testes of the ozone-exposed and control rats were not different with regard to morphology or motility of sperm, but sperm concentration was 17% lower in the ozone-exposed rats.

Sokol et al. (2006) retrospectively studied the relationship between air pollution and human sperm quality over a 2-year period in Los Angeles, California. A linear mixed-effects model was used to study average sperm concentration and total motile sperm count for each donation (more than 5,000 semen samples) from each study participant (48 donors). The model indicated a statistically significant negative correlation between ozone concentration 0-9, 10-14, and 70-90 days before sperm donation and average sperm concentration. Other pollution measures did not correlate with differences in sperm quality. The average daily ozone concentration during the study was 0.022 ± 0.009 ppm. Bonde (2007) indicated that welders who have an occupational exposure to ozone have been reported not to have lower sperm counts.

Immunotoxicity

The immune system is a sensitive target for ozone-induced toxicity (Gilmour et al. 1993a; Gilmour et al. 1993b; Gilmour and Selgrade 1993; Ryan et al. 2002; Selgrade et al. 1988). Ozone exposures at high ambient concentrations (0.08-0.22 ppm) have been shown to induce adverse effects on the local airway mucosal and systemic immune systems in laboratory animals and in hu-

mans. The most sensitive effects include inhibition of bacterial phagocytosis by alveolar macrophages (Devlin et al. 1991; Driscoll et al. 1987; Van Loveren et al. 1988), production of proinflammatory cytokines and mediators (Balmes et al. 1996; Becker et al. 1991; Devlin et al. 1991; Driscoll et al. 1993; Driscoll et al. 1987; Jaspers et al. 1997; Scannell et al. 1996; Torres et al. 1997), and recruitment of inflammatory cells into the lung (Devlin et al. 1991; Koren et al. 1989) and the nasal airways (Graham et al. 1988; Graham and Koren 1990; Harkema et al. 1987). The ozone-induced effects could influence the development of CD4+ T_H2 lymphocytic cytokine responses in allergic airway diseases, such as asthma and allergic rhinitis. Mice exposed to ozone at 0.13 ppm had enhanced allergic sensitization (Osebold et al. 1988), and atopic asthmatic human subjects exposed at 0.12 ppm had increased bronchial responsiveness to allergens (Molfino et al. 1991). In that regard, epidemiologic studies support the experimental findings (Bascom 1996). Asthmatic children living in the inner city of Atlanta had more emergency-room visits on days when ozone concentrations were greater than 0.11 ppm (White et al. 1994).

Genotoxicity

Several in vitro and in vivo studies have been conducted to investigate the genotoxicity and mutagenicity of ozone (Victorin 1996). The research includes in vitro mutagenicity tests in a variety of cell types (bacteria, yeast, plants, human cell lines, and other mammalian cells) and in vitro assays for chromosomal alterations in cells from laboratory animals exposed to ozone at higher than ambient concentrations. Some of the studies have shown that ozone is genotoxic and mutagenic. Collectively, the data from the genotoxicity studies suggest that ozone is at most a weak mutagen, but more data are needed to draw definitive conclusions. However, the reactive, gaseous, and toxic nature of ozone makes it difficult to conduct interpretable studies in those test systems.

Carcinogenicity

In a National Toxicology Program chronic bioassay study, male and female rats and mice were exposed to filtered air or ozone at 0.12, 0.5, or 1.0 ppm 6 h/day, 5 days/week for 2 years or a lifetime (Boorman et al. 1994; Herbert et al. 1996). The results in male and female F344/N rats showed no evidence of carcinogenic activity. In male B6C3F1 mice, there was equivocal evidence of carcinogenic activity. There was some evidence of carcinogenic activity in female B6C3F1 mice only at the highest concentration (1.0 ppm). Other lung-tumor development studies that exposed rats, hamsters, or mice chronically to ozone at up to 0.8 ppm for less than their lifetime were either negative or ambiguous for ozone-induced carcinogenicity (Hassett et al. 1985; Ichinose and Sagai 1992; Last and Warren 1987; Witschi et al. 1993). Thus, ozone has been

shown to be a weak pulmonary carcinogen only in female mice at one concentration and in only one long-term inhalation study. EPA and the International Agency for Research on Cancer have not provided any classification regarding ozone's carcinogenic potential.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

Because ozone is a highly reactive gas, it has a negligible half-life, and its uptake is limited to the air-liquid interface lining the mucosal membranes of the respiratory tract. In resting subjects, 40-50% of inhaled ozone is absorbed in the nasopharyngeal airways with nasal breathing or in the mouth and pharynx with oral breathing. The conducting airways remove 90% of the remainder of the inhaled ozone that reaches the lower respiratory tract. Therefore, about 95% of the total inhaled ozone is removed in the respiratory tract (Asplund et al. 1996; Gerrity et al. 1995; Gerrity et al. 1988; Hu et al. 1992a; Hu et al. 1992b). The efficiency of ozone uptake varies directly with concentration and inversely with breathing rate (Gerrity et al. 1988). With increased ventilation rates, there is a decrease in both upper and lower airway absorption that results in more penetration of ozone into the lung (Hu et al. 1992a; Hu et al. 1992b).

Mathematical models of ozone dosimetry in the respiratory tract have estimated that the rate or amount of ozone uptake in the lung would be greatest in the centriacinar regions. The mathematical predictions for the primary intrapulmonary site of ozone-induced toxicity (Miller et al. 1985; Overton et al. 1987) support the numerous experimental-animal studies that have identified site-specific, ozone-induced lesions in this distal region of the respiratory tract. Experimental dosimetry studies with ^{18}O-labeled ozone have also shown that exercising humans had ^{18}O concentrations in their BAL fluid 4-5 times greater than those in the BAL fluid of similarly exposed resting laboratory rats (Hatch et al. 1994). The results of that comparative dosimetry study are consistent with pulmonary physiology studies that suggest that ozone has greater detrimental effects on the lung function of humans than in animals (Costa et al. 1989; Overton et al. 1987).

Acute responses to controlled exposures to ozone cause alterations in lung function, airway caliber, breathing pattern, respiratory symptoms, and airway inflammation. More than one biologic mechanism appears to mediate the responses to ozone exposure. Broadly categorized, the ozone-induced alteration mechanisms are due to neural or inflammatory mechanisms. Several experimental studies in animals and humans have shown that the reduction in pulmonary function with acute ozone exposure is mediated through the parasympathetic system. Ozone stimulates vagal afferents, including C fibers and rapidly adapting receptors, and this results in vagal reflexes that cause increases in airway resistance and frequency of respiration, symptoms of respiratory irritation, and a decrease in tidal volume (Beckett et al. 1985; Gertner et al. 1983a; Gertner et al.

1983b; Gertner et al. 1983c; Lee et al. 1979; Passannante et al. 1998; Schelegle et al. 2001; Schelegle et al. 1993).

Airway inflammation caused by inhaled ozone is a secondary response to toxicant-induced damage to the epithelial cells lining the luminal surface of the respiratory tract. The extreme reactive nature of ozone with the fluid lining the respiratory tract (epithelial lining fluid, or ELF) makes it unlikely that it passes unreacted into the airway lining cells and causes direct cytotoxicity (Pryor 1992). Ozone is more likely to react with lipids high in unsaturated fatty acids in the ELF or in the outer epithelial cell membranes (lipid peroxidation). Ozonation in the airway lumen, which also has high water content, produces aldehydes, hydroperoxides, and small amounts of ozonides (Driscoll et al. 1993; Frampton et al. 1999a; Frampton et al. 1999b; Leikauf et al. 1993; Pryor et al. 1995a; Pryor et al. 1995b). The ozonation products stimulate airway epithelial cells to release a variety of proinflammatory agents, including eicosanoids, platelet-activating factor, reactive oxygen species, and inflammatory cytokines (Leikauf et al. 1995a; Leikauf et al. 1995b; Pryor et al. 1995a; Schelegle et al. 1989). Ozone-exposed epithelial cells release inflammatory mediators, such as IL-6, IL-8, and fibronectin (Devlin et al. 1994). Cytokines and chemokines released from the injured epithelium recruit neutrophils and monocytes and macrophages into the airways. The activated inflammatory cells release additional mediators that may amplify the inflammatory response and promote later airway structural and functional alterations. Ozone-induced inflammation may directly amplify oxidative damage to the airway tissues due to ozone. It takes several hours for the inflammatory cascade to develop after the start of acute exposure when initial pulmonary function and respiratory symptoms may have abated (Blomberg et al. 1999; Foster et al. 2000; Schelegle et al. 1991). The presence of inflammatory cells, such as PMNs, and inflammatory mediators in the BAL fluid of exposed subjects are important indicators of acute airway injury (Balmes et al. 1996; Foster and Stetkiewicz 1996; Koren et al. 1989).

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

A few organizations have established or proposed acceptable inhalation exposure limits or guidelines for ozone. Selected values are summarized in Table 9-3.

COMMITTEE RECOMMENDATIONS

The committee's recommendations for EEGL and CEGL values for ozone are summarized in Table 9-4. The current and proposed U.S. Navy values are provided for comparison.

TABLE 9-3 Selected Inhalation Exposure Levels from the NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level	Reference
Occupational			
ACGIH	TLV-TWA (heavy work)	0.05 ppm	ACGIH 2001
	TLV-TWA (moderate work)	0.08 ppm	
	TLV-TWA (light work)	0.10 ppm	
	TLV-TWA (2-h, all work types)	0.2 ppm	
NIOSH	REL-Ceiling	0.1 ppm	NIOSH 1997
OSHA	PEL-TWA	0.1 ppm	29 CFR 1910.1000
Submarine			
NRC	EEGL		NRC 1984
	1-h	1 ppm	
	24-h	0.1 ppm	
	CEGL		
	90-day	0.02 ppm	

[a]The comparability of EEGLs and CEGLs with occupational-exposure and public-health standards or guidance levels is discussed in Chapter 1 ("Comparison with Other Regulatory Standards or Guidance Levels").
Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; NIOSH, National Institute for Occupational Safety and Health; NRC, National Research Council; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

TABLE 9-4 Emergency and Continuous Exposure Guidance Levels for Ozone

Exposure Level	U.S. Navy Values (ppm)		Committee Recommended Values (ppm)
	Current Values	Proposed Values	
EEGL			
1-h	1	0.3	0.5
24-h	0.1	0.1	0.1
CEGL			
90-day	0.02	0.02	0.02

Abbreviations: CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level.

1-Hour EEGL

There is a preponderance of strong dose-response data in the scientific literature on short-duration ozone exposure (hours) in human populations of similar age and sex as submariners, and the committee derived the 1-h EEGL from the weight of evidence from the controlled human studies. Clinical research has demonstrated that healthy young men (18-34 years old) at rest (Folinsbee et al. 1978; Horvath et al. 1979) or performing moderate to heavy intermittent exercise (DeLucia and Adams 1977; Folinsbee et al. 1978; McDonnell et al. 1983) or continuous exercise (Adams et al. 1981; Adams and Schelegle 1983; Folinsbee and Horvath 1986) will develop marked decrements in pulmonary function and symptoms of breathing discomfort, such as chest tightness and cough, when exposed to ozone at less than 1 ppm for 1-2.5 h. Collectively, the studies of exercising healthy men have clearly demonstrated that 1 h of continuous exercise or 2-2.5 h of intermittent exercise increases the deleterious pulmonary-function responses to acute ozone exposure. However, in determining the 1-h EEGL for ozone, the committee assumed that most submariners would have VE equivalents closer to "rest" than to the "moderate-to-heavy" exercise paradigms used in the experimental studies and protocols reviewed here because of the confined conditions on the submarine. The lowest ozone concentration at which modest reductions in FVC and FEV_1 have been reported in nonexercising young men after 2 h of controlled exposures is 0.5 ppm (Folinsbee et al. 1978; Horvath et al. 1979). A concentration of 0.5 ppm was used by the committee as the starting point for the derivation of the 1-h EEGL. Because the controlled human studies used short exposure durations and age classes of interest, no further adjustments to the 1-h EEGL were needed for these specific areas. Variability in sensitivity to low ozone concentrations for that short exposure in low to moderate activity was assumed to be minimal, and an intraspecies adjustment was not considered to be warranted. Therefore, the committee determined that a 1-h exposure to ozone at 0.5 ppm would not impair a submariner's ability to conduct normal or emergency activities.

24-Hour EEGL

There have been no human studies of controlled ozone exposures for 24 h. The committee's determination of a recommended 24-h EEGL was based on the weight of evidence from the controlled human studies at low ozone concentrations (0.08-0.12 ppm) for durations of 4-8 h with a range of exercise loads (Folinsbee et al. 1988; Horstman et al. 1990; McDonnell et al. 1991). In those studies, ozone exposures caused dose-dependent symptoms of cough and chest discomfort, increases in airway responsiveness to methacholine challenge, and consistent but transient decrements in pulmonary function, such as FEV_1 and FVC. Further analysis of the data suggests that the ozone-pulmonary response

relationship plateaus after a 6.6-h exposure protocol. Therefore, further decrements in respiratory function of functional and operational significance with an extended exposure up to 24 h are not expected, and the committee did not consider that a time adjustment factor was warranted. The committee acknowledged that the response database exhibits population variability in ozone-induced changes in respiratory function. However, it concluded that the observed degree of change would be clinically or operationally insignificant for low to moderate activity in a submariner population. Therefore, response variability in sensitivity to the low ozone concentrations in submariners in low to moderate exercise for a 24-h exposure was assumed to be low, and no intraspecies adjustment was applied. The committee concluded that exposure to ozone at 0.1 ppm during a 24-h period should not impair a healthy submariner from conducting normal or emergency activities.

90-Day CEGL

There have been no 90-day controlled human exposures to ozone. However, the 90-day exposure study of macaques conducted by Harkema and colleagues (Harkema et al. 1987; Harkema et al. 1993) demonstrated that exposures at 0.15 and 0.30 ppm (6 h/day, 5 days/week) resulted in conspicuous morphologic but subclinical airway injury and remodeling in the nose and lung. Although the reversibility of the airway lesions in monkeys has not been determined, similar nasal airway lesions induced by ozone in laboratory rats have been shown to persist, although markedly attenuated, at least 3 months after the end of a 90-day exposure (Harkema et al. 1999). Therefore, the committee used a concentration of 0.15 ppm as a starting point for deriving the recommended 90-day CEGL. Because the morphology of the upper and lower respiratory tract of the macaque closely resembles that of humans (Tyler 1983), an interspecies uncertainty factor of 1 was used in the committee's determination. An uncertainty factor of 10 was used to adjust from a lowest observed-adverse-effect level to a no-observed-adverse-effect level. That resulted in a recommended 90-day CEGL for ozone of 0.02 ppm, which is well below the EPA NAAQS concentration of 0.08 ppm and within estimated background concentrations of outdoor ozone in the United States.

DATA ADEQUACY AND RESEARCH NEEDS

There is a lack of data on personal exposure of submariners to ozone and other oxidant gases. The committee suggests that the Navy consider conducting exposure studies designed to determine the personal exposure of submariners to ozone during their short- and long-term tours of duty.

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10

Surface Lead

This chapter summarizes relevant toxicologic data on lead poisoning in adult humans. Selected chemical and physical properties and toxicokinetic and mechanism-of-action data are also presented. The committee could not recommend surface-lead exposure guidance levels, but it did endorse the monitoring of submariner blood lead concentrations to determine whether surface lead contamination onboard submarines is resulting in appreciable exposure of crews. Blood lead concentrations in the context of occupational lead exposures are therefore discussed. Adequacy of available data for defining submarine surface-lead exposure levels and research needed to define more clearly the issue of potential lead exposure among submariners are also outlined.

PHYSICAL AND CHEMICAL PROPERTIES

Elemental lead is a member of periodic table group IVB. Common commercially important forms of inorganic lead include elemental lead, lead acetate, lead azide, lead bromide, lead chloride, lead chromate, lead fluoroborate, lead iodide, lead molybdenum chromate, lead nitrate, lead oxide, lead phosphate, lead styphnate, lead sulfate, and lead sulfide. Metallic lead is a blue-grey material that is solid at room temperature. Physicochemical properties of lead are presented in Table 10-1.

OCCURRENCE AND USE

Lead is a ubiquitous element in the environment and biologic systems (Goyer 1996). Exposure to lead can occur from ingestion (for example, through the consumption of contaminated food, water, or soil), inhalation, and, in the

TABLE 10-1 Selected Physical and Chemical Data on Elemental Lead

Synonyms and trade names	NA
CAS registry number	7439-92-1
Molecular formula	Pb
Atomic weight	207.2
Boiling point	1,740°C
Melting point	327.4°C
Flash point	NA
Density	11.34 g/cm^3 at 20°C
Vapor pressure	1.77 mm Hg at 1000°C
Solubility	Salts have variable solubility in water; elemental lead is soluble in hot or concentrated mineral acids

Abbreviations: NA, not applicable or not available
Source: Data from Budavari et al. 1989.

case of organic forms of lead, dermal absorption (Bolger et al. 1996). Historically, elemental lead has been an important component of solder, brass, bronze, and other alloys. Lead is found in electric-cable insulation, and the oxide is found in some paints, inks, glass, crystal, plastics, textiles, and ceramics. At least half the elemental lead used worldwide is in lead-acid batteries (ATDSR 1999). Lead is present in stainless steel—such as that used in food-handling equipment and surfaces—as an unintentional contaminant at not more than 0.1% by weight (Precision Specialty Metals 2003; Nucor 2007). A variety of occupations are associated with lead exposure, including those involved in lead smelting, battery manufacturing, firing ranges, welding, construction, and demolition. Occupational exposure is often the most significant source of exposure of adults (Shannon 1998).

SUMMARY OF TOXICITY

The toxicology, epidemiology, and clinical presentation of lead intoxication have been reviewed (EPA 1986; ATSDR 1999). Lead is a cumulative poison that is poisonous in all forms, its elimination from the body is slow, and consequences of exposure are varied and can be severe (Gosselin et al. 1984). Generally, only 5-10% of ingested lead is absorbed from the adult human gastrointestinal tract. Lead may dissolve to an appreciable degree in the acid environment of the stomach, greatly increasing its absorption. Absorption of lead from the gastrointestinal tract is facilitated by the same mucosal transport proteins that mediate calcium transport (Fullmer 1992). Smaller lead particles are more readily absorbed from the gastrointestinal tract. In the blood, about 95% of

the lead is associated with the red blood cells (RBCs), so measurement of blood lead concentrations is preferred to measurement in serum or plasma. Lead has a half-life of about 30 days in adult blood. Once absorbed, it readily passes membrane barriers, such as the blood-brain barrier. Distribution is primarily to the kidney cortex, liver, and bone; bone contains up to about 90-98% of the total body lead burden. Lead is excreted by the kidney, but the elimination rate varies, depending on the tissue that absorbed the lead.

Lead perturbs multiple enzyme systems, especially ones that contain sulfhydryl groups or are zinc-dependent. Signs of lead toxicosis are generally associated with the nervous, hematologic, and urinary systems (Table 10-2). Blood lead concentration has proved to be a valuable biomarker of exposure. The U.S. Environmental Protection Agency (56 Fed. Reg. 26460[1991]) summarized key aspects of the relationship between circulating lead concentration and adverse health effects (see Table 10-2). Although a valuable indicator of exposure, blood lead concentration does not indicate the length of exposure or the total amount of lead deposited in the body.

Effects in Humans

Sufficient data on the toxicity of lead were available to allow the committee to focus strictly on the human clinical literature. One of the best characterized and most sensitive adverse effects of lead is inhibition of the serum enzyme delta aminolevulinic acid (ALA) dehydratase (Godwin 2001). Lead-induced inhibition of ALA dehydratase can result in increased elimination of ALA in the urine. Determination of urinary ALA excretion has some merit as a diagnostic test for lead poisoning; however, urinary ALA may be unreliable when the daily intake of lead is small. Lead also inhibits ferrochelatase, an enzyme in the heme-biosynthesis pathway. Inhibition of this enzyme results in decreased heme production and accumulation of protoporphyrin in the RBCs of poisoned people (Godwin 2001).

Adverse effects on the nervous system also occur after acute or chronic lead poisoning. Fulminant encephalopathy, wrist drop, and peripheral neuritis are signs of neurotoxicity (plumbism) in adults. Neurologic manifestations of plumbism occur when blood lead concentrations approach or exceed 100-120 μg/dL. Encephalopathy can quickly progress, people going from full consciousness to convulsions and terminal coma within hours. Reduced sensory and motor nerve-conduction velocities can be observed when blood lead concentrations exceed 40-70 μg/dL (Araki et al. 2000; Triebig et al. 1984). There is no clear evidence, however, that adult blood lead concentrations under 30 μg/dL impairs peripheral nerve function (Araki et al. 2000).

TABLE 10-2 Blood Lead Concentrations and Associated Observed Effects in Exposed Men

Blood Lead Concentration (μg/dL)	Observed Effect			
	Cardiovascular, Hematologic, Heme Synthesis	Nervous System	Renal System	Reproductive System
100-120	—	Encephalopathy	Chronic nephropathy	—
80	Frank anemia	—	—	—
60	—	—	—	—
50	—	Subencephalopathic signs	—	Altered testicular function
40	Increased urinary ALA and coproporphyrins	Peripheral neuropathy	—	—
30	Increased blood pressure	—	—	—
25-30	Increased protoporphyrin concentrations	—	—	—
15-20	—	—	—	—
<10	ALA dehydratase inhibition	—	—	—

Abbreviation: ALA, delta-aminolevulinic acid.
Sources: Adapted from 56 Fed. Reg. 26460 (1991); Shannon 1998.

Hypertension has been associated with acute and chronic occupational lead exposure. Overall, the effect of increased blood lead concentration on blood pressure is still the subject of debate because many studies demonstrating an association failed to account for the influence of tobacco-smoking or ethanol consumption (Staessen et al. 1995). Another common criticism of the studies concerns the relatively small numbers of men in each group (under 100), and not all the clinical investigations have confirmed the association. Surveys of 7,371 British men 40-59 years old found that alcohol consumption, body-mass index (BMI), and tobacco-smoking had a greater influence than blood lead on systolic blood pressure (Pocock et al. 1988). Policemen (n = 89) with blood lead concentrations of 30 μg/dL or higher had a significant increase in systolic pressure even after adjustment for tobacco-smoking, BMI, and age (Weiss et al. 1988). In another study that adjusted for potential confounders, systolic blood pressure of 53 male lead workers (blood lead concentrations, 44-51 μg/dL) was

significantly higher than that of 52 workers without lead exposure (blood lead concentrations, under 20 μg/dL) (de Kort et al. 1987). Lead-exposed construction workers developed clinical hypertension when blood lead concentrations increased from 48-50 to 85-120 μg/dL (Pollock and Ibels 1986; Marino et al. 1989). After controlling for exercise, tobacco and alcohol consumption, age, and educational level, Parkinson et al. (1987) could not detect a significant association between increases in diastolic or systolic blood pressure and blood lead concentrations in 270 white male lead-battery plant workers (blood lead concentration, 40 ± 13 μg/dL) and 158 similar males (blood lead concentration, 7 ± 5 μg/dL) who did not work with lead. Pirkle et al. (1985) suggested that a relationship of blood lead concentration to blood pressure was more evident at lower than at higher blood lead concentrations. Overall, there is suggestive evidence of an increase of 1.0-2.0 mm Hg in systolic blood pressure and 0.6 mm Hg in diastolic pressure associated with a doubling of blood lead concentration (Staessen et al. 1995; Victery et al. 1985). Staessen et al. (1995) concluded that increased blood pressure associated with increased low-level lead exposure was "unlikely to entail any public health implication in terms of hypertension-related complications." Excessive urinary lead concentrations (100 to over 250 μg/dL) in a group of retired lead workers were associated with a significant excess risk of cerebrovascular accident (Dingwall-Fordyce and Lane 1963).

Lead is also a recognized nephrotoxin. Two distinct renal syndromes are associated with excessive lead exposure, one acute and temporary and the second chronic and progressive (Gosselin et al. 1984). Acute plumbism can result in reversible impairment of proximal tubular function (Tepper 1963). Prolonged lead-induced damage to the human kidney is associated with interstitial fibrosis with atrophy and dilation of the tubules. It is difficult to relate current blood lead concentrations to reduced kidney function because they do not necessarily correlate with past lead exposures. Duration of lead exposure is an important determinant of potential renal toxicity (Lilis et al. 1968), and relatively low blood lead concentrations in an affected person may reflect a long interval between the last lead exposure and when blood lead concentrations were measured. In general, the risk of chronic nephropathy is increased when blood lead concentrations are over 60 μg/dL (ATSDR 1999).

Renal biopsy of men with pyelonephritis and blood lead concentrations of 70-138 μg/dL found diffuse interstitial or peritubular fibrosis, sclerotic and obliterated glomeruli, proximal tubular degeneration characterized by mitochondrial swelling, and deposition of eosinophilic dense-staining granular inclusion bodies (Pollock and Ibels 1986). Renal biopsy of 12 men with blood lead concentrations between 40 and over 80 μg/dL found characteristic focal interstitial nephritis (Wedeen et al. 1979).

Male Reproductive Toxicity

At blood lead concentrations of 66 μg/dL or higher, lead can act directly on the testes, as evidenced by reduced testosterone synthesis and induction of peritubular testicular fibrosis (Braunstein et al. 1978; Cullen et al. 1984; Rodamilans et al. 1988a,b). Although a number of laboratory animal models have documented the gametotoxic consequences of acute and chronic lead exposure (Stowe and Goyer 1971; EPA 1986), only data relevant to men and the adverse effects of lead on human male fertility are considered here.

Lin and associates (1996) found reduced fertility in 4,256 adult males who were occupationally exposed to lead and had blood lead concentrations of 25 μg/dL or higher, compared with 5,148 matched controls. Although Lin et al. (1996) concluded that men with the highest cumulative exposure had the greatest reductions in fertility, the study failed to account for contraception use or marital status. Gennart et al. (1992) found that fertility decreased with duration of lead exposure in a group of 74 male lead factory workers (mean age, 39 years) who had a mean blood lead concentration of 46 μg/dL. However, the fact that no independent assessment of the worker's wives was carried out by the authors reduces confidence in that finding. Others (Coste et al. 1991; Bonde and Kolstad 1997) failed to document any associations between blood lead concentrations of less than 40 to 60 μg/dL or 39 μg/dL and live-birth rates per couple among 229 French and 1,349 Danish battery workers, respectively.

Ng et al. (1991) studied circulating testosterone, prolactin, lutenizing hormone (LH), and follicle stimulating hormone (FSH) in 122 lead-battery workers (mean blood lead concentration, 35 μg/dL) compared with 49 controls (blood lead concentration, 8.3 μg/dL). Smokers displayed reduced prolactin concentrations, and workers aged 40 years old or older had reduced testosterone concentrations. Among lead workers, circulating LH and FSH were increased.

Among 150 lead workers examined by Lancranjan et al. (1975), a reduction in sperm vitality (asthenospermia) was associated with blood lead concentrations of 53-74 μg/dL. At least five studies (Alexander et al. 1996; Assennato et al. 1987; Chowdhury et al. 1986; Lerda 1992; Wildt et al. 1983) have documented significant reductions in sperm count and increased numbers of abnormal sperm in men with blood lead concentrations of 40 μg/dL or higher.

Genotoxicity

Lead-exposed workers with blood lead concentrations of 28.2 to 65.5 μg/dL had an increased incidence of micronuclei in peripheral lymphocyte and clastogenic and aneugenic effects in peripheral lymphocytes (Palus et al. 2003). An increased number of chromatin defects were observed in sperm collected from men with blood lead concentrations of 45 μg/dL or higher (Bonde et al. 2002).

Carcinogenicity

The International Agency for Research on Cancer (IARC 1987) considered the results of bioassays of parenteral and oral lead (for example, lead acetate and lead phosphate) as sufficient evidence of carcinogenicity in animals. At least two groups have published the results of mortality analyses of U.S. workers in lead smelters and battery plants (Cooper et al. 1986; Selevan et al. 1984). There was a nonsignificant increase in respiratory tract cancer (36.9 cases expected compared with 41 observed), but excess renal cancers (2.9 expected and six observed) and bladder cancers (4.2 expected and six observed) were seen in those employed in smelters (Selevan et al. 1984). There was a significant excess of stomach cancers (20.2 expected and 34 observed) and respiratory tract cancers (93.5 expected and 116 observed) in lead workers (Cooper et al. 1986). However, the smelter and battery-plant workers were also exposed to other carcinogenic materials and processes. IARC (1987) considered the evidence of human carcinogenicity of inorganic lead inadequate.

TOXICOKINETIC CONSIDERATIONS

Depending on the physical characteristics of a particular lead-containing material, substantial differences in relative bioavailability can exist. The bioavailability of lead is a function of its chemical form, route of exposure, and physical state. The bioavailability of the lead in lead-acid batteries, cable coverings, ammunition, solder, caulking, paint, and dust depends in large part on particle size.

Percutaneous uptake of lead acetate through intact skin of human volunteers was negligible (0-0.3%) (Moore et al. 1980).

Deposition of inhaled lead also depends on particle size. Particles deposited in the upper airway are cleared and swallowed. As particle size increases from less than 0.05 μm to 0.05-0.5 μm, the fraction deposited in lung declines from 34-60% to 10-30% (Booker et al. 1969; Chamberlain et al. 1975; Gross 1981; Hursh and Mercer 1970; Morrow et al. 1980). As particle size increases, impaction and sedimentation increase; 28-70% deposition rates were seen in metal scrap yard and lead-battery plant workers (Mehani 1966). Uptake of inhaled lead also depends on the inhalable fraction of the material.

Lead absorption from the gastrointestinal tract is influenced by diet, age, particle size, dose, and calcium and iron nutritional status. Most adults absorb about 7-15% of the soluble lead found in foods. Chemical forms that predominate under alkaline conditions (for example, lead carbonate) are more soluble in gastric contents and are more readily absorbed from the stomach than less soluble forms (for example, lead sulfate). Uptake from the gastrointestinal tract occurs primarily in the duodenum (Mushak 1993). Absorption of soluble lead (consumed as either the nitrate or the acetate) was increased in fasting adults

compared with the amount that was absorbed when the same dose was consumed with food (4-8%) (Rabinowitz et al. 1980; James et al. 1985). Fractional lead absorption from metallic particles in the rat gastrointestinal tract decreased as particle size increased from 5 μm to 200 μm in mean diameter (Barltrop and Meek 1979). Gastrointestinal absorption of lead (consumed as the acetate) by adult volunteers was reduced from 63% of the ingested dose to 10% when the same quantity was consumed with calcium or phosphate salts (Heard and Chamberlain 1982).

Bioavailability can differ among white lead (basic lead carbonate), red lead (Pb_3O_4), basic lead sulfate, or lead chromate. The small size of ingested lead sulfate particles contributed to a higher relative absorption from the rat gastrointestinal tract (164%) than lead acetate, but absorption of lead from lead chromate particles of similar size was 56% less than from lead acetate (Barltrop and Meek 1975).

The pharmacokinetics of lead in healthy people can be described by using a three-compartment model (Rabinowitz et al. 1976). Elimination from the first compartment (blood) has a half-time of about 35 days. Nearly all (90-99%) circulating lead is found in RBCs complexed with hemoglobin (Hb); the remainder is bound to albumin and gamma-globulin. Because lead binding to Hb is capacity-limited, the plasma lead fraction increases as blood lead increases. In career lead employees with blood lead concentrations of 55-60 μg/dL, circulating lead concentrations declined to 40-50 μg/dL within 4-6 weeks of work cessation, but further reductions over the next 18 months were imperceptible (O'Flaherty 1986).

The second compartment—represented by bile, hair, nails, saliva, sweat, digestive secretions, and soft tissues—has a similar elimination half-time. Soft-tissue lead exists primarily bound to cytosolic proteins, including metallothionein and high-affinity cytosolic binding proteins. The latter are thought to be associated with the formation of intranuclear inclusion bodies in the epithelium of the renal proximal tubule (Moore and Goyer 1974).

A third (deep) human compartment—consisting of mineralized structures, including bone and teeth—contains the greatest fraction of the lead body burden. The lead elimination half-time from bone is 2-3 decades. Trabecular bone has a relatively rapid turnover and faster lead elimination than cortical bone. Lead in bone can contribute about 50% of current blood lead (Goyer 1996). Some 17% of blood lead in excess of 50 μg/dL found in macaques originated from bone lead that had accumulated over 11 years of lead exposure (O'Flaherty et al. 1998).

Voluntary ingestion of lead at 0.3-3.0 mg/day (as lead acetate in drinking water) for 16-208 weeks by adults was followed by fecal elimination of over 90% of the administered dose (Kehoe 1987). Lead is excreted in urine, and urinary lead concentration has been used to gauge occupational lead exposure (biological exposure index, 150 μg/g of creatinine) (ACGIH 1994), but the rate of urinary lead elimination is not proportional to blood lead concentration.

Using a physiologically based pharmacokinetic lead model applied to a 20-year-old man employed 40 h/week with occupational lead exposure for 10 years and accounting for dietary and drinking-water lead exposure, O'Flaherty (1993) found that blood lead tripled over that period. For a 45-year-old man having blood lead of 55-60 μg/dL after 25 years of employment and exposure to lead at 84 $\mu g/m^3$ in workplace air, only 4% of total skeletal lead had been eliminated 18 months after removal from work, and 26% of bone lead was removed after 10 years (O'Flaherty 1993).

MAXIMAL INORGANIC SURFACE LEAD CONCENTRATIONS FROM OTHER ORGANIZATIONS

A number of organizations have promulgated or otherwise established maximal surface lead concentrations (Table 10-3).

COMMITTEE RECOMMENDATIONS

The civilian occupational regulations cited in Table 10-3 reflect common work practices associated with the lead industry. They include physically separate eating areas, clothing-changing areas to prevent cross-contamination, separate shower facilities, vacuuming, downdraft booths, specific cleaning methods, and enforceable limits on eating, drinking, tobacco-smoking and tobacco-chewing, and other hand-to-mouth behaviors. The civilian workplace practices are not practical for submariners that reside, work, eat, and sleep in an enclosed and isolated environment.

TABLE 10-3 Selected Maximal Surface Lead Concentrations

Organization	Type of Level/Activity	Maximum Concentration	Reference
State of California	Welding, cutting, scraping, sanding, surface coating	0.06% dry weight	8 CCR 1532.1
DOL	Surfaces	All surfaces shall be maintained as free as practicable of accumulations of lead	1910.1025(h)(1)
HUD-EPA	Residential floors	40 $\mu g/ft^2$	24 CFR 35.1350
NSF	Residential food-zone materials	0.06% lead	NSF/ANSI 51-2002

Abbreviations: ANSI, American National Standards Institute; DOL, Department of Labor; EPA, Environmental Protection Agency; HUD, Department of Housing and Urban Development; NSF, National Sanitation Foundation

Sufficient information concerning the likely human exposures arising from surface lead contamination onboard a submarine was not provided to the committee. An increase in blood lead concentration due to ingestion of a contaminated material, such as dusts found on surfaces, depends on the rate at which the contaminant is ingested (μg/g per day), the concentration of lead in the contaminant (μg/g), and a coefficient relating lead ingestion from a contaminant to blood lead concentration (μg/dL per μg/day) (Stern 1996). Additional consideration of inhalation exposure may also be required if the source of the lead on the contaminated surface is airborne. Thus, data needed to derive a surface lead concentration include characterization of crew exposure, exposure routes, sources of concern, physical and chemical forms, particle size, and bioavailability of the lead onboard the submarine. Lacking such data, the committee could not develop useful surface lead exposure concentrations. The committee endorses monitoring of submariner blood lead concentrations to determine whether surface lead contamination onboard submarines is resulting in appreciable crew exposure.

Occupational-lead risk characterizations are based predominantly on monitoring of blood lead concentrations in workers. The identification of a blood lead value that will protect submariners during their military careers is a prerequisite of any recommendation concerning lead limits for surface or airborne lead-containing paint, dust, or other materials. It is important to determine whether submariner urinary lead, zinc protoporphyrin, or blood lead values are higher than background concentrations for U.S. adults (Pirkle et al. 1994). Blood lead screening values and promulgated blood-lead worker-removal regulations are available from a number of sources (ACGIH 2003; CDC 1991; Title 8 California Code of Regulations Section 1532.1).

Wipe sampling and other techniques to assess surface contamination on work surfaces can provide useful information about worksite lead hazards. A few surface-contamination concentration guidelines have been published, but typical concentration limits must be established for a specific task (OSHA 1999).

DATA ADEQUACY AND RESEARCH NEEDS

No data were available to the committee on the physical nature, chemical identity, routes of exposure, or bioavailability of the surface lead materials of concern. To carry out submarine-specific lead health-risk analyses, data concerning generation, location, dispersion, and extent of onboard lead contamination, including the lead concentration in submarine drinking water, must be available. Available methods for site-specific human health risk assessment for lead-containing dust require rigorous estimates of the quantity of dust ingested daily. In the present circumstance, it appears unlikely that published estimates of lead house-dust exposure (Clark et al. 1995; Wang et al. 1995) could be applied with confidence to a submarine.

No data concerning inhalation exposure to lead onboard a submarine were available. Submarine air is one source that could contribute to a submariner's blood lead concentration (Snee 1981). It is well known that factors other than air lead influence blood lead concentration (Bishop and Hill 1983).

No data concerning urinary lead or blood lead concentrations of submariners were available to the committee. Thus, it is not clear whether significant exposure of the crew to lead occurs. Individual blood lead concentration is generally correlated with the duration of exposure and how much time has passed since termination of exposure (O'Flaherty et al. 1982). At the outset, however, it must be recognized that individual submariner blood lead concentrations reflect not only the combined occupational and residential lead exposures as a result of active duty but environmental lead exposures while the submariners are not engaged in submarine operations (O'Flaherty 1993). Lead-exposed people who have higher rates of hand-to-mouth behavior often have higher lead intake; individual hand-to-mouth lead exposure patterns can result in higher blood lead concentrations in those people than in people who do not eat or smoke in the same lead-containing environment.

It is important to establish whether submariner blood lead concentrations differ from those of civilian adults and active military personnel not engaged in submarine operations who live in the United States. One potential avenue that the committee highly recommends and that could assist in the definition of submarine-associated lead exposure is determination of crew urinary lead or blood lead concentrations before submarine deployment followed by identical measurements on completion of typical tours of duty. If individual submariners with increased blood lead concentrations are identified, identification of the lead sources during deployment or as a result of on-shore activity (such as pottery and hobbies) is necessary.

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11

Toluene

This chapter summarizes relevant epidemiologic and toxicologic studies of toluene. 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 committee considered all that information in its evaluation of the Navy's current and proposed 1-h, 24-h, and 90-day exposure guidance levels for toluene. The committee's recommendations for toluene exposure guidance levels are provided at the conclusion of this chapter with a discussion of the adequacy of the data for defining the levels and the research needed to fill the remaining data gaps.

PHYSICAL AND CHEMICAL PROPERTIES

Toluene is a flammable liquid at room temperature with a benzene-like odor (Budavari et al. 1989). The odor threshold has been reported to be 2.9 ppm (ATSDR 2000). Selected physical and chemical properties are presented in Table 11-1.

OCCURRENCE AND USE

Toluene is an important industrial chemical. It is used as a blending component for automotive fuels, as a chemical intermediate, and as a solvent primarily for paints and coatings and also for inks, adhesives, and pharmaceuticals.

Toluene is a common contaminant of outdoor and indoor air. The Agency for Toxic Substances and Disease Registry (ATSDR 2000) reported that toluene concentrations in suburban and urban air range from 1.3 to 6.6 ppb. Indoor air concentrations are often higher than outdoor air concentrations. The primary

TABLE 11-1 Physical and Chemical Properties of Toluene

Synonyms	Methylbenzene; phenylmethane
CAS registry number	108-88-3
Molecular formula	C_7H_8
Molecular weight	92.13
Boiling point	110.6°C
Melting point	−95°C
Flash point	4.4°C (closed cup)
Explosive limits	NA
Specific gravity	0.866 at 20°C/4°C
Vapor pressure	28.4 mm Hg at 25°C
Solubility	Very slightly soluble in water; miscible with alcohol, chloroform, ether, acetone, glacial acetic acid, carbon disulfide
Conversion factors	1 ppm = 3.77 mg/m^3; 1 mg/m^3 = 0.27 ppm

Abbreviations: NA, not available or not applicable.
Sources: Vapor-pressure data from HSDB 2006; all other data from Budavari et al. 1989.

source of toluene in outdoor air is motor-vehicle emissions; contributors to indoor air concentrations include emissions from household products and cigarette smoke. The toluene emission factor for cigarettes was reported as 80 µg/cigarette (ATSDR 2000).

Sources of toluene in a submarine include paints and coatings (Crawl 2003). The committee notes that cigarette-smoking is also a likely contributor to toluene concentrations in a submarine. Raymer et al. (1994) reported the results of air sampling conducted during the missions of two submarines. The fan room, galley, and engine room in each submarine were sampled over 6 h. Sampling indicated toluene concentrations of 11 ppb in the fan room, 11 ppb in the galley, and 19 ppb in the engine room of one submarine and 14 ppb in the fan room, 14 ppb in the galley, and 27 ppb in the engine room of the other submarine. A similar sampling exercise (two submarines, three locations, and sampling duration of 6 h) was reported by Holdren et al. (1995). Toluene concentrations in one submarine ranged from 122 to 137 ppb and from 241 to 342 ppb, depending on the sampling method, and in the other submarine from 14 to 21 ppb and from 17 to 23 ppb, depending on the sampling method. The committee notes that the results presented by Raymer et al. (1994) and Holdren et al. (1995) represent one-time sampling events in four submarines. Whether the reported concentrations are representative of the submarine fleet is not known, particularly inasmuch as few details were provided about the conditions in the submarines when the samples were taken.

SUMMARY OF TOXICITY

Toluene is a central nervous system (CNS) depressant and, at very high concentrations, can be irritating to the eyes. Consequences of accidental or intentional inhalation include renal toxicity, cardiac arrhythmias, blood dyscrasias, hepatomegaly, and developmental toxicity (ACGIH 1998, 2001, 2007). Sufficiently high concentrations of toluene vapor can produce euphoria; with increasing concentration, stupor, unconsciousness, or coma can occur with little accompanying irritation. The literature contains many descriptions of toluene narcosis and intoxication and the multiorgan sequelae of acute or chronic abuse. Deaths have occurred among abusers, who may expose themselves to acute toluene concentrations as great as 10,000 ppm (Press and Done 1967). Exposure to toluene at the high concentrations encountered in situations of abuse can lead to liver and kidney failure and multifocal leukoencephalopathy. In less affected people, toluene abuse has been reported to cause cognitive dysfunction. This review does not cover the topic of toluene abuse in great depth, because studies of persons known or suspected to have engaged in solvent abuse provide little quantitative information regarding dose-response relationships, and substance abuse is not a relevant model for exposure conditions expected onboard a modern submarine. For a review of the effects of exposure to toluene under conditions of abuse, see Schaumburg (2000).

The database available for characterization of toluene toxicity is large and includes considerable quantities of human and animal data suitable for derivation of exposure guidelines. Many toxicologic reviews are available and include evaluations by the NRC (1966, 1978, 1987; Garcia 1996), ATSDR (2000), the International Agency for Research on Cancer (IARC 1989, 1999), the American Conference of Governmental Industrial Hygienists (ACGIH 1998, 2001, 2007), the National Toxicology Program (NTP 1990), CIIT (Gibson and Hardisty 1983), and the U.S. Environmental Protection Agency (EPA 2005, 2007a).

Toluene is readily absorbed from the respiratory and gastrointestinal tracts and distributed throughout the body, accumulating in tissues with high lipid content. Results of many of the older studies, earlier than about 1960, are now thought to be compromised because of impurities, such as benzene, in the toluene test articles and the limited accuracy of the analytic techniques in use at the time (Neubert et al. 2001a). More recent clinical and epidemiologic studies that involve a variety of toluene exposures are considered more relevant to guideline development. Numerous studies conducted with rodents address neurotoxicity, and data from well-conducted mouse and rat lethality studies are available.

CNS depression by and metabolism of inhaled toluene are well documented and understood. Specific sensitive populations are not identified in the literature, because the primary mechanism of toxicity (CNS depression) is the same in all mammalian species and the toluene vapor concentrations at which CNS depression occurs do not differ greatly among individuals.

Controlled studies with volunteers indicate that blood and brain concentrations reach a steady state rapidly. As a consequence, effects observed during the

first hour of an exposure do not increase in severity when the exposure extends over several hours.

Toluene is not a primary mucous membrane irritant, and adaptation to both the odor and the potential drying effects of toluene on mucous membranes occurs. Complaints of eye and nose irritation have been reported in some controlled studies in which subjects were exposed at concentrations of 100 ppm or more for several hours.

Available human and animal data are considered insufficient to support an estimation of carcinogenic potential in humans (IARC group 3 compound, "not classifiable as to its carcinogenicity to humans"; EPA class D compound, "not classified" as to its carcinogenicity). ACGIH (2007) has concluded that toluene is "not classifiable as a human carcinogen." Extensive and well-conducted studies specifically designed to evaluate toluene carcinogenicity have found no association between cumulative toluene dose (as ppm-years) and standardized mortality ratios for multiple anatomic sites or respiratory tract cancers in humans or for carcinogenic activity by standard measures in chronic and subchronic studies of male and female mice and rats (NTP 1990).

Effects in Humans

Accidental Exposure

There are several case reports of accidental occupational exposure that resulted in intoxication, manifested as narcotic effects (muscular weakness, incoordination, and mental confusion). Early reports suggesting bone marrow toxicity were confounded by exposure to benzene. High exposures encountered in cases of intentional solvent abuse have caused deaths, usually associated with cardiac arrythmia and CNS depression. Severe renal acidosis has also been reported in those patients. The toxicity of toluene in humans has been reviewed by NRC (1981, 1987; Gracia 1996), Cohr and Stockholm (1979), the World Health Organization (WHO 1985), Cosmetic Ingredient Review (CIR 1987), ATSDR (2000), and EPA (1990).

Two men using toluene to remove excess glue from tiles in an empty swimming pool were exposed to toluene at greater than 1,842 ppm in air for 2 and 3 h (Meulenbelt et al. 1990). Toluene concentrations were measured at the pool edge with a Drager tube 3 h after the workers were rescued. It is assumed that toluene concentrations at the pool bottom, where the workers were found, exceeded 1,842 ppm in light of the vapor density (toluene vapor density relative to air) of 3.1. When found, both workers were disoriented; one was unable to walk or sit, and the other experienced difficulty when attempting to walk. Physical examinations 1 h after they were found revealed mucosal irritation of the eyes, slurred speech, headache, paresis, and amnesia. The patient exposed for 3 h had an excessive anion gap and sinus bradycardia. The second, exposed for 2 h, complained of headache, and clinical examination revealed sinus tachycardia

and a slightly excessive anion gap. Neither patient exhibited abnormalities in liver function or hematologic measures. Blood toluene concentrations 2 h after exposure were 4.1 and 2.2 mg/L in the 3-h and 2-h patients, respectively. The most striking effect was the increased anion gap in both patients, which the authors attributed to a high plasma concentration of toluene metabolites (benzoic or hippuric acid) or distal tubular acidosis. Both patients recovered without permanent sequelae.

Longley et al. (1967) reported two cases of accidental occupational exposure to toluene at high concentrations. In one case, workers spraying an antirust paint in an enclosed space (ballast tank) aboard a commercial ship were overcome by toluene vapors from the paint formulation. During the incident and rescue operations, at least 17 men exhibited signs and symptoms of dizziness, collapse, unconsciousness, severe mental confusion, amnesia, and illogical behavior. All affected workers recovered fully within 30 min after breathing oxygen. No estimate of toluene exposure concentrations was reported. In the second case, the hold of a merchant ship was mistakenly sprayed with an undiluted insecticide mixture containing malathion (20%), piperonyl butoxide (8%), pyrethrum (1.5%), and toluene (to 100%) (Longley et al. 1967). Effects exhibited by workers and rescuers could not be attributed to toluene exposure alone. Everyone involved recovered without persistent effects after leaving the vessel.

Experimental Studies

Numerous studies have been conducted with healthy human subjects exposed in controlled settings to toluene at monitored concentrations for various periods. Studies performed in the 1940s are now considered compromised by impurities (for example, other solvents, including benzene) and poor analytic characterization of exposure concentrations, but multiple recent well-conducted clinical studies are eminently suitable for exposure guideline estimations (Table 11-2).

More than 300 people have been evaluated in clinical studies involving toluene exposure at 40-800 ppm, and several thousand have been surveyed in occupational monitoring studies involving toluene exposure at up to 1,500 ppm. Those populations were composed of healthy people and represent a broad spectrum of uptake rates (sedentary, working, and exercise conditions). Although many clinical studies used a concentration of 100 ppm, the addition of exercise to the protocol in the studies of Astrand et al. (1972), Baelum et al. (1990), and Rahill et al. (1996) more than doubled the blood concentration—to that greater than would result from exposure at 200 ppm with subjects at rest (Astrand et al. 1972; Veulemans and Masschelein 1978). Baelum et al. (1990) investigated peak exposures of 300 ppm (14 times during a 7-h exposure at a mean concentration of 100 ppm) with exercise (950-100 W) undertaken for 15 min during three of the peak exposures. Astrand et al. (1972) also incorporated exercise into 200-ppm exposures.

TABLE 11-2 Sensory and Neurobehavioral Effects of Toluene in Short-Term, Controlled Human Studies

Concentration (ppm)	Exposure Duration	Subjects and Effects	Reference
10, 40, 100	6 h	16 men, 21-32 years old Slight irritation of eyes and nose at 100 ppm; no effect on mood, fatigue, or sleepiness; increase in occurrence of headache, dizziness, and feeling of intoxication, rated slight to moderate; no effect on lung function or nasal mucous flow; no significant effect on performance on 8 tests of visual perception, vigilance, psychomotor function, and higher cortical function (five-choice, rotary pursuit, screw-plate, Landolt's rings, Bourdon Wiersma, multiplication, sentence comprehension, and word memory); 40 ppm is NOAEL for tested effects	Andersen et al. 1983
40 3, 30-min peaks to 110	4 h (each of 2 sessions separated by 1 week) Over 4 h (1 session)	12 men, 20-50 years old No effects on measures of motor performance, attention, perceptual coding and memory, or mood and affect; positive correlation between results of finger-tapping test with alternate hands and blood toluene concentrations at end of 4 h	Lammers et al. 2004, 2005a
50[a]	3 h	10 men, 20 women, 19-45 years old No subjective symptoms; no abnormal episodic LH secretion profile in females or males; "subtle effects" on LH secretion in males and females in luteal phase (clinical significance unclear)	Luderer et al. 1999
50	4.5 h	20 nonsmoking men, 30.5 ± 5.2 years old Sleepiness measured after exposure with Pupillographic Sleeping Test and Pupillary Unrest Index did not show a toluene-exposure effect (for example, no increased sleepiness); questionnaire scores for "unpleasant smell" significantly different from control; nonsignificant throat-irritation scores	Muttray et al. 2005
80	4 h	8 men, 22-50 years old No impairment in neurobehavioral tasks	Cherry et al. 1983

(Continued)

TABLE 11-2 Continued

Concentration (ppm)	Exposure Duration	Subjects and Effects	Reference
80	4 h	16 men, 23-38 years old No differences in subjective symptoms between control and exposed group; no impairment in tests of simple reaction time, short-term memory, or choice reaction time; no effect on heart rate	Olson et al. 1985
80	4.5 h	12 men, 22-44 years old Increase in subjective symptoms (nausea, headache, irritation) but rated negligible; no impairment in tests of simple and choice reaction time, color-word vigilance, or memory; no effect on EEG or sleep latency; "weak" depression of heart rate during sleep latency test (disappeared during performance testing)	Iregren et al. 1986
100	3.5 h	18 No behavioral deficits in psychomotor tests	Winneke 1982
100	4 h	30 men and women No serious impairment in series of neurobehavioral tests (choice response time and pattern recognition); significant impairment in one measure of visual-vigilance test	Dick et al. 1984
100	6 h	6 men and women, 27-38 years old No significant effect on lung function (subjects exercised for 30 min); slight effects on some multitask and neuropsychologic tests (increased latency, but not accuracy, on neurobehavioral tasks)	Rahill et al. 1996
100	6.5 h	43 male printers and 43 male nonprinter referents, 29-50 years old; 4 groups tested: 2 exposed and 2 controls Irritation of eyes, nose, and throat (no annoyance or nausea); sleepiness, fatigue, and lower performance on 4 of 10 tests (3 tests evaluated visual perseverance, and the other manual dexterity); complaints of low air quality and strong odor; no changes in kidney function	Baelum et al. 1985

100	1, 3, 7.5 h, over several days	10 men and 9 women No decrement in psychomotor tests on first day of exposure; slight decrement in women on alertness test and deleterious change in 1 of 2 men in visual evoked response at 7.5 h/day, 5 days/week; increase in eye, nose, and throat irritation at 100 ppm 3 h/day, 5 days/week	Stewart et al. 1975
100 100 (TWA; varied with 15-min peaks to 300 ppm every 30 min)	7 h (3 15-min exercise periods with load of 50-100 W; both exposures)	32 men and 39 women, 31-50 years old Sensory irritation of nose and lower airways, but not eyes, in toluene-exposed groups; slight decrease on 1 of 4 psychomotor performance tests; no differences in symptoms or performances between groups exposed to constant and varied toluene concentrations	Baelum et al. 1990
75 150	7 h over 3 days 7 h over 3 days	42 male and female students, 18-35 years old Mean 7% decrement on several neurobehavioral tests at 150 ppm; slight increases in headache and eye irritation exhibited dose-response relationship; sleepiness on first day; CNS effect demonstrated by dose-response relationship in number of times subjects slept; no clear pattern of neurobehavioral effects found; variation in control data across 3 days greater than solvent effect	Echeverria et al. 1989, 1991
100[a], 200[a]	30, 60 min	11 men, 18-29 years old; 4 women, 27-46 years old No difference in heart rate, pulmonary ventilation, oxygen consumption, or blood lactate either at rest or during a work load of 50 W	Astrand et al. 1972

(Continued)

TABLE 11-2 Continued

Concentration (ppm)	Exposure Duration	Subjects and Effects	Reference
100, 200	3, 7 h with 1-h break	23 naive men, average 23 years old Decrease in pulse rate at 200 ppm for 3 h; tendency to prolonged reaction time at 200 ppm; at 100 ppm, no significant change from control in pulse rate, diastolic or systolic blood pressure, flicker value, or reaction time; no clear dose-response relationship	Ogata et al. 1970
100[a] 300[a] 500[a] 700[a]	Successive 20-min exposure periods (one 5-min break); total 85 min	12 men, 20-35 years old At 100 ppm, no effect on reaction time or perceptual speed; at 300 ppm, increase in simple reaction time; at 500 ppm, increase in complex reaction time; at 700 ppm, decrease in perceptual speed at end of exposure; no effect on heart rate during total exposure; 1 of 12 subjects able to distinguish between control and toluene exposures	Gamberale and Hultengren 1972
200, 400, 600, 800	7-8 h	2 subjects Transitory mild throat and eye irritation and slight exhilaration at 200 ppm; metallic taste, transitory headache, lassitude, inebriation, and slight nausea at 800 ppm; threshold for "steadiness" task, 800 ppm	Carpenter et al. 1944
220[b] 427[b]	15 min	6 subjects At 220 ppm, all subjects willing to work for 8 h, negligible sensory symptoms; at 427 ppm, 3 of 6 subjects willing to work for 8 h; 2 subjects reported slight "lightheadedness"; 1 reported "stuffy, drowsy feeling"	Carpenter et al. 1976
200	6 h	5 men, resting Increase in pulse rate; no changes in respiration rate, galvanic skin reflex, or EEG.	Suzuki 1973
240	Three 30-min sessions	11 men, 20-21 years old Impaired vigilance in third session; decreased fatigue during second session	Horvath et al. 1981

[a]Subjects exposed via mouthpiece.
[b]Measured as toluene in "toluene concentrate."
Abbreviations: CNS, central nervous system; EEG, electroencephalography; LH, luteinizing hormone; NOAEL, no-observed-adverse-effect level; TWA, time-weighted average.

Although slight irritation involving the eyes and nose in humans was reported in several studies at 80-200 ppm (Table 11-2), toluene is not a primary respiratory irritant, as evidenced by the high RD_{50} value (the concentration associated with a 50% depression in respiratory rate) of 5,300 ppm in male Swiss-Webster mice (Nielsen and Alarie 1982). Complaints increased among the controls, especially in studies with long exposure durations. Other studies reported exposures at 80-100 ppm to be nonirritating (Stewart et al. 1975; Cherry et al. 1983; Olson et al. 1985; Rahill et al. 1996). The value of 200 ppm is far below concentrations reported to cause frank CNS effects. No CNS effects were reported at 80-100 ppm in studies by Winneke (1982), Cherry et al. (1983), and Stewart et al. (1975); effects were minor in other studies at 100-700 ppm (Gamberale and Hultengren 1972; Dick et al. 1984; Baelum et al. 1990). There were no biologically significant pulmonary or cardiovascular effects at 100 and 200 ppm for up to 6 h (Astrand et al. 1972; Suzuki 1973) and no indications of kidney damage (Nielsen et al. 1985). Exposures at 100 ppm in the study by Stewart et al. (1975) were repeated for 5 days with no greater effects. Thus, the highest no-observed-adverse-effect levels (NOAELs) for more than mild sensory discomfort and other than subtle CNS effects were 100 ppm for 7.5 h (Stewart et al. 1975), 200 ppm for 60 min with exercise (Astrand et al. 1972), 300 ppm over 15 min with exercise (Baelum et al. 1990), and 700 ppm for 20 min (Gamberale and Hultengren 1972).

Muttray et al. (2005) assessed the potential for acute (4.5 h) toluene exposures to induce sleepiness in a two-period crossover design (50-ppm toluene alternating with air only) exposure-chamber experiment with 20 healthy nonsmoking men (mean age, 30.5 ± 5.2 years). Quantitative measurement of the degree of "sleepiness" was performed with the Pupillographic Sleepiness Test and Pupillary Unrest Index, in which pupillary diameter oscillations were monitored (Muttray et al. 2005). Compared with the results of air-only exposure, parametric crossover analysis showed no effects of toluene exposure. Muttray et al. (2005) concluded that acute toluene exposure at 50 ppm did not increase sleepiness.

Studies of toluene exposure in combination with other solvents or alcohol reported delayed metabolism of toluene (Dossing et al. 1984), but there were no additive effects in neurobehavioral tests (Cherry et al. 1983).

Complaints of sensory irritation and differences in neurobehavioral tests have been reported in some occupational monitoring studies (Iregren 1982; Foo et al. 1990; Deschamps et al. 2001; Neubert et al. 2001a). Exposures in those situations were usually at or below the workplace guidelines (now 50-100 ppm but up to 200 ppm earlier) and have ranged up to 300-500 ppm for short durations during the workday (Deschamps et al. 2001). The 20-min NOAEL exposure at 700 ppm by Gamberale and Hultengren (1972) indicates that 700 ppm (after 20 min at 500 ppm, which itself followed successive 20-min exposures at 100 and 300 ppm) may be a threshold for CNS depression. Frank effects were reported at 800 ppm in the studies of von Oettingen et al. (1942a,b) and Carpenter et al. (1944), but the studies suffered from inferior analytic techniques and

potential benzene contamination of the test article. The clinical study of Gamberale and Hultengren (1972) with support from the clinical and occupational studies of von Oettingen et al. (1942a,b), Carpenter et al. (1944), and Wilson (1943) established that 700 ppm for a short duration (20 min) may be a threshold for decreased ability to complete complex tasks in a timely manner, whereas 800 ppm for 3-8 h may result in nausea and incoordination sufficient to impair escape (von Oettingen et al. 1942a,b; Carpenter et al. 1944).

Occupational and Epidemiologic Studies

Occupational studies have focused primarily on CNS impairment. Although exposure concentrations and durations are usually not well characterized, the studies provide information about the more common toxic effects. Interpretation of the results of many occupational studies is confounded by coexposure to other solvents, alcohol use, and age of participants (Table 11-3).

Wilson (1943) surveyed the effects of toluene at various concentrations in workers at a large industrial plant. About 1,000 workers were exposed at 50-1,500 ppm for 1-3 weeks. About 10% of the employees exhibited symptoms severe enough to require examination at a local hospital. Employees were grouped according to the concentration of toluene at their job sites as measured with a combustible-gas indicator. In workers exposed to toluene at 200 ppm, the most common complaints were headache, lassitude, and loss of appetite; workers exposed at 200-500 ppm complained of more pronounced headache, lassitude, and anorexia. The latter workers also complained of nausea, a "bad taste" in the mouth, loss of coordination, impaired reaction time, and momentary loss of memory. At measured concentrations greater than 500 ppm, the major complaints were nausea, headache, dizziness, anorexia, palpitation, and weakness. Physical and laboratory examinations of the roughly 60 workers exposed at 200 ppm were negative, and no significant physical or laboratory findings were noted in the 30 workers exposed at 200-500 ppm. On physical examination of the remaining 10% (exposed at measured concentrations greater than 500 ppm), loss of coordination, impaired reaction time, and skin petechiae were observed. Laboratory investigations of those patients revealed low red-blood-cell counts and leukopenia; in two of the patients, a bone-marrow biopsy revealed aplastic anemia. Workers who were hospitalized were treated symptomatically, and no deaths occurred. The Wilson (1943) results illustrate confounding by the presence of other workplace solvents or such contaminants as benzene; toluene alone is not known to cause hematopoietic toxicity.

Greenberg et al. (1942) identified time-weighted average (TWA) toluene exposures at 100-1,100 ppm (most were at 500 ppm) in a workplace survey of 106 painters employed from 2 weeks to 5 years in an aircraft factory. Other solvents were present in the paint mixtures. A symptom survey for headache, sore throat, and weakness found no increase in complaints. In 30% of the workers,

TABLE 11-3 Effects of Toluene in Occupational Settings

Concentration (ppm)	Time	Subjects and Effects	Reference
≤200 200-500 >500	8 h/day, 1-3 weeks	Industrial-plant workers; results compromised by presence of other solvents At ≤200 ppm, headache, lassitude, and loss of appetite observed; at 200-500 ppm, severe headache, nausea, anorexia, incoordination, increased reaction time, and memory loss; at >500 ppm, nausea, headache, weakness, anorexia, palpitation, low RBCs, leukopenia, and some aplastic anemia (indicative of nontoluene-solvent exposure) observed	Wilson 1943
100-1,100 (TWA); most ≤ 500	2 weeks-5 years	Aircraft-factory painters; results compromised by presence of other solvents Enlarged liver, increased lymphocyte count, and increased mean corpuscular volume	Greenberg et al. 1942
≥101	8-h workshift monitoring (undefined exposure duration over working life)	Industrial workers in printing, paint production, surface coating, painting, and shoe-making facilities; 4 Chinese cities Dizziness, floating sensations, nausea; no eye, nose, or throat irritation	Ukai et al. 1993
30.6 (average)	14.8 years	Auto painters Deficits in intelligence, memory, and performance test battery	Hanninen et al. 1976
60-100	40 months	Female shoe workers Neurologic and muscular deficits	Matshushita et al. 1975
117 (average)	22 years	Male rotogravure workers No clinically significant neurophysiologic or autonomic nervous system deficits; some workers complained of "memory disturbance"	Juntunen et al. 1985

(Continued)

TABLE 11-3 Continued

Concentration (ppm)	Time	Subjects and Effects	Reference
150 in 1974; 50 in 1979	Multiple years	Printers No significant difference from control in 9 of 10 tests of mental and physical dexterity; increase in simple reaction time in toluene-exposed group	Iregren 1982
9-467 (workplace monitor: 9-48 ppm for 3.5-5 h, 50-300 ppm for 15 min)	≥5 years	Male and female workers not exposed to other solvents Mucosal irritation significantly greater in toluene group; no differences from controls for such cognitive-function tests as simple reaction time; toluene-exposed workers scored significantly higher than controls on vocabulary tests	Deschamps et al. 2001
50-100 (TWA) at time of study; 6 h/shift	≥20 years	German rotogravure-factory workers No alteration from referent group on standard tests of psychophysiologic and psychomotor functions; all scores of toluene-exposed group within referent range	Neubert et al. 2001a,b
Maximal (regulatory) concentrations of 200 in 1985; 100 (1985-1993), 50 (1993-present); median printer exposure measured in workplace, 25 at time of study (maximum, 216)	≥20 years	German rotogravure printers and helpers Examined blood pressure, color vision, clinical chemistry, and hormone concentrations; no evidence that long-term occupational exposure was "convincingly associated" with chronic adverse health effects or altered surrogate markers	Gericke et al. 2001
45 ± 17	Work lifetime (average 21.2 years)	47 healthy adult workers in German rotary printing plants; average subject age, 42.9 years; sex not reported; 18 of 47 smokers No health effects observed in self-reported diseases (such as loss of appetite and pain), sensory function (vibration and hearing thresholds and color discrimination), cognitive function (memory), psychomotor function (manual dexterity)	Seeber et al. 2005

9 ± 7	work lifetime (average 21.3 years)	39 healthy adult workers in German rotary printing plants; average subject age, 45.6 years; sex not reported; 19 of 39 smokers No health effects observed in self-reported diseases (such as loss of appetite and pain), sensory function (vibration and hearing thresholds and color discrimination), cognitive function (memory), psychomotor function (manual dexterity)	Seeber et al. 2005
26 ± 19	5.5 years (average)	59 healthy adult workers in German rotary printing plants; average subject age, 31.4 years; sex not reported; 20 of 59 smokers No health effects observed in self-reported diseases (such as loss of appetite and pain), sensory function (vibration and hearing thresholds and color discrimination), cognitive function (memory), psychomotor function (manual dexterity)	Seeber et al. 2005
2 ± 3	6.6 years (average)	47 healthy adult workers in German rotary printing plants; average subject age, 33.2 years; sex not reported; 11 of 47 smokers. No health effects observed in self-reported diseases (such as loss of appetite and pain), sensory function (vibration and hearing thresholds and color discrimination), cognitive function (memory), psychomotor function (manual dexterity)	Seeber et al. 2005

Abbreviations: RBC, red blood cell; TWA, time-weighted average.

the liver was enlarged and both lymphocyte count and mean corpuscular volume were increased. Other solvents were present in the workplace, so those changes could not be attributed solely to toluene.

Juntunen et al. (1985) examined 43 male rotogravure printers with long-term (up to 22 years) occupational toluene exposure for clinical, neurophysiologic, neuropsychologic, and auditory effects and subjective symptoms. Results were compared with those in 31 matched control subjects. Neurologic examinations included physical coordination, reflexes, language and memory functions, computerized axial tomography of the brain, electrocardiography, and electroencephalography. The estimated average long-term workplace toluene concentration was 117 ppm. Of the symptoms tabulated, only the incidence of "memory disturbance" was significantly increased. Overall, the examinations failed to reveal any statistically significant differences between the groups. The authors concluded that there were no clinically significant adverse effects on the nervous system under the study conditions examined.

Ukai et al. (1993) conducted a factory survey in China, using personal sampling for workplace toluene exposure, questionnaires on subjective symptoms, and clinical evaluation of hematology, serum biochemistry, and urinary hippuric acid concentration. Workers were also given a neurologic evaluation. When they were compared with workers without toluene exposure, hematologic and clinical-chemistry measures were essentially normal. A dose-dependent increase in some subjective symptoms among toluene-exposed workers was observed with a threshold concentration at 100 ppm for 22 subjective symptoms. At 101 ppm, complaints of dizziness, "floating" sensations, and nausea (but not eye, nose, or throat irritation) were associated with a significant dose-response relationship.

Deschamps et al. (2001) tested cognitive function in 72 workers (42 men and 30 women) exposed to toluene (but no other solvent) at 9-467 ppm for at least 5 years. A general questionnaire on subjective symptoms was also administered. Personal samplers worn during the workshift indicated mean 3.5- to 5-h exposures at 9-48 ppm in several factories and mean exposures at 50-300 ppm in 15-min samples from pathology laboratories. Exposed workers were compared with 61 matched workers who had no known toluene exposure. The mean age of all workers was 43 years, and the mean duration of chronic exposure of the toluene-exposed workers was 20 years. Workers were tested 48 h after removal from exposure; the absence of toluene in the alveolar air of the subjects ensured that the solvent had been eliminated from the blood. Tests consisted of vocabulary, simple reaction time, digit symbol, digit span forward and backward, continuous tracking, color word vigilance, and switching attention. Except for the vocabulary test in which toluene-exposed workers scored significantly higher than the control group, there were no significant differences between groups in any of the tests. Of six subjective symptoms, only mucosal irritation had a significantly increased score (1.54 vs 1.37).

Iregren (1982) evaluated performance of a group of 34 toluene-exposed printers on a battery of psychologic tests and compared the results with test

scores from a group of spray painters exposed to a mixture of solvents and with a control group. The toluene-exposed printers differed significantly from the control group on only one of 10 tests addressing mental and physical dexterity. Simple reaction time was increased in the printer group compared with the control group (printers, 276 msec; controls, 238 msec). Although workplace air concentrations were not specifically stated, the authors indicated that exposures had decreased from about 150 ppm in 1974 to 50 ppm in 1979. The tests were administered at the end of the week that followed the last workday.

Neubert et al. (2001a) evaluated toluene-exposed employees at 12 German rotogravure factories. The study involved 1,290 exposed workers (1,178 males and 112 females) and 200 controls in a multicenter, controlled, blinded field study with the specific goal of evaluating dose-response relationships. Examinations included subjective self-rating of feeling and standard tests of psychophysiologic and psychomotor functions (verbal memory span, visuomotor performance, immediate visual memory, combined auditory and visual vigilance, and critical flicker-fusion frequency). Subjects were tested before and 0.5 h after a 6-h workday, and blood toluene concentrations were measured before and after the workshift. Air concentrations were monitored by continuous sample collection over the workshift with portable personal monitors. Age and alcohol consumption were recognized as confounding factors and were taken into account. Neither blood toluene concentrations of 0.85-1.70 mg/L nor TWA ambient-air concentrations of 50-100 ppm were clearly associated with alterations in results of psychophysiologic and psychomotor performance or increased subjective complaints in male volunteers. Compared with the referent group, no higher frequencies of headache or other unpleasant sensations were reported with toluene exposures up to 75-100 ppm. All test scores of toluene-exposed persons were within the reference range. There were too few subjects to support conclusions concerning males exposed at more than 100 ppm (blood toluene concentration, greater than 1.70 mg/L).

A subgroup of the printers and their helpers (1,077 male subjects) that participated in the Neubert et al. (2001a) study described above was further evaluated after long-term exposure to toluene (Gericke et al. 2001). The referent group (109 subjects) was selected from the paper industry. The length of exposure of some of the toluene-exposed and referent workers was 20 years. Exposure duration and gross extent of exposure were taken into account. Blood pressure, pulmonary function, color vision, clinical chemistry, hormone concentrations, and subjective symptoms were tallied. Trends showing reduced performance in the digit-symbol and visual-reproduction tests were correlated with age in both the printers and the referents. Although insomnia, dry mucous membranes, and allergies were higher in the toluene-exposed group than in the referent group, the frequency of these complaints and complaints of headache, nausea, loss of appetite, and gastrointestinal distress did not correlate with the duration or extent of toluene exposure. There was no association between circulating liver glutamine oxaloacetic transaminase and glutamic pyruvic transaminase with the length or extent of toluene exposure or age. A small upward trend

was observed for serum cholesterol, but it appeared to be age-related. Renal function as measured by creatinine clearance was unaffected. Follicle stimulating hormone, luteinizing hormone, and testosterone concentrations were not affected by exposure. Higher systolic blood pressure correlated weakly with toluene exposure of longer than 20 years, but confounding factors associated with hypertension were not taken into account. Overall, no clear adverse effects could be verified in this study of long-term occupational exposure. The authors point out the limitations of their study, including the reversibility of effects that may have occurred during past exposures at higher concentrations and the healthy-worker effect.

Seeber et al. (2005) studied 192 workers in German rotary-printing plants who had undergone "long" (about 20 years) or "short" (5.5-6.6 years) exposure to toluene in various portions of the plants. The authors pointed out that the long-term workers had been historically exposed at much higher concentrations (140 ppm vs about 40 ppm currently for the "high" group; and 20 ppm vs about 5 ppm currently for the "low" group). The resulting four exposure-duration groups were made up of people exposed at 45 ppm for 21.2 years, 9 ppm for 21.3 years, 26 ppm for 5.5 years, and 2 ppm for 6.6 years. Smoking was represented in each subject population (see Table 11-3), as was alcohol consumption. Odds ratios were used to evaluate a number of measures: sensory function (vibration and hearing thresholds and color discrimination), cognitive function (memory), and psychomotor function (manual dexterity). For all measures evaluated, results do "not support the hypothesis that there are health effects of toluene at current exposures of about 25 ppm or lifetime weighted exposures of about 46 ppm" (Seeber et al. 2005). Furthermore, "evidence for neurobehavioral effects due to long-term toluene exposure below 50 ppm was not established" (Seeber et al. 2005).

Monitoring studies indicate that workers have been historically and routinely exposed at 32 ppm (range, 0.1-457 ppm; Neubert et al. 2001b), at 26 and 45 ppm (26 ± 19 ppm and 45 ± 17 ppm; Seeber et al. 2005), at a TWA of 63-118 ppm (range, 5-353 ppm; Ovrum et al. 1978), at 132 ppm (range, 66-250 ppm; Zavalic et al. 1998), at 100-440 ppm with peaks at 200-500 ppm (Eller et al. 1999), at 200 ppm (Forni et al. 1971), and at 200-800 ppm (Parmeggiani and Sassi 1954). In most cases in which psychomotor tests were administered, tests were given before the workday began, so acute changes were not measured. In most of the studies, only subtle differences in neurologic measures, such as alterations in the visual evoked response, or small impairments of reaction time were found in comparisons with controls (Abbate et al. 1993; Boey et al. 1997; Eller et al. 1999; Murata et al. 1993; Vrca et al. 1995; 1997a,b; Yin et al. 1987; Zavalic et al. 1998). No changes from baseline in any sensory measure, cognitive function, or psychomotor function were noted by Seeber et al. (2005). Irritation of the conjunctiva and upper respiratory tract was found in one of 11 workers exposed at 200-800 ppm (Parmeggiani and Sassi 1954). In some studies, the incidence of sore throat was greater than in matched control groups. Chronic occupational exposure to toluene at routine concentrations in workplace air has

not resulted in serious kidney damage (ATSDR 2000). An acute exposure of flexoprint workers at about 100 ppm for 6.5 h failed to show significant changes in β-microglobulin or albumin compared with air-exposed controls (Nielsen et al. 1985).

Occupational studies suggest that chronic toluene exposure may be associated with hearing loss (reviewed in ATSDR 2000; Morata et al. 1997; Chang et al. 2006) but these studies do not always account for noise-induced hearing damage. In a study (Chang et al. 2006) of adhesive plants where "noise only" reference occupational populations were also examined, the "noise + toluene" population exhibited hearing losses of a type and magnitude similar to those in the "noise only" group (both groups had been employed for more than 20 years), but the prevalence of hearing loss was significantly greater ($p < 0.001$) in the "noise + toluene" group even at the lowest occupational toluene exposure concentration of 33 ppm. In studies of 333 rotogravure-printing workers (Schaper et al. 2003) and 192 rotary-printing plant workers (Seeber et al. 2005), exposure at less than 50 ppm could not be related to ototoxicity.

In animal models, toluene is associated with damage to outer-ear hair cells in rats exposed at 1,400 ppm 14 h/day for 8 days (Johnson and Canlon 1994). Guinea pigs have not been shown to exhibit solvent-induced hearing loss (Campo et al. 1993); this may be due to significant and large differences in toluene uptake and metabolism between the rat and guinea pig (lower in the guinea pig). The result is lower toluene body burdens for the guinea pig (Campo et al. 2006), which are surmised to induce few or no ototoxic effects.

One evaluation of color-vision impairment in toluene-exposed workers (Zavalic et al. 1998) determined that alcohol use and age were confounding factors. Potential mechanisms of toluene-related effects on hearing and color vision are further discussed under "Toxicokinetics and Mechanistic Considerations."

Effects in Animals

Acute Toxicity

Lethality and sublethality data on the rat (Pryor et al. 1978; Cameron et al. 1938; Hinman 1987; Kojima and Kobayashi 1973; Smyth et al. 1969; Wada et al. 1989; Mullin and Krivanek 1982; and Lammers et al. 2005b), the mouse (Moser and Balster 1985; Bruckner and Peterson 1981a,b; Bushnell et al. 1985; Bonnet et al. 1979; and Svirbely et al. 1943), and the dog (Ikeda et al. 1990) are available. On the basis of LC_{50} values, the mouse is slightly more sensitive to the effects of toluene than the rat. For the mouse, LC_{50} values ranged from 38,465 ppm for 10 min to 5,320 ppm for 7 h. Highest nonlethal exposures were 12,000 ppm for 20 min for the mouse (Bruckner and Peterson 1981a) and 5,000 and 6,250 ppm for 2 h for the rat (Kojima and Kobayashi 1973; Mullin and Krivanek 1982).

Because few human experimental data on neurotoxicity at concentrations below those causing frank narcosis are available, the extensive animal database characterizing neurobehavioral effects has been examined for application to the current analysis. The experimental-animal dataset is large (especially on the laboratory rat) and this necessitates presentation of selected studies representative of the body of available data (see Table 11-4).

Toluene, like many other CNS depressants and anesthetics, generates an initial excitatory stage followed by narcosis. Except for increased activity, experimental toluene concentrations below 1,000 ppm have little or no effect on gross manifestations of animal behavior (NRC 1981) (see Table 11-4). At 2,000 ppm, motor activity and the rate of response in neurobehavioral tests are increased. Higher concentrations suppress activity. In neurotoxicity tests, CNS depression increases motor activity and response rates (excitation) at low concentrations and decreases activity and responses at higher concentrations (Moser and Balster 1981, 1985; Wood et al. 1984). Increases in activity with no or minor decrements in accuracy on tasks occurred in rats and mice at 1,000-2,000 ppm (Mullin and Krivanek 1982; Kishi et al. 1988; Wood and Cox 1995). Mice exposed at about 2,000 ppm for short periods began to fail equilibrium tests in some studies (Moser and Balster 1985; Tegeris and Balster 1994) but exhibited increased activity in others (Kishi et al. 1988; Wada 1997). Mice exposed at 5,200 ppm became immobile in 45 min and lost consciousness in 1.5 h (Bruckner and Peterson 1981a). Those neurologic deficits are similar to ones observed in humans. The onset of neurobehavioral deficits is not readily observable in rodents, so extrapolation to humans is difficult. Furthermore, the increase in activity exhibited by rodents exposed to toluene at low concentrations is not readily observed in humans.

In general, acute exposures to toluene at high concentrations have produced conflicting data in animal studies, some reporting significant neurobehavioral effects and others no significant effects. None of the animal studies reproduced effects observed in humans exposed to toluene at high concentrations for extended periods (Schaumburg 2000). Furthermore, toluene at concentrations that produced unconsciousness in experimental animals has failed to produce residual organ damage (Svirbely et al. 1943; Bruckner and Peterson 1981b; NTP 1990).

Repeated Exposure and Subchronic Toxicity

Taylor and Evans (1985) subjected adult female cynomolgus macaques to 50-min, head-only exposures to toluene at 0, 100, 200, 500, 1,000, 2,000, 3,000, or 4,500 ppm and simultaneously tested for delayed matching-to-sample behavior as a measure of cognitive function. Monkeys were exposed singly at each concentration, and each monkey was tested twice at each concentration. The procedure took 6 weeks. Previously, two of the monkeys were exposed at

TABLE 11-4 Neurobehavioral Effects of Acute Toluene Inhalation Exposure in Rats

Concentration (ppm)	Duration	Effects	Reference
150	0.5, 1 h	Stimulatory effect, multiple schedule performance	Geller et al. 1979
150	2, 4 h	Reduced performance	
0, 1,200, 1,600, 2,000, 2,400	70 min	Signal-detection task; concentration-related reduced attention and increased response time; no effect on false hits; rats sleepy but arousable at 2,400 ppm	Oshiro and Bushnell 2004
178, 300, 560	2 h	Increased activity (for reward)	Wood and Cox 1995
1,000, 1,780	2 h	Increased activity, then return to control rate	
3,000	2 h	Increased activity, then decrease below control rate	
800	4 h	Threshold, decreased unconditioned reflex[a]	Mullin and Krivanek 1982
1,340	1 h	EC_{50}, most sensitive unconditioned reflex[a]	
3,200	2 h	Decreased conditioned-avoidance response	
125, 250, 500	4 h	Decreased conditioned-avoidance responses	Kishi et al. 1988
1,000, 2,000	4, 2 h	Increased incorrect responses and reaction time	
4,000	4 h	Excitation, increased response rate, ataxia	
1,000, 1,500, 2,000	1 h	Initial decrease in detection of auditory signals at all concentrations followed by return to control levels	Bushnell et al. 1994
1,000	4 h	Little effect on avoidance responses	Shigeta et al. 1978
3,000	4 h	Changes in response pattern	
1,000, 1,780, 3,000	2 h	1,780 and 3,000 ppm: increased concentration-dependent rates of response to food reward in spite of electric-shock punishment	Wood et al. 1984
3,000	4 h	Ataxia	
1,000	4 h	No change in behavior (number of rearings)	Takeuchi and Hisanaga 1977
2,000	4 h	Increased rearings and seizures	
4,000	4 h	Excitation followed by narcosis	
2,000	4 h	Increased number of lever presses to avoid shock, no change in avoidance behavior	Harabuchi et al. 1993
4,000	4 h	Increased number of lever presses to avoid shock, decrease in avoidance response	
1,700	4 h	No decrease in activity after exposure	Miyagawa et al. 1986
3,400	4 h	Activity decreased by 31% followed by recovery	
5,100	4 h	Inactivity followed by partial recovery	
2,000	4 h	Increased locomotor activity	Wada et al. 1989
4,000	4 h	Decreased conditioned-avoidance responses	
6,000, 8,000	4 h	Decreased conditioned-avoidance responses, ataxia, narcosis	

(Continued)

TABLE 11-4 Continued

Concentration (ppm)	Duration	Effects	Reference
1,333, 2,667	7.5 h	Effects on visual discrimination, increased motor	van
8,000	Peak	activity, return to baseline on following day	Asperen et al. 2003
2,500	1 h	No effect on motor activity during exposure	Hinman
5,000	1 h	Increased locomotor activity	1987
10,000	1 h	Increased activity followed by slight decrease	
15,000	1 h	Increased activity followed by cessation	

[a]Unconditioned reflex tests evaluated locomotor activity, coordination, corneal reflex, and righting reflex; EC_{50} determined for test failure (Mullin and Krivanek 1982).
Abbreviations: EC_{50}, statistical determination of the effect concentration in 50% of the sample population.

100 ppm and one monkey at 1,000 ppm 6 h/day, 5 days/week for 90 days. Toluene concentration was monitored continuously. Responses at 100 and 200 ppm were similar to those during the control sessions. Responses at 500 and 1,000 ppm were nonsignificantly lower than control responses. Cognitive function was impaired at 2,000 ppm as indicated by an increase in response time and a decrease in matching accuracy. Response time at 4,500 ppm increased by 0.26 sec over the control response time, and monkeys failed to respond during the second half of the session. Most monkeys remained awake at 4,500 ppm but failed to respond. The effect was characterized by the study authors as an attentional deficit with no specific memory effect.

In some cases, behavioral measurements (particularly of olfactory sensitivity to toluene) can be demonstrated as positively associated with histopathologic changes, such as altered cell density and epithelial thickness in olfactory neuroepithelium (Jacquot et al. 2006). Jacquot et al. (2006) compared T-maze sensitivity to toluene odor with nasal-cavity neuroepithelial changes in naive female OF-1 mice during each week of repeated exposure to toluene at 1,000 ppm 5 h/day, 5 days/week for 4 weeks.

Atay et al. (2005) exposed 4-week-old Swiss albino BALB/c mice repeatedly to toluene at 300 ppm 6 h/day for 8 weeks to evaluate potential changes in bone mineralization. Dual x-ray absorptiometry of the right femoral neck allowed accurate measurement of bone mineral density (BMD) and bone mineral content (BMC). In the toluene-exposure group, statistically significant ($p < 0.05$) decrements in both BMD and BMC were observed in comparison with the controls. Atay and co-workers are uncertain about the mechanisms whereby toluene-vapor exposure alters bone metabolism.

Jenkins et al. (1970) exposed Sprague-Dawley rats to inhaled toluene repeatedly or continuously to evaluate long-term effects on mortality, body weight, and hematology. A group of 15 male and female rats was exposed to toluene at 1,085 ppm 8 h/day, 5 days/week for 6 weeks and a group of 13 male and female rats continuously exposed at 107 ppm for 90 days. The control group

was composed of 14 rats. No rats died during the repeated exposure. Two rats died during the continuous exposure (one on day 28 and the second on day 56). Rats in both treatment groups gained more weight than the control group; however, starting weights were not balanced: both treated groups weighed more than the control group. There were no apparent effects on hemoglobin, hematocrit, or leukocyte count.

The NTP (1990) reported subchronic inhalation-exposure studies of groups of 10 male and 10 female F344/N rats and 10 male and 10 female $B6C3F_1$ mice exposed to toluene vapor at 0, 100, 625, 1,250, 2,500, or 3,000 ppm (rats) or 0, 312, 625, 1,250, 2,500 or 3,000 ppm (mice) 6.5 h/day, 5 days/week for 15 weeks (rats) or 14 weeks (mice).

In the rats, eight of the 10 males exposed at 3,000 ppm died during week 2; there were no other deaths in any other exposure group among the males and no deaths at any concentration among the females throughout the study. Final body weights were lower, and ataxia was noted in the 2,500- and 3,000-ppm groups. Dyspnea as a clinical sign was observed in all exposed groups "except males exposed at 3000 ppm and females exposed at 1250 ppm" (NTP 1990, p. 34). Relative weights of multiple organs in males and females were increased at 2,500 and 3,000 ppm, as were the kidney and liver weights in males at 1,250 ppm. The NTP (1990) authors did not consider any observed differences in hematologic measures or serum chemistry to be biologically significant. There were no treatment-related effects on sperm count or the estrous cycle.

In the mice, death occurred swiftly at 3,000 ppm in both sexes, and all females at this concentration were dead by week 2. Deaths also occurred among females exposed at 625 ppm or higher in a roughly dose-dependent manner. Final body weights were lower in all exposed groups. Dyspnea occurred only at 2,500 and 3,000 ppm. Relative organ weights were greater in populations exposed at 625 ppm or higher. The NTP (1990) authors did not consider any observed differences in hematologic measures or serum chemistry to be biologically significant. There were no treatment-related effects on sperm count or the estrous cycle.

Chronic Toxicity

Gibson and Hardisty (1983) evaluated the chronic toxicity and carcinogenicity of inhaled toluene in male and female F344 rats exposed at 0, 30, 100, or 300 ppm 6 h/day, 5 days/week for up to 2 years. Groups of 120 animals of each sex were exposed with interim sacrifices at 6, 12, and 18 months. Each animal was examined for clinical changes, and selected animals were examined further for ophthalmologic, hematologic, urinary, or clinical blood chemistry effects. Although female rats exposed at 100 and 300 ppm exhibited slightly (but significantly) reduced hematocrit and the females exposed at 300 ppm exhibited slightly (but significantly) increased mean corpuscular hemoglobin concentration, the authors did not appear to consider these findings as biologically

significant. They concluded that there were no treatment-related effects on hematology or clinical chemistry measures and that no tissue or organ lesions were attributable to toluene treatment. The observed low incidence of "proliferative, degenerative and inflammatory" lesions was similar in the control and toluene-exposed groups and was not considered attributable to treatment; the authors observed that such lesions were similar to those expected spontaneously. Thus, there were no increases in the incidences of neoplasms in treated rats compared with air controls.

IARC (1989) considered that the Gibson and Hardisty (1983) exposure regimen evaluated toluene at concentrations that may have been too low. As a consequence, the NTP (1990) conducted a second series of oncogenic bioassays in rats and mice exposed at higher concentrations. Groups of 60 male and 60 female F344/N rats and 60 male and 60 female $B6C3F_1$ mice were exposed to toluene at 0, 600, or 1,200 ppm (rats) or 0, 120, 600, or 1,200 ppm (mice) 6.5 h/day, 5 days/week for up to 2 years. The results revealed no evidence of carcinogenicity compared with concurrent controls. Mild degeneration of the nasal-cavity olfactory and respiratory epithelium was observed at 600 and 1,200 ppm, and inflammation and metaplasia of nasal mucosa was observed in exposed female rats. The NTP authors noted that the spectrum of lesions was "not unusual" during inhalation studies of organic solvents and was representative of a generic solvent effect rather than a toluene-specific effect (NTP 1990, p. 43). The NOAELs for carcinogenicity and survival were both 1,200 ppm in rats and mice.

Reproductive Toxicity in Males

Although toluene is reported to produce teratogenic effects in the infants of women who have abused it (Schaumburg 2000), male-mediated effects on reproduction have not been convincingly demonstrated. Most human studies characterizing potential reproductive effects are occupational studies in which mixed exposures cannot be eliminated or studies of persons known or suspected to have abused solvents. Such studies provide little quantitative information regarding dose response and will not be considered further in the present analysis.

In a study of 1,077 male rotogravure workers compared with a referent group of 109 male workers in the paper industry, Gericke et al. (2001) found no effect of chronic (at least 20 years) toluene exposure on follicle-stimulating hormone (FSH), luteinizing hormone (LH), or testosterone concentration; workplace toluene exposure concentrations monitored in 1993-1995 were 50-100 ppm (TWA) for a portion (24%) of the study participants; the remainder were exposed at lower concentrations (Neubert et al. 2001b). Gericke et al. (2001) and Neubert et al. (2001b) both acknowledge that the estimation of chronic individual exposures for past decades is undefined and variable. Luderer et al. (1999) examined the reproductive endocrine effects of acute exposure on 10 males and 20 females by mouthpiece exposure at 50 ppm for 3 h; no alterations in serum gonadotrophins, including LH and FSH, resulted; however, subtle ef-

fects on LH secretion were observed in male participants and in luteal-phase females. The clinical relevance of the findings is not clear. Luderer et al. (1999) observed no change in male blood testosterone concentration in the exposure regimen described.

More complete data are available on laboratory animals. Male F344/N rats and $B6C3F_1$ mice evaluated during the toluene-inhalation studies of the NTP (1990) exhibited no compound-related effects on sperm count or motility when exposed at up to 3,000 ppm for 14 weeks or up to 1,200 ppm for 2 years. There were no histopathologic lesions observed in the epididymes, prostate, or testes of male mice or rats. The relative weights of the right testis of male rats exposed at 2,500 ppm or higher for 15 weeks were increased 15-24% compared with the control; although the changes were statistically significant, the NTP did not consider the difference to be biologically meaningful.

Male Sprague-Dawley rats exposed at 600 ppm 6 h/day for 90 days exhibited a slight decrease (13%) in sperm count; when exposed at 2,000 ppm under the same protocol, the sperm count decreased significantly by 26% and the weight of the epididymes decreased by 15% (Ono et al. 1996); the changes had no effect on mating performance or fertility as characterized by fertility and copulation indexes.

Immunotoxicity

Numerous investigators have noted that results of many early toluene studies (for example, those conducted before about 1985) have been confounded by the presence of benzene (a bone-marrow suppressant) as a contaminant (NTP 1990; NRC 1987; EPA 1985, 1987, as cited in ATSDR 2000). In those earlier times, solvent-extraction procedures were such that industrial toluene often contained some benzene (percentage not reported). Most later studies performed in North America and Europe include purity characterization as part of the experimental protocol. Burns-Naas et al. (2001) note that toluene possesses some immunomodulating activity but that "when compared to benzene" toluene has "little to no effect on immunocompetence." Because of assumed competition for metabolic enzymes, Burns-Naas et al. (2001) point out that toluene exposure can attenuate benzene immunotoxicity. The following immunotoxicity analysis is drawn from summaries prepared by the NTP (1990), ATSDR (2000), and Gibson and Hardisty (1983).

During the 14-week and 15-week inhalation studies of the NTP (1990), male and female F344/N rats and $B6C3F_1$ mice (exposed to toluene at 0, 100, 625, 1,250, 2,500, or 3,000 ppm 6.5 h/day, 5 days/week) exhibited no "biologically meaningful" differences in multiple hematologic measures, including counts of leukocytes, lymphocytes, reticulocytes, and eosinophils. The NTP (1990) authors observed an insignificant decrease in leukocyte count in female rats exposed at 1,250 ppm or higher. An additional NTP study for 15 months (rats exposed at 0, 600, or 1,200 ppm and mice exposed at 0, 120, 600 or 1,200

ppm) led the NTP (1990) to conclude that there were no compound-related effects on any of the hematologic measures. There were no histologic changes in the rat or mouse spleen or thymus at any exposure concentration during the 14-week and 15-week studies nor in the thymus of rats and mice exposed at concentrations at up to 1,200 ppm for 24 months (NTP 1990). The incidence of spleen pigmentation in male mice increased at 120 ppm or higher in the 24-month study; the biologic significance of the observation is not known and was not linked to any immunologic end point by the NTP (1990); it is mentioned here for completeness.

The long-term CIIT studies of male and female F344 rats exposed to toluene at 0, 30, 100, or 300 ppm for up to 24 months also examined multiple hematologic measures, including total and differential leukocyte counts. At study termination, leukocyte values were not different from those of the controls.

Variable response of toluene-exposed CD-1 mice to *Streptococcus zooepidemicus* challenge has been documented (Aranyi et al. 1985, as cited in ATSDR 2000). A single 3-h exposure to toluene at 2.5-500 ppm was associated with significant increases in susceptibility to *S. zooepidemicus* infection, as was serial exposure to toluene at over 100 ppm 3 h/day for 4 weeks. "Pulmonary bactericidal activity" was decreased after toluene exposure at 2.5 ppm and 100-500 ppm but not 5-50 ppm.

Genotoxicity

Toluene has undergone extensive in vitro and in vivo genotoxicity characterization; the large body of evidence indicates that toluene exhibits no genotoxic activity (NTP 1990). The few positive studies (rat in vivo studies by Dobrokhotov 1972; Liapkalo 1973; Sina et al. 1983; Dobrokhotov and Enikeev 1977, all as cited in NTP 1990 and EPA 2007a) have confounding factors—such as protocol artifacts, test-article purity, and presence of other solvents—that limit study reliability and relevance (NTP 1990). Summarized below are selected studies previously reviewed in CIR (1987, as cited in EPA 2007a), NTP (1990), ATSDR (2000), and EPA (2007a,b).

Negative cellular assays reviewed include reverse mutation or growth inhibition due to DNA damage in *Salmonella typhimurium* (with and without activation), mitotic gene conversion and mitotic crossover in *Saccharomyces cerevisiae*, chromosomal aberrations in bone marrow cells of laboratory rodents, and chromosomal aberrations or sister-chromatid exchange (SCE) in cultured human lymphocytes (EPA 2007a and NTP 1990). Reviewed studies in mouse lymphoma L5178Y cells, Chinese hamster ovary cells, *Bacillus subtilis* and *Escherichia coli* were all negative for genotoxicity (EPA 2007a).

Mouse in vivo studies of toluene have been negative for micronucleus induction, sperm-head abnormalities, dominant lethal mutations, SCE, and chromosomal aberrations (reviewed in EPA 2007a).

Carcinogenicity

IARC has published evaluations of toluene twice, most recently in 1999 (IARC 1989, 1999). In both cases, IARC has classified toluene as a group 3 compound ("not classifiable as to its carcinogenicity to humans") on the basis of "inadequate evidence" in humans and experimental animals. Human evidence evaluated by IARC (1999) included results of eight studies: three cohort studies of Swedish rotogravure printing workers by Svensson et al. (1990), U.S. aircraft maintenance workers in Utah by Blair et al. (1998), and U.S. shoe-manufacturing workers in Ohio by Walker et al. (1993); three nested case-control studies of Texas petrochemical plant workers by Austin and Schnatter (1983), U.S. rubber workers by Wilcosky et al. (1984), and U.S. nuclear facility workers by Carpenter et al. (1988); and two case-control community studies of brain cancer in the male workers of Lund, Sweden by Olsson and Brandt (1980) and multiple cancers in male residents of Montreal, Canada by Gérin et al. (1998). IARC (1999) considers the findings of those epidemiologic studies to be only weakly consistent and compromised by multiple workplace exposures and the small numbers sampled. IARC notes further that cancers observed at most anatomic sites were not significantly associated with toluene exposures in any human studies examined.

Experimental laboratory animal evidence includes the inhalation-exposure studies of F344 rats by Gibson and Hardisty (1983; the CIIT study previously discussed) and F344 rats and B6C3F_1 mice by NTP (1990). Under conditions of the 2-year inhalation-exposure protocol (for example, 6.5 h/day, 5 days/week for 52 weeks), the NTP (1990) states that there was "no evidence of carcinogenic activity" in male or female F344/N rats exposed to toluene at 600 or 1,200 ppm or in male or female B6C3F_1 mice at 120, 600, or 1,200 ppm.

The most recent EPA assessment of toluene carcinogenicity for lifetime exposure characterizes toluene as a class D compound, "not classified" as to carcinogenicity (revision of February 1994; IRIS accessed December 2004). The basis of that determination is the stated lack of human data and "inadequate animal data" and the observation that toluene was not genotoxic in the majority of assays available for examination. Laboratory animal evidence presented to support the EPA classification included the CIIT chronic vapor-inhalation study of F344 rats (CIIT 1980) and dermal-application studies of mice: chronic exposure of three times a week (Poel 1963), two times a week for 50 weeks followed by observation for 1 year after exposure termination (Coombs et al. 1973), two times a week for 56 weeks (Doak et al. 1976), and two times a week for 72 weeks (Lijinsky and Garcia 1972), all as cited by EPA (2007b). Cellular-assay data evaluated included much of what is summarized in the genotoxicity analysis above. The EPA assessment also considered in vivo evaluations of potential chromosomal aberration in the lymphocytes of workers exposed to toluene (only) (Maki-Paakkanen et al. 1980; Forni et al. 1971, as cited in EPA 2007b).

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

An extensive database evaluating the uptake, metabolism, distribution, and toxic mechanisms of toluene is available and has been reviewed by the NTP (1990), IARC (1989, 1999), NRC (1987), Garcia (1996), and EPA (2007a).

Toxicokinetics

Toluene is a CNS depressant and is readily absorbed by the blood after pulmonary exposure. Although inhalation is the principal route of exposure, absorption can also take place through the skin or alimentary tract; dermal absorption approximates 1% of that from respiratory absorption (Kezic et al. 2000, as cited in EPA 2007a). Pulmonary retention of toluene was 50% in healthy male subjects as measured by inspired and expired air concentrations when it was inhaled at 50 ppm at a 50-W workload or at 80 ppm in sedentary conditions (Lof et al. 1990, 1993, as cited in EPA 2007a). Because of the dependence of toluene uptake on respiratory rate, uptake is greater during exercise than at rest (Astrand et al. 1972; Veulemans and Masschelein 1978; Carlsson and Lindqvist 1977; Carlsson 1982, all as cited in EPA 2007a); however, once steady state is achieved, blood concentrations are relatively independent of respiratory rate. Toluene can be detected in human blood within 10-15 sec of an exposure; at air concentrations as low as 100 or 200 ppm, toluene reaches 60% of maximal arterial concentrations within 10-15 min (Astrand et al. 1972; Benignus 1981, all as cited in EPA 2007a). When monitored subjects were sedentary and exposed at 80 ppm for 4 h, 90% of the final 4-h blood concentration of toluene was attained after 2 h (Hjelm et al. 1988; Lof et al. 1990, 1993, all as cited in EPA 2007a); when subjects were exercising, a steady state was achieved more rapidly. After a 90-min exposure of seven human subjects to toluene at 50 ppm, Benoit et al. (1985, as cited in EPA 2007a) reported that retention was 83% at steady state as measured by elimination in exhaled air.

Toluene is highly lipophilic, so its distribution to body tissues and organs depends on the lipid content of the tissue, blood flow to the tissue, metabolic rate, and exposure duration. After absorption, toluene is rapidly distributed to highly vascularized tissues, such as the liver, kidney, and brain; accumulation in the brain is rapid because of that organ's high lipid content (Bruckner and Warren 2001). Toluene is eventually taken up and stored in adipose tissues; consequently, obese people tend to accumulate more toluene than do people with low body fat (Carlsson and Lindqvist 1977, as cited in EPA 2007a). Male Sprague-Dawley rats that inhaled ^{14}C-labeled toluene at 550 ppm for 1 h exhibited a higher concentration of labeled material in adipose tissue than in any other tissue examined: adipose tissue, 87 µg/g; adrenal, 56 µg/g; kidney, 55 µg/g; liver, 21 µg/g; and brain, 15 µg/g (Carlsson and Lindqvist 1977). Other metabolic studies of ^{14}C-labeled toluene in rats indicate that initial ^{14}C activity in the brain was highest in the lipid-rich medulla and pons, followed by the midbrain (10-min

exposure at an unstated toluene vapor concentration; Gospe and Calaban 1988, as cited in ATSDR 2000).

Lof et al. (1993) found that toluene elimination from the blood of nine human volunteers after exposure to toluene at 53 ppm for 2 h was triphasic, with three half-lives of 3, 40, and 738 min. Presumably, the longer half-life represents the mobilization of toluene from adipose tissue given its high lipophilicity. Brugnone (1985) reported half-lives of 31.8 and 29 min in vessel-rich tissue and muscle, respectively, and 36 and 2.7 h in fat and vessel-poor tissues. After the first 20-30 min of inhalation exposure, the ratio of toluene concentration in the blood and brain is constant until exposure termination (Benignus et al. 1984).

Absorbed toluene is rapidly and extensively metabolized, primarily in the liver (Low et al. 1988; ATSDR 2000). Metabolism to benzoic acid and later conjugation to form hippuric acid is the primary pathway of toluene detoxification and elimination. In humans, oxidation of the methyl group by the hepatic cytochrome isozyme CYP2E1 in humans yields benzyl alcohol (Liira et al. 1991; Nakajima et al. 1997), which is rapidly oxidized by alcohol dehydrogenase to benzaldehyde. Benzaldehyde is converted to benzoic acid by aldehyde dehydrogenases. About 75-80% of the absorbed dose of toluene is ultimately metabolized to benzoic acid. Benzoic acid is primarily conjugated with glycine and excreted in the urine as hippuric acid; smaller amounts of benzoic acid are excreted as the sulfate or glucuronide conjugate. Toluene can also be hydroxylated to form o-, m-, or p-cresols, which are conjugated with sulfate or glucuronide and are also excreted in the urine. The cresols are considered minor urinary metabolites. The remainder of the absorbed dose of toluene (about 18%) is eliminated via the lungs as unchanged toluene. Studies with rat liver microsomes indicate that metabolic pathways are the same in humans and rats. Because of population variability in the time course and yields of metabolic reactions, monitoring of the urinary metabolites hippuric acid and cresols are best considered qualitative, rather than quantitative, markers of toluene exposure (Andersen et al. 1983; Baelum et al. 1987; Hasegawa et al. 1983).

Toluene concentrations in blood and tissues are proportional to alveolar air concentrations. Numerous investigators have reported on exposure and alveolar toluene concentrations in relation to tissue concentrations during controlled exposures of human subjects (Astrand et al. 1972; Gamberale and Hultengren 1972; Veulemans and Masschelein 1978; Carlsson 1982; Hjelm et al. 1988; Tardif et al. 1991) and animal models (Benignus 1981; Benignus et al. 1984; Bruckner and Peterson 1981a; Tardif et al. 1992; van Asperen et al. 2003). Several studies measured toluene concentrations in the rodent brain. With the exception of concentrations greater than 5,200 ppm in a mouse study (Bruckner and Peterson 1981a), all the studies demonstrated a linear relationship between blood concentration and concentrations in atmospheric and alveolar air. Where several studies involving the same species can be compared, there is relatively good agreement among peak blood concentrations. In general and for similar air concentrations, peak blood concentrations are inversely related to body size.

Empirical data on humans and rodents indicate that venous blood concentrations rapidly increase to a plateau during the first 15 min of vapor exposure, which is followed by minimal increases in blood concentration with continuing exposure (Tardif et al. 1993, 1995). Available human and animal data demonstrate that increasing toluene exposure concentration positively correlates with increase in venous blood concentration. The data indicate that concentration, not duration, is a prime determinant of toluene-induced CNS toxicity. Evidence indicates that toluene irritation of mucous membranes depends on toluene concentration rather than exposure duration. It would thus be inappropriate to apply a time-scale adjustment to exposure guidelines on the basis of narcosis or sensory irritation.

Mechanisms of Toxicity

Toluene is considered primarily a CNS depressant, although it can also cause ocular irritation at high concentrations. CNS depression and narcosis are thought to involve reversible interaction of toluene (not its metabolites) and lipid or protein components of nervous system membranes. Bruckner and Warren (2001) summarized several theories on mechanisms of action: a change in membrane fluidity that alters intercellular communication and normal ion movements, interaction with hydrophobic regions of proteins that alters membrane-bound enzyme activity or receptor specificity, enhancement of the neurotransmitter gamma-aminobutyric acid (GABA) receptor function, and activation of the dopaminergic system. For repeated exposure, two toxic mechanisms have been proposed for CNS effects (ATSDR 2000): interaction of toluene with membrane proteins or phospholipids in brain cells may change activities of enzymes involved in synthesis or degradation of neurotransmitters, and toluene may change neurotransmitter binding to membrane receptors. Toluene is highly lipophilic and, as a nonpolar planar molecule, behaves as an anesthetic and dissolves in the interior lipid matrix of a membrane. Increasing toluene concentrations are thought to produce membrane expansion and changes in membrane structure and fluidity. After acute exposure, toluene diffuses out of the membrane, original integrity is regained, and functional characteristics can be restored (ATSDR 2000).

The molecular mechanisms of action for hearing loss and potential color-vision impairment are poorly understood (ATSDR 2000). Toluene exposure may lead to a loss of outer hair cells in the ear; there may also be neural-cell membrane effects. Color-vision impairment may involve toluene interference with dopaminergic mechanisms of retinal cells or demyelinization of optic nerve fibers (see Zavalic et al. 1998; Gericke et al. 2001).

At the exposure concentrations found in cases of life-threatening toluene abuse, distal renal tubular acidosis has been reported and manifested by generalized muscle weakness, neuropsychiatric derangements, and other effects (Streicher et al. 1981; Batlle et al. 1988; Marjot and McLeod 1989). The disorder re-

sults from the inability of the distal tubule of the nephron to secrete hydrogen ions through the active transport pathway of the kidney tubule, which leads to metabolic acidosis and production of alkaline urine. The high anion gap of the blood may be due to accumulation of the acidic toluene metabolites benzoic acid and hippuric acid.

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

A number of organizations have established or proposed inhalation exposure levels or guidelines for toluene. Selected values are summarized in Table 11-5.

COMMITTEE RECOMMENDATIONS

The committee's recommendations for EEGL and CEGL values for toluene are summarized in Table 11-6. The current and proposed U.S. Navy values are provided for comparison.

The literature contains an abundance of human-exposure data derived from multiple clinical, monitoring, and metabolic studies carried out at toluene concentrations less than 1,000 ppm for various exposure durations and thus suitable for direct estimation of exposure guidelines. Several hundred adults were subjects of the clinical studies summarized in Table 11-2, and several thousand workers were monitored in the occupational studies summarized in Table 11-3; it is rare to have access to such a robust human dataset for analysis. Some studies (von Oettingen et al. 1942a,b; Wilson 1943; Greenberg et al. 1942; Carpenter et al. 1944) were not used in the following assessment, because of the strong likelihood that the toluene test article was contaminated with other solvents (not unusual before 1960), the potential for exposure to multiple solvents in the workplace, and acknowledgment of the poor analytic detection capability for vapor exposures during the 1940s. The dataset that was considered includes human populations made up of healthy people representing a broad spectrum of activities (and thus uptake rates), ranging from sedentary to working and exercise conditions; individual differences in metabolic rates are also present (Gamberale and Hultengren 1972; Veulemans and Masschelein 1978; Brugnone et al. 1986; Hjelm et al. 1988). Although slight respiratory irritation was reported in several studies at 100 and 200 ppm, that toluene is not considered to be a respiratory irritant is supported by the high RD_{50} value, 5,300 ppm (Nielsen and Alarie 1982).

TABLE 11-5 Selected Inhalation Exposure Levels for Toluene from the NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	20	ACGIH 2007
NIOSH	REL-TWA	100	NIOSH 2005
	REL-STEL	150	
OSHA	PEL-TWA	200	29 CFR 1910.1000
	PEL-CC	300	
	PEL-MP	500	
Spacecraft			
NASA	SMAC		Garcia 1996
	1-h	16	
	24-h	16	
	30-day	16	
	180-day	16	
Submarine			
NRC	EEGL		NRC 1987
	1-h	200	
	24-h	100	
	CEGL		
	90-day	20	
General Public			
ATSDR	Acute MRL	1.0	ATSDR 2000
	Chronic MRL	0.08	
NAC/NRC	AEGL-1 (1-h)	200	EPA 2007c (Interim)
	AEGL-2 (1-h)	1,200	
	AEGL-1 (8-h)	200	
	AEGL-2 (8-h)	650	

[a]The comparability of EEGLs and CEGLs with occupational-exposure and public-health standards or guidance levels is discussed in Chapter 1 ("Comparison with 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; CC, ceiling concentration; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; MP, maximum peak; 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; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

TABLE 11-6 Emergency and Continuous Exposure Guidance Levels for Toluene

Exposure Level	U.S. Navy Values (ppm)		Committee Recommended Values (ppm)
	Current	Proposed	
EEGL			
1-h	200	150	200
24-h	100	50	100
CEGL			
90-day	20	16	20

Abbreviations: CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level.

1-Hour EEGL

On the basis of the human data summarized in Tables 11-2 and 11-3, the highest concentrations that resulted in minimal effects after exposure for about 1 h were 200 ppm for 60 min (Astrand et al. 1972) and the serial concentrations (in successive 20-min periods) of 100-700 ppm over 85 min in Gamberale and Hultengren (1972). The exercise regimen incorporated into the study of Astrand et al. (1972) took into account the physical stress that may occur during an emergency situation onboard a submarine.

The Astrand et al. (1972) study of 15 subjects (11 men and four women) noted no treatment-related effects on heart rate, pulmonary ventilation, oxygen consumption, or blood lactate; subjective symptoms were not reported. Subjects were exposed via mouthpiece and exercised during exposure periods on a bicycle ergometer at intensities of 50 W or 75 W.

The neurobehavioral effects observed by Gamberale and Hultengren (1972) in 12 male subjects were subtle and reversible. The gradual changes observed were from no effect on reaction time or perceptual speed after a 20-min exposure at 100 ppm to an increase in simple reaction time after 20 min at 100 ppm and 20 min at 300 ppm (an average of 200 ppm for 40 min) to an eventual decrease in perceptual speed after successive 20-min exposures at 100, 300, 500, and 700 ppm (with one 5-min break presumably at 0 ppm). The latter serial-exposure regimen is equivalent to an average concentration of 376 ppm over 85 min. The degree of effect observed in Gamberale and Hultengren (1972) is minimal and reversible and should not interfere with performance of critical tasks during an emergency onboard.

Empirical data and pharmacokinetic modeling in humans and rodents indicate that rapid increases in blood concentration during the first 15-20 min of exposure are followed by minimal increases with continuing exposure (Gamberale and Hultengren 1972; Tardif et al. 1993; 1995). Toluene reaches an asymptote in the blood within 20-30 min (Gamberale and Hultengren 1972; Carlsson 1982), and a steady state between blood and brain would be attained in a similar

period (Benignus et al. 1984). With exercise during exposure, the steady-state concentration could be attained more quickly. Thus, effects are not expected to become more severe in the period from attainment of steady state to the termination of exposure at 1 h.

The preponderance of data from clinical studies indicates that even a multiple-hour exposure to toluene at about 200 ppm would result in few substantial effects of concern for emergency exposure (for example, see Ogata et al. 1970; Suzuki 1973). The TWA of 376 ppm for the 85-min serial-exposure study of Gamberale and Hultengren (1972) indicates that a 1-h EEGL of 200 ppm would be protective. The weight of evidence from the extensive clinical dataset and the clinical studies of Astrand et al. (1972) and Gamberale and Hultengren (1972) have been used to derive the 1-h EEGL of 200 ppm. The use of human-subjects data obviates the application of an interspecies uncertainty factor. Furthermore, at such low and nonnarcotic concentrations, there is little justification for application of an intraspecies uncertainty factor.

24-Hour EEGL

The clinical human-exposure studies of Andersen et al. (1983), Baelum et al. (1985, 1990), Nielsen et al. (1985), Ogata et al. (1970), and Stewart et al. (1975) were evaluated to determine the 24-h EEGL (see Table 11-2). With the exception of Stewart et al. (1975) who used serial exposures at 100 ppm for a maximum of 7.5 h over multiple days, all the studies were of single exposures at 100 ppm for multiple hours (range, 3-7 h). That exposure duration is sufficient to allow blood concentrations of toluene to maximize and attain a steady state between blood and brain concentrations.

The Baelum et al. (1990) study included exercise at a load of 50-100 W and two exposure regimens: one with a constant 100-ppm exposure for 7 h and one with exposure at a 100-ppm TWA with 15-min peaks to 300 ppm every 30 min for 7 h. Effects noted were minor and included sensory irritation of nose and lower airways but not eyes, increase in dizziness and feeling of intoxication, and a slight decrement in one of four psychomotor performance tests. No differences were observed in symptoms or performance between groups exposed to toluene at constant and varying concentrations. The study design was an improvement over that of Baelum et al. (1985) and Nielsen et al. (1985), which tested groups of previously (occupationally) exposed rotogravure printers. After exposure of naive male students at 100 ppm, Ogata et al. (1970) observed no change from control in pulse rate, diastolic or systolic blood pressure, flicker value, or reaction time. After 6 h at 100 ppm, Andersen et al. (1983) reported slight irritation of the eyes and nose and some increase in occurrence of headache, dizziness, and "feelings of intoxication" but no effect on sleepiness, fatigue, lung function, nasal mucus flow, or performance on eight psychomotor tests. By the end of each exposure duration, subjects reported air-quality deterioration and odor in most of the studies.

The effects are minimal and reversible and should not interfere with performance of critical tasks during an emergency onboard. Furthermore, effects do not appear to increase in severity significantly with repeated equivalent doses of similar duration (Stewart et al. 1975).

A time-scaling adjustment is not applied in this case, for several reasons: a steady state in the blood is achieved, no greater effects were observed after repeated exposure, CNS and irritation effects of toluene are known to be concentration-dependent rather than time-dependent, and adaptation and acclimatization are associated with the irritation caused by toluene. The preponderance of clinical human data on minimal effects at 100 ppm after exposure for multiple hours and repeated exposure supports the selection of 100 ppm as the 24-h EEGL. The use of human-subjects data obviates the application of an interspecies uncertainty factor. Again, at such low and nonnarcotic concentrations, there is little justification for application of an intraspecies uncertainty factor.

90-Day CEGL

Estimation of a 90-day CEGL is on the basis of long-term occupational and epidemiologic evidence (see Table 11-3). The most completely characterized occupational assessments are of German rotogravure-factory workers, as documented in Gericke et al. (2001), Neubert et al. (2001a,b), and Seeber et al. (2005). About 1,500 volunteers in 12 factories were evaluated by Gericke et al. (2001) and Neubert et al. (2001a,b), and 192 workers in an unreported number of rotary-printing facilities were evaluated by Seeber et al. (2005). Although the investigators acknowledge the inability to determine toluene exposures over several decades, maximally exposed people are known to be printers and their helpers. Study participants in that occupational category had been employed for at least 240 months. The purity of toluene used was high (over 99.98%), and the authors state that benzene contamination has not been a problem since 1960 (Neubert et al. 2001a). Since 1993, the maximal allowable workplace concentration of toluene permitted by the Federal Republic of Germany has been 50 ppm (Gericke et al. 2001). The Neubert et al. (2001a,b) studies measured ambient air concentrations (the TWA for 6-h shift) of 50-100 ppm in the workplaces of the majority of printers. Nevertheless, neither those air concentrations nor blood toluene concentrations of 850-1,700 µg/L in the highest toluene-exposure group was "convincingly associated" with subjective complaints or alteration from the referent group on standard tests of psychophysiologic and psychomotor functions; all scores of the toluene-exposure group were within the referent ranges (Neubert et al. 2001a,b). The standard tests administered evaluate short-term and visual memory, vigilance, CNS-depressant effects, and dimensions of neuroticism. Some printers are exposed at concentrations greater than 100 ppm, but Neubert et al. (2001a,b) consider the dataset on that group to be too small "for reaching conclusions."

If straight-line extrapolation is assumed for the psychophysiologic and psychomotor functions tested, a concentration of 50-100 ppm over a 6-h shift is about equivalent to continuous exposure at 12.5-25 ppm for 24 h during the multiple years described in the occupational studies of Neubert et al. (2001a,b). Workers exposed at 26 ± 19 ppm for multiple years exhibited no change from controls in several sensory and cognitive functions and psychomotor measures (Seeber et al. 2005).

Given that the rotogravure workers evaluated by Neubert et al. (2001a,b), Gericke et al. (2001), and Seeber et al. (2005) have been exposed to toluene at concentrations greater than 25 ppm for multiple years and exhibit no adverse effects in standard and well-conducted tests of cognition, vigilance, and CNS effects; the weight of evidence from other occupational studies; and the toxicokinetics of toluene, the committee recommends a 90-day CEGL of 20 ppm, the same value as recommended by NRC (1987). The committee notes that there is little justification for an intraspecies uncertainty factor because the CEGL is based on studies that evaluate large populations and thus incorporate variability and susceptibility that might be observed in the submariner population.

DATA ADEQUACY AND RESEARCH NEEDS

Because toluene is a common solvent, its effects on humans have been extensively studied. Numerous controlled human-exposure studies assess end points meeting the EEGL definition. Animal neurotoxicity studies are extensive, and supporting animal data were in reasonable agreement with values based on human studies. Toluene is fatal to humans only after exposure to extremely high concentrations (greater than 10,000 ppm), and deaths most often occur in cases of solvent abuse.

The anesthetic effects and metabolism of toluene are well documented and characterized. Although specific sensitive populations are not identified, the mechanism of action of CNS depression is the same in all mammalian species, and the concentration at which this effect occurs after toluene inhalation does not differ greatly among individual humans.

Although empirical data on toluene toxicity in humans and animals are abundant, few experimental dose-response data on human serial exposures are available. This is especially true for long-term human exposures encompassing multiple days or weeks. Several well-conducted chamber studies have involved human volunteers, but exposure concentrations were limited to concentrations that produce little if any impairment or anesthesia in humans and to exposure durations of less than 12 h. On the basis of existing human studies that describe effects over time and the fact that blood and brain concentrations reach a steady state rapidly, effects observed during the first hours of an exposure are relevant for exposures up to 24 h. Nevertheless, better characterization could be obtained from studies of a larger range of nonanesthetic concentrations for longer continuous exposure durations.

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12

Xylene

This chapter summarizes the relevant epidemiologic and toxicologic studies of xylene. Xylene is usually found as a mixture of three isomers: *m*-xylene, *o*-xylene, and *p*-xylene, with the *m*- isomer predominating. In this profile, the term xylene refers to the mixture of isomers unless otherwise stated. Small amounts of benzene and ethyl benzene may be present in technical formulations of xylene, but these chemicals are not considered in this document.

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

PHYSICAL AND CHEMICAL PROPERTIES

Xylene is a colorless, combustible, sweet-smelling liquid (NRC 1984). The odor threshold for xylene has been reported to range from 0.09 to 0.4 ppm. Selected physical and chemical properties are listed in Table 12-1.

OCCURRENCE AND USE

Xylene is a component of gasoline, a raw material in the production of many industrial chemicals, and a solvent (Cannella 1998). Xylene has been measured in outdoor and indoor air. The Agency for Toxic Substances and Disease Registry (ATSDR 2005) reported concentrations of 1-30 ppb in outdoor

TABLE 12-1 Physical and Chemical Data on Xylene

Synonyms and trade names	Dimethylbenzene (1,2-, 1,3-, or 1,4-); xylol; *m*-xylene (*m*-isomer); *o*-xylene (*o*- isomer); *p*-xylene (*p*- isomer); methyl toluene
CAS registry number	1330-20-7 108-38-3 (*m*- isomer) 95-47-6 (*o*- isomer) 106-42-3 (*p*- isomer)
Molecular formula	C_8H_{10}
Molecular weight	106.16
Boiling point	137-140ºC
Melting point	No data on mixture −47.4ºC (*m*- isomer) −25ºC (*o*- isomer) 13-14ºC (*p*- isomer)
Flash point	29 ºC
Explosive limits	NA
Specific gravity	0.864 at 20ºC/4ºC
Vapor pressure	6.72 mm Hg at 21ºC
Solubility	Practically insoluble in water; miscible with absolute alcohol, ether, many other organic liquids
Conversion factors	1 ppm = 4.34 mg/m^3; 1 mg/m^3 = 0.23 ppm

Abbreviations: NA, not available or not applicable.
Sources: Vapor pressure from ATSDR 1995; specific gravity from HSDB 2005; all other data from Budavari et al. 1989.

air and 1-10 ppb in indoor air. Major sources of xylene in outdoor air are emissions from vehicles, chemical plants, and paints. Sources in indoor air are cigarette smoke and consumer products. Xylene has been detected in some foods.

Sources of xylene in a submarine are paints and coatings (Crawl 2003). Holdren et al. (1995) reported the results of air sampling at three locations conducted over 6 h during the missions of two submarines. Data were provided on *p*-xylene and *o*-xylene. Sampling indicated concentrations of *p*-xylene of 15.6-20.2 ppb in one submarine, depending on the collection method and location, and concentrations of 7.5-12 ppb in the other submarine, depending on the collection method and location. Concentrations of *o*-xylene ranged from 28.5 to 38.6 ppb, depending on the collection method and location on one submarine, and from 6.3 to 16 ppb on the other submarine, depending on the collection method and location. Raymer et al. (1994) reported the results of a similar sampling exercise (two submarines, three locations, and sampling duration of 6 h).

Reported concentrations of a "xylene isomer" were 4.6 and 12 ppb in the fan rooms, 6.9 and 16 ppb in the galleys, and 6.9 and 23 ppb in the engine rooms. Data were also provided on a "xylene isomer + $C_{10}H_{22}$ isomer," but the proportions of the two components in the mixture were not stated. The committee 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 inasmuch as few details were provided about the conditions on the submarines when the samples were taken.

SUMMARY OF TOXICITY

Inhaled xylene is rapidly absorbed and metabolized. It is excreted almost exclusively in the urine of humans as methylhippuric acid isomers but in the urine of animals as methylhippuric acid isomers and toluic acid glucuronides. Elimination is biphasic; the elimination half-life for the first phase is about 1 h, and that for the second is about 20 h.

Xylene is an irritant of eyes and mucous membranes at sufficiently high concentrations and predominantly a central nervous system (CNS) depressant. No substantive differences in potency among the isomers have been identified after inhalation exposure. Acute and longer-term exposures to xylene result in irritation of the eyes, nose, and throat. Early symptoms of CNS disturbances are headache, nausea, fatigue, irritability, dizziness, vertigo, impaired concentration, and confusion. Reproductive or developmental toxicity has not been observed in males exposed to xylene. Xylene is neither genotoxic nor classified as a human carcinogen by the International Agency for Research on Cancer (IARC) or the U.S. Environmental Protection Agency (EPA).

Effects in Humans

The clinical toxicology of xylene exposure has been summarized (see ACGIH 2001; ATSDR 1995; EPA 2003, 2005), and only data relevant to derivation of submarine EEGL and CEGL values are discussed below.

Accidental Exposures

Of three men exposed to xylene at an estimated concentration of 10,000 ppm for at least 15 h, one died, and the other two were admitted to a hospital unconscious (Morley et al. 1970). The three men had been employed to paint a double-bottom tank in the engine room of a ship with a paint containing 34% solvent (by weight), of which 90% was xylene and only a trace was toluene. The men started work at 10:30 a.m. and were found unconscious at 5 a.m. the next day. The deceased exhibited pulmonary congestion. One of the patients recovered consciousness after admission and was confused and amnesic, had slurred

speech, and was ataxic on walking. Within 24 h after admission, he was fully conscious and alert; by 48 h, the ataxia had disappeared. A slight nonsignificant increase in serum transaminase occurred over 48 h before returning to normal. On admission, the other patient was unconscious and hypothermic and exhibited medium-grade moist rales in the lungs. He regained consciousness after 5 h of treatment with tracheal aspiration and oxygen but remained amnesic for 2-3 days. Renal damage was evident in an increase in blood urea from 59 mg/100 mL to 204 mg/100 mL measured 3 days after admission. Slight hepatic impairment was observed with a rise in serum transaminase to 100 IU over 48 h and thereafter a return to normal.

A variety of signs and symptoms have been identified in case reports of humans exposed to xylene as a paint component or laboratory solvent, including eye irritation, irritation of the nose and throat, headache, dizziness, vertigo, nausea, vomiting, and signs similar to those of slight drunkenness (Goldie 1960; Klaucke et al. 1982). In one report, workers noticed an unusual odor 15-30 min before onset of symptoms; exposure concentrations (of unknown duration) were estimated to be as high as 700 ppm (Klaucke et al. 1982).

Experimental Studies

Groups of six or seven volunteers were exposed for 15 min once a day to air containing mixed xylenes at 110, 230, 460, or 690 ppm (Carpenter et al. 1975a,b). Volunteers recorded responses at 1-min intervals throughout the exposure. At 110 ppm, one of six subjects experienced throat discomfort; at 230 ppm, one of seven subjects reported eye irritation, tears, and dizziness or light-headedness. None of those exposed at 110 or 230 ppm complained of throat irritation. At 460 ppm, four of six subjects reported mild eye irritation, and one of the four left the chamber; a fifth reported mild dizziness; and the sixth recorded mild nose and throat irritation. Exposure at 690 ppm resulted in dizziness or light-headedness in four of six subjects (mild in three, and a slight loss of balance in the fourth). The authors concluded that xylene at 100 ppm would not be objectionable to most people; the volunteers did not believe that xylene at 690 ppm could be tolerated over an 8-h workday.

Male college volunteers were exposed to mixed xylenes at 0, 100, 200, or 400 ppm for 30 min (Hastings et al. 1986). The effects of exposure to xylene were mild. Eye irritation was reported by 56%, 60%, 70%, and 90% of the subjects at 0, 100, 200, and 400 ppm, respectively. No definitive increase was noted in the exposed groups compared with the control group in nose or throat irritation, eye blinks per minute, respiration rate, or performance of behavior tasks.

Groups of five healthy volunteers were exposed to xylene at 0, 100, or 300 ppm for 70 min on one of 3 days (Gamberale et al. 1978). In a second experiment, eight of the volunteers who participated in the first study were exposed to xylene at 300 ppm for 70 min; they exercised (at 100 W) on a bicycle for the first 30 min of exposure. Although a slight increase in frequency of headache,

sickness, and intoxication was noted, the numbers of subjects with these complaints were not indicated. The authors stated that most volunteers reported no or negligible subjective symptoms and that xylene exposure at rest did not significantly affect the results of performance tests of subjects exposed at 100 or 300 ppm. Xylene exposure combined with 100-W exercise, however, impaired performance on all tests.

Healthy male volunteers were exposed in random sequence to air containing toluene at 100 ppm, xylene at 100 ppm, or a mixture of toluene at 50 ppm and xylene at 50 ppm, or to control air for 4-h sessions; exposure sessions were separated by 7-day intervals (Dudek et al. 1990). A battery of nine psychologic tests was administered 1 h before exposure, at the beginning of exposure, and 3 h into exposure. The 3-h xylene exposure significantly increased simple reaction time and choice reaction time; no significant effects were observed in the other seven tests.

Male student volunteers were divided into three groups and exposed to *m*-xylene at a fixed concentration of 200 ppm, *m*-xylene at a basal concentration of 135 ppm with 20-min peak concentrations of 400 ppm at the beginning of morning and afternoon sessions, or control air (Seppalainen et al. 1989; Seppalainen et al. 1991; Laine et al. 1993). Exposures were for 3 h in the morning and 40 min in the afternoon with a 40-min break between morning and afternoon exposures. The subjects were exposed either at rest or with 10 min of exercise (at 100 W) at the beginning of each exposure session; exposure occurred on 6 separate days with a minimum of 5 days between exposures. Xylene exposure at rest did not result in any consistent effects on visual evoked potentials (VEPs), but exposure with exercise resulted in a minor statistically significant decrease in VEPs in two subjects given xylene at fluctuating concentrations of 400 ppm (the second condition described above). No exposure-related changes were noted in brainstem auditory evoked potentials, but the peak exposure concentration (400 ppm) resulted in decreased body sway in both sedentary and exercising subjects.

Six of 17 healthy male volunteers were exposed to *m*-xylene at 100 ppm for 3 h in the morning with hourly peaks of 200 ppm and for 3 h in the afternoon at 200 ppm with hourly peaks of 400 ppm (Savolainen and Linnavuo 1979). A 1-h break separated the exposure sessions. Body balance was not affected by the morning exposure, but impairment of body balance was noted in subjects during the afternoon session. However, tolerance of xylene has been observed with continuing exposure (Riihimaki and Savolainen 1980; Savolainen and Riihamaki 1981).

Using a similar experimental design, Savolainen and co-workers (1984, 1985a, 1985b) exposed nine male volunteers to *m*-xylene at a fixed concentration of 200 ppm *m*-xylene or a basal concentration of 135 ppm with 20-min peak concentrations of 400 ppm at the beginning of the morning and afternoon sessions. The subjects were exposed when sedentary or with 10 min of exercise (at 100 W) at the beginning of each exposure session. Exposures occurred at 6-day intervals during 6 successive weeks; end points monitored were body sway along the anteroposterior and lateral axis, simple and choice reaction times, and

auditory and visual stimuli. The anteroposterior axis was impaired at peak exposure with opposite results when body sway was measured along the lateral axis. No consistent significant effects were noted on reaction times, and effects were observed only at peak exposure (400 ppm).

Six male volunteers were exposed to *m*-xylene at 100 or 200 ppm 6 h/day, 3 days/week for 2 weeks (Savolainen et. al. 1979, 1980; Riihimaki and Savolainen 1980). In one experiment, a peak concentration of 400 ppm was used. Significant increases in reaction time and some impairment of equilibrium were observed during the first week of exposure at 100 ppm. Those transient effects reappeared during the second week at higher concentrations. There were no changes in dexterity or visual functions.

In four male volunteers exposed to *p*-xylene at 70 ppm for 4 h, there was no effect on choice reaction time, simple reaction time, or short-term memory performance measured during exposure at 1, 2, and 4 h (Olson et al. 1985). In addition, no changes from baseline were noted in measured heart rate or subjective symptoms as reported on a questionnaire administered at exposure termination.

Twenty-three male volunteers, divided into four or five per group, were exposed to *m*-xylene, *p*-xylene, or toluene at 100 or 200 ppm for 3 or 7 h with a 1-h break at some point after the beginning of exposure (Ogata et al. 1970). Xylene exposures did not significantly affect blood pressure, pulse rate, flicker value, or reaction time as measured at the beginning and end of exposure.

Nine male students were exposed to *m*-xylene at 200 ppm 4 h/day once a week for 6 consecutive weeks with a 6-day interval between successive exposures (Savolainen et al. 1981; Seppalainen et al. 1983). Xylene exposure did not result in any marked adverse effects, but a slight improvement was observed in performance as measured by a decrease in body sway, a shortened reaction time, and an increase in the critical flicker fusion thresholds. No significant effects in pattern VEP were observed.

Twelve healthy male volunteers (four groups of three) were exposed at rest to xylene at 200 ppm for 5.5 h on 2 days separated by a week (Laine et al. 1993). There were no effects on body sway or reaction times or on auditory, visual, and associative signals.

Nine males and seven females were divided into three "daily groups" and exposed at rest to *p*-xylene vapors for 1, 3, or 7.5 h daily (Hake et al. 1981). All subjects were exposed to xylene at 100 ppm on 5 consecutive days in the first week. The males were exposed at 20 ppm in the second week, at 150 ppm in the third week, and at fluctuating concentrations of 50-150 ppm (time-weighted average [TWA], 100 ppm) during the fourth week (5 days/week). Toxicity was evaluated with neurologic, cardiopulmonary-function, cognitive, and subjective tests. In males, eye irritation was noted during the weeklong exposures seven times at 100 ppm, eight times at 150 ppm, and three times during the 4 control days. One subject wearing contact lenses in the 7.5-h exposure noted eye irritation almost every day; another complained twice at 100 ppm and three times at 150 ppm. No irritation was noted by the males during any 3-h exposure, al-

though one complained of eye irritation during the 1-h exposure to 150 ppm. The exposures did not result in any significant neurologic, cardiopulmonary, or cognitive abnormalities in the males or females. Irritation reported by the female volunteers was confined to the nose and throat.

It is known that xylene vapor may be absorbed through the skin (Kezic et al. 2000, as cited in Kezic et al. 2004). Recent experimental determinations of percutaneous absorption in male human volunteers (21-53 years old) after a maximal exposure duration of 180 min (range, 20-160 min) have been incorporated into an estimate of the dermal contribution during whole-body xylene exposure (Kezic et al. 2004). If it is assumed that the exposed area of skin of a clothed person is 1.24 m^2, 58% of total adult body surface (Loizou et al. 1999, as cited in Kezic et al. 2004), the dermal contribution during an assumed 3-h whole-body exposure would be 0.2% (Kezic et al. 2004). That contribution to whole-body absorption is negligible. Therefore, the present analysis focuses on vapor inhalation as the primary exposure route of concern.

Occupational and Epidemiologic Studies

The most frequent symptoms reported in workers exposed to xylene are headache, fatigue, lassitude, irritability, nausea, anorexia, and flatulence (Gerarde 1960). Workers exposed to commercial grade xylene vapors at over 200 ppm have complained of nausea, vomiting, heartburn, and loss of appetite (Browning 1965). Uchida et al. (1993) reported a significant increase in the prevalence of eye, nose, and throat irritation, anxiety, forgetfulness, inability to concentrate, and dizziness in workers chronically exposed to mixed xylene vapors. The exposure intensity was broken into 1-20 ppm and greater than 21 ppm, with a geometric mean concentration of 14 ppm and an average exposure of 7 years. The symptoms were reported in a questionnaire survey of 175 workers whose exposure to xylene was at an average of 21 ppm. No abnormalities were seen in physical examination, clinical chemistry, or hematology. It is unclear whether the worker complaints reported resulted from short-term exposure at peak concentrations of xylene. Furthermore, no day-to-day or week-to-week data analysis or acute exposure excursions were presented (workers were stated to have been exposed to xylene at up to 175 ppm). Separate groups were not adequately described in the study, nor was there a concentration-dependent increase in symptoms (Table 12-2).

Effects in Animals

Several reviews of the animal-toxicity data on xylene are available (see ATSDR 1995; EPA 2003, 2005). Because primarily human data were used to derive the EEGL and CEGL values here, the relevant supporting animal data are only briefly discussed below.

TABLE 12-2 Effects of Xylene in Controlled Human Studies

Concentration (ppm)	Time	Isomer	Subjects and Effects	Reference
110, 230, 460, 690	15 min daily	Mixed	Healthy subjects (groups of 6 or 7) At 110 ppm, 1 of 6 had throat discomfort; at 230 ppm, 1 of 7 had eye irritation, tears, dizziness or light-headedness; at 460 ppm, 4 of 6 had mild eye irritation, 1 of 6 had mild dizziness, 1 of 6 had mild nose and throat irritation; at 690 ppm, 4 of 6 had dizziness or light-headedness	Carpenter et al. 1975b
0, 100, 200, 400	30 min	Mixed	Healthy male students (50) Eye irritation reported by 56% of controls, 60% of subjects exposed at 100 ppm, 70% of subjects at 200 ppm, and 90% of subjects at 400 ppm; no definitive increase noted between exposed and control groups in nose or throat irritation, eye blinks per minute, respiration rate, or performance of behavioral tasks	Hastings et al. 1986
0, 100, 300	70 min	NA	Healthy subjects (groups of 5) No reported effect on 5 performance tests.	Gamberale et al. 1978
300 with exercise	70 min		Healthy subjects (8 subjects from first study) Exposure combined with 100-W exercise impaired performance on all tests	
100	3 h	NA	Healthy males (10) Decreased performance in simple and choice reaction times; no significant effects on other 7 psychologic tests	Dudek et al. 1990
135-400	3 h in morning, 40 min in afternoon	*m*-	Healthy males (9) No consistent effects on VEPs while at rest; exposure with exercise resulted in minor statistically significant decrease in VEP values in two subjects given fluctuating concentrations of 400 ppm; no exposure-related changes noted in brainstem auditory evoked potentials, but peak exposure concentration (400 ppm) resulted in decreased body sway in both sedentary and exercising subjects	Seppalainen et al. 1989

(Continued)

TABLE 12-2 Continued

Concentration (ppm)	Time	Isomer	Subjects and Effects	Reference
200-400	4 h	*m*-	Healthy subjects (9) Effects of short-term exposure were minor, and no deleterious effects noted	Seppalainen et al. 1991
200	5.5 h	*m*-	Healthy males (12) No effect on body sway or reaction times, or auditory, visual, and associative Signals	Laine et al. 1993
100-400	3 h in morning, 3 h in evening	*m*-	Healthy males (6 of 17 volunteers) Body balance not affected by exposure in morning but affected by afternoon Exposure	Savolainen and Linnavuo 1979
100-400	3 h in morning, 3 h in evening	*m*-	Healthy males (6 of 17) Tolerance of xylene for effects of body sway and balance was observed with continuing exposure	Riihimaki and Savolainen 1980; Savolainen and Riihamaki 1981
135-400	4 h	*m*-	Healthy males (9) Anteroposterior axis impaired at peak exposure with opposite results when body sway was measured along lateral axis; no consistent significant effects noted on reaction time, and effects observed only at 400 ppm	Savolainen et al. 1984, 1985a, 1985b
70	4 h	*p*-	Healthy males (4) No effect on choice reaction time, simple reaction time, short-term memory, heart rate, or subjective symptoms	Olson et al. 1985
100, 200	3 or 7 h with 1-h break	*m*-, *p*-	Healthy males (23), 4 or 5 per group *m*- or *p*-xylene exposure had no effect on blood pressure, pulse rate, flicker value, or reaction time	Ogata et al. 1970

200	4 h/day for 6 weeks	*m*-	Healthy males (9) No marked adverse effects but slight improvement in performance as measured by decrease in body sway, shortened reaction time, and increase in critical flicker fusion thresholds; no significant effects on pattern VEP	Savolainen et al. 1981; Seppalainen et al. 1983
20, 100, 150	1, 3, or 7.5 h daily	*p*-	Healthy males (9) and females (7) In males, eye irritation noted 7 times at 100 ppm, 8 times at 150 ppm, and 3 times on control days; no irritation noted by males during any 3-h exposure, although one complained of eye irritation during 1-h exposure at 150 ppm; no significant neurologic, cardiopulmonary, or cognitive abnormalities in males or females; irritation in female subjects confined to nose and throat	Hake et al. 1981

Abbreviations: NA, not available; VEP, visual evoked potentials.

Acute Toxicity

The 4-h LC_{50}s of xylene range from 3,907 to 11,000 ppm in rats and mice (EPA 2005). In rats exposed to mixed xylene for 4 h, no adverse effects were noted at 580 ppm, and the poor coordination noted after a 2-h exposure at 1,300 ppm was reversible after exposure termination (Carpenter et al. 1975b). Exposure at 1,600 ppm for 4 h resulted in hyperactivity, fine tremors, and unsteadiness (Bushnell 1989). Female rats exposed to *p*-xylene at 1,000, 1,500, or 2,000 ppm for 4 h developed increased serum enzyme activities indicative of acute hepatic damage (Patel et al. 1979). A minimal narcotic concentration of *m*-xylene in the rat was 2,100 ppm for 4 h of exposure (Molnár et al. 1986).

Repeated Exposure and Subchronic Toxicity

Rats, guinea pigs, monkeys, and dogs that were exposed to *o*-xylene at 78 ppm for 90 days or at 780 ppm for 6 weeks had no significant changes in body weight or hematology (Jenkins et al. 1970). In a study in which rats and dogs inhaled mixed xylene at 180, 460, or 810 ppm for 13 weeks, there were no detectable changes in body weight, hematology, blood chemistry, urinalysis, organ weights, or histopathology at any of the concentrations tested (Carpenter et al. 1975a,b). In another study, however, when rats were exposed to mixed xylenes at 690 ppm 8 h/day, 6 days/week for 110-130 days and rabbits were exposed at 1,200 ppm 8 h/day, 6 days/week for 40-50 days, some of the animals showed signs of hind limb paralysis and weight loss (Fabre et al. 1960).

Chronic Toxicity

Rats exposed to *o*-xylene at 1,090 ppm 8 h/day, 7 days/week for 6-12 months had decreased body weight, increased absolute and relative liver weight, and induction of enzymes of the hepatic mixed-function oxidase system (Tátrai et al. 1981). In another study, rats were exposed to mixed xylenes at 0, 140, 350, or 920 ppm 8 h/day, 7 days/week for 6 weeks and then 5 days/week for 6 months (Ungváry 1990). The author did not consider exposure to those concentrations to have produced any significant adverse effects. Rats that inhaled *m*-xylene at 100 ppm for 6 months or at 1,000 ppm for 3 months were evaluated with a rotarod test to measure motor coordination (Korsak et al. 1992). Rats exposed at 1,000 ppm had about 60% failures in the test compared with 35% in the 100-ppm rats and none in the controls. Furthermore, exposure at 100 ppm resulted in nearly a 50% reduction in spontaneous motor activity. In another study by Korsak et al. (1994), *m*-xylene at 50 ppm 6 h/day, 5 days/week for 3 months was considered a no-observed-adverse-effect level (NOAEL) for the rotarod test.

Reproductive Toxicity in Males

The male reproductive system appears not to be a primary target of xylene toxicity. For example, no effects were observed in the testes, accessory glands, or circulating male hormone concentrations in male Sprague-Dawley rats exposed to mixed xylene at a high dose (1,000 ppm) for 61 days (Nylen et al. 1989). And no animal studies have shown any evidence of developmental toxicity when males were exposed to xylene (reviewed in EPA 2005).

The human workplace data regarding the reproductive and developmental effects of xylene are complicated by concurrent exposures to other solvents, small numbers of subjects tested, and absence of quantified exposure concentrations (summarized in ATSDR 1995).

Immunotoxicity

Workers exposed to xylene have manifested decreased lymphocytes and serum complement (Moszczynsky and Lisiewicz 1983, 1984). However, the workers were exposed concurrently to other chemicals, most notably to the trace quantities of benzene often present in technical-grade xylene.

Genotoxicity

Mixed xylene and the individual isomers have been tested for genotoxicity in a variety of in vitro and in vivo assays (reviewed in ATSDR 1995). Results of the various assays indicate that in vitro or in vivo exposure to mixed xylene and xylene isomers is not genotoxic or clastogenic. Furthermore, all studies evaluated by the GENETOX panel were negative except for one on which no conclusion was drawn (GENETOX 1992). Xylene was not mutagenic in bacterial test systems or cultured lymphoma cells and has not induced chromosomal aberrations or sister-chromatid exchanges in Chinese hamster ovary cells or cultured human lymphocytes (EPA 2005). Xylene has not induced chromosomal aberrations in rat bone marrow or in micronuclei in mouse bone marrow or caused sperm-head abnormalities (reviewed in EPA 2005).

Carcinogenicity

Animal studies have not assessed carcinogenicity of inhalation exposure to xylene; rather, xylene has been administered orally (Maltoni et al. 1985; NTP 1986) or dermally in an initiation-promotion study (Pound 1970). The National Toxicology Program (NTP 1986) found no significant dose-related neoplastic effects in male or female F344/N rats or B6C3F1 mice exposed at up to 500 mg/kg per day (rats) or 1,000 mg/kg per day (mice) 5 days/week for 103 weeks.

Although xylene is not classified as a human carcinogen, some human occupational studies have suggested that it is associated with an increased risk of

cancer (EPA 2005). However, the studies are weakened by small sample sizes, lack of quantified exposure concentrations, or concurrent exposures to other solvents. In addition, there are inconsistencies in cancer expression between studies. Animal carcinogenicity studies have been limited to equivocal oral exposure (Maltoni et al. 1985; NTP 1986) and a dermal initiation-promotion study (Pound 1970). IARC (1999) has concluded that there is inadequate evidence of the carcinogenicity of xylene and therefore states that xylene is not classifiable as to its carcinogenicity in humans (IARC 1999). EPA (2003) considers the data inadequate for an assessment of the carcinogenic potential of xylene.

TOXICOKINETIC AND MECHANISTIC CONSIDERATIONS

Inhaled xylene is rapidly absorbed from the lungs into the blood and is distributed to the kidneys, brain, subcutaneous body fat, bone marrow, spinal cord and spinal nerves, liver, and nasal mucosa. It is rapidly eliminated from those tissues with the exception of fat (Bergman 1983; Carlsson 1981; Kumarathasan et al. 1997). Circulating xylene is associated primarily with serum proteins in human blood (Riihimaki et al. 1979). The primary metabolic pathway in humans is side-chain dehydroxylation by hepatic mixed-function oxidases to toluic acids, which are then conjugated with glycine to form methlyhippuric acid isomers that are excreted in the urine (Ogata et al. 1980; Tardif et al. 1989). In animals, xylene is excreted in the urine as methylhippuric acid isomers and toluic acid glucuronides (Ogata et al. 1980). It has been estimated that excretion of xylene in air and urine has an initial half-life of 1 h followed by a slow phase with an estimated 20-h half-life (Riihimaki et al. 1979; Riihimaki and Savolainen 1980).

Xylene is an irritant of eyes and mucous membranes. Regarding systemic effects, the most prominent and consistent in humans and animals is CNS disturbances. CNS toxicity resulting from exposure to xylene at high concentrations has been attributed to its liposolubility (Desi et al. 1967; Gerarde 1959; Savolainen and Pfaffli 1980; Tahti 1992). Although the mechanism of action remains unknown, it has been suggested that xylene acts like other general anesthetic agents by disturbing the action of proteins essential for normal neuronal function (ATSDR 1995). Others have suggested that metabolic intermediates may be responsible for the toxic effects of xylene (Savolainen and Pfaffli 1980).

INHALATION EXPOSURE LEVELS FROM THE NATIONAL RESEARCH COUNCIL AND OTHER ORGANIZATIONS

A number of organizations have established or proposed inhalation exposure levels or guidelines for xylene. Selected values are summarized in Table 12-3.

TABLE 12-3 Selected Inhalation Exposure Levels for Xylene from the NRC and Other Agencies[a]

Organization	Type of Level	Exposure Level (ppm)	Reference
Occupational			
ACGIH	TLV-TWA	100	ACGIH 2001
	TLV-STEL	150	
NIOSH	REL-TWA	100	NIOSH 2005
	REL-STEL	150	
OSHA	PEL-TWA	100	29 CFR 1910.1000
Spacecraft			
NASA	SMAC		Garcia 1996
	1-h	100	
	24-h	100	
	30-day	50	
	180-day	50	
Submarine			
NRC	EEGL		NRC 1984
	1-h	200	
	24-h	100	
	CEGL		
	90-day	50	
General Public			
ATSDR	Acute MRL	2.0	ATSDR 2005
	Intermediate MRL	0.6	
	Chronic MRL	0.05	
NAC/NRC	AEGL-1 (1-h)	130	EPA 2005
	AEGL-2 (1-h)	920	
	AEGL-1 (8-h)	130	
	AEGL-2 (8-h)	400	

[a]The comparability of EEGLs and CEGLs with occupational-exposure and public-health standards or guidance levels is discussed in Chapter 1 ("Comparison with 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; CEGL, continuous exposure guidance level; EEGL, emergency exposure guidance level; 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; REL, recommended exposure limit; SMAC, spacecraft maximum allowable concentration; STEL, short-term exposure limit; TLV, Threshold Limit Value; TWA, time-weighted average.

COMMITTEE RECOMMENDATIONS

The committee's recommendations for EEGL and CEGL values for xylene are summarized in Table 12-4. The current and proposed U.S. Navy values are provided for comparison.

1-Hour EEGL

Xylene vapor at low concentrations is an irritant of eyes and mucous membranes, and at higher concentrations it results in signs of CNS depression. For CNS effects, several acute studies in volunteers reported that exposures to xylene at 70-400 ppm for up to 4 h either failed to affect performance of subjects on neurobehavioral tests (Olson et al. 1985; Gamberale et al. 1978; Hastings et al. 1986; Seppalainen et al. 1989; Seppalainen et al. 1991) or actually improved performance (Laine et al. 1993; Savolainen et al. 1984, 1985b). But, other studies reported a correlation between acute exposure to *m*-xylene at 100-400 ppm for up to 4 h and impaired performance (Savolainen et al. 1979, 1980, 1984, 1985a; Savolainen and Linnavuo 1979; Savolainen and Riihimaki 1981; Dudek et al. 1990; Gamberale et al. 1978; Seppalainen et al. 1983, 1989, 1991). In six volunteers exposed to *m*-xylene at 100 or 200 ppm 6 h/day, 3 days/week for 2 weeks, significant increases in reaction time and some impairment of equilibrium were observed, but the effects were transient, and no changes were noted in manual dexterity or visual function (Savolainen et al. 1979, 1980; Riihimaki and Savolainen 1980). Dizziness occurred in one of six subjects exposed to xylene at 460 ppm for 15 min (Carpenter et al. 1975a), and there was no impairment in performance tests in 15 men exposed at 299 ppm for 70 min (Gamberale et al. 1978) or among 10 men who inhaled xylene at 369 ppm for 30 min (Hastings et al. 1986). Nevertheless, xylene exposure at 100 ppm for 3 h resulted in reduced performance in two (simple reaction and choice reaction times) of nine psychologic tests (Dudek et al. 1990). Results on those two, however, were not affected in other studies conducted at equivalent or higher concentrations of

TABLE 12-4 Emergency and Continuous Exposure Guidance Levels for Xylene

Exposure Level	U.S. Navy Values, ppm		Committee Recommended Values, ppm
	Current	Proposed	
EEGL			
1-h	200	100	200
24-h	100	100	100
CEGL			
90-day	50	50	50

Abbreviations: CEGL, continuous exposure guidance levels; EEGL, emergency exposure guidance level.

xylene and durations of exposure (Hastings et al. 1986; Olson et al. 1985; Laine et al. 1993; Savolainen et al. 1985b). Thus, although five of the 24 neurologic performance and cognitive tests performed by various investigators showed effects, overall the results were inconsistent.

The 1-h EEGL is based on notable discomfort in humans in the form of eye irritation. Eye irritation was reported in four of six subjects exposed to mixed xylene at 460 ppm for 15 min and in one of seven at 230 ppm; no eye irritation was noted at 110 ppm (Carpenter et al. 1975b). Slight eye irritation was noted in humans exposed to mixed xylene at 100, 200, or 400 ppm for 30 min (Hastings et al. 1986). Irritation was noted in 56% of the controls, 60% of those exposed at 100 ppm, 70% at 200 ppm, and 90% at 400 ppm. Males exposed to *p*-xylene at 100 or 150 ppm 7.5 h/day for 5 consecutive days reported eye irritation seven times and eight times during 5 days of exposure, respectively (Hake et al. 1981). One volunteer wearing contact lenses reported eye irritation almost every day. No irritation was reported during any 3-h exposure, although one subject complained of eye irritation during a 1-h exposure to *p*-xylene at 150 ppm. On the basis of those data and a weight-of-evidence approach, a 1-h EEGL of 200 ppm was selected. That exposure concentration resulted in minimal or no eye irritation in 1-h exposures in numerous controlled studies that evaluated xylene-induced eye and throat irritation. Thus, the committee concluded that there was little justification for an intraspecies uncertainty factor.

24- Hour EEGL

Xylene-induced ocular irritation depends heavily on concentration and much less on exposure duration. Humans exposed to *p*-xylene at 20, 100, or 150 ppm 7.5 h/day for 5 weeks reported eye irritation seven times during exposure at 100 ppm, eight times at 150 ppm, and three times during the 4 control days between exposures (Hake et al. 1981). The irritation was minimal and intermittent, and there were no significant neurologic, cardiopulmonary, or cognitive abnormalities. Therefore, a 24-h EEGL of 100 ppm was selected. The committee concludes that the very mild and transient irritation observed in experimental subjects in successive exposures at that concentration would not impair crew performance, cause irreversible harm, or have any delayed health effects. The human data, collectively, support a 24-h EEGL of 100 ppm. Because minimal or intermittent irritation in humans was the end point for the 24-h EEGL and no other effects were noted, an intraspecies uncertainty factor was not justified.

90-Day CEGL

A 90-day CEGL of 50 ppm for xylene is supported by human data. Acute and short-term repeated human exposure to xylene has not reported ocular or respiratory tract irritation at concentrations less than 50 ppm. Furthermore, sev-

eral studies evaluating more than 2 dozen neurologic performance and cognitive tests have shown no adverse effects of exposure at below 50 ppm (see Table 12-2). Only five of the more than 24 tests have shown effects, and all of those were highly inconsistent. Chronic human data are limited to a study by Uchida et al. (1993) that identified a "concentration-related" increase in eye irritation, sore throat, and a "floating sensation" during the workshift. As discussed above, it is unclear whether the worker complaints resulted from short-term exposure at peak concentrations of xylene. Furthermore, no day-to-day or week-to-week data analysis or acute exposure excursions were presented (workers were stated to have been exposed to xylene at up to 175 ppm). Separate groups were not adequately described in the study, nor was there a concentration-dependent increase in symptoms. Thus, the data as presented by Uchida and co-workers are inadequate to derive air exposure guidance levels for xylene. Animal studies support a 90-day CEGL of 50 ppm. Using three 90-day exposure studies that included three animal species, the committee calculated 90-day CEGL values of 47 ppm, 48 ppm, and 260 ppm (Ungvary 1990; Carpenter et al. 1975b; Jenkins et al. 1970). Those values were calculated by identifying the NOAEL in each study and applying an interspecies uncertainty factor of 3 on the basis of a range of a factor of 2-3 in the minimal alveolar concentration of volatile anesthetics in humans (EPA 2005). The committee found that continuous exposure to xylene at 50 ppm for 90 days would result in minimal eye or throat irritation, if any, and that there is no evidence of systemic effects in humans exposed at that concentration. Therefore, the committee saw little justification for the addition of an intraspecies uncertainty factor and concluded that a 90-day CEGL of 50 ppm would not degrade crew performance or produce immediate or delayed adverse health effects.

DATA ADEQUACY AND RESEARCH NEEDS

The human data for determining 1- and 24-h EEGLs are fairly robust, although many of the studies did not specifically report sensory irritation as an end point or as a symptom. A study designed to determine the presence and degree of eye and throat irritation for exposure of 1 and 24 h would improve the level of confidence in the EEGL and CEGL values. Epidemiologic investigations of workers exposed to xylene or longer-term controlled exposure studies of xylene at 25-75 ppm would benefit derivation of the 90-day CEGL.

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Appendix

Biographic Information on the Committee 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 of the National Toxicology Program at the National Institute of Environmental Health Sciences. He has served two terms as a member of the National Research Council Committee on Toxicology and on several other committees, including the Subcommittee on Manufactured Vitreous Fibers. He received his DVM from Ohio State University and his MS in pathology from Michigan State University. He completed his residency in comparative pathology at the Armed Forces Institute of Pathology.

RAKESH DIXIT is head of toxicology with MedImmune/AstraZeneca Biologics. Previously he was a senior director of toxicology with Johnson and Johnson and associate director in Safety Assessment for Merck Research Laboratories, where he conducted safety-assessment studies. His research interests include safety and 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 of *Toxicology Applied Pharmacology* and *Journal of Toxicology and Environmental Health and Methods*. Dr. Dixit served on the National Research Council Subcommittee on Jet Propulsion Fuel 8 and continues to serve on the Committee on Acute Exposure Guideline Levels. He has over 60 peer reviewed publications and book chapters. He has more than 100 invited presentations in the national and interna-

tional meetings. Dr. Dixit received his PhD in toxicology and biochemistry from University of Lucknow with research work at Case Western Reserve University. He is board-certified in toxicology by the American Board of Toxicology since 1992.

DAVID DORMAN is associate dean for research and graduate studies in the College of Veterinary Medicine at North Carolina State University. The primary objective of his research is to provide a refined understanding of chemically induced neurotoxicity in laboratory animals that will lead to improved assessment 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 DVM from Colorado State University. He completed a combined PhD and residency program in toxicology at the University of Illinois at Champaign-Urbana and is a diplomate of the American Board of Veterinary Toxicology and the American Board of Toxicology.

MAUREEN FEUSTON is associate vice president for drug-safety evaluation at sanofi-aventis Inc. Before joining sanofi-aventis, 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 in the Teratology Society. She has also served on the National Research Council Subcommittee on Reproductive and Developmental Toxicants. She received her PhD 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 DVM from MSU and his PhD in comparative pathology from the University of California, Davis.

HOWARD KIPEN is a professor 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 benzene, asbestos, and particulate air

pollutants. He has served as a member or chair of several Institute of Medicine committees, including the Committee on the Persian Gulf Syndrome Comprehensive Clinical Evaluation Program. He received his MD from University of California at San Francisco and his MPH 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 toxic, pathologic, and immunologic effects of toxic substances and the effects of environmental contaminants on tumor growth and immunity. He is a former member of the National Research Council Committee on Toxicology and several of its subcommittees, including the Subcommittee on Immunotoxicity and the Subcommittee on Zinc Cadmium Sulfide. He serves on the Institute of Medicine Committee on the Assessment of Health Effects of Vietnam Veterans. He received his DVM from Washington State University and his PhD in pathology from the University of Wisconsin.

JOHN O'DONOGHUE is an adjunct associate professor of environmental medicine at the University of Rochester School of Medicine and Dentistry. He was the director of the Health and Environment Laboratories and vice president for health, safety, and environment at Eastman Kodak Company until his retirement in 2004. His research interests include neurotoxicology and toxicologic pathology. Dr. O'Donoghue has served on several National Research Council committees, including the Committee on Toxicology and the Subcommittee on Toxicological Hazard and Risk Assessment. He received his VMD and PhD 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 experience 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 serves on the National Research Council Subcommittee on Spacecraft Exposure Guidelines and served on the Subcommittee on Submarine Escape Action Levels and the Subcommittee on Copper in Drinking Water. She received her PhD 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. She has been involved in the development of reference doses, acute exposure guideline levels, and other decision criteria for chemical-warfare agents. Dr. Watson has also interpreted and

applied toxicologic information on hazardous materials to meet community emergency preparedness planning and training needs. She has served on numerous National Research Council committees—including the Committee on Toxicology, the Subcommittee on Toxicological Hazard and Risk Assessment, and the Subcommittee on Guidelines for Military Field Drinking Water Quality—and the Institute of Medicine Committee to Survey the Health Effects of Mustard Gas and Lewisite. She received a PhD from the School of Agriculture at the University of Kentucky and an undergraduate degree in entomology from Purdue University.

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,

[1]Stedman, L.S. 1982. Stedman's Medical Dictionary, 24th Ed. Baltimore, MD: Williams and Wilkins.

[2]See http://www.acgih.org for more information.

[3]Auletta, C.S. 1995. Acute, subchronic, and chronic toxicology. Pp. 69-127 in CRC Handbook of Toxicology, M.J. Derelanko and M.A. Hollinger, eds. Boca Raton: CRC Press.

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 ability of some sensory receptors to modify the response to repeated or continued stimuli.[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.

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.

CEGL *See* continuous exposure guidance level.

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

[5]Hawley, G.G. 1977. The Condensed Chemical Dictionary, 9th Ed. New York: Van Nostrand Reinhold Company.

[6]See http://www.atsdr.cdc.gov/ for more information.

Ceiling concentration A concentration that shall not be exceeded during any part of a working exposure.[7]

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."[8]

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."[9]

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.

COPD *See* chronic obstructive pulmonary disease.

DBNP 2,6-Di-tert-Butyl-4-Nitrophenol.

EEGL See emergency exposure guidance level.

Electrostatic precipitator A system to clear particles and aerosols from air.[10]

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.

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[7]NRC (National Research Council). 2002. Review of Submarine Escape Action Levels for Selected Chemicals. Washington, DC: National Academy Press.

[8]NRC (National Research Council). 1988. Submarine Air Quality. Washington, DC: National Academy Press.

[9]Beers, M.H., and R. Berkow, eds. 1999. The Merck Manual of Diagnosis and Therapy, 17th Ed. Whitehouse Station, NJ: Merck Research Laboratories.

[10]Hagar, R. 2003. Submarine Atmosphere Control and Monitoring Brief for the COT Committee. Presentation at the First Meeting on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, January 23, 2003, Washington, DC.

EPA U.S. Environmental Protection Agency.

Forced expiratory volume at one second (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.[1]

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.[1]

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.

HCFC Hydrochlorofluorocarbons.

IARC (International Agency for Research on Cancer) IARC is an agency of the World Health Organization. IARC's carcinogenicity classifications are as follows:[11]

> 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.

[11]See http://www.iarc.fr/pageroot/GENERAL/indexgen.html for more information.

Irritant A toxicant that exerts deleterious effects by causing inflammation of tissues on contact. Irritants can act on the respiratory system and cause pulmonary edema at high concentrations. At low concentrations, most effects are reversible with cessation of exposure.[12]

Irreversible harm Permanent damage or injury to health. Emergency exposure guidance levels (EEGLs) are designed to avoid or prevent irreversible harm.

LC_{01} Statistical determination of the lethal concentration for 1% of the sample population.

LC_{50} Statistical determination of the lethal concentration for 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.[13]

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."[14]

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)."[15]

MRL *See* minimal risk level.

NAAQS *See* national ambient air quality standards.

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[12]Hodgson, E., R.B. Mailman, and J.E. Chambers, eds. 1988. Dictionary of Toxicology. New York: Van Nostrand Reinhold Company.

[13]EPA (U.S. Environmental Protection Agency). Glossary of IRIS terms. Integrated Risk Information System, U.S. Environmental Protection Agency. [Online]. Available: http://www.epa.gov/iris/gloss8.htm.

[14]NRC (National Research Council). 2000. Toxicological Effects of Methylmercury. Washington, DC: National Academy Press.

[15]ATSDR (Agency for Toxic Substances and Disease Registry). 2003. ATSDR Glossary of Terms [Online]. Available: http:// www.atsdr.cdc.gov/glossary.htm.

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."[16]

NH_3 Ammonia.

NIOSH National Institute for Occupational Safety and Health.

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."[15]

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."[17]

OR Odds ratio.

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[16]EPA (U.S. Environmental Protection Agency). 2003. National Ambient Air Quality Standards (NAAQS). Office of Air and Radiation, U.S. Environmental Protection Agency. [Online]. Available: http://www.epa.gov/air/criteria.html.

[17]IUPAC (International Union of Pure and Applied Chemistry). 1993. Glossary for chemists of terms used in toxicology. Pure Appl. Chem. 65(9):2003-2122. [Online]. Available: http://www.sis.nlm.nih.gov/Glossary/main.htm.

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.[18]

Pb Lead.

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."[19] 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."[20]

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."[21]

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."[21]

REL *See* recommended exposure limit.

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[18]See http://www.osha.gov/ for more information.

[19]29 CFR 1910.1200.

[20]NIOSH (National Institute for Occupational Safety and Health). 2003. NIOSH Pocket Guide to Chemical Hazards. [Online]. Available: http://www.cdc.gov/niosh/npg/pgintrod/html.

[21]EPA (U.S. Environmental Protection Agency). 1989. Glossary of Terms Related to Health, Exposure, and Risk Assessment. EPA/450/3-88/016. Air Risk Information Support Center, U.S. Environmental Protection Agency, Washington, DC.

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.[22]

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."[20]

Short-term public emergency guidance levels (SPEGLs) SPEGLs are "suitable concentrations for single, short-term, emergency exposures, of the general public."[23]

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)."[24]

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[22]Mausner, J.S., S. Kramer, and A.K. Bahn. 1985. Epidemiology: An Introductory Text, 2nd Ed. Philadelphia: W.B. Saunders Company.

[23]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.

[24]NRC (National Research Council). 1994. Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Vol. 1. Washington, DC: National Academy Press.

SMR *See* standardized mortality ratio.

SPEGL *See* short-term public emergency guidance levels.

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.

STEL *See* short-term exposure limit.

Subchronic exposure An exposure lasting 2-13 weeks or 10% of the test animal's lifespan.[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.[7]

2190 TEP Turbine oil.

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."[18]

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-hour workday and a 40-hour workweek without adverse effect.[18]

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.