Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants

Volume 3

SUBCOMMITTEE ON SPACECRAFT MAXIMUM ALLOWABLE CONCENTRATIONS

COMMITTEE ON TOXICOLOGY

BOARD ON ENVIRONMENTAL STUDIES AND TOXICOLOGY

COMMISSION ON LIFE SCIENCES

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Preface

The National Aeronautics and Space Administration (NASA) is aware of the potential toxicological hazards to humans that might be associated with prolonged spacecraft missions. Despite major engineering advances in controlling the atmosphere within spacecraft, some contamination of the air appears inevitable. NASA has measured numerous airborne contaminants during space missions. As the missions increase in duration and complexity, ensuring the health and well-being of astronauts traveling and working in this unique environment becomes increasingly difficult.

As part of its efforts to promote safe conditions aboard spacecraft, NASA requested the National Research Council (NRC) to develop guidelines for establishing spacecraft maximum allowable concentrations (SMACs) for contaminants, and to review SMACs for various spacecraft contaminants to determine whether NASA's recommended exposure limits are consistent with the guidelines recommended by the subcommittee. In response to this request, the NRC first developed criteria and methods for preparing SMACs for spacecraft contaminants, published in its 1992 report *Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants*. Since then, the Subcommittee on Spacecraft Maximum Allowable Concentrations has been reviewing NASA's documentation of chemical-specific SMACs as described in the Introduction to this volume. This report is the third volume in the series *Spacecraft Maximum Allowable Concentrations for Space Station Contaminants*. The first volume was published in 1994 and the second in 1996.

The subcommittee gratefully acknowledges the valuable assistance provided by the following personnel from NASA and its contractors: Dr. John James, Dr. Martin Coleman, Mr. Jay Perry, Mr. Kenneth Mitchell (all from NASA), Dr. King Lit Wong (U.S Department of Commerce, Patent and Trademark Office), Dr. Hector Garcia, and Dr. Chiu Wing Lam (both from Krug International). The subcommittee is grateful to astronaut Dr. Mary Cleave for sharing her experiences. The subcommittee also acknowledges the valuable assistance provided by the Johnson Space Center, Houston, Texas, for providing a tour of their facilities. Linda Leonard was the senior project assistant. Ruth Crossgrove edited the report. The subcommittee particularly acknowledges Dr. Kulbir Bakshi, program director for the Committee on Toxicology, and Dr. Margaret McVey, project director for the subcommittee, for bringing the report to completion.

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SPACECRAFT MAXIMUM ALLOWABLE CONCENTRATIONS FOR SELECTED AIRBORNE CONTAMINANTS:

Introduction

Construction of the International Space Station (ISS)—a multinational effort—is expected to begin in 1997 and, in its present configuration, is expected to carry a crew of four to eight astronauts for up to 180 days. Because the space station will be a closed and complex environment, some contamination of its internal atmosphere is unavoidable. Several hundred chemical contaminants are likely to be found in the closed-loop atmosphere of the space station, most at very low concentrations. Important sources of atmospheric contaminants include off-gassing of cabin materials, operation of equipment, and metabolic waste products of crew members. Other potential sources of contamination are releases of toxic chemicals from experiments, manufacturing activities performed on board the space station, and accidental spills and fires. The water recycling system has also been shown to produce chemical contaminants that can enter the cabin air. Therefore, the astronauts potentially can be chronically exposed to low levels of airborne contaminants and occasionally to high levels of contaminants in the event of accidents, such as a leak, spill, or fire.

The National Aeronautics and Space Administration (NASA) seeks to ensure the health, safety, and functional abilities of astronauts and to prevent the exposure of astronauts to toxic levels of spacecraft contaminants. Consequently, exposure limits need to be established for continuous exposure of astronauts to spacecraft contaminants for up to 180 days (for normal space-station operations) and for short-term (1-24 hr) emergency exposures to high levels of contaminants.

Federal regulatory agencies, such as the U.S. Occupational Safety and Health Administration (OSHA) and the U.S. Environmental Protection Agency (EPA), have not promulgated exposure limits for the durations of exposures encountered in the space station or for conditions of microgravity. In 1972, the National Research Council's Committee on Toxicology (COT) first recommended maximum levels for continuous and emergency exposures to spacecraft contaminants (NRC, 1972). However, that early report did not provide documentation of toxicity data or the rationale for the recommended exposure levels. Toxicity data for most of the compounds were not well developed at that time, and the risk-assessment methods were rudimentary. Over the past several years, COT has recommended emergency exposure guidance levels (EEGLs) and continuous exposure guidance levels (CEGLs) for many chemical substances for the U.S. Department of Defense (NRC, 1984a,b,c,d; 1985a,b; 1986; 1987; 1988). However, EEGLs and CEGLs are not available for most spacecraft contaminants. Because of the experience of COT in recommending EEGLs and CEGLs, NASA requested that the NRC establish guidelines for developing spacecraft maximum allowable concentrations (SMACs) that could be used uniformly by scientists involved in preparing SMACs for airborne contaminants and review the SMACs for individual contaminants to ascertain whether they are consistent with the guidelines.

SMACs are intended to provide guidance on chemical exposures during normal operations of spacecraft as well as emergency situations. Short-term (1 to 24 hr) SMACs refer to concentrations of airborne substances (such as a gas, vapor, or aerosol) that will not compromise the performance of specific tasks by astronauts during emergency conditions or cause serious or permanent toxic effects. Such exposures might cause reversible effects, such as mild skin or eye irritation, but they are not expected to impair judgment or interfere with proper responses to emergencies. Long-term (up to 180 days) SMACs are intended to avoid adverse health effects (either immediate or delayed) and to prevent decremental change in crew performance under continuous exposure to chemicals in the closed environment of the space station for as long as 180 days.

In response to NASA's request to establish guidelines for developing SMACs and to review SMAC documents for selected spacecraft contaminants, NRC assigned the project to the COT, which convened the

Subcommittee on Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants. The subcommittee included experts in toxicology, epidemiology, medicine, physiology, biochemistry, pathology, pharmacology, neurotoxicology, industrial hygiene, statistics, and risk assessment. The subcommittee prepared *Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants* (NRC, 1992). That report provides guidance for deriving SMACs from available toxicological and epidemiological data. It also provides guidance on what data to use, how to evaluate the data for appropriateness, how to perform risk assessment for carcinogenic and noncarcinogenic effects, and how to consider the effects of physiological changes induced by microgravity that might enhance the susceptibility of astronauts to certain spacecraft contaminants. The executive summary of that report is contained in Appendix A of this volume.

SUMMARY OF REPORT ON GUIDELINES FOR DEVELOPING SMACS

As described in Appendix A, the first step in establishing SMACs for a chemical is to collect and review all relevant information available on a compound. Various types of evidence are assessed in establishing SMAC values for a chemical contaminant. These include information from (1) chemical-physical characterizations, (2) structure-activity relationships, (3) in vitro toxicity studies, (4) animal toxicity studies, (5) human clinical studies, and (6) epidemiological studies. For chemical contaminants, toxicity data from human studies are most applicable and are used when available in preference to data from animal studies and in vitro studies. Toxicity data from inhalation exposures are most useful for setting SMACs for airborne contaminants because inhalation is the most likely route of exposure.

For most chemicals, actual human toxicity data are not available. Therefore, toxicity data from studies conducted in animals are extrapolated to estimate the potential toxicity in humans. Extrapolation requires experienced scientific judgment. The toxicity data from animal species most representative of humans in terms of pharmacodynamic and pharmacokinetic properties are used for determining SMACs. If

data are not available on which species best represents humans, the data from the most sensitive animal species are used to set SMACs. Safety or uncertainty factors are commonly used when animal data are used to estimate a safe level for humans. The magnitude of uncertainty factors depends on the quality of the animal data used to determine the no-observed-adverse-effect level (NOAEL). Conversion from animals to humans is done on a body-weight or surface-area basis. When available, pharmacokinetic data on tissue doses are considered for use in species interconversion.

Based on the review of the toxicity data and the use of appropriate safety factors, SMACs for different exposure periods are developed, and a rationale is provided for each recommendation. One- or 24-hr emergency SMACs are derived from acute exposure toxicity studies whenever possible. Development of 1- or 24-hr SMACs usually begins with providing a SMAC for the shortest exposure of 1 hr. Values for 24-hr SMACs might necessitate using Haber's law (an effect level is directly proportional to exposure concentration multiplied by time, or $C \times T = k$) when applicable. Detoxification or recovery and data available on 24-hr exposures are taken into account in modifying Haber's law. The subcommittee and NASA recognize the limitations associated with Haber's law and use it in accordance with the NRC (1992) guidelines for developing SMACs.

When data from chronic exposure studies are available, they are used to derive 7-, 30-, or 180-day SMACs, and safety factors are applied as needed. For substances that affect several organ systems or have multiple effects, all end points—including reproductive (in both sexes), developmental, carcinogenic, neurotoxic, respiratory, and other organ-related effects—are evaluated, the most important or most sensitive effects receiving the major attention. With carcinogenic chemicals, quantitative carcinogenic risks are estimated, and the SMAC is set so that the estimated increased lifetime risk of a neoplasm is no more than 1 in 10,000 exposed persons. When a substance is known to cause an effect that will be aggravated by microgravity, additional safety factors are used.

REVIEW OF SMAC REPORTS

As NASA began developing chemical-specific SMACs, COT con-

vened the Subcommittee on Spacecraft Maximum Allowable Concentrations to review the NASA reports for consistency with the 1992 NRC guidelines (see Appendix A). The SMAC reports are prepared by NASA scientists or contractors. The first SMAC report, published in 1994, addresses 11 compounds: acetaldehyde, ammonia, carbon monoxide, formaldehyde, Freon 113, hydrogen, methane, methanol, octamethyltrisiloxane, trimethylsilanol, and vinyl chloride. Volume 2, published in 1996, covers an additional 12 compounds: acrolein, benzene, carbon dioxide, 2-ethoxyethanol, hydrazine, indole, mercury, methylene chloride, methyl ethyl ketone, nitromethane, 2-propanol, and toluene. This report, Volume 3, addresses another 12 compounds: bromotrifluoromethane (Halon 1301), 1-butanol, *tert*-butanol, diacetone alcohol, dichloroacetylene, 1,2-dichloroethane, ethanol, ethylbenzene, ethylene glycol, glutaraldehyde, trichloroethylene, and xylene.

The SMAC reports are intended for use by engineers in developing design criteria for the ISS. The SMAC reports will also be applicable to the space shuttle, because the recommended SMACs will cover exposure times that are of interest to the space-shuttle program—1-hr and 24-hr SMACs for emergencies and 7-day and 30-day SMACs for continuous exposures.

The subcommittee's review of the SMAC reports prepared by NASA and NASA's contractors involved both oral and written presentations to the subcommittee by the authors of the reports. The subcommittee provided advice and recommendations for revisions to ensure consistency with the NRC (1992) SMAC guidelines. The subcommittee concludes that the SMAC reports on 12 spacecraft contaminants presented in Appendix B of this report are consistent with the 1992 NRC guidelines.

The subcommittee recognizes that many factors, such as the changes in normal human physiological and biochemical processes associated with spaceflight, are not fully understood and could warrant revisions of proposed SMAC values as additional scientific data become available. Because of the enormous amount of data presented in the SMAC reports, the subcommittee could not verify all the data. The subcommittee relied on NASA scientists for the accuracy and completeness of the toxicity data cited in the SMAC reports. Although individual data points were not verified by the subcommittee, the subcommittee agrees with the rationale for the proposed SMAC values.

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Appendix A

GUIDELINES FOR DEVELOPING SPACECRAFT MAXIMUM ALLOWABLE CONCENTRATIONS FOR SPACE STATION CONTAMINANTS: Executive Summary[1]

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

GUIDELINES FOR DEVELOPING SPACECRAFT MAXIMUM ALLOWABLE CONCENTRATIONS FOR SPACE STATION CONTAMINANTS:

Executive Summary

The National Aeronautics and Space Administration (NASA) is preparing to launch a manned space station by the mid-1990s. Because the space station will be a closed complex environment, some contamination of its atmosphere is inevitable. Several hundred chemicals are likely to be found in the closed atmosphere of the space station, most in very low concentrations. Important sources of atmospheric contaminants include metabolic waste products of crew members and off-gassing of cabin materials and equipment. Release of chemicals from experiments performed on board the space station is also a possible source of contamination, and the water reclamation system has the potential to introduce novel compounds into the air. NASA is concerned about the health, safety, and functional abilities of crews exposed to these contaminants.

This report, prepared by the Committee on Toxicology of the National Research Council's Board on Environmental Studies and Toxicology, is in response to a request from NASA for guidelines to develop spacecraft maximum allowable concentrations (SMACs) for space-station contaminants. SMACs are used to provide guidance on allowable chemical exposures during normal operations and emergency situations. Short-term SMACs refer to concentrations of airborne substances (such as gas, vapor, or aerosol) that will not compromise the performance of specific tasks during emergency conditions lasting up to 24 hr. Exposure to 1- or 24-hr SMACs will not cause serious or permanent effects but may cause reversible effects that do not impair judg-

ment or interfere with proper responses to emergencies such as fires or accidental releases.

Long-term SMACs 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 for as long as 180 days. Chemical accumulation, detoxification, excretion, and repair of toxic insults are thus important in determining 180-day SMACs.

ENVIRONMENTAL CONTROL AND LIFE-SUPPORT SYSTEM

The environmental control and life-support system (ECLSS) of the space station is designed to control temperature, humidity, and composition of space-station air, including CO_2 removal; recover water; dispose of waste; and detect and suppress fires. Fires are a great potential hazard and much attention has been given to suppressing them. A fire suppression system is available, but if all else fails, an escape vehicle can be used. A subsystem of the ECLSS, the atmosphere revitalization system, which includes a mass spectrometer called the major constituent analyzer, will analyze cabin air for O_2, N_2, H_2, CO, H_2O, and CH_4 in all areas of the habitation and laboratory modules. A design criterion for the atmosphere revitalization subsystem is the maintenance of space-station exposure levels below the 180-day SMACs under normal conditions.

MODIFICATION OF CONTAMINANT TOXICITY BY ENVIRONMENTAL FACTORS

The special conditions of the space environment must be taken into account in defining spacecraft contaminant exposure limits. Deposition of particles is clearly different and lung function and the toxic potential of inhaled particles may be different under microgravity conditions than under full gravity conditions, as on earth.

Astronauts will be physically, physiologically, and psychologically

compromised for the following reasons: loss of muscle and bone mass, altered immune system, cardiovascular changes, decreased red-blood-cell mass, altered nutritional requirements, behavioral changes from stress, fluid shift in the body, altered hormonal status, and altered drug metabolism. These changes could be important factors in disease susceptibility.

The physiological changes noted in spaceflight to date demonstrate that the astronaut is in an altered homeostatic state and thus may be more susceptible to toxic chemicals. How this altered state modifies reactions to chemicals in the space-station environment is not fully known. The physiological changes induced in the space crew are important and their impact must be taken into account in developing SMAC values for various contaminants.

SOURCES AND TYPES OF DATA FOR ESTABLISHMENT OF SMACS

The subcommittee recommends the use of data derived from a number of sources in establishing SMAC values. These sources provide information on a variety of health effects including mortality, morbidity, clinical signs and symptoms, pulmonary effects, neurobehavioral effects, immunotoxicity, reproductive and developmental toxicity, pathology, mutagenicity, carcinogenicity, and biochemical and enzyme changes.

Chemical–Physical Characteristics of Toxicants

The chemical and physical characteristics of a substance provide valuable information on potential tissue dosimetry of the compound within the body and on its likely toxic effects. Preliminary estimates of the toxic potential of new chemicals also may be derived from known toxicities of structurally similar, well-investigated compounds. However, additional uncertainty (safety) factors must be applied to arrive at safe levels for those congeners that have no dose-response data from intact animals.

In Vitro Toxicity Studies

Useful information can be obtained from studies conducted to investigate adverse effects of chemicals on cellular or subcellular systems in vitro. Systems in which toxicity data have been collected include isolated organ systems, single-cell systems, and tissue cultures from multicellular organisms maintained under defined conditions or from functional units derived from whole cells. In vitro studies can be used to elucidate the toxic effects of chemicals and to study their mechanism of action.

Animal Toxicity Studies

The data necessary to evaluate the relationship between exposure to a toxic chemical and its effects on people are frequently not available from human experience; therefore, animal toxicity studies must be relied on to provide information on responses likely to occur in humans.

The usefulness of animal data depends in part on the route of exposure and species used. Although inhalation studies are most relevant in assessing the toxicity of atmospheric contaminants, data from skin absorption, ingestion, and parenteral studies are also useful. The relevance of animal data to humans may be limited by the absence of information on affected target organs and knowledge of metabolic pathways and pharmacokinetics in animals and humans.

Clinical and Epidemiological Observations

In establishing SMACs for chemicals, dose-response data from human exposure should be used whenever possible. Data from clinical inhalation exposures are most useful because inhalation is the most likely route of exposure. Human toxicity data also are available from epidemiological studies of long-term industrial exposures, from short-term high-level exposures following accidents, or from therapeutic uses of some pharmaceutical agents. Some of these data provide a basis for estimating a dose-response relationship.

Epidemiological studies have contributed to our knowledge of the health effects of many airborne chemical hazards. The limitations of epidemiology stem from its use of available data. The accuracy of data on health outcomes varies with the source of the information, and records documenting historical exposure levels are often sparse. For example, mortality information derived from death certificates is sometimes inaccurate, and exposure information collected from administrative purposes is limited. Despite these limitations, if the populations studied are large enough and have been exposed to high enough doses over a sufficient period to allow for the expression of disease, epidemiological studies usually provide valuable information on the effects of exposure in humans without resorting to cross-species extrapolation or to exposing humans in an experimental situation to possible injuries from chemical hazards.

Pharmacokinetics and Metabolism

Evaluation of the health effects of any chemical in a given environment is greatly facilitated by an understanding of its physiological disposition in the body. Many chemicals require some form of metabolic activation to exert their toxic effects. The formation of reactive metabolites may depend on the level of exposure and the pharmacokinetics of the chemical. Modern pharmacokinetic studies can provide physiologically based models describing disposition of chemicals within organs and tissues in the body. The space station is a closed system with limited capacity to clear the air of chemical vapors; the crew contributes to the removal of the chemicals from the air through sequestration and metabolism.

Toxic metabolites may be highly reactive chemically. These metabolites are biologically reactive intermediates that may covalently bind to nucleic acids or proteins that in turn, may alter DNA replication or transcription. In addition to formation of reactive metabolites, metabolic activity also may lead to formation of species of active oxygen that may damage nucleic acids or proteins or cause lipid peroxidation. The resulting health effects may range from direct, short-term target-organ toxicity to carcinogenesis.

Biological Markers

Biological markers are indicators of change within an organism that link exposure to a chemical to subsequent development of adverse health effects. Biological markers within an exposed individual can indicate the degree of exposure to a pollutant and may provide evidence of the initial structural, functional, or biochemical changes induced by the exposure and, ultimately, the biochemical or physiological changes associated with adverse health effects.

Biological markers can be divided into three classes:

1. Biological markers of exposure to pollutants may be thought of as "footprints" that the chemical leaves behind upon interaction with the body. Such markers contain the chemical itself or a metabolic fragment of the chemical and thus are usually chemical-specific.
2. Biological markers of the effects of exposure include the totality of subclinical and clinical signs of chemically induced disease states. The markers of greatest interest are those that are early predictors of serious effects or late-occurring effects. Such markers would be useful in determining what levels of pollutants in the space station can be tolerated without causing irreversible deleterious health effects.
3. Biological markers of increased susceptibility to the effects of exposure to pollutants could be used to predict which persons are most likely to be at excess risk as space-station crew members.

RISK ASSESSMENT (DEVELOPMENT OF EXPOSURE CRITERIA)

The assessment of toxicants that do not induce carcinogenic or mutagenic effects traditionally has been based on the concept that an adverse health effect will not occur below a certain level of exposure, even if exposure continues over a lifetime. Given this assumption, a reference dose intended to avoid toxic effects may be established by dividing the no-observed-adverse-effect level by an appropriate uncertainty factor or set of factors.

For carcinogenic effects, especially those known to be due to direct mutagenic events, no threshold dose may exist. However, when carci-

nogenesis is due to epigenetic or nongenotoxic mechanisms, a threshold dose may be considered. Attempts to estimate carcinogenic risks associated with levels of exposure have involved fitting mathematical models to experimental data and extrapolating from these models to predict risks at doses that are usually well below the experimental range. The multistage model of Armitage and Doll is used most frequently for low-dose extrapolation. According to multistage theory, a malignant cancer cell develops from a single stem cell as a result of a number of biological events (e.g., mutations) that must occur in a specific order. Recently, a two-stage model that explicitly provides for tissue growth and cell kinetics also has been used in carcinogenic risk assessment.

The multistage model, characterized by low-dose linearity, forms the basis for setting SMACs for carcinogens. Low-dose linearity is generally assumed for chemical carcinogens that act through direct interaction with genetic material.

ISSUES IN MAKING RECOMMENDATIONS FOR THE ESTABLISHMENT OF SMACS

A number of issues need to be considered in developing recommendations for establishing SMACs. These issues include (1) translating animal toxicity data to predict toxicities in humans; (2) determining 30- or 180-day SMACs for carcinogens based on toxicological or epidemiological studies that often involve long-term or lifetime exposure; (3) considering limits set by the Occupational Safety and Health Administration, the American Conference of Governmental Industrial Hygienists, and the National Research Council in developing SMACs; (4) evaluating the toxicities of mixtures; and (5) modifying risk assessments based on the altered environment in the microgravity of space.

Appendix B

REPORTS ON SPACECRAFT MAXIMUM ALLOWABLE CONCENTRATIONS FOR SELECTED AIRBORNE CONTAMINANTS

B1 Bromotrifluoromethane (Halon 1301)

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PHYSICAL AND CHEMICAL PROPERTIES

Synonyms:	Trifluorobromomethane, Freon 1301, Freon 13B1, Fluorocarbon 1301
CAS number:	75-63-8
Formula:	$CBrF_3$
Molecular weight:	148.9
Melting point:	-167.7°C
Boiling point:	-57.8°C
Vapor pressure:	Exists as a gas at ambient temperature
Concentration conversion at 25°C:	1 ppm = 6.1 mg/m^3; 1 mg/m^3 = 0.16 ppm

OCCURRENCE AND USE

Bromotrifluoromethane, a gaseous fluorocarbon, is commonly known as Halon 1301. It is widely used as a fire-extinguishing agent, especially in computer and high-technology facilities (Holness and House, 1992). Because it is effective and residue-free, Halon 1301 is used in the space-shuttle fire-suppression system. Three fixed fire extinguishers

are positioned in the avionic bays, and three portable extinguishers are located in the crew module; the fixed and portable extinguisher tanks each contain 1.73 and 1.25 kg of Halon 1301, respectively (M. Hoy, NASA Fire Detection and Suppression Branch, Crew and Thermal Systems Division, personal commun., 1993). Halon 1301 has been detected, probably from small leaks in the tanks, in the in-flight air samples in about two-thirds of the shuttle missions flown to date. Detected concentrations ranged from 2.3 to 77 mg/m^3 for the first 24 missions (Coleman, 1988); in more recent missions (STS-26 to 52, missions after the Challenger accident), concentrations were generally below 10 mg/m^3, and half of those were below 1 mg/m^3. None of the extinguisher tanks has ever been discharged during a mission. However, in the event of a fire or a false alarm in the avionics bay, all three tanks could be discharged, resulting in a cabin concentration of Halon 1301 at 1% (10,000 ppm, 61,000 mg/m^3). This low concentration of halogenated methane cannot be removed effectively by activated charcoal and would remain in the confined environment of the spacecraft. The crew could be exposed to Halon 1301 for up to 24 h before the shuttle could return safely to earth.

Current environmental policy in the United States calls for phasing out chlorofluorocarbons (CFCs). Although the National Aeronautics and Space Administration (NASA) is phasing out noncritical uses of CFCs, Halon 1301 will continue to be used in the shuttle fire-suppression system. It will not be used in the space station, because the air-revitalization system planned for the station could not remove this compound efficiently (M. Hoy, NASA Fire Detection and Suppression Branch, Crew and Thermal Systems Division, personal commun., 1993). However, if Halon 1301 is discharged in the shuttle while it is docked with the station, the gas could diffuse into the station, and crews could be exposed to low concentrations of Halon 1301 for extended periods during their tours of duty.

TOXICOKINETICS AND METABOLISM

Toxicokinetics

An inhalation study sponsored by NASA was conducted with eight pairs of human subjects exposed to 1% (10,000 ppm) Halon 1301 for

24 h; toxicokinetics were studied. Blood concentrations of Halon 1301 increased rapidly and approached a steady state within 2 h of starting the exposure; the steady-state concentration was approximately 3-4.5 μg/mL (Lam et al., 1993). Postexposure elimination of the compound was biphasic with average half-lives of 4.5 min for richly perfused tissues and 200 min for poorly perfused tissues (chiefly fat). Six hours after the cessation of exposure, the blood concentration was about 10% of the steady-state concentration. The end-tidal-breath, blood, tissue, and fat partition coefficients were estimated to be 17, 1, 0.5, and 33, respectively. Blood concentrations of Halon 1301 were also determined in humans by Harrison et al. (1982) in six volunteers exposed at 7% for 3 h. The mean venous concentrations determined at 30 min, 90 min, and 3 h were 25-29 μg/mL and did not seem to depend on exposure duration. In a study of dogs exposed at 5.0%, 7.6%, and 10% for 20-40 min, venous blood Halon 1301 concentrations were roughly proportional to the inhalation concentrations; the corresponding blood concentrations were 14.6, 28.4, and 32.1 μg/mL (Mullin et al., 1979). Five minutes after the exposure was terminated, blood concentrations decreased to about one-third of the plateau value. When rabbits were exposed to 5% Halon 1301 for 30 min, blood concentrations of Halon 1301, measured 10-30 min after exposure, generally varied from 10 to 15 μg/mL (Griffin et al., 1972). These data show that regardless of the species, the blood at the steady state took up Halon 1301 at about 3-4 μg/mL for each 1% airborne Halon 1301 exposure. Griffin et al. (1972) further concluded that Halon 1301 in the blood was not cumulative in rats exposed for 23 h/d for 30 d.

Tissue concentrations and uptake and elimination kinetics of Halon 1301 in the heart and brain (two major target organs) were investigated in rats exposed to 71-76% Halon 1301 for 5 min (Van Stee and Back, 1971a). The Halon 1301 concentration in the brain was found to be approximately 50% greater than that in the blood; the concentrations in the heart and the blood were about the same. The uptake and elimination kinetics of Halon 1301 in the heart and brain, both richly perfused organs, was similar to that in the blood.

Metabolism

Many halogenated hydrocarbons are known to be biotransformed to

toxic metabolites, which might include free radicals, by mixed-function oxidases in the liver. It is generally agreed that metabolic activation of these compounds is required for producing hepatotoxicity or carcinogenicity (Andrews and Snyder, 1991). Halon 1301, a trifluorinated bromomethane, on the other hand, is metabolically inert. No reports have indicated that Halon 1301, per se, is hepatotoxic in animals exposed to even very high concentrations. Bromide, a potential product of Halon 1301 metabolism, was not detected in serum from human subjects exposed to 7% Halon 1301 (70,000 ppm) for 3 h (Harrison et al., 1982). Liver functions and enzymes of these subjects were not altered. No changes in serum liver enzymes were also observed in another study of eight human subjects exposed to 1% Halon 1301 for 24 h (Calkins et al., 1993). In animal studies, liver morphology and enzymes were found to be normal in monkeys exposed to 5-20% Halon 1301 for 2 h. Van Stee and Back (1971b) exposed 30 mice to 80% Halon 1301 for 5 h/d for 3 consecutive days. The hexabarbital sleeping times and zoxazolamine paralysis times determined in these exposed mice were found to be no different from those in controls. When rats were exposed to 5% Halon 1301 for 30 d (23 h/d), there was no increase in the rate of excretion of fluoride ion in the urine (Griffin et al., 1972). These studies indicate that Halon 1301 is not metabolized in the body or that the metabolism is insignificant.

TOXICITY SUMMARY

Owing to its inertness, Halon 1301 is low in toxicity (Reinhardt and Reinke, 1972). The toxicity of Halon 1301 has been extensively reviewed (DuPont, 1971; NAS, 1972; Haskell Laboratory, 1974, 1978; Van Stee, 1974; NRC, 1978, 1984; Graham, 1981). At relatively high concentrations, Halon 1301 exerts toxic effects primarily on the central nervous system (CNS) and the cardiovascular system (NRC, 1984) at relatively high concentrations. A number of studies have been conducted on human subjects exposed to Halon 1301 at concentrations ranging from 0.1% to 17% (NRC, 1984). The major CNS symptoms, observed mainly at concentrations of a few percent or higher, were light-headedness, dizziness, and/or disturbances in motor coordination (Hine et al., 1968; Call, 1973; Harrison et al., 1982; D.G. Clark, ICI Ltd., unpublished data, 1970, cited in Graham, 1981). Cardiovascular effects, seen

in controlled exposures only at concentrations of 10% or more, included increased heart rate, depressed T wave, and/or premature ventricular contraction (Hine et al., 1968). The cardiovascular effects probably are due in part to the sensitization of the heart to epinephrine, a phenomenon that can be induced by exposures to high concentrations of hydrocarbons and halogenated hydrocarbons (Van Stee and Back, 1969; Hanig and Herman, 1991).

Acute and Short-Term Exposures

Human Exposures

CNS Effects

An accidental discharge of 1200 lb of Halon 1301 for 30 s was triggered by a fire alarm in a hospital facility. The system was designed to provide a Halon 1301 concentration of 5% at equilibrium. Of the 22 workers present at the scene, 12 left after the incident and 10 remained. Some of these workers could have been exposed briefly to concentrations much higher than 5% if they were near the discharge source. Halon 1301 was exhausted with high-volume ventilation equipment after the firemen arrived. No information is available on how long it took to remove Halon 1301 after it was discharged or how long the workers were exposed to it. The CNS symptoms reported by these 22 workers were light-headedness (77%), headache (45%), and disorientation (36%) (Holness and House, 1992). Fatigue, numbness, and anxiety were also reported; however, these symptoms might have been due to the apprehension of the accident itself rather than to the effect of Halon 1301. Some subjects also showed signs of cardiovascular toxicity, which is discussed below in the section on cardiac toxicity.

In a controlled study by D.G. Clark (ICI Ltd., unpublished data, 1970, cited in Graham, 1981), human subjects were exposed to 10%, 12%, or 15% Halon 1301 for 1 min. Severe dizziness and marked paresthesia were observed in subjects exposed to 15%. These symptoms were concentration-dependent. At 10%, slight dizziness and mild paresthesia were reported by half of the test subjects.

In another study, dizziness, faintness, or drowsiness was also observed in six of eight human subjects exposed to 7% Halon 1301 (Call,

1973). Behavioral tests conducted during and after the exposures showed that reaction time was significantly increased when these subjects performed a complex reaction-time task; however, maze-tracking tasks were not affected by Halon 1301. Subjects exposed to 4% Halon 1301 also showed performance decrements, although to a lesser degree.

The effect of Halon 1301 on sensory-motor test performance was also evaluated in groups of four or six subjects exposed to 1.25%, 2.5%, 5%, and 10% Halon 1301 for 22 min in an inhalation chamber (Hine et al., 1968, 1969). The subjects were aware of their exposure to Halon 1301 but did not know the exposure concentration; no control (air only) exposure was included. Slight performance decrements, as compared with pre-exposure results, were observed at 1.25% and 2.5% but not at 5%. Marked decrements were observed when subjects were exposed to 10% Halon 1301. Two of the four subjects exposed to 1.25% and all six subjects exposed to 10% indicated a feeling of light-headedness after 3 to 5 min of exposure; one subject experienced a buzzing in his ears in addition to tingling of extremities.

In the same study, 10 subjects were exposed to Halon 1301 at concentrations of 5-16% by masks (exposure length was not specified). Three subjects breathing 14.4-16.9% felt impending unconsciousness; most of the subjects inhaling 15% felt markedly confused. All subjects recovered from their CNS symptoms within 20 min of the exposure. Headache occurred in two subjects during exposures and persisted for 12 h thereafter.

In a well-controlled study, six human subjects were exposed in a single-blinded fashion for 3 h to 7% Halon 1301 or 7% sulfur hexafluoride (as heavy gas control) on different days; O_2 was added to achieve an O_2 concentration of 20% in the chamber (Harrison et al., 1982). Subjects were also given two 3-h air-only exposures. When exposed to Halon 1301, the subjects experienced mainly transient light-headedness and mild euphoria. Visual and vestibular functions were not impaired. Psychological performance tests showed that reaction time, scores, and number correct were significantly affected; the results on 18 of the 20 tests showed impairment. The authors equated the effects of Halon 1301 with awakening at 3:00 to 5:00 in the morning and a blood alcohol concentration of 90 mg/100 mL. The subjects were aware of the dense gas but could not distinguish between Halon 1301 and sulfur hexafluoride.

A similar study was conducted on subjects who were all exposed to

1% and 2% Halon 1301 for 6 h (Strong, 1987). In two of the four tests, subjects were affected by Halon 1301 exposure; performance decrements, which were only detected at one of the three test points during the 6-h exposure, were about 4%. Statistically, only one of these decrements is significant. This transitory decrement was considered mild and physiologically insignificant. Strong also noted performance fluctuations of 11.6%, 9.5%, and 7.5% from subject variation, daily variation, and diurnal variation alone, respectively.

Because no studies had been conducted in which humans were exposed to Halon 1301 for more than several hours, NASA sponsored a double-blind human inhalation study in which four pairs of subjects were each exposed for 24 h to Halon 1301 or air (NASA, 1989; Calkins et al., 1993). Six cognitive performance assessments and a motor function test, which produced 13 measurements of accuracy and reaction time, were administered before, during, and after the inhalation exposures. Of the 13 measurements, only two (from the same cognitive test) showed a statistically significant decrement with a magnitude of about 4% of the baseline values. Performance decrement was not dependent on the duration of exposure. No symptoms (such as headache, light-headedness, or irritation) were observed. The subjects were unable to tell whether they had been exposed to Halon 1301 or air.

Cardiac Effects

Cardiac effects could result from exposures to high concentrations of halogenated hydrocarbons, which are known to sensitize the heart to epinephrine (Hanig and Herman, 1991). Thus, in the NASA-sponsored inhalation study, electrocardiograms (EKGs) were continuously monitored throughout the 24-h study; no EKG abnormality was observed even during the 15-min light exercise to stimulate the release of epinephrine. Negative EKG results were also observed in human subjects exposed to 7% Halon 1301 for 3 h or to 1% or 2% Halon 1301 for 6 h (Harrison et al., 1982; Strong, 1987). Call (1973) also reported no EKG changes during and after exposure in eight human subjects exposed to either 4% or 7% Halon 1301 for 3 min. No effects on cardiac rhythm were observed in three men exposed to 1%, 3%, 5%, or 10% Halon 1301 for 3.5 min (Reinhardt and Stopps, 1966).

Hine et al. (1968, 1969) reported that exposing human volunteers to

5% or 10% Halon 1301 for up to 20 min produced no cardiac effects. However, one subject did develop a spontaneous cardiac arrhythmia after inhaling 14% Halon 1301 for 5 min. The symptoms disappeared within 2 min of discontinuation of inhalation. D.G. Clark (ICI Ltd., unpublished data, 1970, cited in Graham, 1981) observed increased heart rates and T-wave depression in subjects exposed to 10%, 12%, or 15% Halon 1301 for 1 min. Recovery was rapid and complete within 5 min of the exposure. Cardiac effects have not been detected in subjects exposed at lower concentrations.

The cardiovascular toxic signs and incidence reported by hospital workers exposed to a nominal concentration of 5% Halon 1301 during an accidental discharge were shortness of breath (36%), chest tightness (36%), chest pain (9%), and fast heart rate (45%) (Holness and House, 1992). At the concentrations to which these workers were exposed, cardiac effects would not be expected unless the workers were apprehensive during the accident. In fact, it was reported that a majority of these workers were anxious. Anxiety or fright is known to trigger epinephrine release. Halon 1301 has been shown to sensitize the heart to epinephrine (Van Stee and Back, 1969; Mullin et al., 1979; Clark and Tinston, 1982).

Irritation

In the accidental discharge of Halon 1301 (Holness and House, 1992), all 22 workers complained of throat irritation, and more than half reported eye and nose irritation. The authors attributed the irritation to possible contaminants in the Halon 1301 discharge system. Hine et al. (1968, 1969) reported that two subjects experienced slight eye irritation at exposure concentrations of 5% and 10%; another subject reported moderate eye irritation at 10%, and one reported severe nasal irritation at 10%. The subjects also reported a "bromine odor." The authors noted that a space heater was located near the area where the Halon 1301 was released and that the odor and irritation could be attributed to the thermal decomposition products of Halon 1301. In two well-controlled studies, irritation was not detected by six subjects exposed to 7% for 3 h (Harrison et al., 1982) or by eight subjects exposed to 1% for 24 h (Calkins et al., 1993). It seems that the irritation reported in those two studies was not due to Halon 1301 itself.

Animal Exposures

CNS Effects

According to Carter et al. (1970), monkeys became depressed, tranquilized, and lethargic and shiver when exposed to Halon 1301 at concentrations of 20% or higher. In operant-trained monkeys exposed to 10.5-42% Halon 1301, 20-25% caused performance decrements and higher concentrations caused a complete disintegration of operant behavior. No performance decrements were detected at exposure concentrations less than 20%.

Beagles became anxious and agitated and developed generalized tremors within 1-3 min of exposure to 20% Halon 1301 or more (Van Stee and Back, 1969). The severity of symptoms increased with increased concentrations; when the concentration reached 50% or higher, some dogs developed epileptiform convulsions. Anesthetized dogs and monkeys showed electroencephalographic changes 2-3 min after exposure to 70-80% Halon 1301 (Van Stee et al., 1970). Similar results in unanesthetized dogs were observed by Hine et al. (1968, 1969). Exposing eight dogs to 40% Halon 1301 for 55 min resulted in tremors, howling, dyspnea, and salivation. Eight dogs exposed to 20% Halon 1301 showed tremors but no other toxic signs. All animals returned to normal approximately 20 min after exposure. Dogs exposed to 10% Halon 1301 showed no toxic signs (Hine et al., 1968; Van Stee and Back, 1969).

CNS effects were also observed in rats exposed to 30-53% Halon 1301 for 10 min (Clark and Tinston, 1982). The EC_{50} for inducing tremors of the limbs, ataxia, or loss of righting reflex was 42%.

However, tremors were not observed by McHale (1972) in 10 rats exposed for 1 h to either 77% or 56% Halon 1301. The animals did exhibit initial hyperactivity and subsequent hypoactivity, increased rate of respiration, abdominal breathing, slight-to-moderate ataxia, and a slight bluish tint to the skin. All animals appeared normal during the 14-d postexposure observation period.

Cardiac Effects

Halon 1301 sensitizes the heart to epinephrine. Mullin et al. (1979)

exposed anesthetized dogs to 5%, 7.5%, 10%, and 20% Halon 1301 and gave them intravenous injections of epinephrine at concentrations of 8-10 μg/kg; the incidences of marked cardiac arrhythmias were 0, 5.5%, 11.5%, 28.6%, and 61.5%, respectively. Clark and Tinston (1982) reported similar findings. The EC_{50} for cardiac sensitization in dogs exposed to Halon 1301 and given epinephrine was 20%.

Effects on the cardiovascular system could also be produced in dogs not treated with epinephrine, but these animals were exposed at higher concentrations of Halon 1301. Van Stee and Back (1969) observed an increase in heart rates in dogs exposed to 20-30% Halon 1301 or more. Increases in concentrations beyond 40% caused T-wave alterations and unifocal and multifocal ventricular arrhythmias. Spontaneous arrhythmias developed in all dogs within 5 to 40 s of exposures to 20-80% Halon 1301 (Van Stee and Back, 1969). Further study (Van Stee and Back, 1971c) of anesthetized monkeys exposed to 30% Halon 1301 yielded similar results; spontaneous arrhythmias followed moderate hypotension, all of which might occur during the first 5 min of exposure.

Histopathology and Mortality

An extensive toxicity study of a commercial-grade Halon 1301 (purity 98.8%) was conducted by Treon et al. (1957a) using groups of 31 animals per dose (10 mice, 10 rats, 6 guinea pigs, 4 rabbits, and 1 cat). No compound-related deaths were observed when these animals were exposed for 7 h once or twice at 4.4-4.7% Halon 1301; however, five daily exposures (7 h/d) to the same concentration (4.4%) killed one mouse. No animals died when they were exposed only once for 7 h to 8.8% Halon 1301, but one animal died after two daily exposures, and six died after five daily exposures. Concentrations around 18% killed one animal after one 7-h exposure and seven after two 7-h exposures. Concentrations at 35.9-37.4% killed 0, 5, or 23 animals after exposures of 1.3, 3.5, or 7 h, respectively. The lethality of this commercial product seemed to depend on concentration and exposure length. The causes of death attributed to Halon 1301 exposures were acute hemorrhagic pneumonitis, acute pulmonary edema, and degenerative change of the liver and kidneys. Several animals died from extraneous causes, such as generalized or localized infection (pneumonia, peritonitis, or parasitic infection). Apparently, no control animals were included in the study.

The impurities in this commercial-grade Halon 1301, determined by infrared spectrometric analysis of fluorine, included trifluoromethane, dichlorodifluoromethane, dibromodifluoromethane, bromodifluoromethane, and 1-bromo,1-,1-,2-,2-,2-pentafluoroethane. No information was presented on whether there were nonfluorinated impurities.

In a similar study by Treon et al. (1957b), no deaths occurred from one 7-h exposure and two 7-h exposures to purified Halon 1301 (purity greater than 99.85%) at concentrations of 32% and 17%. According to DuPont (1971), the purity of Halon 1301 marketed since 1958 is superior to that of the sample used by Treon et al. (1957a). The current DuPont specification (DuPont, 1971) and the U.S. military specification on Halon 1301 (MIL-M-12218C of 1981) set a purity minimum of 99.6% for Halon 1301 (Batt, 1988).

To find out whether the impurities caused animal deaths, DuPont investigated the toxic effects of impurities in Halon 1301 on six groups of rats (10 per group) exposed for 4 h to either one of the four samples containing 80% Halon 1301. One sample was highly purified; the other three contained "maximal permissible concentrations" (allowed by product specifications) of one or more of the impurities that might be present in the commercial product, which was the same product used by Treon et al. (1957a). Animals showed toxic signs, but none died. An additional study of 10 other rats exposed to an 80% Halon 1301 containing all possible impurities produced three deaths from marked pulmonary congestion and edema (Waritz, 1968). It appeared that the impurities could be the main cause of the animal death.

The toxicity of Halon 1301 (probably the purified product) was also evaluated by Paulet (1962) in large groups of animals in acute- and repeated-exposure studies. Exposing groups of 10 mice to 30%, 40%, 50%, and 60% Halon 1301 and 30 mice to 80% Halon 1301 for 2 h produced no deaths. A similar study with four groups of rats exposed to either 30%, 40%, or 80% (two groups) Halon 1301 also had no deaths. All eight rabbits survived a 2-h exposure to either 50% or 80% Halon 1301. Clinical signs, including drowsiness, tremors, or short convulsions, were observed mainly in animals exposed to 80% Halon 1301. Deaths of exposed mice and guinea pigs occurred when the Halon 1301 concentration was increased to 85% (O_2 15% or less). In a repeated-exposure study, 20 mice, 10 rats, and 10 guinea pigs were exposed to 50% Halon 1301 2 h daily for 15 consecutive days; one mouse and one guinea pig died. Paulet (1962) stated that these two

deaths were "not significant," but the possible cause of death was not revealed. Unfortunately, no controls were included in the Paulet (1962) study.

No deaths were observed when three groups of animals, each group consisting of 2 monkeys, 4 rabbits, 6 guinea pigs, and 10 rats, were exposed to Halon 1301 at 10%, 15%, or 20% for 2 h. Results of blood-chemistry tests, including assays for liver enzymes (determined only in the monkeys), and gross necropsy and microscopic examination of major organs including the liver were found to be unremarkable. Eye irritation was observed in one monkey in each exposure group (MacFarland, 1967).

Subchronic Exposures

No human studies and only a few animal studies have been conducted to examine the toxicity of Halon 1301 in exposures lasting longer than 1 w. McHale (1972) exposed 20 rats (10 males and 10 females) and 20 guinea pigs (10 males and 10 females) to 5% Halon 1301 continuously (24 h daily) for 10 d and studied clinical toxic signs, clinical chemistry, gross pathological changes of all organs, and microscopic histopathological changes of selected organs (lungs, liver, heart, kidneys, and spleen) of these animals. The findings showed that Halon 1301 caused no signs of toxicity, except for a statistically significant elevation of white-blood-cell counts in female guinea pigs when compared with air-exposed animals. McHale (1972) stated that some animals had pneumonitis, which could easily account for the elevated leukocyte counts. The results also showed that the mean weights of adrenal glands from Halon-exposed male and female rats were reduced by 83% and 88% compared with those of male and female controls, respectively (Table 1-1).

The reported adrenal-gland weights of the air-exposed control rats (Sprague-Dawley) in McHale's (1972) study were about 4 times greater than those of control rats (Fischer 344) of equal size in two National Toxicology Program studies (NTP, 1986, 1990) (Table 1-1). Adrenal glands, which secrete epinephrine, norepinephrine, and corticosteroid hormones, control many vital functions, such as mood, activity, metabolism, and renal function. If the adrenal glands in Halon-exposed animals had undergone such drastic atrophic changes ($>80\%$ weight re-

TABLE 1-1 Comparison of Rat Adrenal Weights Reported by McHale and NTP

	Male Rats			Female Rats			
Study	Body Wt, g	Organ Wt, mg	Organ/Body Wt, %	Body Wt, g	Organ Wt, mg	Organ/Body Wt, %	Rat Strain
Controls (McHale, 1972)	330 ± 5	180 ± 38	0.060	243 ± 3	170	0.70	SD
Halon exposure (McHale, 1972)	321 ± 4	30 ± 4[a]	0.010	243 ± 3	20[a]	0.10	SD
Controls (NTP, 1986)	359 ± 16	39.9 ± 1.31	0.011	201 ± 10	51.3 ± 0.44	0.23	F344
Controls (NTP, 1990)	357 ± 6	42.7 ± 1.31	0.012	208 ± 5	52.9 ± 0.46	0.25	F344

[a]The author reported the organ weight in grams only up to two decimal places.
SD, Sprague-Dawley; F344, Fischer 344.

duction) in 10 d, toxic signs would certainly have been manifested. However, no clinical signs were observed in the Halon-exposed animals. Moreover, McHale (1972) also observed that adrenal weights in Halon 1301-exposed guinea pigs were not statistically different from those of air-exposed guinea pigs. Therefore, the effects of Halon 1301 on the adrenal glands in rats are questionable, and these results would not be considered in setting the exposure limit.

Griffin et al. (1972) also observed no signs of toxicity, hematological changes, and gross pathological changes in rats exposed to 5% Halon 1301 continuously (23 h/d) for 30 d. The authors indicated that a thorough histological examination was under way at the time of the report. However, no further results have been found on this NASA-sponsored animal study.

Comstock et al. (1953) found no clinical signs in rats and dogs exposed to 2.3% Halon 1301 for 18 w (6 h/d, 5 d/w). Necropsy revealed moderate diffuse congestion of the entire respiratory tract but no other significant pathological changes. It is noteworthy that the Halon 1301 used in this study was not the highly purified grade manufactured after 1958.

Four baboons were exposed to 2.3% Halon 1301 for 30 d (23 h/d), and a match-to-sample discrimination task (a test of memory) was administered (Geller et al., 1981). Reaction time was significantly longer in two baboons during the exposures; however, the number of correct responses was greater during exposures than before, which the authors attributed to learning. They also noticed the presence of ammonia in the chamber during Halon 1301 exposure. Geller et al. (1981) also assessed the long-term effects of Halon 1301 on the hearts of monkeys exposed to 2.8% Halon 1301 for 30 d while confined in restraining chairs. Arterial and venous catheters were implanted in the right common carotid artery and the right internal jugular vein of four of the six cynomolgus monkeys. Available EKG results obtained from chest-wall-implanted EKG leads showed no abnormalities. Pathological results were difficult to interpret because of severe stress, fatal emboli, animal deaths, incomplete result documentation, and no control animals in the study.

Developmental Toxicity

Three groups of 27 pregnant rats were exposed to Halon 1301 at concentrations of 0.1%, 1%, or 5% for 6 h daily on days 6-15 of gestation. The number of implantation sites, resorptions, and live fetuses was not significantly different from that of the control animals. No compound-related clinical signs of toxicity or behavioral changes were observed. Exposure did not affect fetal development as measured by fetal weight and crown-rump length. Three fetuses were found malformed; all three were from dams exposed at 1%. These effects were not considered compound-related. Halon 1301 was not considered a developmental toxicant under these test conditions (NRC, 1984; Haskell Laboratory, unpublished data, cited in NRC, 1984, and Graham, 1981).

Mutagenicity

Halon 1301 was tested in *Salmonella typhimurium* strains TA 1538, TA 1537, TA 1535, TA 100, and TA 98 at concentrations up to 40%. The gas was not mutagenic regardless of whether a rat liver microsomal system was present (Haskell Laboratory, unpublished data, cited in NRC, 1984, and Graham, 1981).

Carcinogenicity

No chronic-exposure studies have been conducted to evaluate the carcinogenic potential of this compound; no evidence of carcinogenic potential has been found (NRC, 1984). Because of its biochemical inertness, Halon 1301 is likely to be noncarcinogenic.

TABLE 1-2 Toxicity Summary of Human Studies

Concentration, %	Exposure Duration	Number of Subjects	Cardiovascular Effects	CNS and Other Effects	Reference
1, 3, 5	3 min	3	None observed	Not reported	Reinhardt and Stopps, 1996
1	24 h	8	None observed	Slight decrement in 2 of 13 cognitive tests	Calkins et al., 1993
1-2	3 h	6	Not detected	No CNS syndromes, minimal decrement in mental performance	Strong, 1987
1.25	22 min	4	Not determined	Slight performance decrement, light-headedness	Hine et al., 1969
2.5	22 min	4 or 6	Not determined	Slight performance decrement	Hine et al., 1969
5 or more	Probably several min or more	22	Shortness of breath, chest tightness, chest pain, fast heart rate	Light-headedness, headache, disorientation, eye and throat irritation	Holness and House, 1992
4 or 7	3 min	8	No changes in EKG	Increased behavioralreaction time	Call, 1973
5	22 min	4 or 6	Not determined	Light-headedness, no performance decrement, slight eye irritation	Hine et al., 1969
7	3 h	6	Not detected	Transient light-headedness, mild euphoria, impairment of mental performance, no irritation perceived	Harrison et al., 1982
10	22 min	6	Not determined	Light-headedness, performance decrement, moderate/severe eye irritation	Hine et al., 1969
14-16	Unspecified	10	Not determined	Feeling impending unconsciousness	Hine et al., 1969

10-17	15-25 min	Unspecified	Flattening of T wave, premature ventricular contractions, tachycardia	Not reported	Hine et al., 1969

TABLE 1-3 Toxicity Summary of Studies of Animals Exposed to Impure Halon 1301

Concentration, %	Exposure Duration	Species	Effects	Reference
		Short-Term Studies		
4.4	1 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: None (1 rabbit died of extraneous causes)	Treon et al., 1957a
4.8	2 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: None (2 rats and 1 mouse died of extraneous causes)	Treon et al., 1957a
4.4	5 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: 1 mouse (3 rabbits died of extraneous causes)	Treon et al., 1957a
8.8	1 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: None (1 rat died of extraneous causes)	Treon et al., 1957a
9.1	2 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Motality: 1 guinea pig	Treon et al., 1957a
8.7	5 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: 4 guinea pigs, 1 rat, 1 mouse	Treon et al., 1957a
18.3	1 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: 1 of 3 rabbits	Treon et al., 1957a
17.3	2 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: 1 rabbit, 6 guinea pigs	Treon et al., 1957a

TABLE 1-3 *(Continued)*

Concentration, %	Exposure Duration	Species	Effects	Reference
35.9	1 × 1.3 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: None	Treon et al., 1957a
36.0	1 × 3.5 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: 5 guinea pigs	Treon et al., 1957a
37.4	1 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: 1 cat, 4 rabbits, 6 guinea pigs, 4 rats, 8 mice	Treon et al., 1957a
		Long-Term Studies		
2.3	18 w (6 h/d, 5 d/w)	Rat, dog	Moderate diffuse congestion of the entire respiratory tract; no other toxic signs	Comstock et al., 1953

TABLE 1-4 Toxicity Summary of Studies of Animals Exposed to Purified Halon 1301

Concentration, %	Exposure Duration	Species	Effects	Reference
		Acute Toxicity Studies		
0.7	1 × 0.3 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: None	Treon et al., 1957b
1.8	1 × 0.3 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: None	Treon et al., 1957b
17.3	2 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: 1 guinea pig died 39 d later	Treon et al., 1957b

32.7	1 × 7 h	1 cat, 4 rabbits, 6 guinea pigs, 10 rats, 10 mice	Mortality: 1 rabbit died 4 d later	Treon et al., 1957b
5-20 + iv epinephrine	60 min	Dog	Incidence of cardiac arrhythmias at 5% (0), 7.5% (5.5%), 10% (11.5%), 15% (28.6%), and 20% (61.5%) Halon 1301	Mullin et al., 1979
10	55 min	Dog	No toxic signs	Hine et al., 1968
20 + iv epinephrine	5 min	Monkey	EC_{50} for cardiac sensitization was 20% Halon 1301	Clark and Tinston, 1982
10.5-20	15 min	Monkey	No performance degradations detected	Carter et al., 1970
20	55 min	Dog	Tremors	Hine et al., 1968
20	Unspecified	Dog	Anxious, agitated, and tremors	Van Stee and Back, 1969
20-25	15 min	Monkey	Significant performance decrements	Carter et al., 1970
20-30	Unspecified	Dog	Increase in heart rate	Van Stee and Back, 1969
20-80	Unspecified	Monkey, baboon	Spontaneous cardiac arrhythmias developed in monkeys and baboons within 5-40 s of exposures; monkeys were depressed, tranquilized, shivering, lethargic	Van Stee and Back, 1969
25-42	15 min	Monkey	Performance greatly impaired in some animals	Carter et al., 1970
30	5 min or more	Monkey	Hypotension, spontaneous arrhythmias	Van Stee and Back, 1971c
30	2 h	Mouse, rat, guinea pig	None observed	Paulet, 1962
40	2 h	Mouse	None observed	Paulet, 1962

TABLE 1-4 *(Continued)*

Concentration, %	Exposure Duration	Species	Effects	Reference
40	55 min	Dog	Dyspnea, salivation, howling, and tremors	Hine et al., 1968
42	10 min	Rat	EC_{50} for CNS effects (tremors of the limbs, ataxia and loss of righting reflex)	Clark and Tinston, 1982
40-60	Unspecified	Dog	Increased heart rate, spontaneous cardiac arrhythmias in most dogs	Van Stee and Back, 1969
50	Unspecified	Dog	Anxious, agitated, tremors	Van Stee and Back, 1969
50	2 h/d, 15 d	Mouse, rat, guinea pig	Mortality: 1/20 mice, 0/10 rats, 1/10 guinea pigs	Paulet, 1962
50	2 h/d, 15 d	Mouse, rat, guinea pig	Mortality: 1/20 mice, 0/10 rats, 1/10 guinea pigs	Paulet, 1962
50	2 h	Mouse, rat, guinea pig, rabbit	Slight behavioral changes rats, guinea pigs, and rabbits; no deaths in 32 animals	Paulet, 1962
60	2 h	Mouse	Slight behavioral changes	Paulet, 1962
56 or 77	1 h	Rat	Initial hyperactivity and subsequent hypoactivity, increased abdominal breathing, slight to moderate ataxia, and slight bluish tint to skin; no animal deaths	McHale, 1972
70-80	>2 min	Dog	Electroencephalographic changes 2-3 min after exposure	Van Stee et al., 1970

80	2 h	Mouse, rat, guinea pig, rabbit	Mice: decreased activity, dyspnea, depression, terminal tremors, abnormal gait, and disequilibrium Rats: drowsiness, slight abnormal gait Guinea pigs: drowsiness, terminal tremors, weak limbs Rabbits: Ataxia, dyspnea, depression, tremors, short convulsion No deaths in 44 animals	Paulet, 1962
80	15 min	Rat	$LC_{50} > 80\%$	Clark and Tinston, 1982
85	2 h	Mouse, guinea pig	Marked depression, tremorsMortality: 8/10 mice (Note: O_2 was 15% or less)	Paulet, 1962
		Subchronic Toxicity Studies		
2.8	23 h/d, 30 d	Baboon	Increased reaction time or accuracy during exposure	Geller et al., 1981
5	24 h/d, 10 d	Rat, guinea pig	No clinical signs, no changes in clinical chemistry, no gross pathology, and no microscopic histopathological changes	McHale, 1972
5	23 h/d, 30 d	Rat	No histological and hematological changes	Griffin et al., 1972

TABLE 1-5 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	1000	ACGIH, 1991
OSHA's PEL	1000	NIOSH, 1990
NRC's 30-min EEL	40,000	NRC, 1984
NRC's 60-min EEL	25,000	NRC, 1984
NRC's 90-d CEL	100	NRC, 1984

TLV, Threshold Limit Value; PEL, permissible exposure limit; EEL, emergency exposure limit; CEL, continuous exposure limit.

TABLE 1-6 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	3500	21,350	Cardiovascular effects
24 h	3500	21,350	Cardiovascular effects
7 d	1800	11,000	CNS effects
30 d	1800	11,000	CNS effects
180 d	1800	11,000	CNS effects

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

The general approach to prepare a SMAC document and the safety factors used to derive the acceptable concentrations (ACs) are outlined in *Guidelines for Developing Spacecraft Maximum Allowable Concentrations for Space Station Contaminants* (NRC, 1992).

Studies Not Considered in Setting ACs

Studies on "Impure" Halon 1301

A study using the commercial grade of Halon 1301 available at that

time (purity 98.3%) revealed that, at exposure concentrations as low as 4.5%, Halon 1301 caused acute hemorrhagic pneumonitis, acute fatty degenerative changes of the liver and kidneys, and death (Treon et al., 1957a). A subsequent study by Treon et al. (1957b) using purified Halon 1301 (purity 99.8%) showed no such toxicity. The commercial grade of Halon 1301 marketed after 1957 was purified. Results from extensive studies conducted thereafter showed no evidence of lung, kidney, and liver damage even at very high exposure concentrations. Well-controlled exposures of humans to 7% Halon 1301 for 3 h or 1% for 24 h produced no signs of pulmonary irritation and no changes in kidney and liver enzymes to reveal organ injury (Harrison et al., 1982; Calkins et al., 1993). Toxicity seen in the early study of Treon et al. (1957a) seems to be due to the impurities in Halon 1301. Therefore, results from this study are not used to set an exposure limit.

Studies on Irritation

As discussed above, Holness and House (1992) attributed the sensory irritation experienced by the hospital workers to contaminants in the Halon 1301 discharge system. Hine et al. (1968) suggested that the eye and nasal irritation reported by some of their human subjects in their study was due to Halon 1301 thermal decomposition products. Two well-controlled studies conducted with 14 subjects exposed to 1% or 7% Halon 1301 revealed no sensory irritation (Harrison et al., 1982; Calkins et al., 1993). Therefore, sensory irritation as a toxicity end point is not considered in setting the SMACs.

Studies on Short-Term Exposures of Animals

Halon 1301 produces CNS and cardiovascular effects in humans and animals. Since many short-term human studies have been conducted to evaluate these effects and toxicity data reveal that animals are not as sensitive to those toxicity end points as humans, short-term animal studies are not considered in setting SMACs. However no human Halon 1301 exposures were tested beyond 24 h; therefore, data obtained from the Griffin el al. (1972) long-term animal pathology study is considered in setting SMACs.

Studies Considered in Setting ACs

Studies on CNS Effects in Humans

Harrison et al. (1982) reported that exposing six human subjects to 7% Halon 1301 for 3 h produced transient light-headedness, mild euphoria, and considerable performance decrements. Further unpublished studies by the same group (Strong, 1987) in subjects exposed to 1% for 6 h produced no overt CNS symptoms and only a very slight cognitive performance decrement. Similar findings were reported by Calkins et al. (1993) on eight subjects exposed to 1% Halon 1301 for 24 h.

Because a slight cognitive decrement is acceptable for both 1-h and 24-h exposures in the spacecraft, the AC for CNS effects is set at 1% or 10,000 ppm.

The Calkins and Strong groups also found the effects of Halon 1301 to be noncumulative; these observations are strengthened by toxicokinetic data. Lam et al. (1993) reported that Halon 1301 concentrations in blood in humans increased rapidly and approached a steady state within 2 h of exposure; the half-life ($t_{1/2}$) of uptake for the fast-perfusion tissues was estimated to be 5 min. Van Stee and Back (1971b) reported that the uptake and elimination kinetics of Halon 1301 in the brain of rats were similar to those in blood. Therefore, prolonged exposure to Halon 1301 is not likely to increase the amount of Halon 1301 in the brain. It is reasonable to assume that the CNS effects of such a stable inert gas depends on its concentration in the brain.

From the results of these cognitive tests and toxicokinetic studies, it can be concluded that the CNS effects of Halon 1301 in subjects exposed for 24 h or longer would be similar. The possible mild cognitive decrement acceptable for a 24-h exposure is not acceptable for longer exposures in the spacecraft. Since the CNS effects observed at 10,000 ppm of Halon 1301 were very mild, a factor of 2 is used to extrapolate from the lowest-observed-adverse-effect level (LOAEL) to a no-observed-adverse-effect level (NOAEL). The ACs for 7-d, 30-d, and 180-d exposures thus are set at 1800 ppm, based on the calculation below:

$$10{,}000 \text{ ppm} \times \frac{1}{2} \times \frac{\sqrt{13}}{10} = 1800 \text{ ppm}.$$

The number 13 in the above equation is the total number of test sub-

jects in the Strong and Calkins studies. (Cognitive test results were available from only seven subjects in the latter study.) According to the National Research Council guidelines (NRC, 1992), a factor equal to the square root of the number of subjects divided by 100 (i.e., $\sqrt{(n/100)}$ or $(\sqrt{n})/10)$) is used to account for the small sample size (n) for estimating a true NOAEL from an experimental NOAEL observed in a human study. The rationale for combining the number of subjects in the Strong and Calkins studies is that both studies measured reaction time and scoring accuracy, even though the cognitive tests used by these two groups were different.

Studies on Cardiovascular Effects in Humans

Hine et al. (1968, 1969) reported that an exposure of humans to 5% or 10% Halon 1301 for up to 20 min produced no cardiac effects. D.G. Clark (ICI Ltd., unpublished data, 1970, cited in Graham, 1981) observed increased heart rates and T-wave depression in subjects exposed to 15%, 12%, and 10% Halon 1301 for 1 min. Harrison et al. (1982) and Calkins et al. (1993) recorded no cardiac changes in humans exposed to 7% Halon 1301 for 3 h or 1% Halon 1301 for 24 h, respectively. It may be concluded that 7% is close to the highest NOAEL for the heart.

As discussed above, Van Stee and Back (1971a) reported that uptake and elimination kinetics of Halon 1301 in hearts of rats were rapid and similar to those in blood. Also as noted above, onset and abolition of cardiac effects, upon initiation and termination of exposure to Halon 1301, were also rapid (within a few minutes) (Hine et al., 1968; D.G. Clark, ICI Ltd., unpublished data, 1970, cited in Graham, 1981). These observations support the contention that the cardiac effects of this inert fluorocarbon gas depend on its concentration in the blood or heart. Lam et al. (1993) observed that Halon 1301 blood concentrations in exposed human subjects increased rapidly and approached a steady state within 2 h of starting the exposure; thus, the cardiac effects would be expected to be relatively independent of exposure time shortly after the initiation of exposure. Therefore, 7% Halon 1301, a NOAEL for a 3-h exposure in the Harrison et al. (1982) study, could be considered a NOAEL for longer exposure times. The ACs for 1-h, 24-h, 7-d, 30-d and 180-d exposures are set at the same value (see below). Because

spaceflight is known to have deleterious effects on heart rhythm, a safety factor of 5 has been included in calculating the safe exposure concentration. The value 6 in the following equation is the number of subjects in the Harrison et al. (1982) study:

$$70{,}000 \text{ ppm} \times \frac{1}{5} \times \frac{\sqrt{6}}{10} = 3500 \text{ ppm}.$$

The safe exposure level is rounded from 3430 ppm.

Studies on Subchronic Exposures in Animals

Comstock et al. (1953) observed moderate diffuse congestion in the respiratory tracts of rats exposed to 2.3% Halon 1301 for 30 d. As discussed above, the Halon 1301 products used before 1957 might have contained toxic impurities, and thus these data are not used to set ACs. The study by Geller et al. (1981) on four baboons exposed to 2.8% Halon 1301 for 30 d (23 h/d) showed that two baboons reacted more slowly but two performed significantly better in a memory test. Ammonia was noted by the authors to be present in the chamber during the exposure. This study does not provide data useful for setting long-duration ACs.

Exposing rats to 5% Halon 1301 continuously (24 or 23 h/d) for 10 d or 30 d produced no observable clinical signs of toxicity, gross pathological changes, or hematological changes (McHale, 1972; Griffin et al., 1972). McHale further reported that microscopic examination of selected organs revealed no lesions. Griffin et al. indicated that a thorough histopathological examination was under way; however, no findings have ever been published. It is reasonable to conclude that 5% Halon 1301 is the NOAEL in rats. An animal-to-human species safety factor of 10 is used to extrapolate a NOAEL to humans. The 30-d and 180-d ACs are set at 0.5% (5000 ppm).

TABLE 1-7 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors: To NOAEL	Uncertainty Factors: Species	Uncertainty Factors: Time	Uncertainty Factors: Space-flight	Acceptable Concentrations, ppm: 1 h	24 h	7 d	30 d	180 d
CNS effects	Human	—	—	1	1	10,000	10,000			
LOAEL = 10,000 ppm (24-h inhalation) (Strong, 1987; Calkins et al., 1993)		2 × √(13/100)[a]	—	1	1			1800	1800	1800
Cardiac effects										
NOAEL = 70,000 ppm (3-h inhalation) (Harrison et al., 1982)	Human	√(6/100)[a]	—	1	5	3500	3500	3500	3500	3500
Histopathology										
NOAEL = 50,000 (23-h, 30-d inhalation) (Griffin et al., 1972)	Rat	1	10	1	1	—	—	5000	5000	5000
SMACs						**3500**	**3500**	**1800**	**1800**	**1800**

[a]Factor 2 for extrapolating from LOAEL to NOAEL; $\sqrt{(n/100)}$ is used to account for small sample size, n.
—, Data not considered applicable to the exposure time.

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B2 1-Butanol

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PHYSICAL AND CHEMICAL PROPERTIES

1-Butanol is a colorless, flammable, volatile liquid with a sweet to rancid odor detectable at a threshold of about 0.8 ppm (2.5 mg/m^3) (Amoore and Hautala, 1983). Russian investigators have reported an odor threshold of 1.2 mg/m^3 (Baikov and Khachaturyan, 1973).

Synonyms:	1-butyl alcohol, *n*-butanol, butyl alcohol
Formula:	$CH_3CH_2CH_2CH_2OH$
CAS number:	71-36-3
Molecular weight:	74.1
Boiling point:	118°C
Melting point:	−89°C
Specific gravity:	0.81
Vapor pressure:	6.5 mmHg at 20°C
Lower explosive limit (air):	1.4% (vol/vol)
Solubility:	Water solubility about 7%, miscible with organic solvents
Conversion factors:	1 ppm = 3.08 mg/m^3 1 mg/m^3 = 0.325 ppm

OCCURRENCE AND USE

1-Butanol is used in cosmetics, flavorings, brake fluids, degreasers, repellants, and as a solvent for many processes; it is used as an extractant in the manufacture of antibiotics, hormones, hop, vegetable oils, and vitamins (WHO, 1987; Lington and Bevan, 1994). This alcohol occurs naturally as a product of carbohydrate fermentation; therefore, it is present in alcoholic beverages, fruits, cheeses, and a variety of other foods (Brandt, 1987). Air inside mobile homes has been reported to contain 1-butanol in samples at a frequency of about 50% and at concentrations up to 0.08 mg/m^3 (Connor et al., 1985). 1-Butanol has been found in about one third of the samples of air from recent space-shuttle flights at concentrations ranging from 0.01 to 1 mg/m3 (James et al., 1994). The primary source of this compound for spacecraft atmospheres is off-gassing from flight hardware; however, a small contribution (about 1 mg/d per human) might come from human metabolism.

TOXICOKINETICS AND METABOLISM

Absorption

1-Butanol is readily absorbed through the skin, respiratory tract, and gastrointestinal tract (WHO, 1987; DiVincenzo and Hamilton, 1979, Rumyantsev et al., 1975). Ästrand et al. (1976) found that 12 human subjects absorbed the alcohol vapor with 37-48% efficiency when inhaled at either 100 or 200 ppm, with or without exercise. After a 30-min exposure at 100 or 200 ppm, the arterial blood concentrations were only 0.3 and 0.5 mg/kg (3 and 5 mg/dL), respectively. Those concentrations doubled after 30 min of additional exposure of subjects exercising at a 50-watt intensity (light physical exercise). The concomitant venous concentrations of 1-butanol were roughly half those reported in arterial blood. In dogs exposed for 6 h at 50 ppm, the uptake by the respiratory system averaged 55% with 1-butanol at 22 ppm consistently found in the exhaled breath (DiVincenzo and Hamilton, 1979). It is noteworthy that in neither experiment could 1-butanol be detected in the blood of humans or dogs following exposures at 50 ppm. In rats exposed at 500 or 2000 ppm for 6 h, the serum concentrations of 1-buta-

nol were undetectable or 0.09 mM (70 mg/dL), respectively (Aarstad et al., 1985).

Distribution

According to an abstract, all butanols studied, including 1-butanol, were freely distributed in the body according to water content of the tissue (Bechtel and Cornish, 1975). In several animal models, 1-butanol is rapidly removed from the blood and distributed to tissue compartments. After oral administration to rats of 1-butanol at 2 g/kg, the blood concentrations reached 19 mg% in 15 min, peaked at 51 mg% after 2 h, and fell off to 18 mg% after 4 h (Gaillard and Derache, 1965). In rats given oral doses at 0.5 g/kg, the peak serum concentration of 240 ppm (19 mg/dL) was reached in 45 to 50 min, and butanol was not detected after 2 to 3 h (detection limit not specified) (Bechtel and Cornish, 1975). These investigators reported that *n*-butylaldehyde was found in the serum as a metabolite. Likewise, male rats given 0.45 g/kg showed a maximum plasma concentration of 70 μg/mL (7 mg/dL) after 1 h and undetectable concentrations after 4 h (DiVincenzo and Hamilton, 1979). The blood concentration in rabbits was about 1.1 mg/mL (110 mg/dL) 1 h after oral administration of the butanol at 2 mL/kg (1.6 g/kg) but had declined to 0.3 mg/mL (30 mg/dL) 7 h after dosing (Saito, 1975).

Elimination

1-Butanol is eliminated from the body by a variety of metabolic steps and routes in animals. Rats orally dosed with 0.45 g/kg of *n*-[1-^{14}C]-butanol excreted 83% of the dose as $^{14}CO_2$ in the expired air, 4% in the urine, and <1% in the feces within 24 h of administration (DiVincenzo and Hamilton, 1979). The major labeled components in the urine were sulfates, glucuronides, and urea. Similarly, in rabbits dosed orally at 16 mmol/3 kg (0.4 g/kg), only 1.8% of the dose was excreted as the glucuronide within 24 h (Kamil et al., 1953). Saito (1975) found that rabbits given 2 mL/kg (1.6 g/kg) eliminated less than 0.5% of the dose as unmetabolized alcohol in the breath or urine during the first 10 h. In

mice given an unspecified dose of *n*-[^{14}C]-butanol, 21% of the label remained after 24 h and 5% after 3 d, indicating accumulation of the isotope (Rumyantsev et al., 1975).

Metabolism

The metabolism of 1-butanol is very similar to that of ethanol. The butanol is metabolized to its aldehyde by hepatic alcohol dehydrogenase (ADH) and also by the cytochrome P-450 system, but not by catalase. The aldehyde is oxidized to the acid, which is further oxidized to CO_2. The relative rates of oxidation of *n*-butrylaldehyde and acetaldehyde by aldehyde dehydrogenase isolated from human liver are comparable over a substrate concentration range of 0.05 to 3 mM (Blair and Bodley, 1969). A small fraction of the alcohol is conjugated in the liver and excreted by the kidneys (see above). The rate of oxidation of 1-butanol by ADH prepared from rat livers was twice the rate measured for ethanol oxidation (Arslanian et al., 1971). In vitro studies of rat-liver microsomes showed that the microsomal alcohol-oxidizing system accounts for non-ADH activity for butanol and other primary alcohols; however, for methanol and ethanol only, the catalase system also seems to be partially responsible for alcohol oxidation. Cederbaum et al. (1978) showed that 1-butanol is oxidized through a hydroxyl-radical-dependent pathway in rat-liver microsomes, but not through a catalase-dependent pathway. Morgan et al. (1982) compared the catalytic activity of various cytochrome P-450 isozymes for oxidation of alcohols in rabbit-liver microsomes after the rabbits had been exposed chronically to ethanol. Of the five isozymes tested, the predominant activity for both ethanol and 1-butanol appeared to be P-450$_{LM3a}$, which was later called P-450IIE1 (Yang et al., 1990). Under the assay conditions used to study P450$_{LM3a}$, the K_m for 1-butanol was one fourth the K_m for ethanol; however, the V_{max} for each alcohol was similar (Morgan et al., 1982).

Because the enzymes involved in metabolism of 1-butanol are similar to those involved in metabolism of ethanol, the kinetics of the oxidations can reasonably be expected to be qualitatively similar. In fact, isolated perfused rat livers have shown that both alcohols are oxidized according to zero-order kinetics above a certain concentration and ac-

cording to first-order kinetics below that concentration (Auty and Branch, 1976). The concentration of butanol in the perfusate for transition from zero to first-order kinetics was approximately 0.8 mM (6 mg/dL). In an experiment to assess the ability of various inhaled butanols to induce cytochrome P-450 in the kidneys, lungs, and livers of rats exposed to 1-butanol at 500 ppm (6 h/d for 5 d) or 2000 ppm (6 h/d, 3 d), the only statistically significant increase after exposure was in the livers after the 2000-ppm exposures (Aarstad et al., 1985). Rumyantsev et al. (1975) reported a biphasic removal of *n*-[^{14}C]butanol from liver and kidney of rats given an oral dose of unspecified concentration; however, specific data and half-lives were not given in the paper.

TOXICITY SUMMARY

Acute and Short-Term Exposures

The short-term effects of airborne exposure to 1-butanol include irritation of mucosal surfaces, depression of the CNS, and ultimately death if extremely high concentrations are involved. Administration of single doses via routes other than inhalation have resulted in hepatotoxicity in rodents.

Irritation

Data on the irritancy properties of 1-butanol have been reported on industrial workers, human test subjects, and rodents. The data do not create a consistent impression of the irritation thresholds either in humans or in rodents.

Results of brief human inhalation tests and industrial experience give conflicting results on the irritation threshold. Nelson et al. (1943) reported that 3- to 5-min exposures of 10 test subjects resulted in mild irritation of nose and throat in the majority of test subjects exposed at 25 ppm and eye irritation in the majority of subjects exposed at 50 ppm, which was considered an "objectionable" concentration. The test subjects indicated that a concentration below 25 ppm would be needed

for an 8-h occupational exposure. Unfortunately, the investigators relied on nominal concentrations, had a subjective assessment method, and did not extend the exposures to determine if the subjects would adapt to the concentrations. In contrast to that result, Ästrand et al. (1976) exposed 12 human subjects for 0.5 to 2 h to 1-butanol at concentrations of 100 or 200 ppm. There was no indication that the subjects found the exposure disagreeable. Tabershaw et al. (1944) surveyed workers at six industrial sites where exposures to 1-butanol ranged from 5 to 115 ppm. "Much" eye irritation was found at four of the six plants, but a threshold for irritation could not be gleaned from the data. The authors concluded that eye inflammation resulted if the workers were exposed at concentrations above 50 ppm. A 10-y study of 15 workers (additional subjects added later) exposed at concentrations ranging from 100 to 200 ppm concluded that eye irritation resulting in corneal inflammation, lacrimation, and photophobia were occasionally encountered in workers exposed at 200 ppm (Sterner et al., 1949). Complaints of irritation in association with exposures at 100 ppm were "rare." Because both studies in workers seemed to use adequate analytical techniques, but were limited by subjective assessments of irritation end points, it is difficult to select one result over the other. It seems prudent to conclude that a concentration of 50 ppm poses some risk of mild irritation in persons who have not adapted to 1-butanol.

Several studies of sensory irritation in rodents have been published. Kane et al. (1980) reported that the RD_{50} (concentration giving a 50% depression in the breathing rate) in mice was 4800 ppm. De Ceaurriz et al. (1981) found an RD_{50} of 1270 ppm in mice and suggested that 127 ppm (10% of the RD_{50}) would be an uncomfortable but tolerable concentration. Kristiansen et al. (1988) estimated a threshold response (RD_0) of 233 ppm and an RD_{50} of 12,000 ppm in mice. Korsak et al. (1993) reported an RD_{50} of 3000 ppm in mice. These data are not particularly useful in suggesting an irritation threshold in humans.

CNS Effects

Narcotic effects have been demonstrated in animals given large liquid doses or high-concentration exposures to the alcohol vapor. Munch (1972) reported that the ND_{50} (dose causing stupor or loss of voluntary movement in half the animals) for a single oral dose to rabbits was 11

mmol/kg (0.8 g/kg). After 12 h of exposure to the vapor at 22 mg/L, 6 of 10 mice were anesthetized; a 23-h exposure at 27 mg/L anesthetized 9 of 10 mice (McOmie and Anderson, 1949). Rumyantsev et al. (1979) reported, without giving exposure times, that 50% and 100% of mice were anesthetized when exposed at 15.1 mg/L and 15.3 mg/L, respectively. In a study of alcohol-induced hypothermia and decreased Rotorod performance in mice given a single oral dose, no effects were observed when blood concentrations were at or below 16 mg/dL. This concentration was achieved either 10 min after a dose of 0.5 g/kg or 40 min after a dose of 1.0 g/kg was administered (Maickel and Nash, 1985). Hypothermia was a more sensitive end point, but was not be considered an adverse effect. An ID_{50} (mean airborne concentration associated with a 50% decrease in immobility in the behavioral despair swimming test) of 620 ppm was found for mice exposed for 4 h (De Ceaurriz et al., 1983). The implications of this result in terms of adverse effects that might occur in humans is unclear.

Light Adaptation

There is some evidence that 1-butanol might affect the ability of the eye to adapt to changes in light intensity. Baikov and Khachaturyan (1973) found that three test subjects exposed for 5 min at 1.2 mg/m^3 had slowed darkness-adaptation rates compared with pre-exposure adaptation rates. A slower rate was not observed during exposures at 0.9 mg/m^3. Similarly, three test subjects exhibited a slower reaction time to light increases after exposures at 0.7 mg/m^3, but not after exposures at 0.5 mg/m^3. At best, the findings can only be considered preliminary because of the incompleteness of the report and the small number of test subjects.

Hepatotoxicity

Damage to the liver has been reported in mice exposed at dosages approaching the LD_{50}. Delayed deaths in mice given an intraperitoneal (ip) injection of 1-butanol (or other butanols) were attributed to development of liver injury (Maickel and McFadden, 1979).

Lethality

Death has been caused in experimental animals by using a variety of routes for administration of 1-butanol. Death was induced in 4 of 10 mice exposed to 1-butanol vapor for 23 h at a concentration of 27 mg/L (McOmie and Anderson, 1949). The LD_{50} (7-d observation) in mice given an ip injection was 0.25 g/kg (Maickel and McFadden, 1979). The rat oral LD_{50} (14-d observation) was reported to be 4.4 g/kg (Smyth et al., 1951). The LD_{50} in male rats was reported to be 2.7 g/kg of body weight (Rumyantsev et al., 1979). In rabbits, the single oral dose giving an LD_{50} has been reported as 47 mmol/kg (3.5 g/kg) or 3 mL/kg (2.4 g/kg) (Munch, 1972; Maickel and McFadden, 1979). In dogs, the blood concentration associated with cardiac arrest was 84 mg/dL (MacGregor et al., 1964).

Subchronic and Chronic Exposures

Data on the long-term effects of 1-butanol are available from inhalation-exposure studies of animals, oral-exposure studies of animals, and epidemiological studies of exposed workers.

Inhalation Exposures

Long-term inhalation-exposure studies of 1-butanol have been of mixed quality and have often been reported in an incomplete format. The adverse effects alleged as a result of inhalation exposure to 1-butanol are widespread in such studies. These reports are in contrast to a subchronic oral-exposure study conducted by Toxicology Research Laboratories in the late 1980s in which no adverse effects except ataxia were found in rats (TRL, 1986). Because the reported effects in the inhalation studies are so diverse and the primary issue is the quality of each study rather than the findings, the studies will be discussed individually.

In an early study by Smyth and Smyth (1928), it was reported that 28 to 64 exposures of guinea pigs to 1-butanol at 100 ppm (4 h/d for 6-7 d/w) caused anemia, lymphocytopenia, hemorrhage, and liver and renal degeneration. Although the test material was once-distilled, no

report of purity or of analytical methods used was given to determine exact exposures. The findings were based on groups of three exposed animals; however, the findings were striking in view of negative findings on other solvents, such as ethyl acetate (200 ppm) and ethanol (3000 ppm). The authors conclude that butyl alcohol should be used with caution and only in small amounts in lacquers. Industrial experience and other testing in rodents suggest that these early results are not representative of the toxicity potential of 1-butanol.

Several continuous inhalation studies have been reported in the Russian literature. The World Health Organization (WHO, 1987) reported that Baikov and Khachaturyan (1973) studied rats exposed for 92 d at 0.03 ppm or 7.1 ppm for a variety of effects. According to the WHO summary, no effects occurred at the low concentration; however, at the high concentration, there was a decrease in blood RNA and DNA, an increase in leukocyte luminescence, and changes in the activity of several enzymes. In fact, the Baikov and Khachaturyan (1973) study cited in the WHO report is a short-term human inhalation study involving 18 subjects exposed at concentrations of 0.5 to 1.2 mg/m^3 (see acute exposures section).

In mice continuously exposed to 1-butanol vapor at 0.8, 6.6, and 40 mg/m^3 for 30 d, Kolesnikov (1975) reported an increased tolerance to the narcotic effects of a single oral dose of 1-butanol (details not given). In addition, the hexenal sleep time was approximately halved in the two highest exposure groups. The erythrocyte acid resistance in rats given the identical exposure concentrations as the mice was different from controls in the middle-exposure group, but the description of the results for other groups indicated that there was no dose response for this end point. This brief report lacks sufficient detail to be useful. For example, there is no description of compound purity, analytical methods (except to say that a GC was used), statistical methods, chamber dynamics, or tabulation of results.

In another Russian investigation, which appears to be the overall study from which the report of Kolesnikov (1975) was extracted, rats and mice were exposed continuously at 0.8, 6.6 and 40 mg/m^3 for 120 d (Rumyantsev et al., 1976). Inconsistent and possibly transient changes were reported in both species when evaluated by the "motor-defensive method." The mid- and high-exposure groups (and possibly the lowest exposure group) had significant decreases in oxygen consumption (magnitude not given) after 120 d, and the blood cholines-

terase activity changed (statistical significance not given) but later returned to control levels in the high-exposure group. Transient changes were noted in alanine aminotransferase (SGPT) and reticulocyte counts. Histopathological changes were noted in rats and included disrupted blood circulation, atelectasis, and pulmonary emphysema. The changes were greatest for the mid- and high-exposure groups, but some changes were noted in the lowest exposure group. In view of results from other investigators (e.g., Baikov and Khachaturyan, 1973), these authors concluded that negative effects were observed at 6.6 mg/m^3 but that the effects at 1 and 0.8 mg/m^3 were not adverse. Unfortunately, this study lacks detail on the test material, analytical methods, chamber dynamics, statistical methods, and tabulation of findings. These results are not consistent with the lack of pulmonary and hematological effects reported by Sterner et al. (1949) in workers exposed at 100 to 200 ppm for up to 10 y.

Oral Exposures

Although the continuous inhalation studies reported by the Russians are not directly comparable to the 13-w oral gavage study, there are striking contrasts in the details reported and the adverse effects found. The oral study involved 92-93 d of daily administration of 1-butanol at 0, 30, 125, or 500 mg/kg to male and female rats (30 per sex per group). The only unequivocal effect induced by the alcohol was ataxia and hypoactivity in the highest exposure group during the last 6 w of the administration (TRL, 1986). No treatment-related effects were noted in the rats given 30 or 125 mg/kg/d. At the interim 6-w sacrifice, a statistically significant decrease of $<5\%$ in red-blood-cell (RBC) indices was noted in females of the highest exposure group, but the effect was not observed in males and and was not present in females sacrificed at the end of the study. The only end point in common with the Russian studies is SGPT changes, which the Russians reported as increased transiently at the 60-d sacrifice in all exposure groups (Rumyantsev et al., 1976). The TRL results showed no changes in this enzyme, which is indicative of liver injury, at either the interim or final sacrifice. Reticulocytes were not counted in the TRL study because the protocol specified that this count would not be done unless there were clear signs of anemia in the test animals.

By estimating the total doses delivered and blood concentrations of 1-butanol in the 120-d Russian study and in the 92-d U.S. study, the inconsistencies in the results become readily apparent. From the results of DiVincenzo and Hamilton (1979), the gavage of 0.5 g/kg should result in a blood concentration of about 7 mg/dL at 1 h, followed by a decrease to undetectable concentrations after 4 h. The inhalation results of Aarstad et al. (1985), showing undetected blood concentrations at exposures of 500 ppm (1500 mg/m^3), suggest that the highest concentration in the Russian studies (40 mg/m^3) would not result in significant concentrations of the alcohol in the blood. In the 92-d rat oral-exposure study, assuming an average body weight of 1/3 kg, the dose delivered was 15,000 mg over the entire study. In the 120-d inhalation study, assuming a daily inhalation volume of 0.15 m^3/d and an uptake of 50%, the cumulative amount delivered to rats exposed to the highest concentration was 360 mg. Thus, the dose delivered to the rats in the oral study was approximately 40-fold greater than the dose delivered in the inhalation study, yet the oral study produced no adverse effects (except narcosis), and the inhalation study suggested many adverse effects. Even though the 92-d study was done using a noninhalation route, it will be used in preference to the 120-d study because the former study is much more thoroughly documented.

Epidemiological Studies in Workers

All epidemiological studies of workers are at least 25 y old and generally do not have the rigor found in more recent studies. A survey of six plants involving several hundred workers in which exposures to 1-butanol varied from 5 to 115 ppm showed that the primary adverse effects were eye inflammation if exposures were above 50 ppm, and systemic effects (headache and vertigo) if the concentrations were above 100 ppm (Tabershaw et al., 1944). It was not apparent that these investigators looked for other adverse effects, such as pulmonary or hepatic damage. Sterner et al. (1949) reported no evidence of pulmonary injury and no hematological effects in workers exposed at 100 to 200 ppm for up to 10 y. Workers exposed at 200 ppm occasionally developed blurring of vision, lacrimation, and photophobia, which became more severe toward the end of the work week. Ophthalmic examination revealed slight-to-moderate corneal edema, with injection and mild

edema of the conjunctiva. In contrast to those findings, Velazquez et al. (1969) reported that 9 of 11 workers exposed for an undisclosed time (presumably years) at roughly 80 ppm showed audiological impairment. Although the audiological impairment was the focus of the report, the clinical examinations of the workers showed much more profound illness including anemia, liver dysfunction, neurological symptoms, and bronchitis (see table for details). Given the lack of detail on exposure monitoring and the unusually ill workers in the latter study, more weight will be put on the results of Sterner et al. (1949).

Genotoxicity

All genetic toxicity tests conducted on 1-butanol have been negative. In the Ames test using *Salmonella typhimurium*, 1-butanol was not mutagenic with or without microsomal metabolic activation (McCann et al., 1975). 1-Butanol was unable to induce a significant increase in sister chromatid exchanges in Chinese hamster ovary cells after 7 d of treatment (Obe and Ristow, 1977). An excess of micronuclei in a Chinese hamster lung cell line (V79) was not induced by exposure to 1-butanol (Lasne et al., 1984).

Reproductive Toxicity

No reports were found that targeted reproductive toxicity as an end point. However, in a study designed to assess reproductive effects, Nelson et al. (1989a) exposed male rats to 1-butanol at 3000 or 6000 ppm 7 h/d for 6 w and reported no adverse effects on the mating capability of the rats.

Developmental Toxicity

The potential for developmental toxicity has been evaluated by inhalation exposure of rats. Only a few minor behavioral or neurochemical effects were reported in the offspring of female rats exposed at 3000 or 6000 ppm (7 h/d) throughout gestation or to offspring of unexposed females mated to males that had been exposed at the same concentra-

tions for 6 w before mating (Nelson et al., 1989b). The same group reported that offspring of female Sprague-Dawley rats exposed at 8000, 6000, or 3500 ppm for 7 h/d during gestational days 1 to 19 showed teratogenicity (rudimentary cervical ribs) only at the highest dose, which was overtly toxic (narcosis, reduced weight gain, death of 2 of 18) to the dams (Nelson et al., 1989a). No statistically significant developmental effects were detected at 6000 ppm, although the fetal weights were below normal and there was an increase in skeletal variants. There were no deviations from normal in offspring of dams exposed at 3500 ppm. Nelson et al. (1989a) concluded that if developmental effects were observed, they would likely occur only in the presence of maternal toxicity.

Interaction with Other Chemicals

Limited data are available on the potential for 1-butanol to increase the toxicity of other chemicals. In Sprague-Dawley rats given an oral dose of 1-butanol at 1.48 g/kg 16 to 18 h before a 2-1/2-h exposure to carbon tetrachloride at 1000 ppm, SGOT (an indicator of liver damage) was elevated about threefold over animals not given 1-butanol but exposed to carbon tetrachloride at 1000 ppm (Cornish and Adefuin, 1967). The effect did not occur when the alcohol was given only 2 h before the inhalation exposure to carbon tetrachloride. Of the four butanols tested at 1.48 g/kg, 1-butanol, was the least effective in potentiating the hepatotoxicity of carbon tetrachloride.

TABLE 2-1 Toxicity Summary

Concentration	Exposure Duration	Species	Adverse Effects	Reference
			Inhalation	
25 ppm	3-5 min	Human (n = 10)	Nose and throat irritation	Nelson et al., 1943
50 ppm	3-5 min	Human (n = 10)	Eye irritation	Nelson et al., 1943
20 to 115 ppm	Workday	Human (several hundred)	Eye iritation, occasional vertigo, eye inflammation, sleepiness	Tabershaw et al., 1944
80 ppm	Years	Human (n = 11)	Hearing deficit (9/11), anemia (5/11), decreased liver function (3/11), neurological symptoms (5/11), chronicbronchitis (11/11)	Valazquez et al., 1969
100 to220 ppm	0.5 to 2 h	Human (n = 12)	No EKG changes, uptake 37-48%	Ästrand et al., 1976
100 to 200 ppm (average)	Up to 10 y	Human (n = 15-56)	Eye irritation, lacrimation and photophobia at 200 ppm, few complaints at 100 ppm, no X-ray evidence of lung injury, no hematological effects	Sterner et al., 1949
0.25 ppm	120 d continuous	Rat, mouse	Decreased oxygen consumption and lung damage	Rumyantsev et al., 1976, 1979
2.1 ppm	120 d continuous	Rat, mouse	Decreased oxygen consumption, lung damage, and thyroid dysfunction	Rumyantsev et al., 1976, 1979
13 ppm	120 d continuous	Rat, mouse	Decreased oxygen consumption, lung damage, and thyroid dysfunction	Rumyantsev et al., 1976, 1979
4900 ppm	Unspecified	Mouse	ND_{50} (anesthesia)	Rumyantsev et al., 1979

5100 ppm	Unspecified	Rat	ND_{100} (anesthesia)	Rumyantsev et al., 1979
6000 ppm	7 h/d, 6 w	Rat (male)	No effect on mating capability	Nelson et al., 1989a
7000 ppm	12 h	Mouse	6/10 anesthetized	McOmie and Anderson, 1949
8600 ppm	23 h	Mouse	9/10 anesthetized, 4/10 dead	McOmie and Anderson, 1949
			Oral	
0.125g/kg/d	92 d	Rat	NOEL for all parameters measured	TRL, 1986
0.5 g/kg/d	92 d	Rat	Ataxia and hypoactivity in last 6 w	TRL, 1986
0.8 g/kg	Singledose	Mouse	ND_{50}	Munch, 1972
1.0 g/kg	Singledose	Mouse	NOEL for Rotorod performance	Maickel and Nash, 1985

TABLE 2-2 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	50 (ceiling), intended to change to 25 ppm	ACGIH, 1995
OSHA's PEL	100 (transitional), 50 ppm ceiling ("final" rule)	U.S. Dept. of Labor, 1995

TLV, Threshold Limit Value; PEL, permissible exposure limit.

TABLE 2-3 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	50	150	Eye irritation
24 h	25	80	Eye irritation
7 d	25	80	Eye irritation, systemic injury
30 d	25	80	Eye irritation, systemic injury
180 d	12	40	Systemic injury

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Acceptable concentrations (ACs) must be set to protect against excess risk of irritation and CNS effects due to exposure to 1-butanol. The data on hepatotoxicity suggest that it does not occur unless exposures approach lethal concentrations, and the data on light adaptation are insufficient for setting an acceptable concentration. Epidemiological data are mixed, and the findings on audiological deficits were not convincing in view of the other health effects detected in the workers. In 1987, WHO considered the available data (including the Russian data) inadequate to assess the human health risks or to set an occupational exposure limit. The 92-d oral gavage study in rats was not available to WHO at the time; however, it is clear that they were not willing to rely

on the Russian data that they had tabulated. Likewise, the rationale below does not rely on the Russian data.

The guidelines provided by the National Research Council were used as a framework for developing the SMACs (NRC, 1992).

Irritation and Inflammation of the Eyes

Based on the information from three studies (Nelson et al., 1943; Tabershaw et al., 1944; Sterner et al., 1949), the threshold for eye irritation is between 50 and 100 ppm (150 and 300 mg/m^3). The weight of evidence suggests that any irritation at 50 ppm will be mild and therefore acceptable for 1-h exposures. The comment by Sterner et al. (1949) that the effects on the eyes become worse during the work week suggests that a 24-h continuous exposure must be below 50 ppm to ensure that no more than mild irritation will occur; 25 ppm was judged to be suitable to achieve that goal. For longer exposures, a 25-ppm limit should protect against irritation indefinitely, because some degree of adaptation could be expected.

CNS Effects

Tabershaw et al. (1944) concluded from their study of workers that systemic effects will not appear until 100 ppm is greatly exceeded, and Sterner et al. (1949) did not report any CNS effects in the workers they studied. Unfortunately, no attempt was made in either study to take objective measurements of performance in the exposed workers. It is clear, however, from animal studies that narcosis or decrements in rotorod performance can be induced by inhalation or other routes of administration, but the findings were not appropriate to use for setting human exposure limits because of the severity of the end points. Any CNS effects in humans would have to be mediated through 1-butanol concentrations in the blood. A stable blood concentration of about 3 mg/dL (arterial) was attained in test subjects after 30 min of inhalation of 1-butanol at 100 ppm (Ästrand et al., 1976). Several animal studies have shown that 1-butanol is 5 to 10 times more effective than ethanol in inducing hypothermia, decreased performance in the tilted-plane test, or respiratory arrest (Maickel and Nash, 1985; Wallgren, 1960; Mac-

Gregor et al., 1964). The threshold blood concentration of ethanol at which performance decrements are not detectable is 50 mg/dL (Kennedy, 1993). Hence, even if 1-butanol were 10-fold more potent in inducing CNS effects than ethanol, the blood concentrations associated with a 100-ppm exposure would be below an effect level. The AC for all exposure times to prevent CNS effects was set at 100 ppm. This approach to setting the AC is in general agreement with the epidemiological reports on exposed workers.

Long-Term Systemic Injury

The 92-d oral-exposure rat data, even though it is not directly pertinent to continuous inhalation exposures, can be extrapolated to a human estimate (TRL, 1986). For reasons discussed in the toxicity section, the Russian data were not used to set ACs. Except for narcosis, rats given 0.5 g/kg/d were without adverse effects. Assuming that the rats' average weight was 0.33 kg and extrapolating on a body-surface basis to 70-kg humans, the equivalent dose for humans is

$$0.5 \text{ g/kg/d} \times 0.33 \text{ kg} \times (70 \text{ kg}/0.33 \text{ kg})^{0.67} = 6 \text{ g/d},$$

as a NOAEL for 92 d of exposure. This can be equated to a human inhalation dose by assuming a 40% uptake of the alcohol and a 20-m^3/d inhalation volume. The airborne concentration necessary to deliver 6 g/d was calculated as follows:

$$C_{NOAEL} = 6000 \text{ mg/d} \div (20 \text{ m}^3/\text{d} \times 0.4) = 750 \text{ mg/m}^3.$$

Even though the dose was scaled from rats to humans, interspecies differences might exist in metabolism and tissue susceptibility, so a species factor is needed. For a 90-d exposure, the AC is

$$\text{AC (90 d)} = 750 \text{ mg/m}^3 \div 10 \text{ (species)} = 75 \text{ mg/m}^3 \text{ (25 ppm)}.$$

That AC also is the AC for 7- and 30-d exposures. Because the oral study lasted only 90 d, the 180-d AC should be set using a time factor of 2 (180/90), which results in a 180-d AC of 40 mg/m^3 (12 ppm).

Spaceflight Effects

The toxic effects induced by 1-butanol are not expected to be increased by the microgravity-induced physiological and biochemical changes in astronauts.

TABLE 2-4 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors			Acceptable Concentrations, mg/m³				
		Species	Time	Small *n*	1 h	24 h	7 d	30 d	180 d
Eye irritation									
Mild irritation at 150 mg/m³ (three studies; see text)	Human, n > 100	1	1	1	150	80	80	80	80
CNS effects									
Epidemiology, no effects (Tabershaw et al., 1944)	Human	1	1	1	300	300	300	300	300
Compared with ethanol (see text)	Human	—	—	—	300	300	300	300	300
Systemic injury									
Oral NOAEL (92 d) (TRL, 1986)	Rat	10	1 or HR	1	—	—	80	80	80
SMACs					**150**	**80**	**80**	**80**	**40**

—, Data not considered applicable to the exposure time; HR, Haber's rule.

RECOMMENDATIONS

The analysis of available data to derive ACs involved unproven assumptions and uncertainties that need to be resolved to improve the data base for setting the SMACs. A short-term human inhalation study is needed to measure the irritation thresholds and uptake of the compound over several hours (at least) and to quantify performance decrements at higher concentrations of exposure. The long-term data are problematic because of the differences reported by Russian investigators when compared with results from this country. A continuous inhalation exposure of rodents for at least 30 d is needed to determine the validity of earlier reports. End points should include those traditionally used in this country and as many of the Russian end points as possible.

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B3 tert-*Butanol*

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PHYSICAL AND CHEMICAL PROPERTIES

tert-Butanol is a crystalline solid below 25°C and a colorless, volatile liquid with a camphorlike odor above 25°C. The odor threshold is 47 ppm (Amoore and Hautala, 1983).

Synonyms:	2-methyl-2-propanol, *tert*-butyl alcohol, tertiary butanol, *t*-butanol
Formula:	$(CH_3)_3COH$
CAS number:	75-65-0
Molecular weight:	74.1
Boiling point:	82°C
Melting point:	25°C
Specific gravity:	0.79
Vapor pressure:	42 mmHg at 25°C
Lower flammability limit:	2.4%
Solubility:	Soluble in water, miscible with ethanol and other organic solvents
Conversion factors:	1 ppm = 3.08 mg/m^3 1 mg/m^3 = 0.325 ppm

OCCURRENCE AND USE

tert-Butanol is used in perfumes, cosmetics, aerosol sprays, paint removers, and defoaming agents; industrially, it is used in production processes, separations, and cleaning and as a gasoline additive and dehydrating agent (WHO, 1987; Lington and Bevan, 1994; NTP, 1994). Analysis of air from recent space-shuttle flights showed that this alcohol is found in about 15% of the air samples at concentrations that sometimes exceed 1 mg/m^3 (James et al., 1994).

TOXICOKINETICS AND METABOLISM

Absorption

The rate of absorption of *tert*-butanol has not been investigated directly; however, studies with other goals suggest that the alcohol is rapidly absorbed by inhalation, intraperitoneal injection, or orally. For example, Bechtel and Cornish (1975) reported that rats given 500 mg/kg orally reached their maximum serum concentration of 450 ppm (36 mg/dL[1]) in 45 to 50 min.

Distribution

The tissue distribution of *tert*-butanol appears to be according to the water content of the tissue (Bechtel and Cornish, 1975).

Elimination

Because the metabolic pattern for *tert*-butanol is qualitatively and quantitatively much different from primary and secondary butanols, its means of elimination differs from that of other butanols. The "major portion" of a 500-mg/kg oral dose given to rats was exhaled as the

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[1]Assumes vol/vol dilution.

unmetabolized alcohol (Bechtel and Cornish, 1975). Metabolites of *tert*-butanol could not be found in the blood of exposed rats, whereas metabolites of other butanols were readily detected. In rabbits given an oral dose of *tert*-butanol at 4 mmol/kg, 24% of the dose was excreted in the urine as a glucuronide, as compared with 14% for *sec*-butanol and 1.8% for *n*-butanol (Kamil et al., 1953).

Because of very slow metabolism (see below), the rate of elimination of *tert*-butanol from blood is much slower than that of most alcohols. The peak blood concentration in rabbits orally administered 2 mL/kg (1.6 g/kg) was 200 mg/dL, but detectable amounts (60 mg/dL) were present 70 h after the dose was given (Saito, 1975). Similarly, Beaugé et al. (1981) reported that female rats given 25 mmol/kg (1.8 g/kg) had blood concentrations of 13.2 mM (100 mg/dL) after 2 h and 11.3 mM (80 mg/dL) after 20 h. Gaillard and Derache (1965) reported that rats given 2 g/kg orally had a blood concentration peak of 124 mg% after 2 h, but after 8 h, the blood concentration had only decreased to 110 mg%.

The rate of *tert*-butanol elimination can be increased by repeated exposure to the alcohol; however, that does not occur quickly in some animal models. Thurman et al. (1980) showed that rats given oral doses of the alcohol every 8 h for 1 d required 26 h after the last dose to eliminate it from the blood to the detection limit, whereas after 2-1/2 d of exposure, the elimination time was only 18 h. McComb and Goldstein (1979a) found that to maintain a blood concentration of *tert*-butanol of about 6 mM (40 mg/dL) in male Swiss-Webster mice over 9 d of continuous inhalation exposure, the exposure concentration had to be increased from 50 μmol/L during day 1 to 120 μmol/L during day 9. In contrast to these findings, female C57BL mice pre-exposed to *tert*-butanol (five doses of 10.5 mmol/kg every 12 h) showed the same rate of elimination up to 12 h after exposure as mice that were not pre-exposed (Faulkner et al., 1989). Because Aarstad et al. (1985) showed that the cytochrome P-450 concentration in rat-liver microsomes was induced by inhalation of 2000 ppm (6 h/d, 3 d), it is reasonable to speculate that the increased rate of elimination in the rat is due in part to enzyme induction.

As would be expected, the slow metabolism of *tert*-butanol leads to prolonged signs of intoxication in animals. The recovery from intoxication (measured by using the tilted plane test) was slowest for *tert*-bu-

tanol, when compared with other butanols administered to rats, at a dose of 16 mmol/kg (1.2 g/kg) (Wallgren, 1960). Seven hours after administration of *tert*-butanol, the rats continued to have a performance decrement of 30%, which was the maximum decrement seen for *tert*-butanol.

Metabolism

Limited data are available on the metabolism of *tert*-butanol from in vivo models and in vitro systems; however, there are no data available on its metabolism in humans. Some animal models indicate that much of the alcohol is eliminated unchanged by respiration, and a lesser amount is conjugated to glucuronic acid and excreted in the kidney (Bechtel and Cornish, 1975; Kamil et al., 1953). In rats given 1 g/kg, the elimination appeared to be first order with a half-life of 9.1 h, and acetone was identified in the blood (Baker et al., 1982). Using radiolabeled doses between 0.75 and 2 g/kg, the investigators found that they could recover 0 to 9.5% (molar basis) of the dose as acetone in the urine and expired air. A small amount of labeled carbon dioxide was also eliminated from rats given a dose of 1.75 g/kg. In mice, data suggest a pseudo-zero-order elimination process after a dose of 5, 10, or 20 mmol/kg (0.4, 0.7, or 1.5 g/kg, respectively) was given by intraperitoneal injection (Faulkner and Hussain, 1989).

In vitro studies of the metabolism of *tert*-butanol have suggested an additional important metabolite of the alcohol. Using rat-liver microsomes, a source of cytochrome P-450-linked electron transport, to generate ·OH from H_2O_2 , Cederbaum and Cohen (1980) showed that *tert*-butanol could be oxidatively demethylated to form formaldehyde. These investigators suggested that hydroxyl radicals are involved as follows:

$$(CH_3)_3\text{–C–OH} + \cdot OH \Rightarrow (CH_3)_2\text{–C=O} + CH_2\text{=O} + \text{other products.}$$

That reaction would explain the production of formaldehyde as well as acetone reported by others. Later studies demonstrated the ability of *tert*-butanol to act as a hydroxyl radical scavenger in vitro (Cederbaum et al., 1983).

TOXICITY SUMMARY

Many studies are designed to understand how *tert*-butanol induces physical dependency; however, they are of little value in demonstrating the toxic effects caused by the alcohol. In short-term experiments, the primary effect is CNS depression, whereas prolonged exposures target the urinary bladder and kidney. Data were found on animal exposures by various routes; however, studies using human test subjects have not been reported.

Acute and Short-Term Exposures

Short-term inhalation studies consist of a report that is not available in detail (Battelle, 1988a) and incidental observations during a developmental toxicity study (Nelson et al., 1989). Unsteadiness was reported in pregnant rats at the end of 7-h inhalation exposures to 2000 ppm, and ataxia was reported at the end of 3500 or 5000 ppm exposures (Nelson et al., 1989). Although the exposures were for 19 d, it is reasonable to assume that the signs of narcosis were observed during or after each acute exposure. Ataxia was noted in 10 out of 10 rats exposed to 900 ppm 6 h/d for 12 d, but mice did not exhibit ataxia until the exposures were at or above 1750 ppm (Battelle, 1988a). A single 7-h exposure to 7000 ppm caused coma in 10 of 10 rats and 10 of 10 mice (Battelle, 1988a). Target concentrations have been reported here for the 12-d Battelle study; however, because analytical concentrations were 20% above target concentrations during a 13-w study reported by the same laboratory (Battelle, 1988b), it is possible that the actual exposures during the 12-d study were 20% above target concentrations.

Acute effects have been reported in animals exposed by noninhalation routes. Ataxia (in 1 of 10) and hypoactivity (in 2 of 10) were reported in male rats given oral doses as low as 1.0% (wt/vol) in drinking water (Lindamood et al., 1992). The ND_{50} dose (causing stupor and loss of voluntary movement in 50% of the animals) was reported to be 19 mmol/kg (1.4 g/kg) in rabbits dosed by oral gavage and the LD_{50} was 48 mmol/kg (3.5 g/kg) (Munch, 1972). Thus, *tert*-butanol was found to be comparable in toxicity to other butanols, which had an ND_{50} range of 11 to 19 mmol/kg and an LD_{50} range of 41 to 66 mmol/kg. Other LD_{50} values include the following: 0.9 g/kg given intraperi-

toneally in mice; 1.5 g/kg given intravenously in mice; and 3.5 g/kg given orally in rats (WHO, 1987). Microencephaly (16% decrease in brain weight) was induced in neonatal rats given *tert*-butanol in a milk formula on postnatal days 4 to 7 (dose, 0.6 to 2.7 g/kg); however, there were no reductions in liver or heart weights (Grant and Samson, 1982).

Even though the liver is not thought to be a target of *tert*-butanol, a single oral dose of 25 mmol/kg (1.8 g/kg) given to female Wistar rats induced a 3-fold accumulation of triacylglycerols in the liver 20 h after dosing (Beaugé et al., 1981). The authors seem to attribute the result to a nonspecific stress effect related to the hypothermia action of this alcohol rather than a specific action of the alcohol.

Subchronic Exposures

Long-term oral and inhalation studies of *tert*-butanol have shown that the urinary bladder and kidney are targets for injury. Considerable differences were found in the responses of different sexes and in the two species of rodents evaluated.

An abstract, data tables, and a pathologist's summary of observations were made available on a subchronic inhalation study contracted by the National Toxicology Program (NTP) and conducted in 1988 by Battelle (1988b). Male and female rats (F344) and mice ($B6C3F_1$) were exposed to *tert*-butanol at 0, 134, 270, 540, 1080, and 2100 ppm for 6 h/d for 65 d with breaks for weekends and holidays. Those average analytical concentrations appear to be about 20% above the target concentrations for *tert*-butanol. Measurements were taken and observations made in the following categories to detect toxic effects: weekly body weights and clinical observations, hematology, clinical chemistry (rats only), urinalysis (rats only), gross pathology, organ weights, and histopathology. The findings on rats were as follows: Exposures did not affect survival or body-weight gains; the hemoglobin, hematocrit, and red-blood-cell (RBC) counts were decreased (amount not given) in males exposed at 1080 and 2100 ppm; there were other scattered changes in clinical laboratory results and increased kidney weights were found in conjunction with increased severity of nephropathy in males (Table 3-1). The Battelle (1988b) investigation concluded that kidneys in the two highest exposure groups of male rats were affected by the

TABLE 3-1 Nephropathy in Male Rats Exposed to *tert*-Butanol

Concentration, ppm	Number examined	Nephropathy	
		Minimal	Mild
0	10	9	0
134	10	5	3
270	10	5	4
540	10	4	6
1080	10	1	9
2100	10	0	10

exposure. The investigators felt that the slight anemia in male rats was of little biological importance. Male rats exposed by inhalation did not show urinary-bladder lesions when subjected to exposures above 800 mg/kg/d, as reported in the 13-w drinking-water study (Lindamood et al., 1992). Nine of 10 female rats exposed at 2100 ppm showed kidney mineralization, but no such lesions were reported in the inhalation controls (Battelle, 1988b). That might have been an oversight because female control rats in the 13-w drinking-water study all showed kidney mineralization (Lindamood et al., 1992). Shifts in the relative counts of white blood cells were reported in female rats exposed by inhalation at 2100 ppm (Battelle, 1988b).

In mice the findings were as follows: the 1080- and 2100-ppm groups showed depressed gains in body weight, few if any clinical signs, no consistent changes in organ weights, and no dose-related gross or microscopic lesions. The high-dose male mice did show a leukocyte shift from lymphocytes to neutrophils and possibly an increased incidence of renal tubule regeneration (4 of 10 in the high-dose group compared with 1 of 10 in the control group) (Battelle, 1988b).

In the 13-w drinking-water study, B6C3F_1 mice and F344 rats of both sexes were given 0, 0.25, 0.5, 1.0, 2.0, and 4.0% (wt/vol) *tert*-butanol in their drinking water (Lindamood et al., 1992). The amount of *tert*-butanol consumed by the rats of both sexes averaged about 250 mg/kg/d (low dose) to 3500 mg/kg/d (high dose). In mice, the low-dose group consumed an average of 320 (males) or 570 mg/kg/d (females) and the high-dose group consumed 6200 (males) or 7500

mg/kg/d (females). All male rats and 6 of 10 female rats in the high-dose group died before the end of the study. In mice, 6 of 10 males and 4 of 10 females in the high-dose group died. Male and female rats had reduced urinary volumes in association with crystaluria (probably uric acid). Lesions consisted of urinary-tract calculi; renal, pelvic, and uretral dilatation; and thickening of the urinary-bladder mucosa. Male rats exhibited hyaline droplet accumulation in renal tubules. That is a lesion characteristic of α2u-globulin nephropathy; however, complexes with that protein were not specifically found in the kidney (Takahashi et al., 1993). The highest no-effect level for urinary-tract lesions was 800 mg/kg/d in male rats (Lindamood et al., 1992).

Chronic Exposures

A 2-y chronic-exposure drinking-water study was conducted in rats and mice as a followup to the subchronic study (NTP, 1994). The dose was adjusted depending on the susceptibility of the specific species and sex of the rodents. Male F344 rats were administered the alcohol at 0, 1.25, 2.5, or 5.0 mg/mL of their drinking water and female rats were given twice those concentrations. Male and female B6C3F$_1$ mice were given concentrations fourfold higher than the male rats. The average daily doses delivered are listed in Table 3-2.

TABLE 3-2 Consumption of *tert*-Butanol in 2-y Rodent Drinking-Water Study

	Average daily amount consumed by animals in groups		
Species, sex	Low dose, mg/kg	Mid dose, mg/kg	High dose, mg/kg
Rat, M	85 (1.25)[a]	195 (2.5)	420 (5.0)
Rat, F	175 (2.5)	330 (5.0)	650 (10.0)
Mouse, M	535 (5.0)	1035 (10.0)	2065 (20.0)
Mouse, F	510 (5.0)	1015 (10.0)	2105 (20.0)

[a]Numbers in parentheses are the concentrations of *tert*-butanol in water (mg/mL).

The survival rates of male rats exposed at 5 mg/mL, female rats at 10 mg/mL, and male mice at 20 mg/mL were significantly below controls. Kidney mineralization and renal tubule hyperplasia were statistically increased in the high-dose group of male rats, but were not increased in any group of female rats. The incidence of transitional epithelial hyperplasia in rat kidney was statistically increased in males exposed at 2.5 and 5.0 mg/mL and in females at 10 mg/mL. The average severity of nephropathy in female rats increased from 1.6 in controls to 2.9 in the high-dose group (one, minimally; two, mildly, and three, moderately). Although the low-dose group of female rats (2.5 mg/mL or 0.175 g/kg/d), with an average severity of nephropathy at 1.9, was statistically above the controls, the slight increase over controls, in the subcommittee's opinion, does not represent an adverse effect of biological significance. The no-observed-adverse-effect level (NOAEL) for nephropathy in female rats was 0.175 g/kg/d. The incidences of thyroid-gland follicular hyperplasia were increased in all male mice and in all mid- and high-dose groups of mice. The incidences of chronic inflammation and transitional-cell hyperplasia of the urinary bladder were increased in the high-dose groups of male and female mice. In the 2-y study, male mice were the most sensitive species and sex for urinary-bladder injury. The NOAEL appeared in these mice when drinking an average of 1035 mg/kg/d. The NOAEL in the 13-w study for the same sex and species was 1570 mg/kg/d; therefore, the incidence and severity of these lesions do not increase much with prolonged exposure (i.e., from 90 to 720 d). Possible neoplastic effects of *tert*-butanol are reported below.

Carcinogenicity

The 2-y drinking-water study (NTP, 1994) demonstrated some potential for *tert*-butanol to induce tumors in some of the test groups. "Some evidence of carcinogenic activity" was reported for the induction of renal tubule adenomas and carcinomas in male rats exposed at average doses of 0, 85, 195, or 420 mg/kg/d. The incidences of adenoma and carcinoma combined were as follows in the four groups (controls to high dose): 8 of 50, 13 of 50, 19 of 50, and 13 of 50, respectively. The decreased incidence in the high-dose group was presumably due to the shorter survival time, which limited the time for tumors to develop.

No evidence of carcinogenic activity was found in female rats. Equivocal evidence of carcinogenicity was reported in male mice because of marginal increases in thyroid adenomas and carcinomas combined. "Some evidence of carcinogenicity" was reported in female mice because of increased incidences of follicular-cell adenomas in the thyroid gland. The incidences reported for controls and the three exposed groups of female mice were 2 of 58, 3 of 60, 2 of 59, and 9 of 59, respectively.

Genotoxicity

Without exception, *tert*-butanol has been negative in tests of its genotoxic potential. It was negative in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 for gene mutation with and without the S9 mix (Zeigler et al., 1987). *tert*-Butanol was negative in a mouse lymphoma mutagenicity test, with and without the S9 mix (McGregor et al., 1988). Galloway et al. (1987) has reported an overall negative result for induction of sister chromatid exchanges in Chinese hamster ovary (CHO) cells with and without S9 activation. One of the four trials (−S9) was weakly positive; the others were totally negative. Similarly, the same study reported a negative result for chromosomal aberrations in CHO cells, with and without S9 activation; however, one trial (+S9) gave an equivocal result. The percent of micronucleated peripheral blood erythrocytes was not increased in male and female mice exposed for 13 w at concentrations up to 40,000 ppm in drinking water (MacGregor et al., 1990).

Reproductive Toxicity

Studies were not found that examined the in vivo functional capacity of the reproductive system after exposure to *tert*-butanol; however, histological evidence from the 2-y drinking-water study gave no indication of reproductive effects (NTP, 1994). No increased incidence of lesions was found in male rats and mice after histopathological examination of the epididymis, penis, preputial gland, prostate, seminal vesicle, and testes. Similarly negative results were found in female rats and mice after histopathological examination of the clitoral gland, ovary, uterus,

and vagina. Evaluation of sperm morphology and vaginal cytology in rats and mice from the 13-w portion of this study revealed that the only exposure-related effect was a prolonged or unclear estrus cycle in the high-dose female mice (NTP, 1994). It has been reported that, unlike ethanol, *tert*-butanol did not affect the in vitro fertilizing capacity of mouse spermatozoa at aqueous concentrations up to 4000 mg/L (Anderson et al., 1982).

Developmental Toxicity

Several studies have been published on the developmental toxicity of *tert*-butanol administered by various routes, including inhalation. Some of the studies were designed to answer questions about the role of acetaldehyde in the developmental toxicity induced by ethanol. The rationale was that if *tert*-butanol, which is poorly metabolized, were found to cause developmental toxicity, then that would be evidence that ethanol itself, rather than its metabolite (acetaldehyde), causes the developmental toxicity. The results are mixed. For example, Daniel and Evans (1982) report evidence that *tert*-butanol, given to mice at concentrations of 0.5, 0.75, and 1.0% (wt/vol) during gestational days 6 to 20, was 5 times more potent than ethanol in inducing delay in postparturition physiological and psychomotor performance scores. That result was taken as evidence that ethanol, not acetaldehyde, was responsible for the developmental effects caused by excess ethanol consumption. In contrast, Faulkner et al. (1989) gave *tert*-butanol at a concentration of 10.5 mmol/kg to CBA/J or C57BL mice on gestational days 6 to 18 and found more resorptions per litter but no decreased body weights or malformations in the fetuses. That result was taken as indirect evidence that acetaldehyde, not ethanol, is the cause of developmental toxicity when ethanol is consumed in excess.

Inhalation data suggest that *tert*-butanol is not selective for developmental toxicity (Nelson et al., 1989). Dams exposed at 2000, 3500, or 5000 ppm for 7 h/d during gestational days 1 to 19 produced fetuses with subnormal weight gain (all three exposures) and increased skeletal variations (top two exposures). Dams in all groups exhibited an unsteady gait toward the end of exposure each day, and the highest exposure group failed to gain weight at the rate of controls (a difference of approximately 70 g after 19 d). Nelson et al. (1989) pointed out that

the developmental effects reported by Daniel and Evans (1982) were elicited after much higher doses that would be difficult to achieve by inhalation. Nelson et al. (1989) concluded that developmental effects would be likely to occur only in the presence of maternal toxicity.

Interaction with Other Chemicals

The potential for *tert*-butanol to affect the toxicity of other chemicals has not been studied extensively. Cornish and Adefuin (1967) showed that carbon tetrachloride-induced liver injury in rats, as measured by increases in serum SGOT, could be increased by oral administration of the alcohol at 1.4 g/kg 16 to 18 h before inhalation of the chlorocarbon at 2000 ppm for 2 h. The activity of the enzyme in the serum of *tert*-butanol-exposed rats was increased approximately 50-fold over that in control rats. The effect could not be demonstrated when the alcohol was given only 2 h before inhalation of the chlorocarbon.

In experiments designed to understand the development of physical dependence on alcohols, McComb and Goldstein (1979b) showed that *tert*-butanol can substitute for ethanol in the induction of the dependent state in mice. The larger alcohol was 4 to 5 times more effective in producing the dependent state than the smaller alcohol (McComb and Goldstein, 1979a).

TABLE 3-3 Toxicity Summary

Concentration	Exposure Duration	Species	Effects[a]	Reference
			Inhalation	
134 to 540 ppm	6 h/d, 65 d	Rat, M Rat, F	NOAEL NOAEL	Battelle, 1988b
450 ppm	6 h/d, 12 d	Rat Mouse	NOAEL NOAEL	Battelle, 1988a
900 ppm	6 h/d, 12 d	Rat Mouse	Ataxia (10/10) NOAEL	Battelle, 1988a
1080 ppm	6 h/d, 65 d	Rat, M Rat, F	Mild nephropathy (anemia) NOAEL	Battelle, 1988b
1080 ppm	6 h/d, 65 d	Mouse	NOAEL (depressed weight gain), 5 males died (probably not compound related)	Battelle, 1988b
1750 ppm	6 h/d, 12 d	Rat Mouse	Ataxia (10/10) Ataxia (6/10)	Battelle, 1988a
2000 ppm	7 h/d, 19 d	Rat, F, pregnant	Marginal unsteadiness (LOAEL)	Nelson et al., 1989
2100 ppm	6 h/d, 65 d	Rats, M	Mild nephropathy (anemia), NOAEL for urinary-bladder injury	Battelle, 1988b
2100 ppm	6 h/d, 65 d	Rats, F	Kidney mineralization, WBC shift, NOAEL for nephropathy	Battelle, 1988b
2100 ppm	6 h/d, 65 d	Mouse	NOAEL (WBC shifts, depressed weight gain), 1 male died (compound related?), (renal tubule regeneration?)	Battelle, 1988b
3500 ppm	6 h/d, 12 d	Rat	Subnormal weight gain, ataxia (10/10), ocular discharge (7/10)	Battelle, 1988a

3500 ppm	6 h/d, 12 d	Mouse	Ataxia (10/10), comatose (8/10)	Battelle, 1988a
3500 ppm, 5000 ppm	7 h/d, 19 d	Rat, F, pregnant	Unsteady gait at end of exposures	Nelson et al., 1989
7000 ppm	6 h	Rat Mouse	Moribund (10/10) Moribund (10/10)	Battelle, 1988a
10,000 ppm	7 h	Rat, F, pregnant	Severe narcosis in all, death in 5/6	Nelson et al., 1989
			Noninhalation[b]	
85 mg/kg/d	2 y	Rat, M	13/50 with kidney tumors, control had 8/50	NTP, 1994
175 mg/kg/d	2 y	Rat, F	NOAEL for nephropathy (1.9 vs. 1.6 severity in controls)	NTP, 1994
330 mg/kg/d	2 y	Rat, F	NOAEL for epithelial hyperplasia in kidney, nephropathy severity = 2.3	NTP, 1994
420 mg/kg/d	2 y	Rat, M	Renal tubule hyperplasia, kidney mineralization	NTP, 1994
440 mg/kg	Single (ip injection)	Mouse, M	LD_{50} (7 d)	Maickel and McFadden, 1979
500 mg/kg/d	13 w	Rat, F	NOAEL for nephropathy	Lindamood et al., 1992; NTP, 1994
535 mg/kg/d	2 y	Mouse, M	Thyroid follicular-cell hyperplasia	NTP, 1994
650 mg/kg/d	2 y	Rat, F	Epithelial hyperplasia in kidney	NTP, 1994
800 mg/kg/d	13 w	Rat, M	NOAEL for urinary-bladder lesions, ataxia 1/10	Lindamood et al., 1992
1015 mg/kg/d	2 y	Mouse, F	Thyroid follicular-cell hyperplasia	NTP, 1994
1600 mg/kg/d	13 w	Mouse, M	NOAEL for urinary-bladder lesions	Lindamood et al., 1992
1500 mg/kg/d	13 w	Rat, F	NOAEL for urinary-tract lesions	Lindamood et al., 1992

TABLE 3-3 *(Continued)*

Concentration	Exposure Duration	Species	Effects[a]	Reference
2065 mg/kg/d	2 y	Mouse, M	Urinary-bladder lesions increased	NTP, 1994
2105 mg/kg/d	2 y	Mouse, F	Thyroid follicular adenoma	NTP, 1994
3600 mg/kg	Single (oral)	Rabbit	LD_{50} (24 h)	Munch, 1972
4400 mg/kg/d	13 w	Mouse, F	NOAEL for urinary-tract lesions	Lindamood et al., 1992

[a]Effects in parentheses were considered to be of little biological significance.

[b]Unless noted otherwise, the noninhalation route was by drinking water.

TABLE 3-4 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	100	ACGIH, 1995
OSHA's PEL	100	U.S. Dept. of Labor, 1995
OSHA's STEL	150	U.S. Dept. of Labor, 1995

TLV, Threshold Limit Value; PEL, permissible exposure limit.

TABLE 3-5 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	50	150	CNS depression
24 h	50	150	CNS depression
7 d	50	150	CNS depression
30 d	50	150	Kidney injury, CNS depression
180 d	40	120	Kidney injury, CNS depression, urinary-bladder injury

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Because no data are available on human exposures to *tert*-butanol, the rationale for acceptable concentrations must depend entirely on data obtained in animal models. In 1987, WHO considered the toxicity data base inadequate for setting occupational exposure guidelines, and in 1989, the Cosmetics Ingredient Review Expert Panel concluded that the available data were insufficient to support the safety of *tert*-butanol as used in cosmetics (Brandt, 1989). Since that time, excellent data have become available on the long-term effects of exposure to this alcohol via the oral route of administration (Lindamood et al., 1992; NTP,

1994). In addition, we were able to use results from an inhalation study (not yet peer reviewed) sponsored by NTP as part of the data base for setting acceptable concentrations (Battelle, 1988a,b). In general, the guidelines promulgated by the NRC (1992) were used to derive acceptable concentrations (ACs) for *tert*-butanol.

CNS Effects

Without human data on the CNS effects of *tert*-butanol, the AC is based on animal data. Nelson et al. (1989) reported "unsteadiness" in pregnant rats at the end of 7-h exposures at concentrations as low as 2000 ppm (6200 mg/m^3); hence, that concentration was taken as a lowest-observed-adverse-effect level (LOAEL). The steepness of the dose-response curve (see Table 3-3) suggests that a factor of only 3, rather than the usual 10, would be adequate to estimate a NOAEL; therefore, the NOAEL was estimated to be 2000 mg/m^3. From the Battelle results (1988a), a NOAEL for ataxia in rats was 450 ppm (1400 mg/m^3) and in mice it was 900 ppm (2800 mg/m^3). Thus, in the most susceptible species (rats), the NOAEL for ataxia appears to be about 1500 mg/m^3. Applying the default species factor of 10, gives an AC for humans of 150 mg/m^3 (50 ppm). This limit is independent of exposure time since blood concentrations stabilize a few hours after the start of an exposure and prolonged exposure leads to reduced blood concentrations for a given airborne concentration (McComb and Goldstein, 1979a).

Renal Lesions and Tumors

A dose-dependent increase in the severity of renal lesions was found in exposed male rats in the drinking-water and inhalation studies (Table 3-1), but was not found not in females or other species (NTP, 1994; Battelle, 1988b). Such lesions are difficult to apply to human risk assessment, because male rats produce α2u-globulin in large amounts, and this protein binds hydrocarbons or their metabolites to yield a complex that is difficult to degrade (Swenberg et al., 1989). The formation of such a complex has not been demonstrated specifically for *tert*-buta-

nol; however, a complex has been demonstrated for structurally similar compounds, such as 2,4,4-trimethyl-2-pentanol (Borghoff et al., 1995). Since humans do not produce this globulin, they should have a markedly reduced risk for hydrocarbon-induced nephropathy compared with male rats (Borghoff et al., 1990). Eventually, the nephropathy (as observed in the 13-w inhalation study) can lead to cell death and carcinogenesis through restorative cell replication (Borghoff et al., 1990), as was evident in the 2-y drinking-water study (NTP, 1994).

The risk analysis for renal injury must consider the fact that nephropathy was reported only in female rats in the 13-w drinking water study, and only in male rats in the 13-w inhalation study. In female rats, the incidence of nephropathy in the drinking-water study showed a NOAEL at an average dose of 0.50 g/kg/d (0.5%) for 13 w (NTP, 1994). The highest inhalation dose to female rats was 2100 ppm (measured average concentration), and that was a NOAEL (Battelle, 1988b). These inhalation exposures were for 6 h, so a 0.3-kg rat inhaling air at 0.15 m^3/d with *tert*-butanol at 6.5 g/m^3 (2100 ppm) with an uptake of 50% would receive a daily dose as follows:

$$D_{inhal} = 6.5 \text{ g/m}^3 \times 0.15 \text{ m}^3\text{/d} \times 1/0.3 \text{ kg} \times 0.50 \times (6 \text{ h}/24 \text{ h}) = 0.4 \text{ g/kg/d}.$$

That dose is less than the oral NOAEL; therefore, it is not surprising that nephropathy was not reported in female rats in the inhalation study. Using the default species factor of 10 and conservatively assuming Haber's rule, an AC (renal injury) is calculated as follows:

$$\text{30-d AC} = 6500 \text{ mg/m}^3 \times 1/10 \text{ (species)} \times (390 \text{ h}/720 \text{ h}) = 350 \text{ mg/m}^3.$$

To extend this NOAEL to shorter or longer exposure times, the highest NOAELs in female rats were compared in the drinking-water studies (Table 3-6). The LOAELs were included in Table 3-6 to indicate that the NOAELs were within a factor of 2 of the lowest-effect level. An eightfold increase in the exposure time (from 13 w to 2 y) resulted in a decrease in the NOAEL of only threefold (from 0.50 g/kg/d to 0.175 g/kg/d).

Hence, an increase in astronaut exposure time from 30 d to 180 d

TABLE 3-6 Effect of Exposure Time on NOAELs for Nephropathy in Drinking-Water Studies

Exposure Length	NOAEL, g/kg/d	LOAEL, g/kg/d	Reference
13 w	0.50	0.76	Lindamood et al., 1992
2 y	0.175	0.33	NTP, 1994

(sixfold) should require only a reduction of threefold in the exposure concentration to be assured of a NOAEL. The 180-d AC was determined to be 120 mg/m^3 (about one third the 30-d AC of 350 mg/m^3). The 7-d (168 h) AC can be set from the female rat data as recommended by the NRC (1992) for extrapolation to shorter exposure times, that is, by not increasing the exposure concentration. Using the default species factor of 10, the 7-d AC was calculated to be 650 mg/m^3 for nephropathy.

Urinary-Bladder Injury

The findings on urinary-bladder injury were analogous to those for nephropathy; lesions were found in the 13-w drinking-water study, but not in the inhalation study. The 13-w drinking water study suggested a NOAEL for urinary-bladder injury of 0.8 g/kg/d (1%) in the male rat, the most sensitive species and sex. This dose is well above that calculated for the highest inhalation dose (0.4 g/kg/d). The highest dose in the 2-y study (0.42 g/kg/d) did not cause urinary-bladder injury in male rats, suggesting that this lesion is more dependent on dose than length of exposure. This conclusion is also reached by inspection of the data on male mice. The NOAEL for 13-w exposures in the drinking-water study was 1.6 g/kg/d, whereas the NOAEL in the 2-y study was 1.0 g/kg/d (a LOAEL was found at 2.1 g/kg/d). These data suggest that using Haber's rule to extrapolate the 13-w inhalation NOAEL of 6.5 g/m^3 to other exposure times would be too conservative. The data suggest that the 13-w inhalation NOAEL would be suitable for 7 and 30 d of exposure, and half that concentration would easily be a NOAEL for 180 d of exposure. Hence, applying the default species factor, the ACs were set as follows:

7-d and 30-d ACs = 650 mg/m^3
180-d AC = 300 mg/m^3.

Thyroid Lesions and Tumors

After review of the possible mechanisms leading to the thyroid adenomas in mice, consideration of the differences between rodent and human thyroid function, and the lack of any lesions in the inhalation studies, it was concluded that the data were inappropriate for human health risk estimates. The observations and rationale are summarized below.

Thyroid lesions were not reported in rats exposed to *tert*-butanol by any route; however, the incidence of follicular-cell hyperplasia statistically increased in male mice given the alcohol in drinking water for 2 y at concentrations of 5, 10, or 20 mg/mL and in female mice given 10 or 20 mg/mL. The incidence of follicular-cell adenomas was increased only in the 20-mg/mL group of female mice. Such adenomas are thought to result from progression of follicular-cell hyperplasia and can progress to carcinomas over time (one male in the 20-mg/mL exposure group had a carcinoma). Such proliferative lesions in mice might not be appropriate as models for human thyroid lesions, depending on the mechanism of injury. These lesions could be due to direct action of *tert*-butanol or a metabolite on follicular-cell DNA, but genotoxicity has not been demonstrated for *tert*-butanol despite extensive testing. Even if direct mechanisms were involved, McClain (1992) has pointed out that the strong promoting effect of thyroid-stimulating hormone (TSH) in rodents is likely to lead to over estimation of the risk in other species with lower TSH levels.

The weight of evidence suggests a nongenotoxic mechanism that might involve the stimulation of excess production of TSH, but humans appear to be less sensitive than experimental animals to TSH-stimulating chemicals (Hill et al., 1989). There are marked differences in thyroid function between humans and rodents, and these differences make the "rodent an inappropriate model for the extrapolation of cancer risk to man for chemicals that operate secondary to hormone imbalance" (McClain, 1992). The serum TSH is many times higher in rodents than in humans, and rodents lack the thyroid-binding globulin found in humans and some other species. These differences suggest a much higher

thyroid-gland activity in rodents when compared with humans (Dohler et al., 1979). These differences in thyroid activity are reflected in the interspecies differences in baseline thyroid tumor incidences. For example, the expected lifetime incidence of thyroid cancer in adult humans is 0.2% (males) and 0.5% (females), whereas the estimated neoplasm incidence at the end of the 2-y drinking-water study in control mice was 3.6% (males) and 5.6% (females) (NCRP, 1989; NTP, 1994).

A final argument against using the thyroid data from the drinking-water study for a human inhalation risk estimate is that no effects on the thyroid were observed in any groups involved in the 13-w inhalation study (Battelle, 1988b). Because the highest concentration in the rodent inhalation study was 2100 ppm, it is very unlikely that a less susceptible species, such as humans, would have a significant risk of thyroid tumors at a 40-fold lower concentration (50 ppm vs. 2100 ppm) than the one that was a NOAEL in rodents.

Spaceflight Effects

The adverse effects resulting from exposure to *tert*-butanol would not be expected to be potentiated by the physiological and biochemical changes caused by spaceflight.

TABLE 3-7 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors			Acceptable Concentrations, mg/m^3				
		Species	Time	To NOAEL	1 h	24 h	7 d	30 d	180 d
CNS effects									
NOAEL, 1500 mg/m^3, 12 × 6 h (Battelle, 1988a; Nelson et al., 1989)	Rat	10	1	See text	150	150	150	150	150
Kidney lesions									
NOAEL, 6500 mg/m^3, 65 × 6 h (Battelle, 1988b)	Rat, F	10	See text	1	—	—	650	350	120
Urinary-bladder lesions									
NOAEL, 6500 mg/m^3, 65 × 6 h (Battelle, 1988b)	Rat, M	10	See text	1	—	—	650	650	300
SMACs					**150 (50 ppm)**	**150 (50 ppm)**	**150 (50 ppm)**	**150 (50 ppm)**	**120 (40 ppm)**

—, Data not considered applicable to the exposure time.

RECOMMENDATIONS

The most important weakness in the data base is the lack of information about the effects of short-term exposures in humans. No data were found on the irritation thresholds or potential performance decrements in humans exposed for several hours to *tert*-butanol. Other alcohols show significant irritant properties and cause readily measured performance decrements. The data base for long-term exposures appears to be relatively complete, particularly in view of the recent completion of a 13-w inhalation study. The study has not been peer reviewed by NTP.

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B4 Diacetone Alcohol

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PHYSICAL AND CHEMICAL PROPERTIES

Synonyms:	Diketone alcohol, 4-hydroxy-4-methyl-2-pentanone
Formula:	$(CH_3)_2C(OH)CH_2COCH_3$
CAS number:	123-42-2
Form:	Colorless, flammable liquid with pleasant odor
Molecular weight:	116.16
Boiling point:	168°C
Melting point:	−43°C
Specific gravity:	0.94
Vapor pressure:	1.2 mmHg at 25°C
Solubility:	Miscible in water
Conversion factors:	1 ppm = 4.76 mg/m^3 1 mg/m^3 = 0.21 ppm

OCCURRENCE AND USE

Diacetone alcohol (DAA) is used as an industrial solvent for nitrocellulose, cellulose acetate, celluloid, pigments, waxes, fats, and oils, and in antifreeze and brake fluid (ACGIH, 1991). The odor threshold has been reported to be near 0.3 ppm (Amoore and Hautala, 1983). Concentrations in the space shuttle are typically below 1 mg/m^3; how-

ever, approximately 10% of the time, DAA concentrations above 1 mg/m^3 were found (James et al., 1994). The source of DAA is probably off-gassing from paint that has not totally cured and possibly off-gassing from hardware.

TOXICOKINETICS AND METABOLISM

Absorption

No data were found on the absorption of DAA.

Distribution

Complete distribution studies are not available on DAA; however, its blood/brain partitioning has been studied because DAA is a metabolite of methyl isobutyl ketone (Granvil et al., 1994). From 15 to 90 min after direct intraperitoneal (ip) administration to mice of DAA at 2.5 mmol/kg of body weight, the concentrations of DAA in blood and brain were comparable. Distribution into other tissues has not been reported.

Elimination

Granvil et al. (1994) showed that the concentrations of DAA in blood in mice declined from about 430 μg/mL 15 min after injection to about 70 μg/mL 90 min after injection. The dose was administered ip at a concentration of 2.5 mmol/kg.

Metabolism

No specific data were found on the metabolism of DAA. Granvil et al. (1994) reported that they did not find any biotransformation products after ip administration of DAA to mice; however, they did not make it clear exactly which possible metabolites could be detected with their analytical procedures.

TOXICITY SUMMARY

Few inhalation toxicity data were available on DAA; however, sufficient reports were found to suggest several target organs for this solvent. The data will be divided into acute and short-term exposure studies (less than 4 w duration) and subchronic and chronic exposure studies (more than 4 w duration).

Acute and Short-Term Exposures

A summary of early studies using various nonhuman species and various routes of administration of DAA suggests that DAA is narcotic and can cause injury to the liver, kidney, or red blood cells (von Oettingen, 1943). Lehman and Flury (1938) were quoted as stating that restlessness, irritation of the mucous membranes, excitement and later somnolence were seen in mice, rats, rabbits, and cats exposed to DAA at 10 mg/L (2100 ppm). That concentration exceeds the saturation concentration of 1600 ppm at 25°C (DHHS, 1988). Smyth and Carpenter (1948) reported that none of the rats (number not specified) died that were exposed to saturated DAA vapor for 8 h.

Human exposures have been conducted with the goal of setting industrial hygiene limits (Silverman et al., 1946). Apparently, about 12 subjects were exposed for 15 min to DAA at various concentrations and asked their opinion on the suitability of 8-h exposures. A majority of the test subjects indicated that they could work an 8-h day even when exposed at 100 ppm (table 2 in the paper says 50 ppm), even though irritation of the eyes and throat occurred in the majority of subjects exposed at 100 ppm. Silverman et al. (1946) considered 50 ppm a more suitable worker exposure concentration. They did not explicitly state that the 50-ppm concentration was a no-effect level.

Subchronic and Chronic Exposures

Only one study could be found on "long-term" toxic effects of DAA, and the duration of exposure of that study was only 6 w (Butterworth et al., 1980). Groups of 12 male and 12 female rats were exposed 6 h/d,

5 d/w for 6 w to DAA concentrations of 0, 230, 1040, and 4500 mg/m^3. The corresponding concentrations of DAA in parts per million were 48, 220, and 950 ppm. The rats were exposed in 1-m^3 chambers with a flow rate of about 0.45 m^3/min. The DAA concentrations were monitored continuously with total hydrocarbon analyzers, and periodic monitoring of acetone, a decomposition product, was done by gas chromatography. Acetone comprised about 4% of the highest exposure and less than 1% of the other exposures. Observations were made in the following categories: daily clinical signs, weekly body weights, terminal urinalysis, terminal hematology, terminal clinical chemistry, gross pathology, organ weights, and histopathology.

The following effects of exposure to DAA were noted:

Clinical signs: After 6-h exposures in the last 2 w of the study, the rats receiving the high dose were slightly lethargic for a few hours.

Clinical chemistry: A 50% increase over controls was noted in levels of plasma lactate dehydrogenase (LDH) measured in females in the high-dose group; however, levels of two enzymes more specific for liver injury were not increased in the exposed groups. Various minor differences in plasma electrolytes were noted, but there was not a dose-effect relationship.

Hematology: The hemoglobin values in high-dose females were 6% above the control values.

Organ weights: Relative to body weights, the livers of males in the mid- and high-dose groups were increased 13% and 24%, respectively. Likewise, the liver weights of females in the mid- and high-dose groups were increased 9% and 12%, respectively. The kidney weights of males and females in the high-dose groups were increased 17% and 7%, respectively.

Histopathology: Eosinophilic hyaline droplets were found in the kidneys of all but one male in the high-dose group. The droplets were not observed in any of the female groups or in the mid- and low-dose groups of males.

This study appears to be a high-quality study with attention paid to randomization of animals, compound purity (99.44%), appropriate air flows and environmental conditions, recovery of test material in the

chambers, thorough statistical analysis, and complete assessments of toxic end points.

Carcinogenicity

No data were found pertaining to the carcinogenic potential of DAA.

Genotoxicity

During an evaluation of several hydrocarbon solvents for genotoxicity, DAA was the only test material to exhibit any sort of positive response. The solvents were tested in bacterial mutation assays, a yeast mitotic conversion assay, and a chromosome assay in rat liver cells in vitro in concentrations of 2000 to 4000 μg/mL (Brooks et al., 1988). None of the solvents, including DAA, induced reverse gene mutations in bacteria or mitotic gene conversion in yeast. DAA caused a small, but insignificant, increase in chromatid damage in the rat liver chromosome assay. The change was not quantitatively related to dose. The damage consisted of a few chromatid exchanges, breaks, and acentric fragments. Brooks et al. (1988) speculated that the apparent clastogenic activity was likely due to the physical action of the solvent or the detergent-like properties of DAA rather than to its ability to interact directly with DNA. That conclusion is supported by the data of Shehab (1980), who found that a 3-h exposure of plant root tips to DAA (1% by weight) caused some chromosomal stickiness, which can result from random chromosomal breakage. Thus, no convincing data exist to support the possibility of genotoxic activity for DAA.

Reproductive Toxicity

No data were found on the reproductive toxicity of DAA.

Developmental Toxicity

No data were found on the developmental toxicity of DAA.

Interactions with Other Chemicals

DAA has been shown to potentiate the hepatotoxicity of chloroform in rats (Vezina et al., 1989). Their test procedure was to administer DAA orally at 0, 3.75, 5.6, or 7.5 mmol/kg and 24 h later give an ip injection of chloroform (0.5 mL/kg) to groups of six rats. The chloroform dose alone caused no apparent liver injury. DAA-potentiated liver damage was assessed by the activity of two transferases in plasma, plasma bilirubin concentrations, liver weights, and histopathological examination. Each of the end points showed a clear dose-response relationship for DAA potentiation of chloroform-induced liver injury. Vezina et al. (1989) concluded that DAA might induce one or more of the microsomal cytochrome P-450 systems that lead to increased production of reactive intermediates from chloroform.

TABLE 4-1 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	50 (TWA)	ACGIH, 1991
OSHA's PEL	50 (TWA)	U.S. Dept. of Labor, 1995
NIOSH's REL	50 (TWA)	U.S. Dept. of Labor, 1995

TLV, Threshold Limit Value; TWA, time-weighted average; PEL, permissible exposure limit; REL, recommended exposure limit.

TABLE 4-2 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	50	250	Mucosal irritation, CNS depression
24 h	50	250	Mucosal irritation, CNS depression
7 d	20	100	Mucosal irritation, CNS depression
30 d	6	30	Mucosal irritation, potential liver injury, CNS depression
180 d	4	20	Mucosal irritation, potential liver injury, CNS depression

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Even though the toxicity data base is limited, several adverse effects of DAA exposure have been clearly demonstrated by the inhalation route. Central-nervous-system (CNS) depression was noted in some of the acute exposure rodent studies and late in the 6-w rat inhalation study (Butterworth et al., 1980). Irritation of the throat, eyes, and nose has been demonstrated in humans (Silverman et al., 1946), and potential liver injury has been found in rats (Butterworth et al., 1980). The kidney injury observed in the high-dose group of male rats by Butterworth and co-workers is similar to a common finding of high concentrations of α2u-globulin present in male rats after prolonged exposure to hydrocarbons. This lesion has not been considered relevant to human risk assessment, because humans have much lower concentrations of α2u-globulin than male rats (Swenberg et al., 1989). The guidelines from the National Research Council were used to develop the rationale for DAA SMACs (NRC, 1992).

CNS Depression

There were no acute inhalation studies suitable for human risk assessment; however, Butterworth et al. (1980) noted that their high-dose group of rats did not exhibit postexposure lethargy until week 5 of the 6-w intermittent 6-h/d exposures. Hence, their highest exposure concentration (950 ppm) was considered a no-observed-adverse-effect level (NOAEL) for CNS effects from short-term exposures. The short-term acceptable concentrations (ACs) were as follows:

1- or 24-h AC = 950 ppm × 1/10 (species) = 95 ppm.

The fact that "lethargy" developed late in the 6-w exposure at the highest concentration suggests that lower limits might be needed for long-term exposure. The mid-dose group (220 ppm) never exhibited any obvious CNS effects during the 6-w exposure (180-h cumulative), so 220 ppm is a logical choice for a NOAEL in rats. The long-term ACs for CNS effects were calculated as follows:

7-d AC = 220 ppm × 1/10 (species) = 22 ppm.
30-d AC = 220 ppm × 1/10 (species) × (180 h/720 h) = 6 ppm.

Although there is some concern that the lethargy observed late in the exposures was not the traditional type of solvent-induced CNS depression, the 6-ppm limit should be a threshold below which no CNS effects can occur no matter how long the exposure lasts. Hence, the 180-d AC was also set at 6 ppm.

Irritation of Eyes and Throat

The human exposure study reported by Silverman et al. (1946) indicated that in the 12 (presumably) test subjects, 50 ppm would be an acceptable industrial concentration to avoid irritation; therefore, that concentration was selected as a NOAEL for irritation. The majority of subjects reported an unspecified degree of irritation at 100 ppm. The exposures were for only 15 min, but that time should have been long

enough to elicit a maximal irritation response in the subjects. Even though slight irritation would be permitted in short-term ACs, 50 ppm was selected as the limit for accidental astronaut exposures (1 and 24 h ACs). For long-term ACs, the risk of irritation must be kept very low, and, because only 12 subjects were tested, the limit must be reduced using an uncertainty factor for the small n as follows:

$$\text{7, 30, 180-d AC} = 50 \text{ ppm} \times \frac{\sqrt{12}}{10} \text{ (small } n \text{ factor)} = 17 \text{ ppm.}$$

This limit should preclude DAA induced irritation from occurring in any large group of astronauts exposed to DAA.

Potential Liver Injury

The hepatomegaly reported in rats exposed at concentrations of 220 or 950 ppm for 6 w (Butterworth et al., 1980) and the ability of DAA to enhance the hepatotoxicity of chloroform suggest that DAA can be a potential hepatotoxicant. Because histopathological changes were not detected in the livers of rats exposed at 950 ppm for 180 h (cumulative), that concentration becomes the NOAEL. No data suggested an approach less conservative than Haber's rule to protect against potential liver injury; therefore, the ACs to avoid liver injury were set as follows:

7-d AC = 950 ppm × 1/10 (species) = 95 ppm.
30-d AC = 950 ppm × 1/10 (species) × (180 h/720 h) = 24 ppm.
180-d AC = 950 ppm × 1/10 (species) × (180 h/4320 h) = 4 ppm.

The liver is a remarkably adaptive organ, and it is unlikely that exposures need to be kept this low to prevent liver injury; however, specific data on DAA were not available to predict the effects of variations in exposure times.

TABLE 4-3 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors			Acceptable Concentrations, ppm				
		Species	Time	Small *n*	1 h	24 h	7 d	30 d	180 d
CNS effects									
NOAEL, 950 ppm, 6 h/d (Butterworth et al., 1980)	Rat	10	1	1	95	95	—	—	—
NOAEL, 220 ppm, 6 h/d, 5 d/w, 6 w (Butterworth et al., 1980)	Rat	10	1	1	—	—	22	6	6
Irritation									
NOAEL, 50 ppm, 15 min, 12 subjects (Silverman et al., 1946)	Human	1	1	√12	50	50	17	17	17
Potential liver injury									
NOAEL, 950 ppm, 120 h cumulative (Butterworth et al., 1980)	Rat	10	HR	1	—	—	95	24	4
SMACs					**50**	**50**	**20**	**6**	**4**

—, Data not considered applicable to the exposure time; HR, Haber's rule.

RECOMMENDATIONS

Given the fairly widespread use of DAA, it is surprising that at least a subchronic inhalation study has not been reported. This type of study would determine whether the risk of liver injury increases with exposure time (Haber's rule) or is negligible below some threshold concentration. Acute inhalation exposures in animals with performance assessments would determine if CNS effects occur that have not been reported. Metabolism studies would be useful to define the metabolites of DAA and to measure the rates of uptake and elimination. On the other hand, astronaut exposures to this chemical will be rare, and an accidental release is unlikely to involve significant exposures to DAA.

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B5 Dichloroacetylene

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PHYSICAL AND CHEMICAL PROPERTIES

Dichloroacetylene (DCA) is a liquid with the following properties (Torkelson and Rowe, 1981):

Synonyms:	1,2-Dichloroacetylene, dichloroethyne
Formula:	$CCl \equiv CCl$
CAS number:	7572-29-4
Molecular weight:	94.9
Boiling point:	Explodes upon boiling
Melting point:	−66°C to −64.2°C
Specific gravity:	0.94
Solubility:	Insoluble in water; soluble in organic solvents
Flash point:	Spontaneously combustible
Conversion factors at 25°C, 1 atm:	1 ppm = 3.9 mg/m^3
	1 mg/m^3 = 0.24 ppm

OCCURRENCE AND USE

DCA is the major product formed from trichloroethylene (TCE) in carbon dioxide scrubbers containing alkaline materials (Saunders, 1967). Although TCE is not used in the spacecraft, it has been found in numerous atmospheric samples collected from the cabin of the space

shuttle (Coleman, 1985) and is a potential source of DCA in spacecraft. In a National Aeronautics and Space Administration (NASA) Toxicology Laboratory experiment, all the TCE disappeared when passed over heated alkaline adsorbent, with at least 75% conversion to DCA (Rippstein, 1980). Alkaline decomposition of 1,1,2,2-tetrachloroethane also results in formation of DCA.

TOXICOKINETICS AND METABOLISM

Absorption

DCA appears to be efficiently absorbed by the respiratory system of rodents; however, toxic effects are not expressed there (Reichert et al., 1984; Kanhai et al., 1989).

Distribution

DCA has marked organotropism for the kidney where it reacts with neutrophils to yield compounds that cause tissue injury (Kanhai et al., 1989).

Elimination

In rats exposed to 36 ppm of a DCA-diethyl ether complex, a large fraction of inhaled DCA is metabolized to *N*-acetyl-S-(1,2-dichlorovinyl)-L-cysteine (*N*-ADCVC) and excreted in the urine. Kanhai et al. (1989) estimated that 10% of the complex introduced into a nose-only inhalation system during 1-h exposures was eliminated within 24 h as *N*-ADCVC in the urine of male rats. The metabolite was postulated to be a detoxification product leading away from pathways that produce compounds capable of acylation of macromolecules in the kidney (Kanhai et al., 1989).

Metabolism

In vitro studies have shown that DCA can be readily metabolized to *S*-(1,2-dichlorovinyl)-glutathione (DCVG) by microsomes from rat liver

and kidney; however, the metabolism is slower in cytosol fractions from the same tissue (Kanhai et al., 1989). Metabolic pathways have been proposed that result in conversion of DCVG to *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC). DCVC could then be cleaved to form reactive metabolites (e.g., enethiols), which lead to acylation of macromolecules. Alternatively, DCVC could be acetylated to form *N*-ADCVC, which is eliminated in the urine of rats (Kanhai et al., 1989).

TOXICITY SUMMARY

Acute Exposures

Human exposures to DCA have occurred as a result of its formation from TCE in closed-circuit anesthesia machines (Defalgue, 1961). These exposures have shown that DCA is neurotoxic to humans, characteristically producing functional impairment of certain cranial nerves, particularly the trigeminal (Carden, 1944; Humphrey and McClelland, 1944; Reichert et al., 1976). Predominant symptoms reported include headache, nausea, and vomiting, which are followed, some hours later, by numbness in the perioral region, anesthesia throughout the facial skin and mucosa, and analgesia or reduced sensitivity over the entire distribution of the trigeminal nerve, with loss of the corneal reflex (Carden, 1944; Humphrey and McClelland, 1944; Reichert et al., 1976). In addition, labial sores, visual disturbances, and impaired taste and smell sensations frequently occur (Carden, 1944; Humphrey and McClelland, 1944; Reichert et al., 1976). Two fatalities occurred among several cases of cranial-nerve palsies attributed to the anesthetic use of TCE in a hospital (Humphrey and McClelland, 1944). The concentration of DCA was not measured in any of the poisonings involving the use of TCE in anesthesia machines (Siegel et al., 1971).

In animals, the principal target organs of acute exposure to DCA are the kidney and, to a lesser extent, the liver and brain. A 1-h exposure of NMRI mice to DCA at a concentration of 101 ppm caused the most marked pathological changes in the kidneys, consisting of extensive necrosis of the distal proximal tubules (Reichert et al., 1975). In the liver, there were fat accumulations, vacuolation, and basophilic cytoplasm. The brain showed generalized tissue edema and axonal swelling, atrophied cells in the brain stem, and shrinkage of ganglion cells.

A 1-h exposure of New Zealand albino rabbits to DCA at 126, 202, or 307 ppm caused extensive necrosis of the collecting tubules of the kidneys, with subsequent tubule calcification and scar formation (Reichert and Henschler, 1978). A slight and transitory increase in blood urea nitrogen (BUN) was evident at 126 ppm, and, at 202 ppm, the increase was large and prolonged, indicating a severe uremia. At 202 and 307 ppm, there were morphological changes in the liver and serum enzyme changes indicative of acute but reversible liver cell damage. At 126 ppm, liver pathological changes were less severe, and marker enzymes for liver damage were normal. The effects on the kidneys and livers of rabbits exposed at 17-23 ppm for 6 h were similar to those at 126 ppm for 1 h (Reichert and Henschler, 1978).

Histological changes in the brains of New Zealand albino rabbits after a 1-h exposure to DCA at 126, 202, or 307 ppm were evident in the sensory cortical regions and were concentration-related (Reichert et al., 1976). The most severe injury was to the sensory trigeminal nucleus, followed in decreasing intensity by the facial and oculomotor nerves and the motor trigeminal nucleus. A 6-h exposure at 17 ppm caused somewhat less severe damage than a 1-h exposure at 126 ppm (Reichert et al., 1976).

Short-Term Exposures

Human exposures to DCA also have resulted from its formation from TCE in environmental control systems containing alkaline, carbon dioxide scrubbers (Saunders, 1967). One serious incident occurred in a test of a sealed environmental chamber (Saunders, 1967). Within 48 h of the start of the test, a distinct, sweet-sour odor developed, and became increasingly irritating and nauseating to the five-man crew. After the third and fourth day, the crew experienced headaches, vomiting, itching around the eyes, sore gums, and painful jaws, and the test was terminated. Shortly thereafter, severe cold sores developed. Analyses of desorbed charcoal samples identified several volatile compounds, including TCE as the most prevalent contaminant, DCA, and monochloroacetylene (MCA). Saunders (1967) attributed the symptoms to DCA, but did not provide any information on its concentration in the chamber. The American Conference of Governmental Industrial Hygienists (ACGIH) cited this report as the reference for its statement that dis-

abling nausea occurred in at least 85% of individuals exposed to DCA at about 0.5 to 1 ppm for prolonged periods (ACGIH, 1991). Apparently, the ACGIH was referring to another incident and provided the wrong reference.

In a simulated flight test, the performance and health of a pig-tailed monkey deteriorated after 2 or 3 d (Saunders, 1969). DCA and TCE were detected at concentrations of 0.1 and 0.3 ppm, respectively. Although Saunders (1969) was of the opinion that DCA was the causative agent, the evidence was weak.

Subchronic and Chronic Exposures

Noncancer Toxicity

The principal target organ of repeated and continuous exposures of the NMRI:O(SD) rat to DCA is the kidney. Exposures to DCA at 15.5 ppm (with TCE at 150 ppm as a stabilizer) for 6 h/d, 5 d/w, for 6 w caused large increases in cytoplasmic and nuclear mass of epithelial cells of proximal convoluted tubules (Siegel et al., 1971). Similar repeated exposures to DCA at 9.8 ppm (with TCE at 50 ppm) produced some nonspecific toxic effects without any morphological changes to the kidneys, and DCA at 2.8 ppm (with TCE at 3.2 ppm) resulted in neither toxic signs nor pathological changes. Continuous exposure of rats at 2.8 ppm (with TCE at 5.3 ppm) for 24 h/d for 90 d caused kidney pathological changes similar to those from repeated exposures at 15.5 ppm (Siegel et al., 1971). In addition, some animals exposed continuously developed weakness in the hind legs and had difficulty walking, indicating neurological effects. The investigators attributed the renal changes to DCA, and not to TCE or acetaldehyde, which was present as an impurity at 4 ppm in the repeated and continuous 2.8-ppm DCA studies (Siegel et al., 1971). As evidence for their conclusions, they cited the work of Prendergast et al. (1967) in which repeated exposures to TCE at 713 ppm and continuous exposure at 35 ppm did not produce any morphological changes in the kidneys or other organs of rats. A continuous 90-d exposure of rats to acetaldehyde at 5 ppm also did not produce renal changes (Siegel et al., 1971).

Similar histopathological changes were observed in the kidneys of NMRI:O(SD) rats exposed to DCA at 4.8 ppm (with ether at 33 ppm as

a stabilizer) for 24 h/d for up to 28 d (Jackson et al., 1971). The renal lesion was observed initially after 4 d of exposure and increased in prevalence and severity up to 28 d of exposure. Of six rats observed for up to 9 d, four showed signs of hind-leg weakness, two had self-inflicted bite wounds, and three died. Renal pathological changes were not present in control animals exposed continuously to ether at 49 ppm for up to 30 d or to 50 ppm for 90 d (Jackson et al., 1971).

Carcinogenicity

A small number of studies suggest that DCA might be carcinogenic to both NMRI mice and Wistar rats. In male and female NMRI mice, exposures to DCA (with acetylene as a stabilizer) at 9 ppm for 6 h/d, 1 d/w, for 12 mo (group 1), 2 ppm for 6 h/d, 1 d/w, for 18 mo (group 2), or 2 ppm for 6 h/d, 2 d/w, for 18 mo (group 3) resulted in apparent increases in the incidence of cystic kidney tumors (Reichert et al., 1984). Unfortunately, this finding was confounded by the presence of numerous oncocytomas in the kidneys of some control groups. Cystadenocarcinomas in male mice were found at an incidence of 12/30 in group 2 (low dose for 1 d/w), but the incidence in group 3 (low dose for 2 d/w) was only 3/30. No adenocarcinomas were reported in female mice. In some cases, lung tumors were more common in controls than in exposed mice. For example, male controls in group 2 had an incidence of lung adenomas of 8/30, whereas the exposed group had an incidence of only 3/30. Malignant lymphomas were much more common in male and female controls for group 1, with incidences of 14/30 and 17/30, respectively, versus incidences of 1/30 and 2/30 in exposed male and female mice, respectively. Harderian gland tumors, commonly seen in this strain of mice, were increased in DCA-exposed mice, but this gland is not present in human beings.

The carcinogenic findings were no clearer in male and female Wistar rats exposed to DCA (6 h/d, 2 d/w, 18 mo). The incidence of renal cystadenomas was higher in exposed males (7/30) than in controls (0/30); however, far more oncocytomas occurred in male controls (14/30) than in exposed males (0/30). In exposed rats, liver cholangiomas increased in both sexes. However, because only one group was exposed, a quantitative risk estimate was not attempted on these tumors. Overall, the data are considered to provide limited evidence of carcino-

genicity, a finding that agrees with the assessment of DCA by the International Agency for Research on Cancer (IARC, 1986).

Genotoxicity

DCA is mutagenic for strain TA100 *Salmonella typhimurium* if the bacteria are suspended in Oxoid medium to promote active bacterial growth (Reichert et al., 1983).

Biological Interactions

Lethality data suggest possible biological interactions between DCA and TCE or ether, but the data are sparse (Siegel et al., 1971). In NMRI:O(SD) rats, the 4-h LC_{50} value for TCE was 12,500 ppm, and the values for DCA in DCA–TCE (1:7) and DCA–ether (1:9) mixtures were 55 and 219 ppm, respectively. Also, in NMRI:ASH and FTD:Hartley guinea pigs, the 4-h LC_{50} value for DCA in a DCA–ether (1:9) mixture was approximately 4 times higher than that for DCA in a DCA–TCE (1:10) mixture.

TABLE 5-1 Toxicity Summary

Concentration	Exposure Duration	Species	Effects	Reference
NS	NS	Human	Headache, nausea, vomiting, trigeminal nerve involvement from TCE in closed-circuit, anesthesia machine,	Defalgue, 1961; Carden, 1944; Humphrey and McClelland, 1944
NS	NS	Human	Fatalities after TCE anesthesia in closed-circuit machine	Humphrey and McClelland, 1944
0.1 ppm	8 d	Monkey	Anorexia, vomiting, difficulty closing mouth	Saunders, 1969
Uncertain	3.5 d	Human	Headache, nausea, vomiting, painful gums and jaw, cold sores	Saunders, 1967
2 ppm (with acetylene)	6 h/d, 1 d/w, 18 mo	Mouse (NMRI)	Limited evidence that DCA might be tumorigenic to the kidney; increase in Harderian gland tumors over controls	Reichert et al., 1984
2 ppm (with acetylene)	6 h/d, 2 d/w, 18 mo	Mouse (NMRI)	Limited evidence that DCA might be tumorigenic to the kidney; increase in Harderian gland tumors over controls	Reichert et al., 1984
9 ppm (with acetylene)	6 h/d, 1 d/w, 12 mo	Mouse (NMRI)	Limited evidence that DCA might be tumorigenic to the kidney; increase in Harderian gland tumors over controls	Reichert et al., 1984
2.8 ppm (TCE at 5.3 ppm)	24 h/d, 90 d	Rat (NMRI:O[SD])	Renal pathological changes, depressed body-weight gain, hind-leg weakness in 2/8, 1/8 blind	Siegel et al., 1971
2.8 ppm (TCE at 3.2 ppm)	6 h/d, 5 d/w, 6 w	Rat (NMRI:O[SD])	No toxic signs or hematological and biochemical changes; no pathological changes	Siegel et al., 1971
4.8 ppm (ether at 33 ppm)	24 h/d, up to 28 d	Rat (NMRI:O[SD])	Renal pathological changes first appear at 4 d, worsen at 28 d; hind-leg weakness in some	Jackson et al., 1971

9.8 ppm (TCE at 50 ppm)	6 h/d, 5 d/w, 6 w	Rat (NMRI:O[SD])	Respiratory distress 5th and 6th w, some blood changes; no renal pathologicalchanges	Siegel et al., 1971
14 ppm (with acetylene)	6 h/d, 2 d/w, 18 mo	Rat (Wistar)	Mixed evidence that kidney tumors were induced; probable increase in liver cholangiomas	Reichert et al., 1984
15 ppm (TCE at 150 ppm)	4 h	Guinea pig (NMRI:ASH or FTD:Hartley)	Half of animals died; convulsions preceded deaths	Siegel et al., 1971
15.5 ppm (TCE at 150 ppm)	6 h/d 5 d/w, 6 w	Rat (NMRI:O[SD])	Renal pathological changes, depressed body-weight gain, some blood changes, respiratory distress 5th and 6th w	Siegel et al., 1971
17-23 ppm	6 h	Rabbit (New Zealand albino)	Renal pathological changes, slight increase in BUN; some liver changes, normal enzymes, histological changes in brain	Reichert et al., 1976; Reichert and Henschler, 1978
19 ppm (with TCE)	6 h	Mouse (NMRI)	Half of animals died	Reichert et al., 1975
52 ppm (ether at 468 ppm)	4 h	Guinea pig (NMRI:ASH or FTD:Hartley)	Half of animals died; convulsions preceded deaths	Siegel et al., 1971
55 ppm (TCE at 385 ppm)	4 h	Rat (NMRI:O[SD])	Half of animals died	Siegel et al., 1971
101 ppm (with TCE)	1 h	Mouse (NMRI)	Marked pathological changes in kidneys; pathological changes in liver and brain	Reichert et al., 1975
124 ppm (with TCE)	1 h	Mouse (NMRI)	Half of animals died	Reichert et al., 1975

TABLE 5-1 *(Continued)*

Concentration	Exposure Duration	Species	Effects	Reference
126, 202, or 307 ppm (with TCE)	1 h	Rabbit (New Zealand albino)	Some deaths at 202 and 307; renal pathological changes at all concentrations, large increase in BUN at 202 and 307, slight at 126; liver pathological changes and enzyme changes at 202 and 307, slight pathological changes and normal enzymes at 126; concentration-related brain histopathological changes	Reichert et al., 1976; Reichert and Henschler, 1978
219 ppm (ether at 1971 ppm)	4 h	Rat (NMRI:O[SD])	Half of animals died	Siegel et al., 1971

[a]NS, not specified.

TABLE 5-2 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	0.1 (ceiling)	ACGIH, 1995

TLV, Threshold Limit Value.

TABLE 5-3 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	0.6	2.4	Neurotoxicity, kidney, and liver
24 h	0.04	0.16	Neurotoxicity, kidney, and liver
7 d	0.03	0.12	Neurotoxicity and kidney
30 d	0.025	0.1	Neurotoxicity and kidney
180 d	0.015	0.06	Neurotoxicity and kidney

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Based on accidental exposures of human beings to DCA during trichloroethylene anesthesia and during closed environmental testing, it is apparent that the principal target organ of DCA is the nervous system for exposures of a few days or less. The human data cannot be used to set safe exposure concentrations because the DCA concentrations were never reported in descriptions of the accidents. DCA has been tested in a number of animal species and has elicited neurotoxicity, nephrotoxicity, hepatotoxicity, respiratory distress, and lethality. The only animal exposures that result in neurotoxicity similar to that found in human beings (trigeminal nerve injury) are those involving rabbits (Reichert et al., 1976). The results of the only cancer study conducted on DCA provided limited evidence of its carcinogenicity; however, the study

protocol was unconventional, and the results are difficult to interpret (Reichert et al., 1984). For these reasons, a cancer risk assessment was not attempted on DCA. Reported respiratory-system injury was probably due to DCA decomposition into phosgene when appropriate stabilizer concentrations were not used during high-concentration exposures (Reichert et al., 1975). This decomposition does not occur at DCA concentrations below a few parts per million even without a stabilizer; hence, respiratory-system injury is not considered in detail below.

Nephrotoxicity

Renal injury appears to be the primary finding in rats (Jackson et al., 1971) and mice (Reichert et al., 1975) exposed to DCA. The lethality seen in mice has been attributed to the severe renal damage caused by DCA; however, this type of injury has not been observed in humans exposed to DCA unless severe neurological symptoms were already apparent (Reichert et al., 1975). Renal injury has been demonstrated in rabbits, and neurotoxicity similar to that in humans has also been shown (see below). The acceptable concentrations (ACs) to prevent nephrotoxicity in humans were estimated from acute (1- and 6-h) exposures in rabbits and longer (2- to 90-d) exposures in rats. Exposure concentrations causing mild-to-moderate effects, as evident by serum urea nitrogen increases and histopathological changes, were reduced by a factor of 10 to reach a no-observed-adverse-effect level (NOAEL) and by a factor of 10 for species differences. Using Haber's rule, which appears to represent the acute data well, the 6-h NOAEL concentration of 0.2 ppm (20 ppm $\div$ 100) was reduced by a factor of 4 to estimate a human 24-h NOAEL of 0.05 ppm. The 1-h AC (renal) was set at 1.3 ppm. Rat data were used in a similar manner to derive ACs (renal) for 7-d, 30-d, and 180-d exposures. A factor of 20 was used to estimate a NOAEL from the 28-d, 5-ppm exposures because 11 of 11 rats showed moderate-to-severe changes (see footnote *a* to Table 5-4).

Hepatotoxicity

The liver injury associated with DCA exposures has been seen in several species, although the extent of injury is typically less severe

than renal injury. Again, rabbit and rat data were used along with factors of 10 for reaching a NOAEL (if appropriate) and for species extrapolation. Haber's rule was used to estimate 24-h and 180-d levels from 6-h and 90-d data, respectively. The 30-d AC was set at 0.1 ppm because 0.1 ppm was determined to be a safe concentration for 7 and 180 d.

Neurotoxicity

Neurotoxic effects in rabbits and rats were used to set ACs that would protect human beings from DCA-induced neurotoxicity. The cranial nerve effects in rabbits are a good model of human neurotoxicity, whereas hind-limb weakness was the major manifestation of the neurotoxicity in rats. The moderate histological effects noted in the rabbits exposed at 126 ppm for 1 h were not considered a lowest-observed-adverse-effect level (LOAEL). Based on the severe effects seen at 202 ppm, a factor of 2 applied to the moderate effects at 126 ppm was estimated to give a LOAEL. The effects reported after 17-ppm exposures for 6 h were mostly mild; hence, the factor of 2 was not applied. From the calculated or observed LOAELs, factors of 10 were used to reach NOAELs and to extrapolate animal data to human. Using the approach recommended by the National Research Council (NRC, 1992) for extrapolating to shorter exposure times, the 7-d and 30-d ACs were determined from the 90-d NOAEL of 0.03 ppm without increasing the concentration.

Lethality

Lethality data were available for mice and rats. Using the NRC-recommended "benchmark" approach, the 95% limit of the LC_{10} was calculated from the dose-response curves in mice exposed for 1 h or 6 h, and these values were reduced by factors of 10 for species extrapolation and setting a NOAEL; a factor of 4 was applied to the 6-h value to determine a 24-h estimate. The resulting values were not used because the estimated concentrations to protect against lethality were far below concentrations estimated to protect against neurotoxicity, the major effect seen in human beings. Estimates for the 7-d, 30-d, and 180-d ACs

(lethality) were based on concentrations that caused no deaths in rats after prolonged exposures. Only a species factor was applied to these "NOAEL" concentrations. This approach led to reasonable ACs (lethality), which were approximately 10-fold above concentrations that protect against sublethal injury to the kidney, liver, or nervous system.

Summary

The SMACs were set to protect against kidney injury and neurotoxicity at each potential exposure time. For 1- and 24-h exposures, hepatotoxicity was also a factor in setting SMACs. Human exposure data were useful only to the extent that they demonstrated that neurotoxicity is the primary effect in humans. Analysis of acute lethality data in mice led to values that were inconsistent with sublethal effects, so the lethality results were not used. Data on carcinogenic potential were not used because there was not an appropriate way to apply the linearized multistage model to the data.

TABLE 5-4 Acceptable Concentrations

Effect, Data, (Number)[a]	Species	Uncertainty Factors: To NOAEL	Species	Time	Space-flight	Acceptable Concentrations, ppm: 1 h	24 h	7 d	30 d	180 d
Nephrotoxicity										
Mild to moderate, 126 ppm, 1 h (1)	Rabbit	10	10	1	1	1.3	—	—	—	—
Mild to moderate, 20 ppm, 6 h (1)	Rabbit	10	10	4 (HR)	1	—	0.05	—	—	—
0/6, 5 ppm, 2 d (2)	Rat	1	10	1	1	—	0.5	0.05	—	—
4/6 (slight), 5 ppm, 7 d (2)	Rat	10	10	1	1	—	—	—	—	—
11/11 (moderate to severe), 5 ppm, 28 d (2)	Rat	20[b]	10	1	1	—	—	—	0.025	—
8/8 (slight), 2.8 ppm, 90 d continuous (cont.) (3)	Rat	10	10	2 (HR)	1	—	—	—	—	0.015
Hepatotoxicity										
Slight, 126 ppm, 1 h (1)	Rabbit	10	10	1	1	1.3	—	—	—	—
Slight, 20 ppm, 6 h (1)	Rabbit	10	10	4 (HR)	1	—	0.05	—	—	—
2/8 (necrosis) 10 ppm, 6 h/d, 5 d/w, 6 w (3)	Rat	10	10	1	1	—	—	0.1	—	—
0/8, 2.8 ppm, 90 d cont. (3)	Rat	1	10	2 (HR)	1	—	—	—	0.1	0.1
Neurotoxicity										
Moderate histopathological changes, 126 ppm, 1 h (4)	Rabbit	20[c]	10	1	1	0.6	—	—	—	—

TABLE 5-4 *(Continued)*

Effect, Data, (Number)[a]	Species	Uncertainty Factors: To NOAEL	Species	Time	Space-flight	Acceptable Concentrations, ppm: 1 h	24 h	7 d	30 d	180 d
Neurotoxicity (continued)										
Mild histopathological changes, 17 ppm, 6 h (4)	Rabbit	10	10	4 (HR)	1	—	0.04	—	—	—
2/8 (severe), 2.8 ppm, 90 d cont. (3)	Rat	10	10	2 (HR)	1	—	—	0.03	0.03	0.015
Lethality										
95% limit of LC_{10}, 25 ppm, 1 h (5)	Mouse	10	10	1	1	0.25[d]	—	—	—	—
95% limit of LC_{10}, 6 ppm, 6 h (5)	Mouse	10	10	1	1	—	0.0015[d]	—	—	—
0/8, 15 ppm, 6 h/d, 5 d/w, 6 w (3)	Rat	1	10	HR	1	—	—	1.5	0.3	—
0/8, 2.8 ppm, 90 d cont. (3)	Rat	1	10	2 (HR)	1	—	—	—	—	0.15
SMACs						**0.6**	**0.04**	**0.03**	**0.025**	**0.015**

[a]Reference key: (1) Reichert and Henschler, 1978; (2) Jackson et al., 1971; (3) Siegel et al., 1971; (4) Reichert et al., 1976; (5) Reichert et al., 1975.

[b]A factor of 2 (5/28) reduces the severe effects to slight, and a factor of 10 reduces the effects to a NOAEL.

[c]A factor of 2 was used to reduce the concentration causing moderate effects to one causing mild effects (LOAEL), and a factor of 10 was used to reduce the LOAEL to a NOAEL.

[d]Calculated values were not used because human exposures clearly show that neurotoxicity occurs before death during exposures of a few days or less.

—, Data not considered applicable to the exposure time; HR, Haber's rule.

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B6 1,2-Dichloroethane

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PHYSICAL AND CHEMICAL PROPERTIES

The compound 1,2-dichloroethane (EDC) is a colorless liquid with an odor characteristic of chlorinated hydrocarbons (ACGIH, 1991).

Synonym:	Ethylene dichloride
Formula:	CH_2ClCH_2Cl
CAS number:	107-06-2
Molecular weight:	99.0
Boiling point:	83.5°C
Melting point:	−35.5°C
Specific gravity:	0.94
Vapor pressure:	87 mmHg at 25°C
Saturated vapor concentration:	114,474 ppm at 25°C
Conversion factors at 25°C, 1 atm:	1 ppm = 4.05 mg/m^3 1 mg/m^3 = 0.25 ppm

OCCURRENCE AND USE

EDC has been used in vinyl chloride manufacture, as a solvent, degreaser, and fumigant (ACGIH, 1991). EDC has been detected at a trace concentration (just enough was collected on Tenax for gas chromatography-mass spectrometry qualitative identification, but not for

quantitative analysis) in air samples taken in a shuttle mission (NASA, 1990). Off-gassing was most likely its source in that mission.

TOXICOKINETICS AND METABOLISM

Toxicokinetics

Reitz et al. (1982) did a toxicokinetic study in rats using the maximally tolerated concentration of EDC (i.e., 150 ppm) from a 2-y bioassay of Maltoni et al. (1980). When the rats were exposed at 150 ppm for 6 h, the blood concentration reached a plateau in about 2 h; this observation indicated that EDC was absorbed readily. When the 6-h exposure ended, the compound was rapidly cleared from the blood with a bi-exponential decay, and the half-lives of the two phases were 6 and 35 min. During the 48 h after the 6-h exposure, only 1.8% of the amount of EDC absorbed by the body (the total body burden) was exhaled unchanged. Much of the total body burden was excreted in those 48 h as metabolites: 84.4% in urine, 7.0% as CO_2, and 1.7% in feces, so that only 4.4% of the total body burden remained in the rat's body after 48 h.

Metabolism

Anders and Livesey (1980) showed that EDC is metabolized by two competing pathways. A proposed metabolic scheme is shown in Figure 6-1 (IPCS, 1987). In one pathway, EDC is oxidized by the cytochrome P-450 system to 2-chloroacetaldehyde, which could react with macromolecules in the cell. 2-Chloroacetaldehyde could be further oxidized by aldehyde dehydrogenase to 2-chloroacetic acid, which is oxidized to 2-chloroethanol or conjugated with glutathione, and be eliminated eventually. Another pathway involves direct conjugation with glutathione to form *S*-(2-chloroethyl)-glutathione, which could either form glutathione episulfonium ion or react with glutathione to form ethane and hydrogen chloride. Glutathione episulfonium ion could react with cellular macromolecules, or it could conjugate with glutathione to form *S,S'*-ethane bisglutathione, which is then eliminated after being transformed into other metabolites.

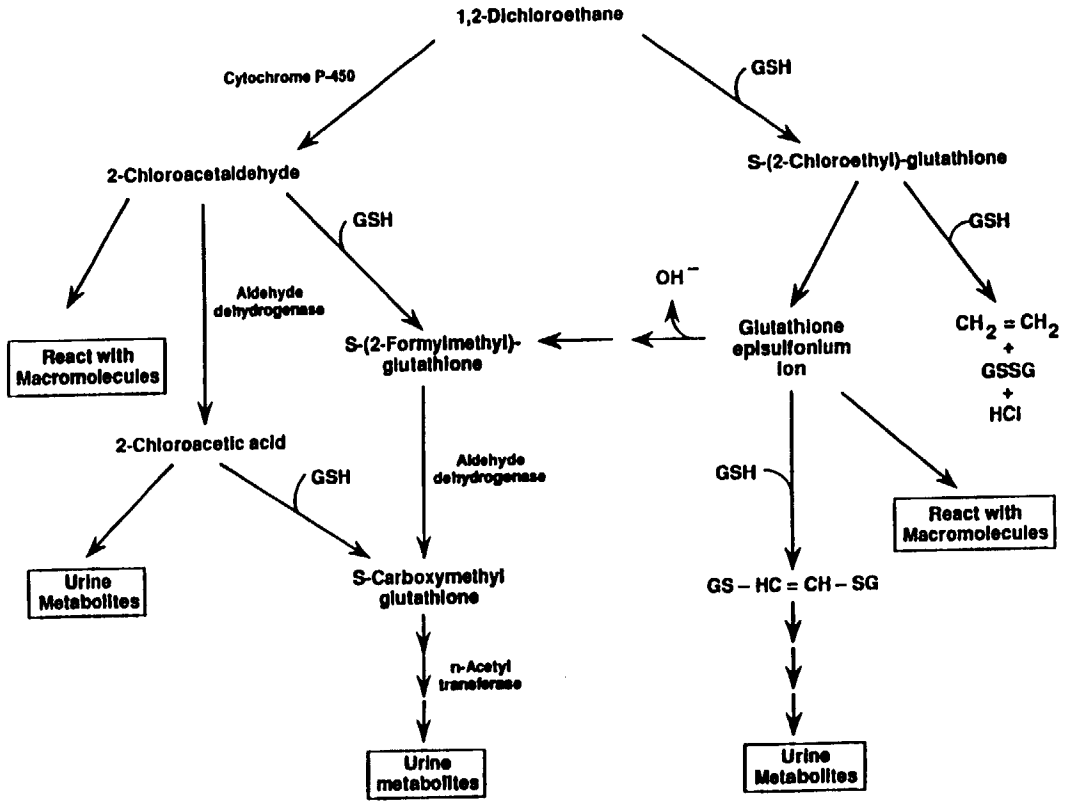

FIGURE 6-1 Proposed metabolic pathways for 1,2-dichloroethane. Source: Adapted from IPCS, 1987.

Because both metabolic pathways generate a metabolite (i.e., 2-chloroacetaldehyde and glutathione episulfonium ion) that could react with macromolecules, it is of interest to compare their genotoxic potency. Storer and Conolly (1985) compared the amount of hepatic DNA damage in EDC-exposed mice pretreated with dimethyl maleate, which depleted glutathione, or piperonyl butoxide, which inhibited the cytochrome P-450 system. They demonstrated that EDC resulted in more hepatic DNA damage during inhibition of the cytochrome P-450 than during depletion of glutathione. Therefore, the metabolites in the glutathione conjugation pathway are more genotoxic than those in the oxidative pathway (Storer and Conolly, 1985). It appears that glutathione episulfonium ion is probably the major genotoxic metabolite of EDC.

The cytochrome P-450 oxidative pathway of EDC metabolism is saturable at lower EDC concentrations than the glutathione conjugation pathway (NRC, 1987). Based on a physiologically based pharmacokinetic model, the cytochrome P-450 oxidative pathway tends to be saturated at an oral EDC dose of about 1 mg/kg in mice (NRC, 1987). In rats, as the EDC dose increased to above 25 mg/kg, there was a transient depletion of glutathione in the liver (D'Souza et al., 1988). Therefore, at oral EDC doses below 1 mg/kg, the model predicts that the amount of glutathione-conjugate metabolite formed in the liver of mice increases linearly with the dose (NRC, 1987). At doses between 1 and 25 mg/kg, the amount of glutathione-conjugate metabolite in the liver does not increase linearly with the dose, but instead it curves upward, probably because of the saturation of the cytochrome P-450 pathway. Between a dose of 25 and 150 mg/kg, however, the amount of glutathione-conjugate metabolite begins to reach a plateau because of glutathione depletion.

TOXICITY SUMMARY

EDC is known to cause death, central-nervous-system (CNS) depression, miscellaneous symptoms, corneal opacity, gastrointestinal (GI) and hepatic toxicity, and impairment of host defense toward microbes. It has also been shown to cause tumors in rodents.

Acute and Short-Term Exposures

The literature on the toxic effects of EDC after a single exposure or after exposures repeated for no more than 7 d is reviewed here.

Lethality

A number of fatal cases of accidental EDC poisoning have been reported in the literature. Most of them involve occupational exposures. These workers were generally found unconscious after an acute exposure to a presumably very high, but unknown, concentration of EDC. Their symptoms and signs could include CNS depression, weakness of the limbs, cyanosis, rales over the chest, tachycardia, jaundice, and anuria. Autopsies showed lung edema and congestion, fatty liver, hepatic necrosis, cavernous formations in liver, hyalinized or swollen glomeruli, swollen renal tubules with the lumen filled with dead cells or hyaline and granular casts, and granular degeneration of renal tubules (Brass, 1949; Hadengue and Martin, 1953; Troisi and Cavallazzi, 1961). In one case, pulmonary edema was the cause of death, and a victim of another case probably died of circulatory collapse (Brass, 1949; Hadengue and Martin, 1953).

Quantitative information is lacking on the lethal concentration of EDC in humans. In contrast, several studies have been done to determine the lethal concentrations of EDC for laboratory animals (Heppel et al., 1946; Spencer et al., 1951; Bonnet et al., 1980). Analyses of these animal data revealed that species differences exist in EDC's acute lethal effect. The species sensitivities ranked in decreasing order are mice, guinea pigs, rabbits, rats, dogs, and cats. Bonnet et al. (1980) showed that the 6-h LC_{50} of EDC was only 262 ppm in mice and 1646 ppm in rats. Heppel et al. (1946) compared the sensitivities of rats, rabbits, guinea pigs, cats, and dogs to acute EDC exposures. They reported that in two daily exposures to EDC at 1000 ppm for 7 h/d, 58% of guinea pigs and 33% of rabbits died, but none of the rats, cats, and dogs died. When the exposures were extended to 7 h/d, 5 d/w for several weeks, 55% of the rats died after 1 w of exposure, but none of the dogs and cats died after 3 w. Because three of six dogs became sick

after 3-4 w of exposure and only one of six cats was affected after 4 w of exposure, cats appeared to be the most tolerant species among the ones tested. Even though rhesus monkeys were also tested in their study, no valid species comparison can be made with monkeys because only two monkeys and no controls were used in the 1000-ppm group.

In addition to species differences, there are sex differences in EDC's lethal effect in some of the species. In an experiment conducted in which rats and guinea pigs were exposed to EDC at 400 ppm, 7 h/d, 5 d/w, for up to 8 w, it took 10 exposures to kill 15 female rats, compared with 40 exposures to kill 15 male rats. It took only 10 exposures to kill eight male guinea pigs but 24 exposures to kill eight female guinea pigs (Spencer et al., 1951). Therefore, female rats are more sensitive than male rats, but the reverse is true in guinea pigs.

Spencer et al. (1951) studied extensively the acute exposure concentrations of EDC that produced lethality in rats. They showed that the acute lethal effect of EDC increased with the product of exposure concentration and duration in rats (Table 6-1).

TABLE 6-1 Lethality of EDC Concentration and Duration of Exposure

Exposure Concentration, ppm	Duration for 0.01% Mortality, h	Duration for 50% Mortality, h
12,000	0.2	0.5
3000	1.0	3.0
1500	2.0	5.5
1000	3.5	7.0

Cause of Death and Internal Injuries

As mentioned above, pulmonary edema and circulatory collapse have been postulated to be the cause of death in humans acutely poisoned by EDC (Brass, 1949; Hadengue and Martin, 1953). Among laboratory animals, rats are the best studied. The cause of death from acute EDC poisoning in rats depends on the exposure concentration. An exposure at 20,000 ppm killed the rats with extremely severe CNS depression in 0.3-0.4 h (Spencer et al., 1951). In contrast, at 12,000 ppm or lower,

Spencer's group found that CNS depression was not the cause of death because no coma or death occurred during the exposure. In rats exposed to EDC at the LC_{50} of 12,000 ppm for 0.5 h, 3000 ppm for 3 h, or 1000 ppm for 7 h, the most pronounced histopathological effect was in the kidney with necrosis and degeneration of the tubular epithelium, interstitial edema, hemorrhage, and congestion. There were also varying degrees of parenchymatous degeneration and hemorrhagic necrosis in the liver, but no fatty degeneration. Based on the lesions described by Spencer et al. (1951), severe renal injuries appeared to cause the death of rats acutely exposed to EDC at 12,000 ppm or lower.

In addition to determining the acute lethal concentrations of EDC, Spencer et al. (1951) also determined acute exposure concentrations that did not produce any internal injuries. They found that the exposure conditions shown in Table 6-2 were devoid of any adverse macroscopic or microscopic effects.

Miscellaneous Symptoms

In nonfatal cases of acute EDC exposure of workers, the symptoms included dizziness, headache or pressure in the head, nausea, vomiting, epigastric cramps, and weakness (Wirtschafter and Schwartz, 1939; Jordi, 1944). Except for dizziness, the symptoms could last for a few days. The victims could also develop hypoglycemia and leucocytosis. Based on the industrial experience in Russia, Rosenbaum (1947) stated that repeated exposures to EDC at 75-125 ppm could result in acute poisonings with the development of dizziness, headache, weakness, mucosal irritation, nausea, and vomiting.

TABLE 6-2 EDC Exposure Conditions Without Adverse Effects

Exposure Concentration, ppm	Exposure Duration, h
12,000	0.1
3000	0.3
1000	1.5
300	3.0
200	7.0

CNS Depression

As discussed previously, EDC could cause unconsciousness at 20,000 ppm during an exposure lasting 0.3-0.4 h in rats. However, Spencer et al. (1951) found that acute exposures of rats to lower concentrations of EDC, such as 12,000 ppm for 1 h or 3,000 ppm for a few hours, resulted in "drunkenness" instead of unconsciousness. Dizziness has been reported in nonfatal cases of acute EDC exposure of workers (Wirtschafter and Schwartz, 1939; Jordi, 1944). The only quantitative data on the depressive effects of EDC in humans were gathered by Borisova (1957, 1960). Borisova measured the light perception threshold in three human subjects during an exposure to EDC at 1 to 12.5 ppm. At 1.5 to 12.5 ppm, the light-perception threshold was reduced in a concentration-dependent fashion, but there was no reduction at 1 ppm. Because Borisova used only three men in this study, the data are not used in setting the SMACs.

Corneal Opacity

A study by Heppel et al. (1944) demonstrated that an exposure of dogs to EDC at 1000 or 1500 ppm for 7 h resulted in bilateral corneal opacity, which cleared up within a week. In repetitive exposures of dogs at 1000 ppm for 7 h/d, 5 d/w, for several weeks, they reported that the corneal opacity increased in intensity during the five exposure days in the first week. The opacity cleared up during 2 d (weekends) of no exposure. As the weekly exposures were repeated, the cornea developed tolerance toward the clouding effect of EDC, and the cornea became almost totally resistant after a few weeks. A similar phenomenon was observed by Heppel et al. (1944) in dogs exposed at 400 ppm for a similar duration. By the fifth week of exposure, 400 ppm was only mildly effective in producing corneal opacity. By the tenth week, the cornea failed to show any cloudiness at all. When Heppel's group exposed 11 species, including dogs, foxes, rabbits, cats, raccoons, guinea pigs, rats, and hogs to EDC at 3000 ppm, only the corneas of the dog and fox were affected. Because corneal opacity has never been documented in accidental and nonaccidental EDC exposures of humans, it is not used as a toxic end point in deriving EDC's SMACs.

Bacterial Respiratory Infection

Female mice exposed to EDC at 10 ppm for 3 h resulted in decreased pulmonary bactericidal activity against inhaled *Klebsiella pneumoniae* and in increased mortality upon inhalation challenge of *Streptococcus zooepidemicus* (Sherwood et al., 1987). During the challenge, the mice inhaled about 20,000 to 40,000 *Klebsiella* or *Streptococcus* bacteria in 30 min. A 3-h exposure to EDC at 5 ppm, however, increased mortality from streptococcal challenge but did not change pulmonary bactericidal activity. A single exposure or five daily 3-h inhalation exposures to EDC at 2.5 ppm failed to produce any change in the mortality from streptococcal challenge and pulmonary bactericidal activity.

Subchronic and Chronic Exposures

Miscellaneous Symptoms

The symptoms of subchronic EDC intoxication resemble those of nonfatal acute EDC poisoning, consisting mainly of CNS and GI symptoms. McNally and Fostvedt (1941) reported intoxication in two workers exposed to EDC on the job. These workers extracted cholesterol from spinal cords by grinding 2500 pounds of spinal cords in 750 to 900 gallons of EDC. They inhaled EDC vapors during centrifugation of homogenized spinal cords to separate the cholesterol and also when they emptied barrels containing the cholesterol. Both of them presented drowsiness, anorexia, nausea, vomiting, and epigastric discomfort. Nystagmus and fine tremor of the tongue developed in one of the workers, and nervousness was detected in the other worker.

Symptom data from studies in which EDC exposure concentrations were measured are summarized as follows. Cetnarowicz (1959) studied Polish workers involved in purifying mineral oil with a solvent containing 80% EDC and 20% benzene. In a study of 10 workers exposed for 2-8 mo to a mixture of EDC at 62-200 ppm and benzene at 3-8 ppm, all workers complained of mucosal irritation, which disappeared as they adapted to it. Six of them developed dizziness, sleepiness, a sweetish aftertaste, dry mouth, nausea, vomiting, and constipation. Three of 10 workers complained of epigastric pain. Among six workers exposed

for 2-8 mo to EDC at 10-37 ppm and benzene at 3-8 ppm, only one complained of the CNS and GI symptoms. Although benzene is known to cause headache, drowsiness, nausea, and loss of appetite (Finkel et al., 1983), the difference in the severity of the symptoms between the two groups can be attributed to the difference in EDC exposure concentrations, because the benzene concentrations were the same in both groups. The data of Cetnarowicz (1959) can be interpreted to mean that the lowering of the EDC exposure concentration from 62-200 ppm to 10-37 ppm decreases the severity of the CNS and GI symptoms of EDC.

Byers (1945) reported that U.S. workers exposed to EDC at concentrations not much higher than 100 ppm for 7.5 h/d developed nausea, vomiting, abdominal pain, lassitude, and malaise in a few hours after they left work. These delayed effects of EDC were reduced somewhat, but not totally eliminated, when the EDC concentration was decreased to 70 ppm.

EDC poisoning was reported in two workers exposed on the job to a measured EDC concentration of 120 ppm for 10 min three to four times a day and also to an estimated concentration of greater than 120 ppm daily for 10-15 min (Guerdjikoff, 1955). After several weeks (3 w for one of the workers), they developed fatigue, irritability, nervousness, anorexia, and epigastric pains. As the exposure continued for 7 or 9 mo, the workers gradually experienced tingling sensations of the eyes, headaches, insomnia, dizziness, slight hand trembling, difficulty in walking, and deviation to the right in a blind walk.

Rosenbaum (1939) presented the industrial experiences of EDC in Russia in the 1930s and 1940s. Without specifying the exact exposure concentrations, Rosenbaum reported that occupational exposures of 90 workers to EDC at below 25 ppm could produce bradycardia, fatigue, insomnia, and headache, but no effects on the blood.

Kozik (1957) studied workers in the aircraft industry in Russia. These workers applied glue containing EDC as a solvent to large rubber sheets. On the basis of the data presented by Kozik, the National Institute of Occupational Safety and Health (NIOSH) estimated that, in the first half of the shift, the time-weighted average (TWA) exposure concentrations of EDC were 28 ppm during glue application and 16 ppm during the period the glue dried (NIOSH, 1976). In the second half of the shift, the TWA exposure concentration of EDC was 11 ppm. Therefore, the EDC TWA exposure concentration for the entire shift

was 15 ppm (NIOSH, 1976). Comparing the morbidity data between the gluers and the machinists, who were not exposed to EDC, Kozik (1957) reported that the EDC exposure increased both the number of cases of acute GI disorders per 100 workers and the number of workdays lost to acute GI disorders per 100 workers. Kozik also measured the hand-eye coordination speed of the gluers and machinists at the start and end of the workday for 14 d in 17 gluers and 10 machinists (as controls). The speed did not differ among the groups. However, more errors were made in the test by the EDC-exposed gluers test than by the nonexposed machinists (error rates of 30% for the gluers and 10% for the machinists).

Brzozowski et al. (1954) studied 118 agricultural workers who used EDC as a fumigant in Poland. These workers were exposed to EDC at a TWA concentration of about 15 ppm and at a maximum concentration of 60 ppm, but they were also subjected to cutaneous exposure due to EDC spilled on their skin and clothes and due to the use of EDC to wash their skin. In 90 of 118 workers, Brzozowski et al. (1954) also detected redness of the conjunctiva and pharynx, burning sensation of the eye, bronchial symptoms, weakness, metallic taste in the mouth, headache, nausea, liver pain, tachycardia, and cough.

Among the studies reported by Cetnarowicz (1959), Byers (1945), Rosenbaum (1939) , Kozik (1957), and Brzozowski et al. (1954), the data of Brzozowski et al. are not used to derive an acceptable concentration (AC) for CNS and GI symptoms because of the confounding effect of cutaneous exposures. The data of the other four studies used to derive a LOAEL for the symptoms are listed in Table 6-3.

TABLE 6-3 Symptoms of Occupational EDC Poisoning

EDC Concentration, ppm	Symptoms Produced in Workers	Reference
Slightly >100	CNS and GI symptoms	Byers, 1945
70	Less severe than >100 ppm	Byers, 1945
<25	CNS symptoms	Rosenbaum, 1939
15	CNS and GI symptoms and signs	Kozik, 1957

According to Byers (1945), the reduction of occupational exposure to EDC from not much higher than 100 ppm to 70 ppm reduced the symptoms of EDC intoxication, indicating that the lowest-observed adverse-effect level (LOAEL) ought to be 70 ppm or lower. The report from Rosenbaum (1939) that occupational exposures to less than 25 ppm still produced some CNS symptoms suggests that the LOAEL should be lower than 25 ppm. Therefore, the TWA exposure concentration of 15 ppm found to cause GI disorders and CNS impairment by Kozik (1957) is selected as the LOAEL for GI and CNS symptoms. The LOAEL of 15 ppm is supported by Cetnarowicz's data that Polish workers exposed to EDC at 10-37 ppm and benzene at 3-8 ppm were less likely to complain of CNS and GI symptoms than those exposed to EDC at 62-200 ppm and benzene at 3-8 ppm.

Gastroenterological and Hepatic Toxicity

As discussed above, Cetnarowicz (1959) reported symptoms in 10 Polish workers exposed to EDC at 62-200 ppm with benzene at 3-8 ppm and in six workers exposed to EDC at 10-37 ppm with benzene at 3-8 ppm. X-ray examinations of these 16 workers showed that six had chronic catarrh of the stomach with atrophy of the mucous membrane and three also had periodic spasm of the pylorus. Those results are evidence of an organic basis for the EDC-induced GI symptoms.

Cetnarowicz (1959) also reported that four of the 10 workers exposed to EDC at 62-200 ppm and benzene at 3-8 ppm for 2-8 mo had minimally enlarged livers, which were tender when palpated. Liver function tests were performed in the workers. Most of the 16 workers (the 62-200-ppm group and the 10-37-ppm group) showed increased urobilinogen levels in the urine. Six of them had reduced serum levels of albumin, but eight of them had increased globulin levels in the serum. The workers had normal blood glucose levels, but the levels in eight of them were slow in returning to normal during the glucose tolerance test. From these tests on liver function, Cetnarowicz (1959) concluded that liver functions were impaired in 50% of the 16 EDC-exposed workers (75% of those in the 62-200 ppm group).

When two monkeys, without any control group, were exposed to EDC at 400 ppm, 7 h/d, 5 d/w, for 8 or 12 exposures, both monkeys developed enlarged fatty liver and degeneration of renal tubules

(Spencer et al., 1951). Heppel et al. (1946) also exposed two monkeys to EDC at 200 ppm, 7 h/d, 5 d/w, for 25 w. These two monkeys appeared to tolerate the exposure well and had satisfactory appetites and weight gains. However, microscopic examination after necropsy revealed fine fat droplets in both the liver and myocardium. The data of Spencer's and Heppel's studies suggested that EDC is a hepatotoxicant in primates, consistent with the results of Cetnarowicz (1959) in human workers. Unfortunately, none of these studies on monkeys or humans provided information on the no-observed-adverse-effect level (NOAEL), which must be determined from experiments involving rodents.

According to Spencer et al. (1951) and Heppel et al. (1946), who exposed rats and guinea pigs to EDC at 0, 100, 200, or 400 ppm, 7 h/d, 5 d/w, for 2-8 w, subchronic EDC exposures at 400 ppm definitely produced fatty liver in rats and guinea pigs. The hepatotoxicity of similar exposures at 200 ppm was only equivocal in these rodent species. However, the 5-d/w exposures at 100 ppm failed to cause any liver or kidney injuries in guinea pigs after 3, 15, or 32 w (Spencer et al., 1951; Heppel et al., 1946). In rats, the 5-d/w exposures at 100 ppm also did not produce any liver or kidney damage after 15 or 30 w. Finally, Cheever et al. (1990) showed that even a chronic exposure of rats to EDC at 50 ppm, 7 h/d, 5 d/w, for 2 y resulted in no histopathological effects. On the basis of the data on the hepatotoxicity of EDC in exposed rodents, the NOAEL for subchronic exposure is 100 ppm and that for chronic exposure is 50 ppm.

Carcinogenicity

The data gathered on rodents exposed to EDC show that tumor development in chronic studies might depend on the route of exposure. In an inhalation study performed by Maltoni et al. (1980), EDC failed to produce tumors after repetitive exposures of Sprague-Dawley rats and Swiss mice at 150 ppm, 7 h/d, 5 d/w, for 78 w. The failure of the inhalation study to yield tumors was not because the exposure concentration was too low. In fact, the repetitive exposures started at 250 ppm, and the concentration had to be reduced to 150 ppm after several exposures because of the severe toxic effects. So there is no doubt that the rats and mice were exposed to EDC at the maximally tolerated concentration.

In contrast, Ward (1980) presented the results of a National Cancer Institute (NCI, 1978) study showing that EDC produced tumors in multiple organs of Osborne-Mendel rats and in $B6C3F_1$ mice administered EDC by gavage in corn oil daily, 5 d/w, for 78 w. The high-dose group of rats received EDC at 100 mg/kg per exposure day for 7 w, 150 mg/kg per exposure day for 10 w, and 100 mg/kg per exposure day for 18 w, followed by cycles of one exposure-free week alternating with 4 w of exposure at 100 mg. The high-dose male mice were given EDC at 150 mg/kg per exposure day for 8 w and 200 mg/kg per exposure day for 70 w, followed by 13 w of no exposure. The high-dose female mice received 250 mg/kg per exposure day for 8 w, 400 mg/kg per exposure day for 3 w, and 300 mg/kg per exposure day for 67 w, followed by 12 w of no exposure. In male rats, EDC produced significant increases in squamous cell carcinoma of the forestomach and hemangiosarcomas of the circulatory system; in female rats EDC exposure significantly increased the incidence of mammary adenocarcinoma. Statistically significant increases of alveolar-bronchiolar adenocarcinoma were produced in both male and female mice. Male mice also developed an increase in hepatocellular carcinomas, and female mice developed endometrial stromal polyps and sarcomas.

The reason for the difference in the carcinogenicity of EDC in the inhalation study of Maltoni et al. (1980) and the gavage study of Ward (1980) is unknown. The difference could be due to the different strains of rats and mice used in the two studies. Another possibility is the different routes of exposures used. No studies have compared the carcinogenicity of EDC in different strains of rats or mice exposed by the same route. The National Toxicology Program did compare the organ toxicity of EDC given in drinking water at 0 to 8000 ppm for 13 w in Sprague-Dawley rats and Osborne-Mendel rats (Brondeau et al., 1983). No EDC-related clinical signs, mortality, or histopathological effects were found in either strain. However, the body-weight gain of Osborne-Mendel rats appeared to be more sensitive to EDC than that of Sprague-Dawley rats. It took a concentration of EDC at 1000 ppm or more or 2000 ppm to retard the growth of female and male Osborne-Mendel rats, respectively, but it took 4000 ppm or more to retard the body-weight gain in Sprague-Dawley rats of both sexes (Morgan, 1991). Because the National Toxicology Program study (Brondeau et al., 1983) was only 13 w long, it did not answer the question of whether the carcinogenicity of EDC in rodents is dependent on strain.

Evidence that the difference in the route of exposure might explain the difference in EDC's carcinogenicity in the inhalation and gavage studies was presented by Reitz et al. (1982). In a pharmacokinetic study, they demonstrated that the peak concentration of EDC in the blood of Osborne-Mendel rats after EDC was given by gavage at 150 mg/kg was about 5 times higher than that observed when EDC was given by inhalation at 150 ppm for 6 h. Moreover, the amounts of DNA binding in the liver, spleen, kidney, and stomach after gavage were about double or triple those after a 6-h inhalation exposure. Finally, the pharmacokinetic model predicted that elimination of EDC is more likely to become saturated when EDC is administered by gavage than by inhalation. Therefore, EDC administered by gavage at the high dose used in the study by Ward (1980) would result in higher concentrations of EDC in the body for a longer duration than when EDC was administered by inhalation exposure at the high dose used by Maltoni et al. (1980).

On the basis of the findings of the NCI (1978), the International Agency for Research on Cancer (IARC, 1979) stated that there is sufficient evidence that EDC is carcinogenic in mice and rats. In the absence of adequate data in humans, it is reasonable to regard EDC exposure as a carcinogenic risk to humans. Similarly, the U.S. Environmental Protection Agency (EPA, 1990) classified EDC as a probable human carcinogen on the basis of the findings of the NCI study in rats and mice (Ward, 1980), despite the lack of human carcinogenicity data.

Genotoxicity

EDC is mutagenic in *Salmonella typhimurium*, it induces sex-linked recessive lethality in *Drosophila melanogaster*, and it produces DNA damage in mice (Rapoport, 1960; Brem et al., 1974; Storer et al., 1984). Therefore, the compound appears to be genotoxic.

Developmental Toxicity

Rao et al. (1980) exposed pregnant rats to EDC at 100 ppm, 7 h/d on days 6 through 15 of gestation and pregnant rabbits at 100 or 300 ppm, 7 h/d on days 6 through 18 of gestation. The exposures failed to

cause any maternal toxicity. They also did not increase the incidence of major malformations, showing that the compound is not teratogenic. There were no adverse effects on mean litter size, fetal body weight, fetal crown-rump length, and incidence of resorptions, indicating that EDC is not toxic to the embryo and fetus. A similar exposure of pregnant rats to EDC at 300 ppm killed two-thirds of the rats, so the teratogenicity of that exposure concentration cannot be evaluated.

Interaction with Other Chemicals

The administration of 0.05% disulfiram in the diet of Sprague-Dawley rats exposed to EDC at 50 ppm, 7 h/d, 5 d/w, for 2 y increased the incidence of intrahepatic bile-duct cholangiomas in male and female rats, mammary adenocarcinomas in female rats, and subcutaneous fibromas and interstitial cell tumors in testes (Cheever et al. 1990). In contrast, inhalation of EDC alone at 50 ppm was not carcinogenic. These results indicate that disulfiram in the diet increases the carcinogenicity of inhalation exposures of EDC.

TABLE 6-4 Toxicity Summary[a]

Concentration, ppm	Exposure Duration	Species	Effects	Reference
1	NS	Human	No change in threshold of light perception in three subjects	Borisova, 1957, 1960
1.5	NS	Human	Threshold of light perception reduced in three subjects	Borisova, 1957, 1960
3	NS	Human	1/20 subjects could smell it	Borisova, 1957, 1960
4.5	NS	Human	6/20 subjects could smell it	Borisova, 1957, 1960
6	NS	Human	13/20 subjects could smell it	Borisova, 1957, 1960
10 to 37 and 3 to 8	2 to 8 mo of on-the-job exposure	Human (workers)	1/6 workers complained of dizziness, sleepiness, dry mouth, nausea, vomiting, constipation, and epigastric pain	Cetnarowicz, 1959
12.5	NS	Human	Threshold of light perception decreased in three subjects	Borisova, 1957, 1960
15	On-the-job exposures of unspecified duration	Human (workers)	Acute gastrointestinal disorders; increased number of errors made in a hand-eye coordination test	Kozik, 1957
15 with cutaneous exposure	On-the-job exposures of unspecified duration	Human (workers)	Redness of the conjunctiva and pharynx, burning sensation of the eye, bronchial symptoms, weakness, metallic taste in the mouth, headache, nausea, liver pain, tachycardia, and cough	Brzozowski et al., 1954
<25	On-the-job exposures of unspecified duration	Human (workers)	Bradycardia, fatigue, insomnia, and headache; no effects on the blood	Rosenbaum, 1939

TABLE 6-4 *(Continued)*[a]

Concentration, ppm	Exposure Duration	Species	Effects	Reference
62-200 and benzene at 3 to 8	2 to 8 mo of on-the-job exposure	Human (workers)	Eye irritation in all 10 workers; 6/10 had constipation, nausea, vomiting, dizziness, and sleepiness; 3/10 complained of epigastric pain	Cetnarowicz, 1959
70 or lower and >100	7.5 h/d, 40 h/w for an unspecified duration	Human (workers)	Nausea, vomiting, abdominal pain, lassitude, and malaise occurred in a few hours after exposure; the effects were less at 70 ppm than >100 ppm	Byers, 1945
75-125	Repeated acute exposures of unspecified duration	Human (workers)	Dizziness, headache, weakness, mucosal irritation, nausea, and vomiting	Rosenbaum, 1947
5, 10, 50 or 150	7 h/d, 5 d/w, 78 w	Rat, mouse	No increases in tumor incidence	Maltoni et al., 1980
25, 75, or 150	6 h/d, 5 or 7 d/w	Rat	No reproductive toxicity	Rao et al., 1980
50	7 h/d, 5 d/w, 2 y	Rat	No change in body weight, no histopathological changes	Cheever et al., 1990
100	7 h/d, 5 d/w, 3 w	Guinea pig	No effects on mortality, growth, organ weight, blood urea nitrogen, blood nonprotein nitrogen, serum phosphatase, plasma prothrombin clotting time, and fatty contents of liver, and no effects found on gross and microscopic morphological examination of tissues	Spencer et al., 1951
100	7 h/d, 5 d/w, 3 w and 4 d	Mouse	No death or adverse effect on body-weight gain	Heppel et al., 1946

100	7 h/d, 5 d/w, 13 w and 4 d	Guinea pig	No histological effects	Heppel et al., 1946
100	7 h/d, 5 d/w, 14 w and 4 d	Rat	No deaths; no effects on body-weight gain or histological effects; only 1/16 females failed to become pregnant; the other 15 females bred successfully and gave birth to healthy rats even after exposures during pregnancy	Heppel et al., 1946
100	7 h/d, 5 d/w, 29 w and 3 d	Monkey (n = 2)	No adverse effects on behavior, general appearance, periodic hematological examination, growth and organ weights; gross and microscopic tissue morphological examination revealed no effects	Spencer et al., 1951
100 or 200	7 h/d, 5 d/w, 30 w and 1 d	Rat	No effects on mortality, growth, organ weight, blood urea nitrogen, blood nonprotein nitrogen, serum phosphatase, plasma prothrombin clotting time, and fatty contents of liver; gross and microscopic morphological examination of tissues revealed no effects	Spencer et al., 1951
100	7 h/d, 5 d/w, 32 w and 2 d	Guinea pig	No effect on mortality, growth, organ weight, blood urea nitrogen, blood nonprotein nitrogen, serum phosphatase, plasma prothrombin clotting time, and fatty contents of liver; and gross and microscopic morphological examination of tissues revealed no effects	Spencer et al., 1951
100	7 h/d, d 6 to 15 or 18 of gestation	Rat, rabbit	No developmental toxicity	Rao et al., 1980
150	4 h	Mouse	No liver necrosis or hepatic DNA damage	Storer et al., 1984
200	7 h/d, 5 d/w, 1 w and 2 d	Mouse	18/20 mice died by the end of seven exposures	Heppel et al., 1946

TABLE 6-4 *(Continued)*[a]

Concentration, ppm	Exposure Duration	Species	Effects	Reference
200	7 h/d, 5 d/w, 17 w and 1 d	Rat	Body-weight loss, crusty materials about the eyes, listlessness, and a generally unkempt appearance; 7/12 Wistar rats died within the full exposure period; 8/12 Osborne-Mendel rats died within 28 exposures; in five Wistar rats narcopsied after 86 exposures, pathological effects were found only in one, consisting of fatty degeneration of renal convoluted tubules	Heppel et al., 1946
200	7 h/d, 5 d/w, 24 w and 4 d	Guinea pig	5/14 died within the exposure period; necropsy of the survivors found nothing in five and pulmonary congestion in the other four (necrosis and hemorrhage were detected in the liver of one and in the adrenal cortex of another)	Heppel et al., 1946
200	7 h/d, 5 d/w, 25 w	Monkey (n = 2, no control)	Tolerated the exposure well with satisfactory appetite and weight gain; both had fine fat droplets in myocardium and liver (one had focal calcification of adrenal medulla)	Heppel et al., 1946
200	7 h/d, 5 d/w, 25 w	Rabbit	No exposure-related deaths (n = 5); no adverse findings upon gross and microscopic examinations; no effect on growth	Heppel et al., 1946
200	7 h/d, 5 d/w, 36 w	Guinea pig	Slight retardation of growth; slight degeneration of liver parenchyma with several fat vacuoles distributed diffusely and a slight rise in the lipid contents; no microscopic changes in the structure of other tissues; no changes in blood urea nitrogen, blood nonprotein nitrogen, serum phosphatase, plasma prothrombin clotting time, and organ weights	Spencer et al., 1951

262	6 h	Mouse	Half the animals died in 14 d	Bonnet et al., 1980
400	7 h/d, 5 d/w, 8 or 12 exposures	Monkey (n = 2)	One became moribund after eight exposures; both developed enlarged fatty liver, degeneration of renal tubules with cast formation in the lumens, and increased plasma prothrombin clotting time	Spencer et al., 1951
400	7 h/d, 5 d/w, 10 or 40 exposures	Rat	0/15 females survived more than 10 exposures; 0/15 males survived more than 40 exposures; 60% mortality in females exposed twice and males exposed three times; rapid loss in body weight, slight increases in liver and kidney weights, slight increase in lipid contents of liver in females, slight clouding and swelling of liver with a few large fat vacuoles mainly in centrilobular location, and no histological changes in kidney or other tissues; no effects on blood urea nitrogen, blood nonprotein nitrogen, serum phosphatase, and plasma prothrombin clotting time	Spencer et al., 1951
400	7 h/d, 5 d/w, 10 or 24 exposures	Guinea pig	0/8 males survived more than 10 exposures; 0/8 females survived more than 24 exposures; rapid loss in body weight, increases in liver and kidney weights, slight-to-moderate clouding and swelling of renal tubular epithelium, slight-to-moderate fatty degeneration in centrilobular areas, and elevated blood urea nitrogen and nonprotein nitrogen; no effects on serum phosphatase and plasma prothrombin clotting time	Spencer et al., 1951

TABLE 6-4 *(Continued)*[a]

Concentration, ppm	Exposure Duration	Species	Effects	Reference
400	7 h/d, 5 d/w, 15 w and 4 d	Rat	9/16 died (seven died after 2-4 exposures); fur roughness, general weakness, and weight loss appeared a few days before death but appeared normal before that; only one of the survivors exhibited slight- to-moderate fatty changes in the liver, kidney, and heart, as well as diffuse myocarditis; remaining survivors had no histopathological changes	Heppel et al., 1946
400	7 h/d, 5 d/w, 16 w	Guinea pig	14/20 died (nine died after 8-14 exposures); fur roughness, general weakness, and weight loss appeared a few days before death but appeared normal before that; slight-to-moderate fatty changes of liver and kidney commonly found in dead animals; slight fatty changes found in the heart in about half of them	Heppel et al., 1946
400	7 h/d, 5 d/w, 19 w and 2 d	Rabbit	5/5 died (four died in the last 2 w of exposure)	Heppel et al., 1946
400	7 h/d, 5 d/w, 35 w	Dog	Appeared not to be affected and had satisfactory appetite and body-weight gain; no abnormalities upon physical examinations of nervous system and eyes; normal hematological results, prothrombin time, plasma total protein, globulin, albumin, nonprotein nitrogen, icterus index, bromsulfalein clearance, and arterial pressure; slight fatty change in the liver of five dogs and the kidney of one	Heppel et al., 1946

500	7 h/d, 5 d/w, 17 w	Rat, guinea pig, rabbit, monkey	Liver and kidney injuries, lung congestion, and high mortality	Hofmann et al., 1971
500	4 h	Mouse	Liver necrosis, but no hepatic DNA damage	Storer et al., 1984
846	4 h	Rat	Liver damage is evidenced by increases in sorbitol dehydrogenase, glutamate dehydrogenase, GOT, and GPT in the serum	Brondeau et al., 1983
1000	3 h	Rat	LC_{001}	Spencer et al., 1951
1000	4 h	Mouse	Liver necrosis and liver DNA damage	Storer et al., 1984
1000	7 h	Mouse	22/22 died	Heppel et al., 1946
1000	7 h	Rat	LC_{50}	Spencer et al., 1951
1000	7 h/d, 1 to 4 d	Guinea pig	36/41 died after 1-4 exposures; lacrimation and inactivity detected during exposure; autopsies showed congestion of the lung and other viscera	Heppel et al., 1946
1000	7 h/d, 5 d/w, 3 w	Rat	20/26 died (14 died after 3-5 exposures, three died after 10 exposures, and three died after 15 exposures); fur roughness, general deterioration, weakness, and crusting about the nose seen during exposure; chronic splenitis; and degenerative and proliferative changes in renal tubular epithelium	Heppel et al., 1946
1000	7 h/d, 5 d/w, 6 w and 1 d	Dog	2/6 died (one after 21 exposures and one after 31 exposures; cloudy corneas and coma in some dogs	Heppel et al., 1946
1000	7 h/d, 5 d/w, 11 w	Cat	2/6 died (one after 34 exposures and one after 43 exposures); all six showed fatty changes and congestion of the liver	Heppel et al., 1946

TABLE 6-4 *(Continued)*[a]

Concentration, ppm	Exposure Duration	Species	Effects	Reference
1000	7 h/d, 5 d/w, 12 w and 4 d	Rabbit	5/6 died (two died after two exposures, two after 15-20 exposures, and one after 43 exposures)	Heppel et al., 1946
1000	7 h/d, 5 d/w, 6 w and 2 d	Monkey (n = 2, no control)	One died after two exposures, showing hemorrhage, necrosis, fatty degeneration of liver; and very slight fatty changes in renal tubular epithelium; the other died after the full exposure showing fatty degeneration of liver, slight fatty changes in kidneys, and focal myocarditis	Heppel et al., 1946
1000	12 h	Rat	$LC_{99.99}$	Spencer et al., 1951
1500	2 h	Rat	$LC_{0.01}$	Spencer et al., 1951
1500	5 h	Rat	LC_{50}	Spencer et al., 1951
1646	6 h	Rat	Half of the animals died in 14 d	Bonnet et al., 1980
3000	1 h	Rat	$LC_{0.01}$	Spencer et al., 1951
3000	2.2 h	Rat	LC_{50}	Spencer et al., 1951
3000	7 h	Rat	$LC_{99.99}$	Spencer et al., 1951
12,000	0.2 h	Rat	$LC_{0.01}$	Spencer et al., 1951
12,000	0.5 h	Rat	LC_{50}	Spencer et al., 1951
12,000	1 h	Rat	22/22 died after the exposure; "drunkenness" but not unconsciousness during exposure	Spencer et al., 1951
22,000	0.2 h	Rat	0/20 died	Spencer et al., 1951
22,000	0.4 h	Rat	20/20 died of CNS depression	Spencer et al., 1951

[a]Only inhalation study results were included.

NS, not specified.

TABLE 6-5 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	10 (TWA)	ACGIH, 1991
OSHA's PEL	50 (TWA)	U.S. Dept. of Labor, 1995
OSHA's STEL	100	U.S. Dept. of Labor, 1995
NIOSH's REL	1 (TWA), 2 (ceiling)	NIOSH, 1978
NIOSH's IDLH	1000	NIOSH, 1978

TLV, Threshold Limit Value; TWA, time-weighted average; PEL, permissible exposure limit; STEL, short-term exposure limit; REL, recommended exposure limit; IDLH, immediately dangerous to life and health.

TABLE 6-6 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	0.4	2	GI symptoms
24 h	0.4	2	GI symptoms
7 d[a]	0.4	2	GI symptoms
30 d	0.4	2	GI symptoms
180 d	0.2	1	Carcinogenesis

[a]The former 7-d SMAC is 10 ppm.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

The SMACs for EDC are derived by consulting guidelines developed by the National Research Council (NRC, 1992). The derivation involves consideration of the important toxic end points: CNS effects, GI symptoms, liver toxicity, impaired host resistance, and carcinogenesis. The acute lethality end point is not used, because toxic end points, such as CNS and GI symptoms and internal injuries, should be more sensi-

tive than the lethality end point. SMACs derived to prevent these toxic end points will also prevent lethality. For each toxic end point, an acceptable concentration (AC) is estimated for a given exposure duration. In the end, the lowest AC for each exposure duration is chosen to be the SMAC for that duration.

CNS Effects

Based on the report by Kozik (1957), 15 ppm is the lowest-observed-adverse-effect level (LOAEL) for CNS effects (e.g., increased errors in a hand-eye coordination test) in workers exposed to EDC. The severity of CNS effects is assumed to be related to the concentration of EDC in the blood. Because Reitz et al. (1980) showed that the blood concentration of EDC in rats reached equilibrium in 2 h during an inhalation exposure at 150 ppm, it is highly likely that the concentration of EDC in the blood also reached equilibrium within several hours of occupational exposure of workers in the studies performed by Kozik (1957). Therefore, the occupational LOAEL of 15 ppm should be a LOAEL for any EDC exposure lasting from 24 h to 180 d. Because the occupational LOAEL is good for 24 h, it should be valid for a 1-h exposure.

An extrapolation factor of 10 is used to estimate the NOAEL from the occupational LOAEL. The LOAEL of 15 ppm is based on a large population of workers (Kozik, 1957); therefore, no adjustment for "small n" is needed.

1-h, 24-h, 7-d, 30-d, and 180-d ACs for CNS depression
= occupational LOAEL × 1/NOAEL factor
= 15 ppm × 1/10
= 1.5 ppm.

Gastrointestinal Symptoms

The LOAEL for GI symptoms is also 15 ppm based on the study of Kozik (1957) in Russian workers. Unlike CNS symptoms, it is not certain whether GI symptoms are totally dependent on blood concentration. As a result, time adjustment is needed to extrapolate from the occupa-

tional LOAEL to a continuous exposure of 7, 30, or 180 d. Kozik did not report how many months or years the Russian workers in his study were exposed to EDC; therefore, the time adjustment is done on a per workweek basis. Due to the uncertainty on the number of hours worked per week in the 1940s and 1950s by Russian workers, the number of work hours is prudently assumed to be 40 h/w.

7-d, 30-d, and 180-d AC for GI symptoms
= occupational LOAEL × 1/LOAEL factor × time adjustment
= 15 ppm × 1/10 × (40 h/w)/(24 h/d × 7 d/w)
= 15 ppm × 1/10 × 0.24
= 0.36 ppm.

Without a better approach, the 1-h and 24-h ACs for GI symptoms are conservatively estimated to be the same as the 7-d AC of 0.36 ppm.

Liver Toxicity

According to the study of Spencer et al. (1951), a 1-h exposure of rats to EDC at 1200 ppm would not produce any liver injuries. So the 1-h AC is derived using 1200 ppm as the NOAEL.

1-h AC for liver toxicity
= 1-h NOAEL × 1/species factor
= 1200 ppm × 1/10
= 120 ppm.

The 7-d, 30-d, and 180-d ACs for liver toxicity also are derived using the data of Spencer et al. (1951). The NOAEL for liver toxicity was determined to be 100 ppm in rats and guinea pigs exposed 7 h/d, 5 d/w, for 15 or 30 w.

180-d AC for liver toxicity
= 30-w NOAEL × 1/species factor × time adjustment
= 100 ppm × 1/10 × (7 h/d × 5 d/w × 30 w)/(24 h/d × 180 d)
= 100 ppm × 1/10 × 0.24
= 2.4 ppm.

30-d AC for liver toxicity
= 15-w NOAEL × 1/species factor × time adjustment
= 100 ppm × 1/10 × (7 h/d × 5 d/w × 15 w)/(24 h/d × 30 d)
= 100 ppm × 1/10 × 0.73
= 7.3 ppm.

For the 24-h and 7-d ACs, the conservative approach of not adjusting for the exposure time is taken.

24-h and 7-d ACs for liver toxicity
= 15-w NOAEL × 1/species factor
= 100 ppm × 1/10
= 10 ppm.

Impaired Host Defense

A 3-h exposure to EDC as low as 5 ppm was reported to increase the mortality of mice challenged with *Streptococcus* via inhalation (Sherwood et al., 1987). Repetitive exposures of mice to 2.5 ppm, 3 h/d, for 5 d failed to affect the host defense against bacterial challenges. Based on the SMAC subcommittee report (NRC, 1992), no interspecies extrapolation factor is needed to derive ACs for the prevention of impaired host defense against pulmonary bacterial infections. However, an uncertainty factor of 3 is used because microgravity is known to impair cell-mediated immunity in astronauts (Taylor, 1993).

1-h, 24-h, 7-d, 30-d, and 180-d ACs for host defense impairment
= 5-h NOAEL × 1/microgravity factor
= 2.5 ppm × 1/3
= 0.8 ppm.

Carcinogenesis

Although EDC has been shown to produce tumors only in a gavage study (Ward, 1980) and not in an inhalation study (Maltoni et al., 1980), the carcinogenicity findings were considered by NIOSH (1978) in recommending an exposure limit of 1 ppm and the Occupational

Safety and Health Administration (OSHA) (U.S. Department of Labor, 1989) in promulgating a permissible exposure limit (PEL) of 1 ppm. EPA (1990) also derived an inhalation tumor risk from the data of the gavage study.

It is important that the SMACs prevent unacceptable risks of tumor development. Even though there is uncertainty about the validity of data extrapolation from gavage and inhalation exposures, the tumor data from the gavage study are used to derive ACs. The 2-5 times difference between gavage and inhalation exposure in peak blood concentrations of EDC and DNA binding in several potential target organs (Reitz et al., 1982) is small compared with the 2 orders of magnitude difference between the statistical sensitivity of an animal bioassay and the tumor risk accepted by the National Aeronautics and Space Administration (NASA). In other words, a negative finding in an animal bioassay in which 50 rodents per group inhaled EDC does not guarantee that inhaled EDC would not produce a significant tumor response if 10,000 rodents were used. Because EDC given by gavage was carcinogenic in about 50 rodents and because the effective dose of EDC given by inhalation was only 2-5 times lower than that given by gavage (Ward, 1980; Reitz et al., 1982), there is a possibility that inhaled EDC is carcinogenic at a risk level of greater than 1 in 10,000.

In contrast, Baertsch et al. (1991) advocated not using the gavage data to estimate the carcinogenic potency of continuous inhalation exposure at a low concentration. Their position was based on their comparison of the amount of EDC absorbed and the amount of DNA binding in the liver and lung in female F344 rats exposed to EDC at 80 ppm for 4 h (i.e., continuous low exposure) or at 4400 ppm for a few minutes (i.e., peak exposure). They showed that the amount of EDC metabolized, which was a measure of the amount of EDC absorbed, in the 12 h after the exposure was 3 times higher in the peak-exposure group than the low-exposure group. The amount of DNA binding in the liver was about 110 times higher in the peak-exposure group than in the low-exposure group. Similarly, the amounts of DNA binding in the lung differed by about 90 times in the two groups. Unfortunately, Baertsch et al. (1991) did not include a gavage-exposure group in their study, so it is difficult to disregard the carcinogenic potential of inhaled EDC on the basis of their data alone. The only conclusion from their study was that DNA binding of EDC depends on the concentration and time exposure profile.

Based on the gavage data of the National Cancer Institute (Ward, 1980) and assuming that equivalent amounts of EDC inhaled per day would produce the same tumor responses, EPA (1990), using a linearized multistage model, estimated that an airborne EDC concentration of 4 $\mu g/m^3$ would yield an excess tumor risk of less than 1/10,000 in a continuous lifetime exposure of humans. Based on the approach of the NRC (1992) (assuming that the carcinogenesis of EDC is a three-step process, the earliest age of exposure is 30 y, and the average life span of an astronaut is 70 y), an adjustment factor of 3728, 871, or 146.7 is needed to compress the lifetime exposure at 4 $\mu g/m^3$ into a much shorter continuous exposure of 7, 30, or 180 d, yielding the same tumor risk.

Some genotoxic carcinogens are known to produce tumors even after a single exposure (Williams and Weisburger, 1985). EDC is genotoxic. Consequently, its carcinogenicity has to be considered in setting the 24-h SMAC. For a 24-h exposure, an adjustment factor of 26,082 is calculated by using the NRC (1992) approach. With these adjustment factors, the EDC exposure concentrations for 24 h, 7 d, 30 d, and 180 d can be calculated and are as follows:

Exposure Duration	Concentration with a 10^{-4} Tumor Risk
24 h	26 ppm
7 d	4 ppm
30 d	1 ppm
180 d	0.2 ppm

Establishment of SMACs

All the ACs derived above are tabulated to show the minimum AC for each exposure duration of interest. The 1-h, 24-h, 7-d, and 30-d SMACs are all set at 0.4 ppm on the basis of the ACs for protecting against GI symptoms. Based on an exposure concentration that will yield a tumor risk of 1/10,000, 0.2 ppm is selected to be the 180-d SMAC.

TABLE 6-7 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors: To NOAEL	Species	Time	Micro-gravity	Acceptable Concentrations, ppm: 1 h	24 h	7 d	30 d	180 d
CNS effects										
LOAEL, 15 ppm, occupational exposure (Kozik, 1957)	Human	10	—	—	—	1.5	1.5	1.5	1.5	1.5
GI symptoms										
LOAEL, 15 ppm, occupational exposure (Kozik, 1957)	Human	10	—	160/40	—	0.4	0.4	0.4	0.4	0.4
Liver toxicity										
NOAEL, 1200 ppm, 1 h (Spencer et al., 1951)	Rat	—	10	—	—	120	—	—	—	—
NOAEL, 100 ppm, 7 h/d, 5 d/w, 15 or 30 w (Spencer et al., 1951)	Rat	—	10	—	—	—	10	10	—	—
NOAEL, 100 ppm, 7 h/d, 5 d/w, 15 or 30 w (Spencer et al., 1951)	Rat	—	10	HR	—	—	—	—	2.4	7.3
Impaired host defense										
NOAEL, 2.5 ppm, 3 h/d, 5 d (Sherwood et al., 1987)	Mouse	—	—	—	3	0.8	0.8	0.8	0.8	0.8
Carcinogenesis										
Bioassay data (Ward, 1980)	Rat, mouse	—	—	NRC[a]	—	—	26	4	1	0.2
SMACs						**0.4**	**0.4**	**0.4**	**0.4**	**0.2**

[a]NRC (1992).
—, Data not considered applicable to the exposure time; HR, Haber's rule.

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B7 Ethanol

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PHYSICAL AND CHEMICAL PROPERTIES

Ethanol is a clear, colorless, flammable liquid with an odor threshold of approximately 80 ppm (0.15 mg/L) (Amoore and Hautala, 1983) and a "minimum identifiable odor level" of approximately 350 ppm (0.66 mg/L) (Scherberger et al., 1958).

Synonym:	Ethyl alcohol
Formula:	CH_3CH_2OH
CAS number:	64-17-5
Molecular weight:	46.07
Boiling point:	78.5°C
Melting point:	−114.1°C
Specific gravity:	0.789 at 20°C
Vapor pressure:	43 torr at 20°C
Solubility:	Miscible with water and most organic solvents
Conversion factors:	1 ppm = 1.88 mg/m^3 = 0.00188 mg/L 1 mg/m^3 = 0.531 ppm = 0.001 mg/L

OCCURRENCE AND USE

Ethanol is used as a fuel additive and in the manufacture of chemicals and medicines. It is used as a solvent in the manufacture of explo-

sives, plastics, resins, cosmetics, adhesives and preservatives. Ethanol is present in alcoholic beverages, which are widely consumed, and most toxicological concerns stem from the consequences of that consumption.

Ethanol has been found in almost every sample of shuttle air, but at concentrations that seldom exceed 0.01 mg/L (James et al., 1994). Concentrations in the Mir space station are often above 0.01 mg/L; however, concentrations as high as 0.107 mg/L have been reported (James and Coleman, 1994). Ethanol enters the spacecraft atmosphere by off-gassing from hardware and from its use as a cleaner and disinfectant in operational procedures. Dilutions of liquid ethanol are frequently used in payload experiments; occasionally, it is used in undiluted form, and as such, it could pose a significant eye hazard upon escape.

TOXICOKINETICS AND METABOLISM

Absorption

The absorption of ethanol has been most thoroughly studied by the oral ingestion route; however, absorption in the respiratory tract has been investigated in a few studies. It is important to compare the relative ability of the two routes of absorption to deliver ethanol to the bloodstream because that is where most of the distribution, metabolism, and excretion of ethanol occurs. Furthermore, specific toxic effects are often reported in terms of their probability of occurrence at known ethanol blood concentrations. Hence, it is important to know the efficiency of the inhalation route in delivering ethanol to the blood.

Orally ingested ethanol is absorbed rapidly from the gastrointestinal tract by simple diffusion; peak blood concentrations are reached in 0.5 to 1.5 h after ingestion has ended. Food in the stomach delays gastric emptying and consequently delays the delivery of ethanol to the small intestine, where absorption is more rapid than it is in the stomach (Rall, 1990). In experimental animals, absorption of an oral dose of 6.4 g/kg has been shown to be much slower in rats than in guinea pigs (Strubelt et al., 1974). That absorption rate results in the maximum blood concentrations in rats being about one third the maximum in guinea pigs; this species difference is less pronounced at lower doses.

In a human inhalation study, Lester and Greenberg (1951) showed

that the respiratory system absorbed about 62% of the ethanol from inspired air at concentrations ranging from 11 to 19 mg/L. This percentage appeared to be independent of ventilation rates, which ranged from 7 to 25 L/min. In the three test subjects, the blood alcohol concentrations averaged 4 mg/dL (0.004%) when inhaling ethanol at 15 to 16 mg/L for 3 h at a ventilation rate of 7 to 8 L/min. In two of the subjects tested at a ventilation rate of 15 L/min, the blood concentrations of ethanol after 3 h were approximately 9 mg/dL (0.009%). The concentration profiles suggested that the blood concentrations had reached equilibrium values during the 3 h of exposure. At a ventilation rate of 22 L/min, the blood concentrations, which averaged about 35 mg/dL (0.035%), did not appear to be at equilibrium after 3 h. The authors concluded that a ventilation rate above 14 L/min would be necessary to achieve a continuously increasing blood concentration of ethanol during an exposure at 15 mg/L for several hours.

In an experiment designed to test the hypothesis that an individual inhaling ethanol vapors from an open liquid source could have significant blood concentrations of alcohol, Mason and Blackmore (1972) showed that, in a warm room with a maximum concentration of 17 mg/L (estimated from the amount of ethanol evaporated from the source), the ethanol concentration in the blood of the four subjects was below 5 mg/dL (0.005%) when tested at various intervals during the exposure. Although the subjects experienced no subjective symptoms of intoxication, those entering the room for the first time found the atmosphere intolerable at the end of the exposure. That information is consistent with the findings of Lester and Greenberg (1951) described above and underscores the difficulty in reaching significant blood concentrations in practical situations where inhalation is the only route of entry. It has been shown in vitro that rat lung tissue can metabolize ethanol to carbon dioxide at a capacity roughly one fifth of that of liver slices (Masoro et al., 1953).

Some inhalation uptake data are available in rodents from experiments that were designed to develop models for evaluation of alcohol dependence and withdrawal. These inhalation models were developed to solve problems associated with variable blood alcohol concentrations after repeated oral or intravenous administrations. After the initial rise in blood ethanol concentration, continued inhalation of ethanol at a fixed concentration will result in gradually decreasing blood concentrations unless alcohol dehydrogenase (ADH) activity is inhibited (e.g., by

pyrazole) or the vapor concentration is increased during the exposure. Mice exposed to ethanol at 12 mg/L for 96 h, with daily injections of pyrazole, showed stable blood concentrations after about 20 h of exposure. The equilibrium blood concentration was approximately 170 mg/dL (0.17%) (Goldstein and Pal, 1971). A survey of rodent exposures to ethanol for up to 21 d suggested that the blood alcohol concentrations varied considerably with species, strain, and sex of the rodent (Goldstein, 1980). When exposures were conducted without an ADH inhibitor, the blood alcohol could be kept reasonably constant by doubling the exposure concentration progressively during the first 10 d of exposure. In rats exposed for 24 h at 28 mg/L, the blood concentration reached 340 mg/dL (0.34%). This exposure conferred tolerance to a hypothermia-inducing injection of ethanol administered 48 h after the exposure (Mullin and Ferko, 1981). Blood alcohol concentrations were stabilized at approximately 150 mg/dL (0.15%) by continuous exposures of rats that were individually exposed to air concentrations of 22 to 28 mg/L on the basis of their blood alcohol concentrations (Rogers et al., 1979).

Distribution

The arterial concentration of ethanol is significantly higher than the venous concentration during the absorption phase (Harger and Forney, 1967; Keiding, 1979). Ethanol diffuses slowly across cellular membranes but passes rapidly into the brain (Crone, 1965). The tissue-to-blood-concentration ratios in cat tissue were found to be about 0.85 in all tissues studied except in fat, where the ratio was only 0.2 (Eggleton, 1940, as cited in Rowe and McCollister, 1982). The placental transfer of ethanol is rapid in the many species studied, and the concentrations follow those in maternal blood (IARC, 1988).

Excretion

Once it reaches the blood, ethanol is removed at a constant rate in a given individual over a wide concentration range (zero-order kinetics); however, there is considerable evidence that the removal rate is lower at very low concentrations of ethanol. Above a peripheral blood con-

centration of about 2 mm (0.009%), the human liver eliminates ethanol at a constant rate; however, below that concentration, the rate of elimination decreases with concentration following Michaelis-Menton kinetics (Keiding, 1979; Bosron et al., 1988). Chronic alcoholics are able to eliminate ethanol from their blood almost twice as fast as the average person (Harger and Forney, 1967).

Ethanol is mainly eliminated from the body by oxidation in the liver to acetate, which is distributed to other tissues and metabolized to carbon dioxide for excretion by the lungs (see Metabolism below). A small fraction of ethanol is excreted without change by the kidneys (0.5 to 2%), lungs (5%), and sweat (0.5%); elimination rates range from 70 to 180 mg/kg·h or 170 to 410 mg/kg·h, depending on the method of calculation (Haggard and Greenberg, 1934; von Wartburg, 1989). A small amount has been reported to be conjugated and excreted in the kidney as ethyl glucuronide (Kamil et al., 1953).

Metabolism

Understanding of Ethanol Metabolism Before 1900

Ethanol is certainly one of the most often studied toxicants and probably one of the earliest to receive serious toxicological evaluation and metabolic study. By 1900, it was known that nearly all of an ingested dose was metabolized by the test animal; this knowledge enabled use of ethanol as a source of energy (Jacobsen, 1952). The accumulation of fat in tissues of animals fed alcohol over long periods of time had also been demonstrated, even when the intake of food in the ethanol-fed animals and control animals was the same. Several investigators showed that the overall metabolic rate of an organism was not altered by ingestion of ethanol (Jacobsen, 1952).

Understanding of Ethanol Metabolism by 1950

Investigations during the next half century resulted in much more detailed understanding of ethanol metabolism in experimental animals and humans. The state of knowledge at that time was reviewed by Jacobsen (1952), and most of the information below is from his review.

Although some controversy existed about the shape of the blood-concentration curve after ethanol administration, the majority of available data indicated a linear decrease in concentration with time. A relatively high preconditioning dose of alcohol causes a second smaller dose to be removed faster than a dose given without preconditioning. With blood alcohol levels in the range of 15 to 94 mg/dL (0.015-0.094%), constant infusion of ethanol was shown to maintain constant blood levels in humans. The capacity to metabolize alcohol appeared to vary less than 25% among individuals. The prevailing opinion at that time was that long-time use of alcohol does not increase the rate of metabolism and that exercise, except for increased evaporation, does not increase the removal rate of alcohol. Starved animals have a lower rate of alcohol metabolism than well-fed animals.

Long before 1950, investigators conducted studies using hepatectomized dogs, liver slices, and perfused livers to show that the liver was the predominant organ to metabolize ethanol. Some metabolism of alcohol appeared to occur in muscles also (Bartlett and Barnet, 1949). Data were not consistent on whether the kidney could metabolize ethanol; however, it was apparent that little, if any, metabolism occurred in brain tissue.

The biochemical pathway for ethanol metabolism was reasonably well demonstrated to be as follows:

$$\text{ethanol} \xrightarrow[1]{} \text{acetaldehyde} \xrightarrow[2]{} \text{acetate} \xrightarrow[3]{} \text{carbon dioxide}$$

Acetaldehyde was found in the blood of animals and humans given sufficient doses of ethanol. Typically, the acetaldehyde concentration was less than 1% of the concentration of ethanol. Premedication with Anabuse was found to increase the blood concentrations of acetaldehyde up to 10 times over those in unmedicated animals after ethanol ingestion. Following up on evidence of the formation of acetate from ethanol, Bartlett and Barnet (1949) used ^{14}C-labeled ethanol (1 g/kg) administered to rats to show that the acetate formed is rapidly oxidized to carbon dioxide, because 75% of the label was recovered in 5 h and 90% was recovered in 10 h.

The enzymes involved in the first step of the pathway were known to be ADH and, to a much reduced extent, catalase. ADH catalyzes the transfer of hydrogen to nicotinamide adenine dinucleotide (NAD+).

Catalase facilitates the transfer of hydrogen from ethanol by reduction of peroxide to water. Jacobsen (1952) speculated that the second step in the pathway was catalyzed by either a flavoprotein or another dehydrogenase, but no evidence indicated which type of aldehyde-oxidizing enzyme might actually be involved.

Present Understanding of Ethanol Metabolism

In general, the view of ethanol metabolism has not fundamentally changed from the pathway illustrated above. There have been significant new insights into ethanol's metabolism that have facilitated an understanding of the pathogenesis of ethanol-induced liver injury and carcinogenesis. Data now available on the location and enzymatic function of subcellular organelles in hepatocytes and how they respond to repeated insults from ethanol present a complex picture; the major features of that picture are described below. In addition, some of the early concepts about ethanol metabolism, especially the degree of variability among individuals, have been corrected by new data. Genetic variations in the enzymes involved in ethanol metabolism control the immediate response to ingested ethanol; however, no studies could be found in which differences in susceptibility to inhaled ethanol were evaluated.

The cascade of effects induced by ethanol can be partially understood by refinements in the pathway shown above. Many of the effects occur as a result of the changed redox state (NADH/NAD ratio). The excess of hydrogen equivalents from ethanol metabolism are used by mitochondria instead of those equivalents originating from the citric-acid cycle by metabolism of 2-carbon fragments from fatty acids. This process facilitates decreased fatty-acid metabolism and causes deposition of dietary fat in the liver. Chronic administration of ethanol to rats or baboons results in attenuation of this change in redox state, so continued accumulation of fat must depend on another mechanism, possibly one involving changes in the mitochondria (Lieber, 1979).

The first reaction in the metabolic pathway is primarily catalyzed by ADH in the cytosol. This step is affected by the presence of acetaldehyde, the transport of reducing equivalents, and the ability of the mitochondria to oxidize those reducing equivalents. ADH activity is affected by hormonal status, dietary status, and age of the subject (IARC, 1988). ADH is a dimer with multiple molecular forms determined by

at least five gene loci (ADH1 to ADH5). An atypical form (ADH2*2) has been identified at a high frequency (33% to 81%) in Asian populations compared with a much lower frequency (1% to 12%) in Caucasian populations (Agarwal and Goedde, 1992). The atypical subunit of ADH2 has a 20-fold higher capacity than the ADH2*1 subunit to oxidize ethanol. That difference might explain the two- to threefold variability in alcohol elimination observed in humans given identical ethanol doses (Bosron et al., 1988).

Two other enzymes have been shown to be capable of catalyzing the first step in the pathway. Cytochrome P-450 (the microsomal ethanol-oxidizing system) depends on nicotinamide adenine dinucleotide phosphate (NADPH), oxygen, and hydrogen to oxidize ethanol to acetaldehyde. One isoenzyme of cytochrome P-450, IIE1, is induced by ethanol, and that induction might explain ethanol's ability to act as a cocarcinogen (see below). In addition, it has been argued that this enzyme is important in ethanol oxidation at high concentrations and may become more important with chronic ingestion (Lieber, 1984). Catalase, a hemoprotein located in the peroxisomes of many tissues, can also catalyze the first reaction, but it depends on a hydrogen peroxide generating system to control the rate (IARC, 1988). When ethanol is present at low concentrations, neither of these enzymes appears to be nearly as important as ADH in ethanol oxidation. In vitro studies of ethanol metabolism in rat and monkey liver slices have shown that more than 89% of the metabolism is due to ADH alone (Havre et al., 1977).

The second step in the pathway is catalyzed by aldehyde dehydrogenase (ALDH), an enzyme present primarily in mitochondria with broad specificity for aldehydes. The mitochondrial form (ALDH2) is thought to be most important in oxidizing acetaldehyde, and because approximately 50% of the Japanese and Chinese lack an active form of this enzyme, they experience much higher blood acetaldehyde concentrations after ethanol ingestion (Bosron and Li, 1986). It was suggested that Asian individuals who exhibit the "flush" reaction to ethanol have the highly active ADH2 and the deficient ALDH2 enzymes (Yoshida et al., 1983). In fact, Shibuya et al. (1989) showed that of nine Japanese who flushed, all were at least heterozygous for the atypical allele (ALDH1*2 or ALDH2*2), and of six who did not flush, five were homozygous for the usual allele (ALDH1*1). Thomasson et al. (1993) showed that the atypical ALDH2*2 in a large population of Chinese men was associated with the most intense flushing (as measured by in-

creased facial blood flow). In individuals with the usual allele, the metabolic rate is very fast, so little acetaldehyde accumulates to reach the blood; however, in those who flush, the concentration of acetaldehyde may reach 100 μm (Bosron et al., 1988). Several isoenzymes of ALDH have been identified, and ALDH is present in tissues other than the liver (IARC, 1988). Acetaldehyde produced in the mitochondria during chronic ethanol intake appears to cause the biochemical changes and ultrastructural damage apparent in test animals and alcoholics (Lieber, 1979).

According to several reviews, the third step occurs mostly outside the liver when acetate is released into the circulation and delivered to peripheral tissues (Lieber, 1979; Weiner et al., 1988; Bosron et al., 1988). No specific data are cited by any of the reviewers to support this conclusion. Early studies using liver slices from rats and ^{14}C-labeled ethanol suggested that the liver alone converted a sizable portion of ethanol to carbon dioxide (Bartlett and Barnet, 1949). A later study in 10 human subjects infused to an average plasma concentration of ethanol at 3.6 mm (0.017%) showed that the output of acetate from the splanchnic area was 50% to 100% of the ethanol disappearance rate (Lundquist et al., 1962); that result was confirmed in another human study (Tygstrup et al., 1965).

TOXICITY SUMMARY

The number of toxicity studies involving ethanol is enormous; however, the goal of this document is to provide a rationale for setting inhalation exposure limits. With this goal in mind, my focus will be on the few inhalation studies conducted with ethanol and on studies by other routes of administration if those studies provide insight into how inhaled ethanol could adversely affect living systems. It is beyond the scope of this document to provide a comprehensive review of ethanol's toxicity by all routes. For example, adverse effects of ethanol on skeletal, cardiac, and vascular smooth muscle (Altura and Altura, 1982; Urbano-Marquez et al., 1989) and on the kidney (Ponticelli and Montagnino, 1979) will not be considered in detail because such effects are seen only in chronic alcoholics, and there is no indication that inhalation exposure would injure these tissues. Also, reports of responses to ethanol vapor at 0.5 ppm (0.001 mg/L) by 3 of 47 chemically sensi-

tive patients are not considered evidence of toxicity in this report. The responses were not applicable to nonsensitive populations, and the effects are hardly adverse (e.g., increased pulse rate) (Rea et al., 1991).

Acute and Short-Term Exposures

Short-term exposures to ethanol are known to induce irritation of mucosal surfaces, central-nervous-system (CNS) depression, and possibly a flush response in certain individuals. The first two effects have been demonstrated by inhalation exposure; however, the flush reaction has only been demonstrated by oral ingestion. The first two effects are thought to be mediated by ethanol, whereas the flush reaction is almost certainly due to acetaldehyde, which accumulates to unusually high concentrations in susceptible individuals.

Neurotoxicity

The mechanism of toxic action of ethanol on the nervous system is believed to depend on its ability to fluidize the bulk lipid in membranes. A correlation between the severity of intoxication and the magnitude of ethanol-induced fluidization of brain membranes has been demonstrated in vitro, and long-sleep strains of mice show greater ethanol-induced fluidization of membranes than short-sleep strains (Wood and Schroeder, 1988). In a refinement of the bulk lipid effect, some of the membrane effects may be explained by differential effects in domains of the membrane defined by their degree of hydrophobicity, orientation (lateral or vertical) or composition (e.g., protein and phospholipid). Changes in membrane fluidity and lipid composition might be related to ethanol sensitivity and tolerance, but that relation has not been convincingly demonstrated (Wood and Schroeder, 1988).

Ethanol causes CNS depression in animals and humans exposed to air concentrations on the order of 10 mg/L and above. Table 55.4 in Rowe and McCollister (1982) summarizes mouse, guinea pig, and rat exposures to ethanol at 6 to 94 mg/L for 1 to 24 h that induce drowsiness, ataxia, narcosis, and death. Most of the data are from Loewy and von der Heide (1918) and suggest that guinea pigs are somewhat less

susceptible than rats. In rats, the no-observed-adverse-effect level (NOAEL) for a 6-h exposure was 6.1 mg/L, whereas in guinea pigs, the NOAEL was 12 mg/L for an exposure of 8 h. The table shows that in mice, guinea pigs, and rats exposed for 7 to 10 h, the concentration × time needed to induce death is about 400 h·mg/L. Quantifying the effect of inhaled ethanol on the human nervous system depends on correlating inhalation exposures with blood alcohol levels (BALs) and then correlating those BALs with specific nervous system effects. This correlation is necessary because the assessment of nervous-system effects in the human inhalation studies is inadequate as compared with the extensive work that has been reported on the effects after oral ingestion. For example, Loewy and von der Heide (1918) reported headache after 33 min of exposure at 2.6 mg/L and a slight daze after leaving the chamber, whereas Lester and Greenberg (1951) reported no CNS effects during 3 to 6 h exposures at 15 mg/L, even in subjects breathing at 24 L/min. The authors of the latter paper, in which subjects were exposed with a head-only hood, attribute the differences in their findings and those of Loewy and von der Heide (1918) to fatigue caused by confinement in the exposure chamber. As discussed in the section Rationale for Acceptable Concentrations, the data from Loewy and von der Heide (1918) are not that different from Lester and Greenberg's (1951) data. The older study was conducted in a sealed static chamber of 8000-L capacity, and the apparent concentrations of ethanol decreased far more rapidly than the authors expected. They attributed the rapid decrease to wall condensation (Loewy and von der Heide, 1918). Both studies suffer from having few test subjects, subjective end points, and no control exposures. Fortunately, Lester and Greenberg (1951) measured BALs and found that, at a light load (15 L/min), the BAL reached 9 mg/dL (0.009%). They reported no CNS effects at that concentration, but their methods to detect such effects were subjective.

It appears that minimal to no effects are detectable in humans with BALs of 50 mg/dL (0.05%), which is compatible with the report of Lester and Greenberg (1951). Flury and Klimmer (1943) indicated that the "beginning of uncertainty" is 0.06%. Small performance decrements (< 10%) were demonstrated in grammatical reasoning, code substitution, choice reaction, and tapping in 18 subjects with BALs of 0.06% after ingestion of 95% ethanol mixed with juice (Kennedy et al., 1993a). In another study with the same group, 20 males with BALs of

0.05% were subjected to a microcomputer performance test and found to be indistinguishable from their controls (Kennedy et al., 1993b). A BAL of 0.075% was found to be an effect level in that study.

Mucosal Irritation

According to Lester and Greenberg (1951), humans experienced smarting of the eyes and nose and coughing when exposed to ethanol at 10 to 20 mg/L. Those symptoms disappeared in 5 to 10 min. Again those data do not fully agree with the older data from Loewy and von der Heide (1918), in which nasal itching was reported in an unaccustomed subject exposed at 6 mg/L after 10 min of a 50-min exposure. They did report that 17 mg/L was at first intolerable to an unaccustomed person, but became tolerable except for continuing eye irritation. Because the 1951 study appears to be more complete than the 1918 study, 10 mg/L is considered the threshold for ethanol-induced irritation. Tolerance develops rapidly, so it is effectively a NOAEL for mucosal irritation.

Flush Response

Symptoms experienced primarily by Asian individuals soon after ingestion of ethanol are called the "alcohol sensitivity syndrome." When these persons consume ethanol at 0.3 to 0.5 mL/kg of body weight, they experience facial flushing, elevation of skin temperature, and an increase in pulse rate (Shibuya et al., 1989). Vasodilation in the neck and chest areas, headache, nausea, hypotension, and extreme drowsiness are also commonly reported (Thomasson, et al., 1993). No data show explicitly that this response has been elicited by inhalation exposure to ethanol; however, the description of the symptoms in Loewy and von der Heide (1918) suggests that they might have observed some of the symptoms of flushing. In test subjects exposed to ethanol vapor, they reported an increased feeling of heat in the forehead and ears, warmth in the head and trunk, and fatigue. It is unlikely that any of the three subjects had flush responses, because only about 10% of Caucasians have them; however, because acetaldehyde mediates the response, it is plausible that during the high-concentration exposures, a

sufficient quantity of acetaldehyde was produced to elicit the response even in those without the flush response.

Subchronic and Chronic Exposures

Hepatotoxicity

Even though long-term oral ingestion of ethanol is hepatotoxic in humans and laboratory animals, there have been no contemporary studies showing lasting hepatotoxicity after repeated or continuous inhalation exposures. There are scattered old reports of studies of workers that suggest that cirrhosis of the liver develops after prolonged exposure to ethanol vapor (see Browning, 1953, 1965), but the accumulated experience in workers exposed to ethanol suggests that liver injury due to ethanol inhalation is rare. In animals, some investigators reported fatty infiltration of the liver in mice repeatedly exposed to high concentrations of ethanol (Weese, 1928, as cited in Rowe and McCollister, 1982), and cirrhosis of the liver was observed by Mertens (1896), as cited by Rowe and McCollister (1982), in rabbits exposed for 25 to 365 d to air saturated with ethanol. In rats exposed to ethanol at 1.4 mg/L for up to 14 d continuously, French and Morris (1972) reported that liver biopsies examined by light and electron microscopy showed no liver damage, although minimal fat accumulation was detected. Finally, it should be noted that liver injury is found in many of the inhalation models used to produce withdrawal effects in rodents. Such models use doses of pyrazole to maintain constant high blood concentrations of ethanol; however, the confounding effects of pyrazole preclude the conclusion that inhaled ethanol alone could induce hepatotoxicity (Goldstein, 1980). In an inhalation study of tolerance in rats exposed at 20 mg/L (initial 8-h 10-mg/L adjustment period) for 26 d, plasma triglyceride concentrations remained normal even though liver triglyceride concentrations increased on d 3, 6, and 9 (Di Luzio and Stege, 1979). In addition, GPT (glutamic pyruvic transaminase or alanine transaminase) activity was above control activity on the same days but returned to the control range by d 26. The authors characterized these changes as mild transient effects observed before adaptation; they found that BAL peaked at about 125 mg/dL (0.125%) on d 9 and had fallen to about 30 mg/dL (0.030%) by d 26.

The effects of oral ingestion of ethanol on the livers of animals and humans has been extensively studied and reviewed by many investigators (Lieber et al., 1975; Forsander, 1979; Ainley et al., 1988; Weiner et al., 1988). Many studies of cell injury show that damage to hepatocytes is mediated by acetaldehyde, which reaches progressively higher plasma (and presumably intracellular) concentrations for a given acute dose in those chronically consuming alcohol. This increasing concentration of the injurious metabolite appears to be a result of increasing production of acetaldehyde (induction of cytochrome P-450 in the smooth endoplasmic reticulum (SER)) and decreasing catabolism of acetaldehyde (decreased mitochondrial ALDH activity). Acetaldehyde is known to cause many adverse effects on biochemical processes and on organelles, including forming complexes with proteins, depleting glutathione, increasing collagen synthesis, increasing lipid peroxidation, inhibiting protein secretion (by interfering with microtubules), and injuring mitochondria and SER. These biochemical and subcellular changes eventually result in gross changes in the liver including fatty liver, hepatitis, and cirrhosis. Acetaldehyde also induces many genetic alterations, which might lead to adverse effects including cancer (IARC, 1988).

Carcinogenicity

The carcinogenicity of ethanol has been reviewed by scientists from many disciplines, thereby increasing the understanding of the many mechanisms through which ethanol might influence the rates of human cancer (Lowenfels, 1975; Seitz and Simanowski, 1986; IARC, 1988; Garro and Lieber, 1990). In 1975, it was clear that alcoholism was linked to cancer at sites where the liquid made direct contact with tissue (oropharynx, larynx, and esophagus) and where it is heavily metabolized to injurious products (liver). The frequent use of tobacco in combination with ethanol tended to confound the contribution from ethanol alone (Lowenfels, 1975). More recent evidence suggests that ethanol acts as a cocarcinogen primarily by inducing cytochrome-P-450-dependent mixed-function oxidase systems that activate such carcinogens as benzo(*a*)pyrene, nitrosamines, and aflatoxins. It is important to note that the induction of microsomal enzymes by orally ingested ethanol occurs in the lung as well as the intestine and liver (Lieber et al.,

1987). In addition to enzyme induction, several mechanisms that might contribute to the carcinogenic properties of ethanol are mitogenic effects, depletion of hepatic vitamin A, glutathione depletion, inhibition of DNA repair, and suppression of the immune system (Garro and Lieber, 1990). Despite the extensive studies of ethanol carcinogenesis, the findings do not tell us whether inhaled ethanol can act as a carcinogen or a cocarcinogen in the lung. Aside from the lung, it is very unlikely that inhaled ethanol would pose a significant cancer risk unless other symptoms of toxicity were present.

Mutagenicity and Genotoxicity

The genotoxicity of ethanol has been studied in many test systems and found to be attributable to ethanol's metabolism to acetaldehyde, which increases the incidence of sister chromatid exchanges (SCE) in bone-marrow cells of rodents exposed in vivo, induces chromosomal abnormalities in embryos exposed in vivo, and causes DNA cross links, chromosomal abnormalities, and SCE in human cells exposed in vitro (IARC, 1988). The important role of acetaldehyde is supported by the observation that without a test system capable of converting ethanol to acetaldehyde, ethanol does not exhibit genotoxicity. It must be noted that alcoholic beverages or their nonethanol extracts are often found to be mutagenic even in the absence of a metabolic system; however, this is attributed to the nonethanol components, which are themselves mutagenic (IARC, 1988).

Most in vitro test systems capable of metabolizing ethanol have given a positive analysis for the genotoxicity of ethanol. In mammalian cells exposed in vitro, the frequency of SCEs was increased when a metabolizing system was present (de Raat et al., 1983; Takehisa and Kanaya, 1983). Human lymphocytes exposed in vitro showed no increased frequency of SCEs at 1% ethanol; however, the frequency of SCEs was increased by the addition of ADH, but reduced by the addition of ADH and ALDH (Obe et al., 1986). Many mammalian in vivo genotoxicity assays have been negative; however, those involving embryonal cells have tended to show SCEs and other genetic abnormalities (IARC, 1988). Dominant lethal mutations have been induced by oral exposure of Sprague-Dawley rats (Klassen and Persaud, 1978), Long-Evans rats (Mankes et al., 1982), and CBA mice (Badr and Badr, 1975). Ethanol

induces aneuploidy in fertilized mouse eggs by directly interfering with the spindle apparatus during the first and second meiotic divisions (Kaufman and O'Neill, 1988). No studies were found that showed genotoxicity induced by inhalation exposure.

Reproductive Toxicity

Many experiments have demonstrated some degree of reproductive toxicity by ethanol (Gavaler and Van Thiel, 1987); however, the doses used are typically 5% or more of the calories consumed or the liquid volume ingested. Such experiments may be pertinent to the human population consuming large quantities of alcoholic beverages, but the findings have little bearing on inhaled ethanol vapor. Structural abnormalities have been produced in reproductive organs of rodents administered ethanol as a major fraction (>10%) of their diet; however, these doses often do not result in serious adverse effects on reproductive performance (Thiessen et al., 1966; Oisund et al., 1978; Mankes et al., 1982). High-concentration exposures of rat offspring to ethanol and its metabolites in utero have resulted in retarded physical and developmental maturation and disturbances in sexual behavior and performance as adults (Leichter and Lee, 1979; Parker et al; 1984). Hormonal changes often accompany morphological changes in rodents given high doses of ethanol. Male Sprague-Dawley rats given ethanol for 5 w (6-10% of a liquid diet) showed adverse effects on testes and a decrease in serum testosterone (Klassen and Pesaud, 1978). Female Wistar rats given ethanol as 36% of calories for 49 d showed a 60% decrease in ovarian weight and hormonal changes (Van Thiel et al., 1978). Despite disturbances in the estrus cycles of Holtzman rats given 5% ethanol for 16 w, there were no adverse effects on fertility or litter size after mating with unexposed males (Krueger et al., 1982).

A few recent studies have addressed the potential for inhaled ethanol to affect reproductive capacity. Male Sprague-Dawley rats exposed up to 16,000 ppm (30 mg/L), 7 h/d, for 6 w were mated 2 d after the exposure to unexposed females; compared with controls, no differences were seen in maternal weight gain, feed intake, water consumption, and number of offspring (Nelson et al., 1985a). As part of this study, it was found that exposure concentrations greater than 11,000 ppm (21

mg/L) were necessary for ethanol to begin accumulating in the blood. Hence, given the high oral doses that do not induce functional reproductive effects, it is not surprising that inhalation of ethanol vapor at 16,000 ppm (30 mg/L) also does not cause reproductive toxicity.

Developmental Toxicity

There is no question that maternal consumption of alcohol can increase the risk of developmental toxicity in offspring. Historically, the capacity for alcohol consumption to cause excess infant mortality and feeble children has been known for centuries (Warren and Bast, 1988). Modern recognition and naming of the fetal alcohol syndrome (FAS) by Jones and Smith (1973) have stimulated research to define the concentrations of alcohol consumption that might cause the syndrome and to better characterize the many adverse effects that are associated with maternal alcohol consumption. Nearly all developmental toxicity studies of ethanol involve oral ingestion; however, a few inhalation studies of rodents have been reported in an effort to address concerns about occupational exposure to ethanol vapor.

The increased risk of fetal abnormalities associated with FAS has been summarized in terms of specific effects and maternal alcohol consumption (Pratt, 1982). Even in offspring of women ingesting less than 10 g of alcohol per day, the risk of congenital malformations, abnormal behavior, and small (body length and body weight) for gestational age is about 10%. At this consumption level, the risk of characteristic FAS was considered very low. In offspring from heavy drinkers (consuming more than 50 g of alcohol per day), the risk of congenital malformation averaged 25%, the risk of mental deficiency averaged 35%, and the risk of small (body length and body weight) for gestational age averaged about 20%. In offspring of heavy drinkers, the risk of FAS was estimated to be 2.5% to 25%. After the review by Pratt (1982), a large study (12,440 women) from two Boston hospitals showed that consumption rates up to 14 drinks per week (average about 20 g/d) did not result in any adverse effects and that only abruptio placenta was observed with increased frequency above that rate (Marbury et al., 1983). However, in a study of 1122 pregnancies in the United Kingdom, intake of more than 10 g of ethanol per day early in pregnancy was found to double the risk of a low-birth-weight baby (Wright et al., 1984). Low

birth weight appears to be one of the most sensitive of the effects associated with FAS.

A diagnosis of FAS requires abnormalities in three specific areas: growth retardation (< 10th percentile), CNS deficits or mental retardation, and facial abnormalities (Warren and Bast, 1988). Immune deficiencies have also been characterized as an important aspect of the clinical problems associated with FAS (Johnson et al., 1981). The prevalence of FAS is approximately 2/1000 live births among Australians, Europeans, and North Americans, with wide variations depending on study site (Abel and Sokol, 1987). It appears that the most critical period for maternal alcohol use in causing FAS is near the time of conception (Ernhart et al., 1987).

Oral studies using rodents have given mixed results on rodents' ability to respond to ethanol in the same way as humans (IARC, 1988). The response of the animal model depends on strain, dose of ethanol, and gestational age at which ethanol is administered. From rodent models, it appears that reduced body weights in offspring are one of the most sensitive indicators. The potential for inhaled ethanol to induce fetotoxicity has been evaluated in a series of rat studies (Nelson et al., 1985a,b, 1988). Using maternal exposures up to 16,000 ppm (7 h/d for 6 w), no effect on behavior of offspring 10 to 60 d old could be demonstrated (Nelson et al., 1985a). Even when exposures were up to 20,000 ppm (7 h/d, gestational days 1 to 19) and the dams were narcotized, no definite increase in structural malformations could be found (Nelson et al., 1985b). In the latter study, offspring from males and females exposed at up to 16,000 ppm (7 h/d for 6 w) were found to perform as well as controls in a battery of neuromotor coordination tests and learning tests; however, a few neurochemical differences between exposed and control offspring were detected (Nelson et al., 1988).

Despite the well-founded concerns about ethanol's ability to induce fetal abnormalities when the mother consumes even modest amounts of alcohol, there is no evidence that fetotoxicity can occur at non-narcotic exposures via the inhalation route.

Interaction with Other Chemicals

Ethanol has been found to potentiate the toxicity of many chemicals;

however, all data obtained were by noninhalation routes of administration of ethanol. Typical studies involved oral ingestion of ethanol coupled in some way to inhalation exposures to industrial chemicals. The summary provided here will be limited to ethanol-mediated effects on the inhalation toxicity of chemicals found at least occasionally in the space-shuttle atmosphere.

A number of studies have described the effect of ethanol on inhaled chlorocarbons. In human volunteers given trichloroethylene (TCE) at 50 or 100 ppm and enough ethanol to produce a BAL of 0.4% to 0.7%, Müller et al. (1975) found that the plasma TCE concentration was approximately 2.5 times higher in persons receiving ethanol than in controls. This result was due to blockage of TCE metabolism to trichloroethanol and trichloroacetic acid, thus increasing the CNS effects of TCE. Acute ethanol intoxication (5 g/kg) increases the incidence of chloroform-induced liver-function abnormalities in mice (Kutob and Plaa, 1962). Ethanol also potentiated the hepatotoxicity of TCE, but not 1,1,1-trichloroethane, in rats exposed to high solvent concentrations 18 h after ingestion of ethanol at 5 g/kg (Cornish and Adefuin, 1966). Interactions of various chlorocarbons have been studied in conditions in which ethanol is administered to rats and the chlorocarbons are administered by inhalation (Ikatsu and Nakajima, 1992).

The toxicity of inhaled aromatic chemicals is affected by concomitant administration of ethanol. Rats exposed to toluene at 2000 ppm (8 h/d for 2 w) and given 6% ethanol in drinking water showed reduced weight gain and abnormal clinical chemistry changes compared with toluene-exposed controls (Pryor et al., 1985). Padilla et al. (1992) showed that *p*-xylene inhaled by rats (1600 ppm, 6 h/d for 8 d) caused a reduction in rapid axonal transport, which was completely preventable by ethanol drinking (10% in water). Ingested ethanol (8 g/kg) and inhaled *m*-xylene (4 h at 6 or 11.5 mmol/m^3) interacted in human volunteers in such a way that metabolic clearance of xylene was decreased and blood acetaldehyde concentrations were transiently increased. That interaction probably caused the dizziness and nausea reported by the test subjects given combined exposures (Riihimäki et al., 1982). In mice inhaling benzene at 300 ppm (6 h/d, 5 d/w, for 9 w), excess erythropoietic disruption (circulating normoblasts) developed in those also given 5% ethanol in drinking water as compared with controls not given ethanol (Baarson and Snyder, 1991).

TABLE 7-1 Toxicity Summary

Concentration, mg/L	Exposure Duration	Species	Blood Alcohol Levels (BALs) and Effects	Reference
			Inhalation	
2.6 (start)	39 min	Human (n = 1)	No BAL, headache, slight stupor after leaving chamber	Loewy and von der Heide, 1918
4.7 (average)	50 min	Human (n = 1)	No BAL, nasal itching, warmth and pressure in head, slightly dazed after leaving chamber	Loewy and von der Heide, 1918
10-20	5-10 min	Human (n = 3)	No BAL, coughing, irritation of eyes and throat	Lester and Greenberg, 1951
9.5-11.5	2 h	Human (n = 2)	NOAEL except temple pressure and slight pain	Loewy and von der Heide, 1918
13.1 (average) 19.7 (start)	1.8 h	Human (n = 2)	Eye pain and pressure, sensation of warmth, fatigue	Loewy and von der Heide, 1918
14-16	3-6 h	Human (n = 3)	BAL = 0.009% at 15 L/min respiration; BAL = 0.004% at 7-8 L/min respiration; BAL = 0.050 % at 24 L/min respiration; no effects	Lester and Greenberg, 1951
17 (average)	64 min	Human (n = 1)	No BAL, nearly intolerable odor, burning eyes, fatigue, warmth in forehead and ears	Loewy and von der Heide, 1918
40	Few min	Human (n = 3)	No BAL, intolerable	Lester and Greenberg, 1951
0.086	90 d, continuously	Rat, guinea pig, rabbit, dog, monkey	No effects detected by limited clinical pathological and histopathological examination; possible mild lung inflammation	Coon et al., 1970

1.4	14 d, continuously	Rat	BAL < detection limit of approximately 0.1 mg/dL, transient physiological dependency, minimal fat accumulation in liver	French and Morris, 1972; Jones et al., 1970
5.6	64 d, 4 h/d	Guinea pig	No effects on blood parameters	Smyth and Smyth, 1928
6.1	6 h	Rat	No intoxication	Loewy and von der Heide, 1918
12.0	8 h	Guinea pig	No intoxication	Loewy and von der Heide, 1918
20	26 d, continuously	Rat	BAL = 0.125% (d 9); BAL = 0.030% (d 26); NOAEL at 26 d for liver and lung injury; transient liver changes on d 3 to 9	Di Luzio and Stege, 1979
41	10 h	Rat, guinea pig	Deep narcosis, death	Flury and Klimmer, 1943
55	7 h	Mouse	Narcosis, death	Flury and Klimmer, 1943
			Oral Administration to Humans	
0.33 g/kg of body weight	—	Human (n = 14)	BAL, 0.30-0.22%; NOAEL on battery of performance tests	Echeverria et al., 1991
Unspecified	—	Human (n = 20)	BAL, 0.05%; NOAEL in microcomputer performance test	Kennedy et al., 1993b
0.66 g/kg of body weight	—	Human (n = 14)	BAL, 0.063-0.054%; 4-16% decrement in 7 of 12 performance tests	Echeverria et al., 1991
Unspecified	—	Human (n = 18)	BAL, 0.06%; approximately 10% decrement in tapping, choice reaction time, code substitute, and grammatical reasoning	Kennedy et al., 1993a

TABLE 7-1 *(Continued)*

Concentration, mg/L	Exposure Duration	Species	Blood Alcohol Levels (BALs) and Effects	Reference
Unspecified	—	Human (n = 20)	BAL, 0.075%; slight effect on microcomputer performance test	Kennedy et al., 1993b
Unspecified	—	Human (n = 12)	BAL, 0.09%; 14 of 47 performance measurements show decrement	Stokes et al., 1994
Unspecified	—	Human (n = 18)	BAL, 0.10%; 10-15% decrement in performance of tapping, choice reaction time, code substitution, and grammatical reasoning	Kennedy et al., 1993a

TABLE 7-2 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	1000 (TWA)	ACGIH, 1995
OSHA's PEL	1000	U.S. Dept. of Labor, 1995
NIOSH's REL	1000	ACGIH, 1991

TLV, Threshold Limit Value; TWA, time-weighted average; PEL, permissible exposure limit; REL, recommended exposure limit

TABLE 7-3 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3 [a]	Target Toxicity
1 h	2000	4000	CNS, flush response, irritation
24 h	2000	4000	CNS, flush response, irritation
7 d[b]	1000	2000	Irritation, hepatotoxicity, CNS effects, flush response
30 d	1000	2000	Irritation, hepatotoxicity, CNS effects, flush response
180 d	1000	2000	Irritation, hepatotoxicity, CNS effects, flush response

[a]1 mg/L = 1000 mg/m^3.

[b]The former 7-d SMAC is 10 ppm.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

The toxic effects of ethanol are unusual in several respects. There is a clearly defined population of flush-response persons who are highly susceptible to oral ingestion of alcohol; however, this group has never been tested for inhalation susceptibility. Long-term ingestion of alcohol (or inhalation in animal models) can lead to physiological dependency, which is evident when the toxicant is withdrawn from the environment.

The more clearly defined effects for which acceptable concentrations can be developed are as follows: neurotoxicity, mucosal irritation, and hepatotoxicity. As explained in the Toxicity Summary, no data show that inhaled ethanol can produce cancer, reproductive effects, or fetotoxicity at concentrations that do not cause short-term effects (e.g., narcosis) in the mother. The guidelines promulgated by the National Research Council will be used to structure the rationale for setting SMACs for ethanol (NRC, 1992).

Neurotoxicity

Estimating the inhaled concentration of ethanol that will not induce neurobehavioral or performance decrements involves the results of two studies. First, the observations of Loewy and von der Heide (1918), who suggested effects at concentrations as low as 2.6 mg/L (2600 mg/m^3), are discounted in favor of the report by Lester and Greenberg (1951). In the older study, individual subjects exposed at 2.6 or 4.7 mg/L were reported to be slightly dazed after leaving the chamber; however, that effect was not reported in subjects exposed to higher concentrations. In fact, two subjects exposed at 9.5 to 11.5 mg/L reported no effects (except temple pressure and slight pain), a result consistent with Lester and Greenberg's (1951) result. In the later study, the BAL after 3 to 6 h of exposure at 15 mg/L was less than 10 mg/dL (0.01%), and no symptoms were reported by the three test subjects. The lack of symptoms is not sufficient to exclude performance decrements, but Kennedy et al. (1993b) observed no performance decrements at a BAL of 0.05% in 20 test subjects. Exposures in rodents show that, at a constant exposure concentration, the blood concentration actually decreases over long periods of exposure, so time effects are unimportant after the first few hours. Noting that the BAL from inhalation of 15 mg/L was less than one fifth the BAL in a study of 20 human subjects showing no performance decrements, the AC was calculated as follows:

$$AC = 15 \text{ mg/L} \times \frac{\sqrt{20}}{10} = 7 \text{ mg/L } (7000 \text{ mg/m}^3).$$

Because performance decrements are not acceptable for even short exposures, this AC applies to both short- and long-term exposures.

Mucosal and Eye Irritation

Lester and Greenberg (1951) reported that transient (< 10 min) coughing and smarting of the eyes and nose occurred at exposure concentrations between 10 and 20 mg/L. Based on that observation in three test subjects, the investigators exposed their subjects to ethanol at 15 mg/L for several hours, which the subjects found easily tolerable at low ventilation rates. Eye pain was not reported by Loewy and von der Heide (1918) in subjects exposed at concentrations below 11.5 mg/L (average); however, it was reported in two subjects exposed at 19.7 mg/L (starting concentration). That result is reasonably consistent with the finding of Lester and Greenberg (1951) that 10 mg/L is the irritation threshold. For short-term exposures, mild irritation is accepted, so the 1-h and 24-h ACs for irritation were set at 10 mg/L. Because the irritation is transient, 10 mg/L might also be taken as a NOAEL for purposes of setting long-term ACs, but the fact that only five subjects (three from the 1951 study and two from the 1918 study) were tested must be considered. The long-term (7-d and 180-d) ACs were set as follows:

$$\text{AC} = 10 \text{ mg/L} \times \frac{\sqrt{5}}{10} = 2 \text{ mg/L}.$$

This concentration should preclude risk of irritation regardless of the length of exposure.

Hepatotoxicity

Di Luzio and Stege (1979) found that Sprague-Dawley rats exposed continuously at 20 mg/L for 26 d did not exhibit any lasting liver damage, although mild transient changes were seen at 3, 6, and 9 d into the study. From this result, the long-term (7-d, 30-d and 180-d) ACs for hepatotoxicity were set as follows:

$$\text{AC} = 20 \text{ mg/L} \times 1 \div 10 \text{ (species)} = 2 \text{ mg/L}.$$

Increases in time of exposure were not considered a factor because tolerance to ethanol develops and BALs decrease as the exposure time

is increased beyond a few days. To induce hepatotoxicity with exposures of less than 24 h, the concentrations would have to be in a range that would cause narcosis; hence, short-term (1-h and 24-h) ACs were not set for liver damage.

Flush Response

No studies have directly measured the dose-response or NOAEL in a population of people known to exhibit the flush response. A NOAEL for flushing was estimated as follows: Inspection of the data of Zeiner et al. (1979) showed that the response was not observed when the breath acetaldehyde concentration (BAC) was below 10 ng/mL. Among those studied, the highest BAC was 60 ng/mL, and all subjects were given ethanol at 0.7 mL/kg. A sixfold reduction (to about 0.1 mL/kg) in the amount of ethanol given can be estimated to reduce the BAC of all subjects to below 10 ng/mL, a concentration at which no flush response would occur. Then, the NOAEL for a 70-kg subject would be as follows:

$$\text{NOAEL} = 0.1 \text{ mL/kg} \times 0.8 \text{ g/mL} \times 70 \text{ kg} = 5.6 \text{ g}.$$

Note that a NOAEL of 0.1 mL/kg is consistent with the dose range of 0.3 to 0.5 mL/kg often given to elicit the flush response in susceptible persons (Shibuya et al., 1989). The BAC profiles (Zeiner et al., 1979) show that the BAC remains above 10 ng/mL for only 2 h after oral ingestion (0.7 mL/kg); hence, one may assume that an inhaled dose must be delivered within 2 h at a threshold concentration that could induce flushing. During that period, an inhalation rate of 15 L/min and 62% uptake would result in the following concentration needed to deliver 5.6 g:

$$\text{AC} = 5.6 \text{ g} \times 0.62/(0.015 \text{ m}^3/\text{min} \times 120 \text{ min}) = 2 \text{ mg/L}$$

That concentration should be a NOAEL and applies to long-term exposures (7 to 180 d). For 1-h and 24-h exposures, some degree of headache and other symptoms associated with the flush response can be tolerated. Doubling the AC estimate to 4 mg/L would result in a 2-h delivered dose of 0.2 mL/kg (assuming a weight of 70 kg), which is

below the typical lowest dose of 0.3 mL/kg used to elicit a flush response. Hence, the 1-h and 24-h ACs were set at 4 mg/L.

TABLE 7-4 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors			Acceptable Concentrations, mg/L				
		Species	Time	Small *n*	1 h	24 h	7 d	30 d	180 d
Neurotoxicity									
NOAEL, 15 mg/L, 6 h (Lester and Greenberg, 1951; Kennedy et al., 1993b)	Human (n = 20)	1	√20/10	1	7	7	7	7	7
Irritation									
NOAEL, 10 mg/L (Lester and Greenberg, 1951; Loewy and von der Heide, 1918)	Human (n = 5)	1	√5/10	1	10	10	2	2	2
Flush response									
NOAEL, 0.2 mL (Shibuya et al., 1989)	Human	1	1	1	4	4	2	2	2
Hepatotoxicity									
NOAEL, 20 mg/L, 26 d continuous (Di Luzio and Stege, 1979)	Rat	10	1	1	—	—	2	2	2
SMACs					**4**	**4**	**2**	**2**	**2**

—, Data not considered applicable to the exposure time.

RECOMMENDATIONS

Despite the general consensus that inhaled ethanol is not harmful except in very high concentrations, no toxicity studies appear to have been conducted according to modern protocols to substantiate this conclusion. Only two studies involving human exposure were found, and they involved very few subjects, were uncontrolled, and relied on subjective reporting of symptoms. A carefully structured human inhalation study, including both flush-response and nonflush-response subjects, would provide a valuable addition to the data base.

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B8 Ethylbenzene

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PHYSICAL AND CHEMICAL PROPERTIES

Ethylbenzene (EB) is a clear, colorless, flammable liquid with an aromatic odor (Windholz, 1976; Sandmeyer, 1981; ACGIH, 1991a).

Synonyms:	Phenylethane, ethylbenzol, ethyl benzene
Formula:	C_8H_{10}; $C_6H_5(CH_2CH_3)$
CAS number:	100414
Molecular weight:	106.18
Boiling point:	136.2°C
Melting point:	−94.9°C
Lower explosive limit:	1.2%
Upper explosive limit:	6.8%
Autoignition temperature:	810°F
Flash point (closed cup):	59°F
Specific gravity:	0.8669 at 20°, referred to water at 4°C
Vapor pressure:	10 mm at 25.9°C
Vapor density:	3.66
Solubility:	Insoluble in water or ammonia; miscible in alcohol, ether, and some organic solvents; soluble in SO_2
Conversion factors at 25°C, 1 atm:	1 ppm = 4.34 mg/m^3 1 mg/m^3 = 0.23 ppm
Human odor threshold:	10 ppm

OCCURRENCE AND USE

EB is present in crude petroleum oil and is produced commercially by the alkylation of benzene with ethylene. Its principal use is as a starting material for the production of styrene by catalytic dehydrogenation. It also is used in gasoline and many industrial solvents. It is present in substantial amounts (~15%) in technical-grade xylene.

EB has been identified as a trace component of many volatile flavor compounds, such as those found in honey, jasmine, papaya, olive oil, and cheese flavors and in the neutral component of roast-beef flavor isolate (Min et al., 1979).

EB is not used in spacecraft during flight; however, occasionally it is found in the spacecraft atmosphere at concentrations of up to 0.12 ppm, probably due to off-gassing from nonmetallic materials (Liebich et al., 1973; Rippstein and Coleman, 1983).

TOXICOKINETICS AND METABOLISM

The rates of absorption, distribution, and excretion of EB are functions of its solubilities in the various compartments of body tissues and fluids. EB is taken up from the lungs at a moderate rate by the blood and tends to partition preferentially into fat, as indicated by its partition coefficients of 28.4, 1.7, and 3790 for blood, water, and oil, respectively (Sato and Nakajima, 1987). Although no studies were found on the rate of uptake of inhaled EB into the blood in humans exposed to known concentrations of EB, a good estimate of the time needed to reach steady-state blood concentrations can be made based on data available for xylene, a structural isomer of EB. For *m*-xylene, the concentration in the blood reached a steady state in about 1 h in eight subjects exposed to EB at 100 ppm (Riihimäki and Savolainen, 1980).

Humans exposed for 8 h to EB at 23, 43, 46, and 86 ppm retain 49-64% of the EB inhaled (Bardodej and Bardodejova, 1970). Only 4-5% of the retained EB is estimated to be exhaled without transformation (Åstrand et al., 1978). The half-life of EB in the exhaled breath is 0.5 to 3 h (Wolf, 1976).

In humans exposed to EB at up to 85 ppm for 8 h, only traces of EB were found in the expired air after termination of exposure, and only a negligible amount of the unchanged hydrocarbon was excreted in the urine (Bardodej and Bardodejova, 1970). Absorbed EB is excreted mainly in the urine as mandelic and phenylglyoxylic acids (Bardodej and Bardodejova, 1970; Engström et al., 1984; Gromiec and Piotrowski, 1984). Urinary elimination of mandelic acid is biphasic, with half-lives of 3.1 and 24.5 h (Gromiec and Piotrowski, 1984). In four volunteers exposed to EB at 150 ppm, mandelic acid and phenylglyoxylic acid amounted to 90% of the metabolites, and 4% consisted of 4-ethylphenol, *p*-hydroxyacetophenone, and *m*-hydroxyacetophenone (Engström et al., 1984).

Rats differ from humans in metabolism of EB, as shown in Table 8-1 (Bardodej and Bardodejova, 1970). It should be noted, however, that the exposure conditions differed: male Wistar rats were exposed at 0, 300, or 600 ppm for 6 h (Engström et al., 1985), and humans were exposed at up to 85 ppm for 8 h. In rats, quantitation of the total output of EB by measurement of urinary metabolites is complicated by the fact that some EB metabolites also are formed endogenously.

The rat study cited above (Engström et al., 1985) also examined elimination kinetics. At 600 ppm, 6% of the amount absorbed (assuming 60% retention) was excreted in the urine during exposure, and 59% was excreted within 48 h of onset of exposure. At 300 ppm, the amounts were 13% during exposure and 83% within 48 h of exposure (Engström, 1984). In rats inhaling ^{14}C-labeled EB at 230 ppm for 6 h, about 80-90% of absorbed ^{14}C label was excreted in the urine and about 10% was exhaled in breath (Chin et al., 1978).

TABLE 8-1 Metabolism of EB in Humans and Rats

Urinary Metabolites	Humans	Rats
Mandelic acid	64%	25%
Phenylglyoxylic acid	25%	—
Methylphenylcarbinol	5%	—
1-Phenylethanol	—	25%
Benzoic acid	—	27%

In rats exposed 6 h/d for 5 d to various mixtures of xylene and EB in air, metabolite excretion rates abruptly increased from the second day onward for rats exposed to a mixture of *m*-xylene at 600 ppm and EB at 200 ppm, an exposure concentration that also increased microsomal drug-metabolizing activity in the liver. That mixture also resulted in interactive metabolism; the metabolism of EB was specifically enhanced at that exposure concentration but not at an exposure concentration of *m*-xylene at 300 ppm and EB at 100 ppm.

TOXICITY SUMMARY

The acute toxicity of EB is low. The main effect of exposure to EB vapors is irritation of eyes, nose, and mucous membranes at concentrations of about 200 ppm; higher concentrations cause central-nervous-system (CNS) depression and transient liver and kidney injury (Von Burg, 1992). Exposure to more than 460 ppm can cause acute poisoning in laboratory animals (Aldyreva, 1983). Toxic signs include irritation of mucous membranes, conjunctivitis, difficulty in breathing, pulmonary irritation, cramps, coordination disorders, narcosis, convulsions, and death due to respiratory center paralysis (Aldyreva, 1983). The main pathological findings are marked edema of brain and lungs, foci of epithelial necrosis in renal tubules, and hepatic dystrophy (Aldyreva, 1983).

Acute and Short-Term Exposures

Lethality

Smyth et al. (1962) reported a 4-h LC_{50} of EB at 4000 ppm for rats. Yant et al. (1930) reported EB vapor to be lethal at 5000 ppm to guinea pigs in 30 to 60 min; 10,000 ppm was lethal in "a few minutes."

Eye and Respiratory Irritation

Six volunteers exposed to EB at 1000 ppm experienced initial severe

eye irritation and lacrimation that decreased after 1 or 2 min to scarcely noticeable (Yant et al., 1930). The same six volunteers found EB at 2000 ppm almost intolerable initially but less irritating upon continued exposure, and one volunteer who remained in the chamber for 5 min found that the eye and throat irritation gradually disappeared, but vertigo developed (Yant et al., 1930). Exposure at 2000 ppm was accompanied by a feeling of constriction of the chest (Yant et al., 1930). Four volunteers exposed for 6 min to EB accumulating in a chamber to a concentration of 2000 ppm noticed moderate nasal irritation and moderate-to-strong eye irritation; all complained of dizziness upon leaving the chamber. Three volunteers entering a 5000-ppm EB atmosphere found it intolerably irritating to the eyes, nose, and throat (Yant et al., 1930).

Bardodej and Bardodejova (1961) investigated the metabolism of inhaled EB in humans. During 8-h exposures to EB vapors, they found that nine subjects exposed at 100 ppm did not complain of any problems, but 11 subjects exposed at 180 ppm complained of irritation of the respiratory tract and conjunctiva.

Yant et al. (1930) reported that groups of three to six guinea pigs exposed to EB at 1000 ppm showed signs of nasal irritation (rubbing nose) after 3 min and signs of eye irritation and lacrimation after 8 min of exposure. Comparing the responses of guinea pigs with those of humans reported by Yant et al. (1930), guinea pigs and humans appear to suffer similar signs at 1000 ppm, but the effects in guinea pigs might be delayed slightly more than those in humans.

CNS Effects

In the report by Bardodej and Bardodejova (1961) in which the metabolism of EB was studied in humans, nine subjects exposed at 100 ppm for 8 h did not complain of any problems, but toward the end of the exposure time at 180 ppm, 11 subjects complained of headaches and sleepiness. Because blood concentrations probably reached steady state early ($\sim$ 1 h) in the exposure, the kinetics suggest that headaches and sleepiness are due to metabolites of EB. Transient sensations of slight drunkenness also were reported.

In the Yant et al. (1930) study of groups of three to six guinea pigs

exposed to various concentrations of EB, a 480-min exposure at 1000 ppm produced only transient slight nasal irritation and slight lacrimation, which disappeared within 30 min, but no vertigo or ataxia. At 2000 ppm, guinea pigs showed signs of moderate eye and nasal irritation after 1 min, vertigo after 390 min, and ataxia after 480 min of exposure (Yant et al., 1930). At 5000 ppm, vertigo was seen after 26 min, ataxia after 30 min, apparent unconsciousness after 160 min, tremors of the extremities after 178 min, and shallow respiration after 215 min of exposure (Yant et al., 1930). At 10,000 ppm, vertigo and ataxia were seen after 4 to 10 min, unconsciousness after 18 min, tremors of the extremities after 5 to 18 min, rapid jerky respiration after 21 min, shallow respiration after 57 min, and very slow gasping respiration after 260 min (Yant et al., 1930).

Molnár et al. (1986) reported that narcosis occurred in groups of eight rats exposed for 4 h to EB at concentrations as low as 2180 ppm but not at 1500 ppm. Concentrations of 400 to 1500 ppm induced a moderate increase in group motility in rats exposed for 1, 2, 3, or 4 h.

Subchronic Exposures

Lethality

Cragg et al. (1989) reported a 4-d LC_{100} of EB at 2400 ppm for 6 h/d for rats and mice and an LC_{80} at 1200 ppm (four of five mice died). Chan (1992) reported a no-observed-adverse-effect level (NOAEL) at 1000 ppm for lethality in rats and mice for 13 w of exposure for 6 h/d, 5 d/w.

NOAELs for All Measured Effects

In rats and mice exposed 6 h/d, 5 d/w, for 4 w, the NOAEL for clinical chemistry, hematological, urological, and histological toxicity was reported to be 382 ppm; in similarly exposed rabbits, the NOAEL was 782 ppm (Cragg et al., 1989). In a 13-w National Toxicology Program (NTP) study of rats and mice, the highest exposure concentration tested (1000 ppm) was found to be a NOAEL for mutagenicity,

clastogenicity, and clinical or histopathological changes; effects observed were increases in weights of kidney, liver, and lung in rats and weights of livers in mice (Chan, 1992).

Elovaara et al. (1985) showed that exposure of rats to EB at concentrations up to 600 ppm for 6 h/d, 5 d/w, for up to 16 w increased the levels of liver proteins and increased the activity of selected liver and kidney enzymes (Elovaara et al., 1985). Glutathione levels remained constant in the liver and increased 30% in the kidney. Ultrastructural changes included slight proliferation of smooth endoplasmic reticulum, slight degranulation and splitting of the rough endoplasmic reticulum, and enlarged mitochondria. These data are not used to set acceptable concentrations (AC) for two reasons: first, all of the changes can be considered adaptive rather than adverse; second, the results could not be easily extrapolated to humans because metabolism of EB in humans has been shown to be qualitatively and quantitatively different from metabolism of EB in rats.

Respiratory Distress, CNS Effects

In a range-finding study, Cragg et al. (1989) exposed male mice, rats, and rabbits (4-5 animals per species) 6 h/d for 4 d to EB at 400, 1200, and 2400 ppm. At 1200 ppm, all rats showed marked clinical signs, including respiratory distress, salivation, prostration, and anogenital staining. The signs were similar in one surviving mouse of five mice exposed at 1200 ppm. No effects were noted in mice or rats exposed at 400 ppm. Rabbits showed no signs of respiratory distress or CNS effects at concentrations up to 2400 ppm (Cragg et al., 1989). In a recent NTP study (Chan, 1992), however, no adverse effects were seen in rats or mice exposed at 1000 ppm (the highest exposure concentration tested) for 6 h/d, 5 d/w, for 13 w (equivalent to ~16 d if exposure were continuous). Because those effects are less sensitive indicators than eye and respiratory irritation in humans, no ACs were set on the basis of those end points. ACs based on eye and respiratory irritation should protect against the more severe effects.

Neurochemical Changes

Exposure of rabbits to EB at 750 ppm for 12 h/d for 7 d caused a

marked depletion of striatal and tubero-infundibular dopamine (Mutti et al., 1988). Similar effects were achieved using mandelic and phenylglyoxylic acid, which are metabolites of EB. Experiments in vitro suggest that dopamine condenses nonenzymatically with reactive carbonylic groups of such α-keto acids, thus becoming ineffective as a neurotransmitter. Although those results might help explain the mechanism of EB's CNS effects, the neurochemical changes are not correlated to a functional deficit; thus, those results cannot be used to set an AC.

Chronic Exposures

Sleepiness, Fatigue, Eye, and Respiratory Irritation and Headache

Bardodej and Bardodejova (1970) measured exposures of industrial workers to EB, styrene, and α-methylstyrene. The study showed that some symptoms were reported by the workers if the exposure exceeded the threshold limit value of 100 ppm for EB. "The main complaints were fatigue, sleepiness, and headache, and mild irritation of the eyes and respiratory tract, particularly in the case of styrene." No detailed data were reported on the range of exposure concentrations, the duration of the exposures necessary to produce the reported effects, how EB concentrations in the workplace were determined, the numbers of individuals exposed, or whether any or all of the symptoms correlated with EB exposure concentrations.

Carcinogenicity

Bardodej and Cirek (1988) conducted biomonitoring of occupational EB exposures for 20 y (1964-1985) in approximately 200 Czechoslovakian production workers. No excess malignancies were seen in the workers for the last 10 y of the study. None of the exposed workers showed excess damage to hematopoiesis or liver tissue (Bardodej and Cirek, 1988). Although levels of urinary metabolites were monitored, atmospheric concentration measurements were not reported except to say that they were below the Czechoslovakian maximum allowable concentration (MAC) (46 ppm time-weighted average) after 1974.

Kidney and Liver Changes

Wolf et al. (1956) reported that a slight increase in liver weights was induced by inhalation of EB by male and female rats at concentrations of 400, 600, and 1250 ppm; by male rats at 2200 ppm; by guinea pigs at 600 ppm, but not at 400 or 1250 ppm; and by monkeys at 600 ppm, but not at 400 ppm, for 7-8 h/d, 5 d/w, for 6 mo (144 d at 2200 ppm). They also reported that slight "cloudy swelling" in the liver occurred in rats inhaling 1250 ppm for 186 d or 2200 ppm for 144 d (Wolf et al., 1956).

In the same study, rats, but not guinea pigs, rabbits, or monkeys, exposed to EB at concentrations of 400, 600, 1250, and 2200 ppm for 186 d (144 d at 2200 ppm) were found to have slightly increased kidney weights. Rats exposed at 1250 or 2200 ppm also exhibited slight "cloudy swelling" of the tubular epithelium of the kidney (Wolf et al., 1956).

Testicular Toxicity

Wolf et al. (1956) exposed 10 to 25 rats to EB at concentrations of 0, 400, 600, 1250, or 2200 ppm; 5 to 10 guinea pigs and 1 rabbit at 0, 400, 600, and 1250 ppm; and 1 to 2 monkeys at 0, 400 or 600 ppm for 7-8 h/d, 5 d/w, for 186 d. In the rabbit and monkey, 600 ppm induced slight histopathological changes described as degeneration of the germinal epithelium of the testes (concentration $\times$ time (C $\times$ T) = 900,000 ppm·h. No male monkeys were tested at exposure concentrations below 600 ppm, but no effects on the testes were seen in one male rabbit exposed at 400 ppm (C $\times$ T = 600,000 ppm·h). No testicular effects were seen in guinea pigs or rats at any of the tested exposure concentrations (up to 2200 ppm in rats). Although these results may be due to species differences in metabolism, it is not known whether the metabolism of humans is more similar to that of monkeys or rabbits than of rats.

The preceding results can be compared with the results of Cragg et al. (1989) who found no gross or microscopic changes in rabbit testes (in five rabbits per group) attributable to EB exposure at concentrations of 382, 782, or 1610 ppm for 6 h/d, 5 d/w, for 4 w (20 expo-

sures) (C × T = 46,000 to 190,000 ppm·h). Although no effects were seen, the total dose in these experiments was 4.7-fold below that used by Wolf et al. (1956), a dose that led to testicular toxicity.

Ivanov (1964, as cited by Cragg et al., 1989) exposed rabbits to EB at 2.3, 23, and 230 ppm for 4 h/d for 7 mo. Further protocol details were not provided. Assuming exposure for 7 d/w, the maximum dose for the rabbits (expressed as C × T) was 190,000 ppm·h.

Genotoxicity

EB injected intraperitoneally into mice did not induce micronuclei in their bone-marrow cells (Mohtashamipur et al., 1985). EB was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA97, and TA98 when tested at doses up to 1000 μg per plate, which was toxic to the bacteria, in the presence or absence of S9 (Dean et al., 1985; Chan, 1992). EB was not mutagenic in strains TA1537 or TA1538, in *Escherichia coli* WP2 or WP2uvrA (Nestmann et al., 1980; Dean et al., 1985), or in *Saccharomyces cerevisiae* JD1 (Dean et al., 1985). EB exposure did not induce sister chromatid exchanges (SCEs) or chromosomal aberrations in CHO cells in the presence or absence of S9 (Chan, 1992), but it did induce a slight increase in SCEs in cultured human lymphocytes in the presence of S9 (Norppa and Vainio, 1983).

Reproductive and Developmental Toxicity

The offspring of rats inhaling EB continuously from gestation d 7 to 15 showed moderately increased rates of malformations at 552 ppm compared with controls and increased rates of skeletal retardation and dead or resorbed fetuses at 138, 276, and 552 ppm (Ungváry and Tátrai, 1985). The offspring of mice inhaling EB at 115 ppm continuously from gestation d 6 to 15 showed only moderately increased rates of malformations compared with controls (Ungváry and Tátrai, 1985). EB caused spontaneous abortion in rabbits exposed at 230 ppm continuously from gestation d 7 to 20 (Ungváry and Tátrai, 1985). No ACs are based on those effects.

Interactions with Other Chemicals

In rats exposed to EB at a concentration of 650 ppm, coexposure to ethyl acetate at 1000 or 4000 ppm lowered the concentrations of EB in the blood by 26% (Freundt et al., 1989). In rats exposed at 180 ppm, coexposure to ethanol at 20 mmol/kg increased the blood concentration of EB by 139%.

TABLE 8-2 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
101	8 h	Human (n = 9)	NOAEL for eye and respiratory irritation, sleepiness, headache, and transient sensations of slight drunkenness	Bardodej and Bardodejova, 1970
150	4 h	Human (n = 4)	No toxic effects reported; study examined urinary metabolites of EB	Engström et al., 1984
184	8 h	Human (n = 11)	LOAEL for eye and respiratory irritation; transient sensations of slight drunkenness and, toward the end of exposure, sleepiness and headache	Bardodej and Bardodejova, 1970
200	NS	Human	Transient eye irritation	Gerarde, 1963
1000	A few min	Human (n = 6)	Initial severe eye irritation and lacrimation, decreasing to very slight within 2 min	Yant et al., 1930
2000	5 min	Human (n = 1)	Initial extreme eye, nose, and throat irritation and feeling of chest constriction, gradually disappearing with development of vertigo	Yant et al., 1930
5000	A few breaths	Human (n = 3)	Intolerable irritation of eyes and nose	Yant et al., 1930
115	6 h/d, 5 d/w	Mouse	Moderately increased fetal malformation	Ungváry and Tátrai, 1985
138	6 h/d, 5 d/w	Rat	Increased "skeletal retardation," dead or resorbed fetuses	Ungváry and Tátrai, 1985
230	6 h/d, 5 d/w	Rabbit	Spontaneous abortion	Ungváry and Tátrai, 1985
382	6 h/d, 5 d/w, 4 w	Rat, mouse	NOAEL	Cragg et al., 1989

TABLE 8-2 *(Continued)*

Concentration, ppm	Exposure Duration	Species	Effects	Reference
400	6 h/d, 4 d	Rat, mouse	NOAEL for salivation, prostration, anogenital staining	Cragg et al., 1989
552	6 h/d, 5 d/w	Rat	Increased rate of fetal malformations	Ungváry and Tátrai, 1985
600	7-8 h/d, 5 d/w, 6 mo	Rat	Clouding and swelling of kidney tubular epithelium and hepatocytes	Wolf et al., 1956
600	6 h/d, 5 d/w, 16 w	Rat	Increased levels of proteins and increased liver and kidney enzyme activities	Elovaara et al., 1985
600	186 d	Guinea pig	No testicular histopathological changes	Wolf et al., 1956
600	186 d	Monkey, rabbit	Slight testicular histopathological changes	Wolf et al., 1956
750	12 h/d, 7 d	Rabbit	Marked depletion of striatal and tubero-infundibular dopamine	Mutti et al., 1988
782	6 h/d, 5 d/w, 4 w	Rabbit	NOAEL	Cragg et al., 1989
1000	6 h/d, 5 d/w, 13 w	Rat, mouse	NOAEL for histopathological and clinical effects	Chan, 1992
1000	3 min	Guinea pig	Nasal irritation—rubbing nose	Yant et al., 1930
1000	8 min	Guinea pig	Eye irritation—squinting and lacrimation	Yant et al., 1930
1000	480 min	Guinea pig	Slight transient nasal irritation and slight lacrimation that disappeared within 30 min	Yant et al., 1930
1200	6 h/d, 4 d	Rat	Salivation, prostration, anogenital staining	Cragg et al., 1989
1200	6 h/d, 4 d	Mouse	LC_{80} (4 of 5 mice died)	Cragg et al., 1989
1250	214 d	Rat	No testicular histopathological changes	Wolf et al., 1956
1500	4 h	Rat	NOAEL for anesthesia	Molnár et al., 1986

1610	6 h/d, 5 d/w	Rabbit	NOAEL for testicular toxicity	Cragg et al., 1989
2000	1 min	Guinea pig (n = 3 to 6)	Eye irritation—squinting and lacrimation	Yant et al., 1930
2000	1 min	Guinea pig (n = 3 to 6)	Nasal irritation—rubbing nose	Yant et al., 1930
2000	390 min	Guinea pig (n = 3 to 6)	Vertigo—unsteadiness	Yant et al., 1930
2000	480 min	Guinea pig (n = 3 to 6)	Static and motor ataxia	Yant et al., 1930
2180	4 h	Rat	LOAEL for anesthesia	Molnár et al., 1986
2200	144 d	Rat	No testicular histopathological changes	Wolf et al., 1956
2400	6 h/d, 4 d	Rabbit	NOAEL for clinical signs	Cragg et al., 1989
2400	6 h/d, 4 d	Mouse, rat	LC_{100}	Cragg et al., 1989
4000	4 h	Rat	LC_{50}	Smyth et al., 1962
5000	26 min	Guinea pig (n = 3 to 6)	Vertigo	Yant et al., 1930
5000	30 min	Guinea pig (n = 3 to 6)	Ataxia	Yant et al., 1930
5000	160 min	Guinea pig (n = 3 to 6)	Apparent unconsciousness	Yant et al., 1930
5000	178 min	Guinea pig (n = 3 to 6)	Tremors of extremities	Yant et al., 1930
5000	215 min	Guinea pig (n = 3 to 6)	Shallow respiration	Yant et al., 1930
8000	4 h	Rat	LC_{50}	Smyth et al., 1962
10,000	4-10 min	Guinea pig	Vertigo and ataxia	Yant et al., 1930
10,000	18 min	Guinea pig	Apparent unconsciousness	Yant et al., 1930
10,000	5-18 min	Guinea pig	Tremors of extremities	Yant et al., 1930
10,000	21 min	Guinea pig	Rapid jerky respiration	Yant et al., 1930
10,000	57 min	Guinea pig	Shallow respiration	Yant et al., 1930
10,000	260 min	Guinea pig	Very slow gasping type of respiration	Yant et al., 1930

NS, not specified.

TABLE 8-3 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV (1985)	100	ACGIH, 1991b
ACGIH's STEL	125	ACGIH, 1991b
NIOSH's REL	100 (TWA)	ACGIH, 1991b
OSHA's PEL	100	ACGIH, 1991b

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

TABLE 8-4 Spacecraft Maximum Allowable Concentrations

Exposure Duration[a]	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	180	780	Eye and respiratory irritation, headache, sleepiness
24 h	60	260	Headache, sleepiness
7 d[b]	30	130	Eye and respiratory irritation, testicular toxicity
30 d	30	130	Eye and respiratory irritation, testicular toxicity
180 d	12	50	Testicular toxicity

[a]Ceiling limits.

[b]The former 7-d SMAC is 20 ppm (86.8 mg/m^3).

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

The evaluation of and setting priorities for the toxicological effects listed in Table 8-2 took into account various factors. For EB, rodent toxicity was not heavily weighted because rat metabolism of EB has

been shown to yield products qualitatively and quantitatively different from those of humans. The human metabolic products of EB, however, caused neurochemical effects in rabbits similar to those caused by EB. Because differences in metabolism in humans and in lower primates have not been reported, data on toxicity in monkeys could not be discounted. Fetal toxicity was not considered in setting ACs because the National Aeronautics and Space Administration will not knowingly send a pregnant astronaut into space.

Of the toxic effects attributed to EB, ACs were not set for the following: lethality (adequate protection would be achieved by ACs for less severe effects); genotoxicity (minimal effect demonstrated; quantitation of dose-response relationship is not currently possible); respiratory distress (adequate protection would be achieved by ACs for less severe effects); neurochemical changes (no correlation to a functional deficit); developmental toxicity (not applicable to astronauts); carcinogenicity (none observed); and kidney and liver changes (demonstrated effects at high exposure concentrations were not judged to be adverse). ACs were set for the remaining effects: eye and respiratory irritation, testicular toxicity, and sleepiness and headache.

Calculation of the highest AC for each major end point and exposure duration is documented below. The resulting ACs for the various end points are listed in Table 8-5. SMAC values set at each exposure duration are based on the end point that yielded the lowest AC at that duration, following the guidelines of the National Research Council (NRC, 1992).

Eye and Respiratory Irritation

Bardodej and Bardodejova (1961) reported that 100 ppm was an 8-h NOAEL for 9 volunteers and 180 ppm was a lowest-observed-adverse-effect level (LOAEL) for 11 volunteers for eye and respiratory irritation and, toward the end of the 8-h exposure, for sleepiness and headache. Because irritation of the eye and respiratory system is generally independent of exposure duration and sleepiness and headache might increase in intensity with longer exposures, these end points will be dealt with separately. Mild eye and respiratory irritation would be acceptable

for emergency situations and the intensity should not increase with longer exposures; therefore, the 1-h and 24-h ACs are set equal to the 8-h LOAEL:

$$\text{1-h and 24-h ACs} = \text{8-h LOAEL} = 180 \text{ ppm.}$$

For exposures longer than 24 h, no irritation is acceptable. Thus, these ACs are based on the 8-h NOAEL and adjusted for the low number of subjects tested:

7-d, 30-d, and 180-d ACs

$$= \text{8-h NOAEL} \times \frac{\sqrt{n}}{10} = 100 \text{ ppm} \times \frac{\sqrt{9}}{10} = 30 \text{ ppm.}$$

Testicular Toxicity

In a study by Wolf et al. (1956), 10-25 rats per group were exposed to EB at 2200, 1250, 600, or 400 ppm, 5-10 guinea pigs per group were exposed at 1250, 600, or 400 ppm, one male rabbit per exposure concentration was exposed at 400, 600, and 1250 ppm, and one male monkey per exposure concentration was exposed at 400 and 600 ppm for 7-8 h/d, 5 d/w, for 186 d. In the rabbit and monkey, EB at 600 ppm for 7-8 h/d, 5 d/w, for 186 d induced slight histopathological changes described as degeneration of the germinal epithelium of the testes. No testicular toxicity was seen in the one male rabbit tested at 400 ppm. No testicular effects were seen in guinea pigs or in rats at any of the tested exposure concentrations (up to 2200 ppm in rats). No other chronic inhalation studies on monkeys have been found that could be used to confirm or discredit these findings. One Russian study (Ivanov, 1964) cited by Cragg et al. (1989) exposed rabbits to EB at 2.3, 23, and 230 ppm for 4 h/d for 7 mo. That study reported hematological changes, changes in blood protein and cholinesterase levels, and dystrophic changes in kidneys and liver but did not mention effects on the testes. It is not known if the testes were examined. Thus, some evidence points to toxicity of EB to the testes, but that toxicity certainly has not been firmly established. Nevertheless, because an effect was

reported in a primate, and at the risk of erring on the conservative side, ACs were calculated by dividing the 400-ppm NOAEL by a factor of 10 for possible species differences. Haber's rule was used to extrapolate from 54 d (i.e., where 7 h/d ÷ 24 h/d × 186 d = 54 d) but was not used to increase ACs for exposures shorter than 54 d.

180-d AC = NOAEL/10 (species) × (54 d/180 d) = 400 ppm/10 × 0.3 = 12 ppm.

30-d and 7-d AC = 400 ppm/10 (species) = 40 ppm.

No ACs were set for 1 h and 24 h because it would require a time extrapolation of greater than 10-fold.

Sleepiness and Headache

The AC for 1-h and 24-h exposures can be set on the basis of the 180-ppm 8-h LOAEL for sleepiness and headache in human volunteers. Although narcosis is not acceptable, slight sleepiness, headache, and fatigue would be acceptable for emergency 1-h and 24-h exposures. Thus, the ACs for a 1-h exposure is set equal to the 8-h LOAEL (which is a NOAEL for 1 h), and the AC for a 24-h exposure is reduced threefold to ensure that the slight headaches do not become severe.

1-h AC = 8-h NOAEL = 180 ppm.
24-h AC = 8-h LOAEL/3 = 180/3 = 60 ppm.

ACs were not set for exposure periods of 7 d or more because that would require more than a 10-fold time extrapolation from the data.

Spaceflight Considerations

None of the toxic effects known to be induced by exposure to EB would be affected by launch, microgravity, or re-entry; thus, no spaceflight factor was used.

RECOMMENDATIONS

- Additional research is needed to confirm or disprove the reported testicular toxicity of long-term exposure to EB in rabbits and possibly in monkeys and the potential of EB to cause testicular toxicity in humans.
- Testing of a large number (near 100) of volunteers for irritation at the 100-ppm exposure concentration for 4 h or more might permit the ACs for 7 d and 30 d to be increased from 30 to 100 ppm.
- Testing of volunteers for more than 8 h at several concentrations is needed to examine the effect of long (greater than 24 h) continuous exposures on headache and sleepiness in humans. Performance testing would be a necessary part of this experiment.

TABLE 8-5 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors: To NOAEL	Species	Time	Space-flight	Acceptable Concentrations, ppm: 1 h	24 h	7 d	30 d	180 d
Eye and respiratory irritation										
LOAEL, 184 ppm, 8 h (Bardodej and Bardodejova, 1970)	Human (n = 11)	1	1	1	1	180	180	—	—	—
NOAEL, 101 ppm, 8 h (Bardodej and Bardodejova, 1970)	Human (n = 9)	0.3 = $\sqrt{(n/100)}$	1	1	1	—	—	30	30	30
Testicular toxicity										
NOAEL, 400 ppm, 7-8 h/d, 5 d/w, 186 d (Wolf et al., 1956)	Rabbit, monkey	1	10	HR	1	—	—	40	40	12
Sleepiness and headache										
8-h LOAEL, 184 ppm (Bardodej and Bardodejova, 1970)	Human (n = 11)	1	1	1 or HR	1	180	60	—	—	—
SMACs						**180**	**60**	**30**	**30**	**12**

—, Data not considered applicable to the exposure time; HR, Haber's rule.

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B9 Ethylene Glycol

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PHYSICAL AND CHEMICAL PROPERTIES

Ethylene glycol is a clear, colorless, odorless, and viscous liquid (ACGIH, 1986).

Synonym:	1,2-Ethanediol
Formula:	$HOCH_2CH_2OH$
CAS number:	107-21-1
Molecular weight:	62.1
Boiling point:	197.6°C
Melting point:	−13°C
Vapor pressure:	0.06 torr at 20°C
Saturated vapor concentration at 20°C:	204 mg/m^3 or 79 ppm
Conversion factors at 25°C, 1 atm:	1 ppm = 2.54 mg/m^3 1 mg/m^3 = 0.39 ppm

OCCURRENCE AND USE

Ethylene glycol is used as an antifreeze, an industrial humectant, and a solvent in paint and in the plastics industry (ACGIH, 1986). In the U.S. space program, it was used as an antifreeze in a payload experiment in one shuttle mission (Lam, 1988), and it was a component inside lithium manganese dioxide batteries used in payload experiments in two

shuttle missions (Lam, 1990, 1991). Ethylene glycol has also been predicted to be found as an off-gas product in the space station, but only in relatively small amounts (Leban and Wagner, 1989). One of the possible reasons is that its vapor pressure is low compared with other organic compounds found in off-gassing.

TOXICOKINETICS AND METABOLISM

Absorption

Marshall and Cheng (1983) exposed rats, nose only, to ^{14}C-ethylene glycol vapor at 32 mg/m^3 for 30 min and found that at least 60% of the chemical inhaled was absorbed. Immediately after exposure, the concentrations of ^{14}C activity in various tissues were compared, and those in the nasal cavity and the trachea were the highest. On the basis of the initial body burden of the ^{14}C activity, Marshall and Cheng (1983) calculated that the rats received a dose of 0.7 mg/kg in the 30-min inhalation exposure at 32 mg/m^3.

They also observed that the plasma concentration of ^{14}C activity peaked 6 h after the 30-min inhalation exposure of rats to ethylene glycol at 32 mg/m^3. Assuming that all the ^{14}C activity was due to ^{14}C-labeled ethylene glycol, Marshall and Cheng (1983) calculated that the peak plasma concentration of ethylene glycol was about 1 μg/mL.

Metabolism

Ethylene glycol is metabolized via oxidation. McChesney et al. (1971) found that, in monkeys exposed orally to ethylene glycol at 1 mL/kg (or 1.1 g/kg), the metabolites included glycolic acid, oxalic acid, CO_2, and a small amount of hippuric acid. The metabolic pathway probably involves stepwise oxidation. McChesney et al. (1972) proposed that ethylene glycol is first oxidized to glycolaldehyde, which is then oxidized to glycolic acid. Oxidation of glycolic acid results in glyoxylic acid. Glyoxylic acid might undergo several reactions, one of which is oxidation by glycolic acid dehydrogenase to oxalic acid (Rich-

ardson and Tolbert, 1961). Another reaction is the formation of formyl-S-CoA and CO_2 (McChesney et al., 1972). According to Richardson and Tolbert (1961), alcohol dehydrogenase and glycolic acid dehydrogenase are involved in the catalysis of some of the reactions in the metabolic pathway.

Excretion

On the basis of data gathered in laboratory animals, ethylene glycol or its metabolites are excreted via three routes: urinary, expiratory, and fecal. In four monkeys given ethylene glycol orally at 1 mL/kg, about 44% of the dose was excreted in the urine within 24 h of the oral exposure, and very little was excreted in the urine in the 24-48 h period (McChesney et al., 1971). Half of the dose excreted in urine was unchanged ethylene glycol, and the balance was metabolites. Urinary metabolites collected for 48 h from one of those monkeys were analyzed. The results revealed that glycolic acid was the most abundant, accounting for 12% of the dose. Only 0.3% of the dose was excreted as oxalic acid in the urine of this monkey. Similar findings were reported by Wiley et al. (1938); in two dogs exposed to ethylene glycol at 5.5 g daily for 5 d, the conversion rate of ethylene glycol to urinary oxalic acid was 0.5%.

There is evidence that the conversion rates of ethylene glycol to oxalic acid in rats and humans differ from the rates in the monkey and two dogs (Wiley et al., 1938; Levy, 1960). In addition to the monkey, McChesney et al. (1971) also studied the urinary excretion of ethylene glycol and its metabolites in six rats exposed orally to ethylene glycol at 1 mL/kg of body weight (or 1.1 g/kg). Rats excreted 56% of the dose in the urine within 24 h of oral exposure, and 32% of the dose was excreted as unchanged ethylene glycol. These rats excreted 2.5% of the dose as oxalic acid in the urine. The conversion rate to oxalic acid in rats is very similar to that in humans who have been found to convert about 2.3% of the ethylene glycol dose to oxalic acid in urine (Reif, 1950).

In the inhalation study of rats conducted by Marshall and Cheng (1983), 63% of the body burden of ethylene glycol was expired as $^{14}CO_2$ within 4 d of the exposure. Urinary excretion was the second most important route of ethylene glycol elimination. The researchers

found that 20% of the initial body burden was excreted unchanged in the urine. Fecal excretion was the least important elimination route, because only 3% of the body burden was eliminated in the feces.

The relative abundance of urinary elimination of ethylene glycol as the unchanged parent compound and metabolites versus CO_2 in expired air is dose-dependent. When rats were injected intravenously with ethylene glycol at 20 or 200 mg/kg, 35% of the dose was excreted in the urine and 39% of the dose was expired as CO_2 (Marshall, 1982). When the dose was increased, however, urinary excretion became more important than CO_2 expiration in eliminating ethylene glycol from the body. At a dose of 1000 or 2000 mg/kg, 56% of the dose was excreted in the urine, and only 26% was expired as CO_2.

Toxicokinetics

Several toxicokinetics studies on ethylene glycol have been reported in the literature, but most of them involved a noninhalation route of exposure. Hewlett et al. (1989) compared the elimination half-lives of ethylene glycol in the plasma of rats and dogs. Rats were gavaged with ethylene glycol at 2 g/kg and the dose for dogs was 1 g/kg. Ethylene glycol was eliminated faster in the rats than the dogs; the half-life in the plasma was found to be only 1.7 h in rats versus 3.4 h in dogs. Comparison of the data of Hewlett et al. (1989) and McChesney et al. (1971) shows that rhesus monkeys tend to eliminate ethylene glycol at about the same rate as dogs. A half-life of 2.7-3.7 h in rhesus monkeys, which were given an oral dose of ethylene glycol at 1 mL/kg of body weight (or 1.1 g/kg), was found by McChesney et al. (1971).

The elimination half-life of ethylene glycol appears to be dose-dependent. A review of the data shown in Table 9-1 indicates that the half-life lengthened when ethylene glycol was administered orally at a higher dose.

The only toxicokinetics study with inhalation exposures to ethylene glycol was one conducted by Marshall and Cheng (1983). In that study, after reaching a peak 6 h following inhalation exposure of rats to ^{14}C-ethylene glycol, the plasma concentration of ^{14}C activity declined monoexponentially for the remaining 4 d, with a half-life of 39 h. Evidently, not all the ^{14}C activity represented ethylene glycol. Hewlett et al. (1989) and Lenk et al. (1989) reported that the half-life of ethylene

TABLE 9-1 Elimination Half-life of Ethylene Glycol

Oral Dose, g/kg	Species	Half-life	Reference
1.1	Monkey	2.7-3.7	McChesney et al., 1971
2	Rat	1.7	Hewlett et al., 1989
3.3-5.5	Rat	4.1-4.5	Lenk et al., 1989
1	Dog	3.4	Hewlett et al., 1989
10.7	Dog	10.8	Grauer et al., 1987

glycol in plasma was only 1.7 h or 4.1-4.5 h in rats given an oral dose of ethylene glycol at 1-2 g/kg or 3.3-5.5 g/kg, respectively. The half-life of the plasma ^{14}C activity measured by Marshall and Cheng (1983) was 10-20 times that of ethylene glycol reported by Hewlett et al. (1989) and Lenk et al. (1989). Therefore, the half-life of 39 h of the plasma ^{14}C activity reflected the elimination of ethylene glycol's metabolites (Marshall and Cheng, 1983). Since Hewlett et al. (1989) reported that the elimination half-life of glycolic acid equaled that of ethylene glycol in rats, the half-life of 39 h of the plasma ^{14}C activity found by Marshall and Cheng (1983) probably represented the elimination kinetics of metabolites other than glycolic acid.

TOXICITY SUMMARY

A review of the literature on the toxicity of ethylene glycol indicates that the three major toxic end points of ethylene glycol poisoning are mucosal irritation, central-nervous-system (CNS) effects, and renal toxicity. In very severe poisoning, ethylene glycol can be lethal. Details of these toxic effects of ethylene glycol are summarized below.

Acute or Short-Term Exposures

Relatively few data are available on the toxicity of ethylene glycol in acute or short-term inhalation exposures. Most of the information on its acute toxicity is derived from oral exposures.

Lethality

Ingestion of ethylene glycol has been known to be lethal in certain cases. The minimum oral lethal dose for average human adults has been estimated to be about 110 g (Scully et al., 1979). Evidence of species differences in ethylene glycol's lethality was presented by Laug et al. (1939). They exposed animals to ethylene glycol via gavage and found that the LD_{50} was 6.1, 8.1, and 14.4 g/kg in rats, guinea pigs, and mice, respectively. In rats and guinea pigs, ethylene glycol was discovered to be more deadly in large adults than small adults. However, body-weight dependency of the lethal effect was not found in mice. Their mortality data are summarized in Table 9-2.

TABLE 9-2 Mortality Data of Laug et al.(1939)

Species	Dose, g/kg	Body Weight, g	Mortality
Rats	5.0	214	3/10
	5.0	380	7/10
Guinea pigs	6.6	268	1/10
	6.6	558	9/9
Mice	13.8	14.6	7/10
	13.8	27.1	6/10

CNS, Cardiopulmonary, and Renal Toxicity

Berman et al. (1957) divided the responses to acute poisoning from ethylene glycol ingestion in humans into three stages. The first stage is characterized by CNS depression clinically similar to ethanol intoxication. This stage appears within half an hour to several days, depending on the amount ingested (Berman et al., 1957; Friedman et al., 1962; Moriarty and McDonald, 1974). Friedman et al. found that, if the dose is high (e.g., 90-120 g in a 17-y-old Caucasian girl), the victim can enter into a coma, convulse, and die. The urine might contain oxalate crystals and albumin. In an autopsy of a victim, Friedman et al. (1962) found oxalate crystals in multiple tissues, most notably in the lumen of

renal proximal tubules, astrocytosis in the cerebral cortex, thalamus, globus pallidus, and the brain stem, together with a focal loss of cerebellar Purkinje cells and centrilobular fatty changes in the liver.

The second stage involves cardiopulmonary dysfunctions. The studies of Berman et al. (1957) and Friedman et al. (1962) revealed that there could be tachypnea, cyanosis, pulmonary edema, bronchopneumonia, left ventricular hypertrophy, myositis resulting in muscle pain, and even death.

The third stage consists primarily of progressive renal impairment. Both studies mentioned above showed that the victim can develop proteinuria, anuria, flank pain, and costovertebral angle tenderness. In a 14-y-old Caucasian boy who died from ingesting 120 g of ethylene glycol, renal biopsy showed focal hydropic degeneration of the proximal tubules with luminal obliteration of many of the tubules, glomeruli with increased density and cellularity, and numerous calcium oxalate crystals in tubular lumens and in some tubular epithelial cells (Friedman et al., 1962).

The histopathological changes in the heart, lung, and kidney, which have been documented in ethylene-glycol poisoning cases in humans, most likely are due to massive doses. Hong et al. (1988) failed to detect any histopathological changes in mice given ethylene glycol at 0, 50, 100, or 250 mg/kg/d for 4 d by gavage. One day after the 4-d exposure, they examined the heart, lung, kidney, urinary bladder, adrenal glands, liver, thymus, spleen, stomach, intestines, uterus, and testes in the mice and found no change in those tissues. However, the researchers found a suppression of granulocyte-macrophage progenitor colony formation in the bone marrow of male mice 14 d after the mice were gavaged with ethylene glycol at 50 mg/kg/d for 4 d. They also reported that a similar gavage at 100 mg/kg/d reduced the cellularity in the bone marrow of the mice after 14 d of exposure, whereas gavage at 50 mg/kg/d had no such effect.

Some investigators have studied the CNS effects of ethylene glycol in laboratory animals. Most of its CNS effects can be characterized as CNS depression. The administration of ethylene glycol by gavage in rats and guinea pigs at fatal or near-fatal doses produced "no narcosis but varying degrees of sluggish depressed functioning" (Smyth et al., 1941). Similarly, Bove (1966) exposed rats to ethylene glycol by oral gavage and found that a dose of 12 g/kg produced marked lethargy in 3

h and death in less than 24 h. However, 9 g/kg caused only moderate lethargy. The only data on the CNS depression effect of inhaled ethylene glycol were gathered by Flury and Wirth (1934). They exposed cats to ethylene glycol at 500 mg/m^3 for a total of 28 h in 5 d. The animals developed a slight narcosis from which they recovered after the exposure.

Metabolic Acidosis and Miscellaneous Signs and Symptoms

In 12 teenagers who drank an unknown amount of antifreeze containing ethylene glycol, leukocytosis and pleocytosis were detected (Moriarty and McDonald, 1974). Nausea, vomiting, and abdominal pain were found in 60% of the patients. Oxalate crystals in the urine and metabolic acidosis were present in 33% and 50%, respectively, of the patients. The investigators reported that the severity of the clinical picture was not correlated with the urinary and blood concentrations of ethylene glycol in these victims.

Metabolic acidosis induced by ethylene glycol was also found in animals by Clay and Murphy (1977). They injected ethylene glycol at 3 or 4 g/kg intraperitoneally into pigtail monkeys. After the injection, a transient narcosis developed. The monkeys recovered from the narcosis for "a period of hours" but entered into comas later. A severe acidosis developed 12-24 h after the injection, with a drop in blood pH by a unit of 0.2-0.3. Hewlett et al. (1989) reported that an oral dose of ethylene glycol at 2 g/kg in rats or 1 g/kg in dogs caused mild acidosis with no sedation.

The amount of oxalic acid formed from ethylene glycol metabolism could not entirely explain the degree of acidosis seen in victims of ethylene glycol poisoning; therefore, the acidosis might be partially due to other acidic metabolites of ethylene glycol (Friedman et al., 1962; Moriarty and McDonald, 1974). Clay and Murphy (1977) showed that a decrease in blood bicarbonate concentrations in pigtail monkeys was associated with an increase in the blood concentration of glycolic acid. They also showed that exposure of monkeys to 4-methylpyrazole, which is an inhibitor of alcohol dehydrogenase, 30 min after the intraperitoneal injection of ethylene glycol shortened the duration of metabolic acidosis induced by ethylene glycol. The evidence suggests that ethyl-

ene glycol causes metabolic acidosis via formation of its metabolites. According to Jacobsen et al. (1984), glycolic acid is important in causing acidosis in ethylene glycol poisoning in humans.

Mucosal Irritation

Wills et al. (1974) exposed 20 men to a mixture of ethylene glycol aerosol and vapor at 30 mg/m^3, time-weighted average (TWA), 20-22 h/d for 30 d. To determine which concentrations were irritating, the experimenters periodically increased the ethylene glycol concentration for up to 15 min (typically the increase occurred immediately after a lunch break when the volunteers returned to the exposure chamber). The responses of the volunteers to these spurts of relatively high exposures are summarized in Table 9-3. In what appears to be an interim report of that study, Harris (1969), one of the investigators, described the results of exposure of male volunteers to aerosolized ethylene glycol for up to 28 d. The data on mucosal irritation responses from that report are also included in Table 9-3.

TABLE 9-3 Responses of Male Volunteers to Ethylene Glycol Exposure

Concentration, mg/m^3	Response	Reference
64	Completely oblivious to the exposure	Harris, 1969
127	Pharyngeal irritation	Harris, 1969
40	Irritation became common	Wills et al., 1974
188	Exposure tolerated for 15 min	Wills et al., 1974
190	Exposure not tolerated when subjects awakened from sleep	Harris, 1969
>200	Exposure not tolerated because of pain in the tracheobronchial tree	Wills et al., 1974
244	Exposure not tolerated for more than 1-2 min	Wills et al., 1974
308	Subjects rushed out of chamber after only one or two breaths	Wills et al., 1974

On the basis of the reports of the above two investigators, an exposure of humans to ethylene glycol at 64 mg/m^3 is nonirritating, but it is irritating at 140 mg/m^3 and could become intolerable extremely rapidly at 308 mg/m^3.

Animal data on the irritation effect of ethylene glycol were gathered in the experiment of Flury and Wirth (1934). Moderate mucosal irritation was discovered in cats exposed to ethylene glycol at 500 mg/m^3 for a total of 28 h in 5 d. When comparing the findings of Flury and Wirth with that of Wills et al. and Harris, the cats appeared not to be affected by ethylene glycol's irritation as much as the humans subjects. It is not certain whether it was due to a species difference in sensitivity or due to a difference in the detection sensitivity of irritation in the two studies.

Subchronic and Chronic Exposures

Ethylene glycol's toxicity in long-term exposures somewhat resembles that in short-term exposures. The major toxic effects in long-term exposures are mucosal irritation, CNS effects, and renal toxicity.

Mucosal Irritation and Eye Toxicity

Mucosal irritation induced by ethylene glycol was studied in subchronic exposures, but the results were mixed. Within 8 d of a 90-d continuous exposure, Coon et al. (1970) detected moderate-to-severe corneal erythema, edema, and discharge in all three rabbits and corneal opacity, with apparent blindness, in 2 of 15 rats exposed at a concentration of 12 mg/m^3. No mention was made of any effects on the eyes of similarly exposed guinea pigs and monkeys (Coon et al., 1970). MacEwen (1969) reported that a 47-d continuous exposure of 30 rats and 20 guinea pigs to ethylene glycol at 12.6 mg/m^3 resulted in no corneal changes. According to MacEwen (1969), four rabbits also were exposed continuously for 17 d. Only minimal cloudiness of the corneal surface was seen in the first 3 d, but no changes occurred afterward. Coon et al. (1970) also reported that exposures of rats, guinea pigs, and rabbits to ethylene glycol at a higher concentration of 57 mg/m^3, albeit only intermittently (8 h/d, 5 d/w, for 6 w), failed to produce any signs

of eye irritation. The absence of positive corneal findings in the exposure at 57 mg/m^3, together with MacEwen's negative findings, casts doubt on whether the signs of eye irritation observed by Coon et al. (1970) in the 12-mg/m^3 groups were chemical-related. Because no eye irritation or corneal damage was reported in human subjects exposed at 30 mg/m^3, TWA, for 30 d (Harris, 1969; Wills et al., 1974), the eye irritation findings of Coon et al. (1970) are not relied on in setting the SMACs.

CNS Effects

Troisi (1950) studied 38 young women workers exposed to a vapor generated by heating a mixture of 40% ethylene glycol, 55% boric acid, and 5% ammonia at 105°C on a table within their reach. Nine of the 38 workers suffered recurrent attacks of loss of consciousness after a few hours of continuous work at that location. The attacks lasted for only 5 to 10 min. These nine workers also developed nystagmus and five of them had lymphocytosis. Among the workers who did not suffer unconsciousness, five had nystagmus. Urinary examinations in all 38 workers were normal. After the installation of a ventilation system for the area, the CNS effects disappeared. Unfortunately, the investigator did not characterize the concentration or composition of the vapor in that study. The mixture was heated at a temperature below boric acid's melting point of 185°C (Sax, 1984); therefore, it is highly unlikely that these workers inhaled boric acid at any significant concentration. Ammonia is not known to cause CNS depression and nystagmus (Wong, 1993). So the CNS effects observed by Troisi were probably due to ethylene glycol alone.

The no-observed-adverse-effect level (NOAEL) for the CNS toxicity of ethylene glycol can be determined from the data of a 30-d study of Wills et al. (1974). They exposed 20 men to ethylene glycol for 20 to 22 h/d for 30 d. The test atmosphere, to which 20 human subjects were exposed, was generated by forcing ethylene glycol aerosol into the cooled air stream of three air conditioners that supplied air to the exposure chamber. Ethylene glycol droplets were collected at various locations in the chamber. Their diameters were 1 to 5 μm as measured under a microscope. Assuming these droplets were all spherical, the mea-

sured diameters should be very close to the aerodynamic equivalent diameter given that the density of ethylene glycol is 1.1 (ACGIH, 1986; Hinds, 1982). Hinds found that these droplets were all in the inhalable range. The analytical method of Wills et al. (1974) could be used to measure ethylene glycol in either aerosol or vapor form. The exposure concentrations reported in the study of Wills et al. (1974) reflected the combined exposure concentration of aerosol and vapor. The weekly mean exposure concentrations were 29, 17, 23, 49, and 31 mg/m^3, yielding a TWA concentration of 30 mg/m^3 for the 30-d exposure. At the midpoint and the end of the exposure, the researchers subjected the men to a battery of psychometric tests designed to evaluate simple reaction time, reaction time with discrimination, visual-motor coordination, perception, and mental ability. No decrements in these neurological functions were detected; therefore, 30 mg/m^3 can be considered the NOAEL on the basis of the CNS effects resulting from a 30-d exposure to ethylene glycol.

Renal Toxicity

As discussed above, renal damage is one of the manifestations of acute intoxication with ethylene glycol. In the study of Wills et al. (1974), a nearly continuous exposure of 20 men to ethylene glycol at 30 mg/m^3 for 30 d failed to produce any adverse changes in kidney functions, as measured by urine specific gravity, serum urea nitrogen, serum creatinine, and creatinine clearance.

Animal data on ethylene glycol's renal toxicity also were found in the literature. Felts (1969) reported that two chimpanzees exposed to aerosolized ethylene glycol at 256 mg/m^3, with none of the aerosol droplets bigger than 5 μm, for 28 d had impaired ability to concentrate urine, which was indicative of distal tubular dysfunction. He then exposed four chimpanzees to the same concentration in a chamber held at an atmosphere of 5 psi, 68% oxygen, and 32% nitrogen for 28 d. No significant changes were detected in the blood urea nitrogen, serum creatinine, insulin clearance, and para-aminohippuric acid clearance; those findings suggest that no renal impairment developed in the four chimpanzees.

In the study by Coon et al. (1970), no histopathological changes

were found, and no changes occurred in the histochemical studies of lactate, isocitrate, succinate, glucose 6-phosphate, and *b*-hydroxybutyrate dehydrogenase in the kidneys and livers of rats, guinea pigs, and rabbits exposed to ethylene glycol continuously at 12 mg/m for 90 d or repeatedly at 57 mg/m^3, 8 h/d, 5 d/w, for 30 d. Wiley et al. (1936) reported that repeated exposures to ethylene glycol at 398 mg/m^3, 8 h/d, 5 d/w, for 16 w, killed 3 of 20 mice and 1 of 10 rats. The exposures, however, caused no histological changes in the surviving mice and rats. From the data on inhalation exposures summarized above, the NOAEL, based on ethylene glycol's renal toxicity in humans, is 30 mg/m^3 for subchronic inhalation exposures.

No chronic inhalation study with ethylene glycol has been found in the literature. However, two chronic feeding studies were done. Blood et al. (1962) fed two male rhesus monkeys with a diet containing 0.2% ethylene glycol and a female rhesus monkey with a diet containing 0.5% for 3 y. Possible calcification of the urinary tract was monitored with x-rays before the exposure and once every 3 mo during the exposure, but no calcification was seen. When the monkeys were sacrificed after 3 y of ethylene glycol exposure, microscopic examination failed to reveal ethylene-glycol-related pathological changes in the kidney, ureter, urinary bladder, liver, esophagus, stomach, intestine, pancreas, heart, spleen, adrenal, pituitary, thyroids, parathyroids, lymph nodes, and bone marrow. Therefore, long-term feeding with a diet containing 0.2% ethylene glycol would have been devoid of any renal toxicity in the three monkeys studied by Blood et al. (1962). According to the National Institute of Occupational Safety and Health (NIOSH), a 1-ppm diet is equivalent to 0.05 mg/kg/d in monkeys (Sweet, 1987). As a result, a 0.2% diet is estimated to yield a daily dose of 100 mg/kg.

DePass et al. (1986a) performed a 2-y feeding study with ethylene glycol at concentrations of 1, 0.2, 0.04, or 0 g/kg/d in rats and mice. Ten animals of each species and sex in each group were sacrificed at 6, 12, and 18 mo into the exposure; the remaining animals were sacrificed at 24 mo. Renal lesions were detected only in male rats in the 1-g/kg/d group. The renal lesions included tubular hyperplasia, tubular dilation, peritubular nephritis, and oxalate crystalluria at 6 mo, chronic nephrosis and oxalate crystalluria at 12 mo, and tubular obstruction by oxalate crystals leading to secondary tubular degeneration and dilation at 18 mo. Because all the male rats in the 1-g/kg/d group died at 18 mo as a

result of oxalate nephrosis, no renal histological data were available at 24 mo. No renal injury was observed in any female rats or mice, as well as male rats in the lower dose groups.

Potential Effect on the Liver

Fatty liver changes have been reported in human victims who died from acute ethylene glycol ingestion. DePass et al. (1986a) also reported detecting mild fatty liver in female rats fed ethylene glycol at 1 g/kg/d for 24 mo. No hepatic histopathological changes were seen in female rats in the 1-g/kg/d group at 6, 12, or 18 mo. Similarly, the male rats were not inflicted with any hepatic histopathological changes. As discussed above, Blood et al. (1962) did not find any liver toxicity in two male monkeys fed a diet containing 0.2% ethylene glycol and 1 female monkey fed a diet with 0.5% for 3 y.

In contrast to the relatively heavy dietary exposures of ethylene glycol in the rat study of DePass et al. (1986a), no signs of liver injuries were found in animals or humans exposed subchronically to ethylene glycol's vapor or aerosol. Wills et al. (1974) found no changes in serum bilirubin, serum aspartate transaminase, alkaline phosphatase, and prothrombin time in 20 men exposed nearly continuously to 30 mg/m^3, TWA. Similarly, Coon et al. (1970) failed to detect any abnormalities on the basis of the serum activities of aspartate transaminase, alanine transaminase, alkaline dehydrogenase, and lactate dehydrogenase in rats, guinea pigs, rabbits, and dogs exposed to ethylene glycol at either 12 mg/m^3 for 90 d or 57 mg/m^3, 8 h/d, 5 d/w, for 30 d. No histopathological changes in tissues, including the liver, that were examined were detected in rats, guinea pigs, and rabbits in the study of Coon et al. (1970) (12 mg/m^3 continuously for 90 d or 57 mg/m^3 repeatedly for 30 d) and in mice and rats in the study of Wiley et al. (1936) (398 mg/m^3, 8 h/d, 5 d/w, for 16 w). Therefore, no evidence is found that inhaled ethylene glycol is hepatotoxic in long-term inhalation exposures.

Potential Hematological Effects

In the 2-y feeding study conducted by DePass et al. (1986a), certain

hematological changes were reported with an unusual time-dependency. Reduction in hematocrit, red-blood-cell counts, hemoglobin concentrations, and neutrophil counts were detected in male rats in the 1-g/kg/d group after 12 mo of oral administration but not after 6, 18, or 24 mo. No significant hematological changes were seen in female rats and mice of both sexes. Because the researchers failed to detect any hematological changes in male rats in the 1-g/kg/d group after the 12-mo result even though ethylene glycol continued to be fed to them for the next 12 mo, it is uncertain whether ethylene glycol is hematotoxic on the basis of that study alone.

In the study of DePass et al. (1986a), even if the hematological changes found in male rats after 12 mo (not at 6, 18, or 24 mo) of ethylene glycol exposure were chemically related, there is no evidence that ethylene glycol has hematological toxicity in primates. Blood et al. (1962) failed to discover any microscopic change in the bone marrow of two male rhesus monkeys fed 0.2% ethylene glycol and one female rhesus monkeys fed 0.5% ethylene glycol for 3 y. Similarly, no hematological changes have ever been demonstrated in humans exposed to ethylene glycol via inhalation. In the study by Wills et al. (1974) of 20 men exposed to ethylene glycol at 30 mg/m^3, venous blood was sampled on d 0, 1, 3, 5, 8, 12, 19, 22, 26, and 29. No changes in hematocrit, hemoglobin concentration, neutrophil counts, and prothrombin time were detected. Therefore, the hematological findings of DePass et al. (1986a) and Hong et al. (1988) might be either peculiar to rodents or produced only by oral exposures at a relatively high dose.

Lethality

Inhalation exposures to ethylene glycol can be lethal. A continuous exposure of eight monkeys to aerosolized ethylene glycol at 500 mg/m^3 for up to 30 w killed six of them (Harris, 1969). Autopsies revealed impacted intestines and oxalate crystals in the kidneys and lungs.

Coon et al. (1970) reported that 30 repeated exposures, 8 h/d, 5 d/w, to ethylene glycol at 10 or 57 mg/m^3 failed to kill any rats, guinea pigs, rabbits, dogs, and monkeys. However, a 90-d continuous exposure to ethylene glycol at 12 mg/m^3 killed some guinea pigs, a rat, and a rabbit as shown in Table 9-4.

TABLE 9-4 Mortality Data from Inhalation Exposure to Ethylene Glycol Study (Coon et al., 1970)

Exposure	Concentration, mg/m^3	Rat	Guinea Pig	Rabbit	Dog	Monkey
Control	0	4/123	0/73	0/12	0/12	0/8
Repeated	10	0/15	0/15	0/3	0/2	0/2
Repeated	57	0/15	0/15	0/3	0/2	0/2
Continuous	12	1/15	3/15	1/3	0/2	0/3

The LC_{20} of 12 mg/m^3 in guinea pigs is not used in setting SMACs because, in the study by Wills et al. (1974), no deaths occurred among 20 men exposed nearly continuously (20 to 22 h/d) for 30 d to 30 mg/m^3, TWA.

Carcinogenesis

A search of the National Library of Medicine's database, Medline, failed to reveal any epidemiological data on tumor incidences associated with ethylene glycol exposures in humans. Only one cancer bioassay is known. The 2-y feeding study by DePass et al. (1986a) showed that ethylene glycol is not carcinogenic up to 1.0 g/kg/d in rats and mice.

Genotoxicity

The lack of carcinogenicity of ethylene glycol appears to be consistent with its lack of genotoxicity. In the studies of McCann et al. (1975), Pfeiffer and Dunkelberg (1980), and Zeiger et al. (1987), ethylene glycol failed to increase mutation frequency in Ames tests. Similarly, McGregor et al. (1991) reported that ethylene glycol tested negative in the L5178Y mouse lymphoma cell forward mutation assay. In the study of McCarrol et al. (1981), ethylene glycol did not appear to cause DNA damage because it did not inhibit the growth of several strains of *Escherichia coli*, which were deficient in DNA repair ability. According to Griffiths (1979), ethylene glycol did not produce aneuploidy in *Neurospora crassa*.

Reproductive Toxicity

Ethylene glycol's reproductive toxicity, or the lack thereof, has been studied using both conventional continuous breeding protocols for more than one mating period (Lamb et al., 1985; DePass et al., 1986b) and a new short-term screening protocol for only one mating period (Harris et al., 1992). In a study with mice, Lamb et al. (1985) found that ethylene glycol might cause reproductive toxicity. Mice were given ethylene glycol in drinking water at concentrations of 0.25%, 0.5%, or 1% for 18 w, starting at age 11 w. Starting 1 w after the beginning of ethylene glycol exposure, pairs of male and female mice were mated for 14 w and then separated for 3 w while still being exposed. In the 1%-dose group, the researchers observed reduced number of litters per mated pair, reduced number of live pups per litter, and reduced live pup weight. The offspring of female mice in the 1%-dose group exhibited unusual facial features, such as wide-set eyes and a short snout, and skeletal defects. The fertility of these offspring was lower than that of controls (61% vs. 80%), but the difference was not statistically significant. It should be noted that the effects found in the 1%-dose group might be related to the general toxicity of ethylene glycol in female mice, because the exposure decreased the body weight of the female mice by about 10% in w 10 (Lamb et al., 1985). Because the maternal weight loss in this study was severe, the presence of maternal toxicity lessened the value of the study in the detection of developmental toxicity. Therefore, the study of Lamb et al. (1985) is not as useful as the study of DePass et al. (1986b), which is described below.

In a three-generation reproduction study in which rats were fed ethylene glycol in the diet at 0.04, 0.2, or 1 g/kg/d, DePass et al. (1986b) reported that ethylene glycol had no reproductive toxicity at doses up to 1 g/kg/d. They also found ethylene glycol to be negative in a dominant lethal test in F_2-generation male rats exposed to the compound in the diet at about 0.04, 0.2, or 1.0 g/kg/d for three generations. The data of Lamb et al. (1985) and DePass et al. (1986b) indicate that ethylene glycol has no specific reproductive toxicity. It might, however, affect the reproductive function in female mice by causing general toxicity.

Harris et al. (1992) developed a new short-term screen for reproductive and developmental toxicity and used it to test four compounds, including ethylene glycol. In part A of the screen, female mice were

gavaged with ethylene glycol in water at concentrations of 0, 250, 700, or 2500 mg/kg/d for 21 d, from study day 0 to 20. Male mice were similarly gavaged from study day 3 to 20. The doses were chosen with the highest dose being about one-third of the LD_{50}. On study days 8 to 12, the exposed females cohabited with exposed males. The animals were sacrificed on study day 21. Microscopic examinations of the liver, kidney, testis, and epididymis in the male mice were essentially negative. It should be noted that the lack of histopathological changes found in the Harris et al. (1992) study supports a similar finding by Hong et al. (1988) in mice gavaged with ethylene glycol at concentrations of 0, 50, 100, or 250 mg/kg/d for 4 d. In the reproductive study by Harris et al. (1992), no significant changes in the testis weight, epididymis weight, number of sperm per gram cauda, and percentage of motile sperm were detected in the male mice. In the female mice, ethylene glycol caused no clinical signs and no change in the fertility index.

In part B of the screen, untreated female and male mice cohabited 3 d to produce time-mated females, which were gavaged with ethylene glycol at the same doses as in part A of the screen on gestational d 8-14 (Harris, et al., 1992). The litters were examined on postnatal d 0, 1, and 4. The exposures to ethylene glycol did not change the number of live pups per litter. However, the exposure at 2500 mg/kg/d reduced the total litter weight on postnatal d 1 and 4. In conclusion, the short-term screen shows that ethylene glycol is, at the most, only very weakly toxic to the reproductive systems in mice.

Developmental Toxicity

Price et al. (1985) studied the developmental toxicity of ethylene glycol by gavaging rats at concentrations of 1250, 2500, or 5000 mg/kg/d and mice at 750, 1500, or 3000 mg/kg/d on gestational d 6-15. They reported that ethylene glycol could cause severe developmental toxicity in rats and mice. Other than a slight retardation of weight gain in the dams, there were no clinical signs of maternal toxicity. In rats, 5000 mg/kg/d induced postimplantation losses. There were also findings indicative of fetal toxicity. Exposure at 5000 or 2500 mg/kg/d reduced fetal body weight in rats, and exposures at all doses had the same effect

in mice. All groups showed increases in the percentage of litters with malformed fetuses and in the percentage of malformed live fetuses per litter. Malformations produced included axial skeletal dysplasia, neural tube closure defects, and craniofacial defects.

In a similar study, Tyl et al. (1993) exposed female rabbits to ethylene glycol via gavage at 0, 100, 500, 1000, or 2000 mg/kg/d on gestational d 6 through 19. The highest dose caused serious maternal toxicity, as manifested by a 42% mortality, several early deliveries, and renal toxicity (a presence of oxalate crystals in the lumen of cortical renal tubules accompanied by tubular necrosis, degeneration, and dilation). The other doses did not cause maternal toxicity. No developmental toxicity was detected in any of the exposure groups. There were no changes in preimplantation loss, postimplantation loss, the number of fetuses, fetal body weight, and the fetal sex ratio per litter. In addition, no increased incidence of malformation was found.

In part A of the short-term screen conducted by Harris et al. (1992), female mice were gavaged with ethylene glycol for 8 d before a 5-d mating period, 5 d during the mating period, and 8 d after the mating; and male mice were gavaged for 5 d before the mating, during the mating period, and 8 d after the mating. The exposure failed to affect significantly the total number of implants per female. However, the ethylene glycol exposure at 2500 mg/kg/d reduced the number of live implants and raised the number of dead implants per female; two of six litters were totally resorbed. Similar exposures at 700 or 250 mg/kg/d had no such effects. Harris et al. (1992) concluded that their short-term screen succeeded in detecting ethylene glycol as a developmental toxicant in mice. Tyl et al. (1993) compared their data with the data in the literature and concluded that the maternal sensitivities of three species to ethylene glycol can be ranked in the following decreasing order: rabbits, mice, and rats. In terms of its developmental toxicity, the ranked order is mice, rats, and rabbits (Tyl et al., 1993).

Interaction with Other Chemicals

Literature on ethylene glycol's interaction with other chemicals is available. Ethanol treatment of a patient who had ingested a large amount of ethylene glycol appeared to prevent organ damage (Stokes

and Aueron, 1980). Evidence indicates that ethanol's therapeutic effect on ethylene glycol poisoning is related to the competitive inhibition of ethylene glycol's metabolism. Jacobsen et al. (1982) reported that a blood ethanol concentration of 70 mg/dL almost completely inhibited ethylene glycol metabolism in a patient with ethylene glycol intoxication. Grauer et al. (1987) found that coadministered ethylene glycol and ethanol in dogs reduced the elimination half-life of ethylene glycol in the plasma from 10.8 h for ethylene glycol only to 6.8 h for ethylene glycol and ethanol.

TABLE 9-5 Toxicity Summary

Concentration, mg/m^3	Exposure Duration	Species	Effects	Reference
30	22 h/d, 30 d	Human	No changes in serum and urininary chemistry; no reduction in psychomotor performance	Wills et al., 1974
64	NS	Human	No irritation	Harris, 1969
127	NS	Human	Pharyngeal irritation	Harris, 1969
140	NS	Human	Eye and respiratory irritation became common	Wills et al., 1974
188	NS	Human	Irritating and tolerated for only 15 min	Wills et al., 1974
190	NS	Human	Subjects awaken out of their sleep; not tolerated	Harris, 1969
244	NS	Human	Not tolerated more than 1 or 2 min	Wills et al., 1974
308	NS	Human	Intolerable after a breath or two	Wills et al., 1974
10 or 57	8 h/d, 5 d/w, 6 w	Rat, guinea pig, rabbit, dog, monkey	No adverse effects	Coon et al., 1970
12	24 h/d 90 d	Guinea pig	3/15 guinea pigs died (vs. 0/73 in the control group)	Coon et al., 1970
12	24 h/d 90 d	Rat, rabbit	Moderate-to-severe eye irritation (3 and 8 d into the exposure in rabbits and rats, respectively); no chemical-induced histological, hematological, or serum chemistry changes; 1/15 rats died (vs. 4/123 in controls) and 1/3 rabbits died (vs. 0/12 in controls)	Coon et al., 1970
256	24 h/d, 28 d	Chimpanzee (n=2)	Slight reduction in kidney's urine concentrating ability; oxalate crystals found in the kidney of one of two chimpanzees	Felts, 1969
350	8 h/d, 16 w	Rat, Mouse	Cecal ulceration, cysts of pericecal lymph nodes	Wiley et al., 1936
500	28 h in 5 d	Rat	Eye and respiratory irritation	Flury and Wirth, 1934

500 to 600	22 h/d, 2 to 3 w	Monkey	Tolerated	Wills et al., 1974

[a]Only the more important results were included.

NS, not specified.

TABLE 9-6 Results of Selected Oral and Parenteral Studies

Dose, g/kg	Exposure Route	Species	Effects	Reference
14.5	Oral	Dogs	Muscular incoordination lasted for 51 h	Kersting and Nielsen, 1969
4.4	i.v.	Dogs	Transient incoordination lasted <6 h	Kersting and Nielsen, 1969
6.6	i.v.	Chimpanzees	Ataxia, coma, and death	Felt, 1969
2.2	i.v.	Chimpanzees	Ataxia, coma	Felt, 1969
2.2	i.v.	Rhesus monkeys	Ataxia for 2 h, recovered afterward	Felt, 1969
1.1	i.v.	Chimpanzees	Ataxia	Felt, 1969
1.1	i.v.	Rhesus monkeys	No behavioral changes	Felt, 1969
2.62 (daily for 10 d)	In drinking water	Rats (male)	No changes in blood urea nitrogen (BUN) and serum creatinine levels; renal histological changes included mild tubular dilatation, intratubular birefringent crystal, and acute inflammation	Robertson et al., 1990
1.34 (daily for 10 d)	In drinking water	Rats (male)	No changes in BUN, but serum creatinine levels increased; crystals in pelvis in 4/10 rats (0/10 in controls); no other renal changes	Robertson et al., 1990
0.65 (daily for 10 d)	In drinking water	Rats (male)	No changes in BUN or renal parameters studied	Robertson et al., 1990

TABLE 9-7 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	50	ACGIH, 1991
OSHA's PEL	50	ACGIH, 1991
NRC's 1-h EEGL	40	NRC, 1985
NRC's 24-h EEGL	20	NRC, 1985
NRC's 90-d CEGL	4	NRC, 1985

TLV, threshold limit value; PEL, permissible exposure limit; EEGL, emergency exposure guidance level; CEGL, continuous exposure guidance level.

TABLE 9-8 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	25	64	Mucosal irritation
24 h	25	64	CNS depression, mucosal irritation
7 d[a]	5	13	CNS depression, renal toxicity
30 d	5	13	CNS depression, renal toxicity
180 d	5	13	CNS depression, renal toxicity

[a]The former 7-d SMAC is 50 ppm or 127 mg/m^3.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

The SMACs are set with the assistance of guidelines provided by the National Research Council (NRC, 1992a). For each important toxic end point, acceptable concentrations (ACs) are established for the exposure durations of interest, i.e., 1-h, 24-h, 7-d, 30-d, and 180-d. These ACs are tabulated and the lowest AC for an exposure duration is selected to be the SMAC.

Mucosal Irritation

As discussed above, Harris (1969) reported that an exposure of men to ethylene glycol at 64 mg/m^3 was nonirritating, and Wills et al. (1974) reported that mucosal irritation, of unspecified intensity, became common in 20 men at 140 mg/m^3. A nonirritating concentration based on the experience of 20 men could still be slightly irritating in sensitive individuals. Nevertheless, because 1-h and 24-h SMACs are for emergencies, a potential for slight nose and throat irritation is acceptable. Consequently, the 1-h and 24-h ACs, based on mucosal irritation, are set at 64 mg/m^3 without any correction for the small sample size. Even if mucosal irritation does occur in a small proportion of individuals at these ACs, it should only be sparse and mild because these ACs are much lower than 127 mg/m^3, the lowest concentration that mucosal irritation has been reported in 20 men (Wills et al., 1974; Harris, 1969).

1-h and 24-h ACs for mucosal irritation
= short-term NOAEL
= 64 mg/m^3.

For the longer-term exposures, however, the ACs should protect against any mucosal irritation. Harris (1969) reported that the 20 men were "completely oblivious" to the 64-mg/m^3 exposure. Unfortunately, Harris did not specify the length of time these men were exposed at 64 mg/m^3. Judging from the description of Wills et al. (1974), it appeared that these men were exposed at 64 mg/m^3 for about 15 min. Because mucosal irritation resulting from ethylene glycol exposure is not expected to increase beyond 15 min, the 15-min NOAEL should also be nonirritating for an exposure lasting 7, 30, or 180 d. The 7-d, 30-d, and 180-d ACs for mucosal irritation are derived from the 15-min NOAEL.

The 15-min NOAEL is estimated from data gathered from only 20 men (Wills et al., 1974; Harris, 1969), so it is possible that some sensitive individuals could feel mucosal irritation at the 15-min NOAEL. The 15-min NOAEL, therefore, is adjusted for the small sample size in the derivation of the ACs by multiplying the NOAEL by the square root of 20 divided by 10.

7-d, 30-d, and 180-d ACs for mucosal irritation
= 15-min NOAEL × $(\sqrt{n})/10$
= 64 mg/m^3 × $(\sqrt{20})/10$
= 28 mg/m^3.

CNS Depression

CNS depression is the major CNS effect of ethylene glycol. Although nystagmus was reported in workers exposed to ethylene glycol, it was found in a co-exposure to other chemicals. Consequently, the acceptable concentrations to be derived here will aim at preventing CNS depression. No time adjustment is needed for the 7-d, 30-d, and 180-d ACs to prevent CNS depression. The reason is that the NOAEL in the study of Wills et al. (1974) was based on a 30-d exposure. CNS depression is believed to be dependent on blood concentration. The blood concentration of ethylene glycol should reach steady state before the midpoint of the 30-d exposure, when the psychometric tests were first used. A NOAEL from that study ought to be able to prevent CNS depression for 7, 30, or 180 d.

7-d, 30-d, and 180-d ACs for CNS depression
= 30-d NOAEL × $(\sqrt{n})/10$
= 30 mg/m^3 × $(\sqrt{20})/10$
= 13 mg/m^3.

The 24-h AC to prevent CNS depression is set using CNS data gathered in animals with acute exposures to ethylene glycol. Kersting and Nielsen (1966) orally administered ethylene glycol to dogs by using ethylene glycol to moisten dry feed. They found that an oral dose of 14.5 g/kg caused muscular incoordination, which lasted as long as 51 h. An oral dose of 4.4 g/kg produced a transient incoordination, which lasted for less than 6 h, in the dogs. Felts (1969) injected primates intravenously with ethylene glycol once.

According to the data of Kersting and Nielsen (1966) in dogs and the data of Felts (1969) in monkeys and chimpanzees, 1.1 g/kg is the lowest dose known to cause CNS depression effects in acute ethylene glycol poisoning. The acute NOAEL is estimated from the LOAEL by applying an uncertainty factor of 10.

NOAEL for acute CNS depression
= LOAEL × 1/NOAEL factor
= 1.1 g/kg × 1/10
= 110 mg/kg.

The 24-h AC for CNS depression is estimated by calculating the inhalation exposure concentration that would yield the acute NOAEL of 110 mg/kg in a 70-kg person breathing 20 m^3 of air in 24 h (NRC, 1992b). Because Marshall and Cheng (1983) reported that rats absorbed about 60% of the inhaled ethylene glycol, that exposure concentration is corrected by the fraction absorbed. An interspecies extrapolation factor of 10 is also applied.

24-h AC for CNS depression
= acute NOAEL × body weight × 1/daily respiratory volume × 1/absorption fraction × 1/species factor
= 110 mg/kg × 70 kg × 1/20 m^3 × 1/0.60 × 1/10
= 64 mg/m^3.

Similarly, the 1-h AC for CNS depression is set using the acute NOAEL of 110 mg/kg, assuming that a 70-kg person breathes 20 m^3 of air in 24 h (NRC, 1992b).

1-h AC for CNS depression
= acute NOAEL × body weight × time adjustment × 1/daily respiratory volume × 1/absorption fraction × 1/species factor
= 110 mg/kg × 70 kg × (24 h/1 h) × 1/20 m^3 × 1/0.60 × 1/10
= 1500 mg/m^3.

Renal Toxicity

The exposure of 20 men to ethylene glycol at 30 mg/m^3, TWA, for 30 d caused no changes in urinary creatinine clearance, serum urea nitrogen, and the urinary specific gravity (Wills et al., 1974). A concentration that did not produce any renal toxicity in 30 d should not be toxic to the kidney for a 7-d exposure. It also should not be toxic to

the kidney for a 180-d exposure, because the body should reach an equilibrium before 30 d in a continuous exposure. Consequently, the ACs for 7, 30, or 180 d can be set at the same concentration.

7-d, 30-d, and 180-d ACs for renal toxicity
= 30-d NOAEL × $(\sqrt{n})/10$
= 30 mg/m^3 × $(\sqrt{20})/10$
= 13 mg/^{3}.

To check the validity of these ACs, the renal toxicity data from the 2-y bioassay of DePass et al. (1986a) are used. Although mice were not affected in that study, rats given ethylene glycol orally at 1 g/kg/d for 2 y developed histological renal injuries. In contrast, a dose of 0.2 g/kg/d was not toxic to the kidney. An airborne concentration of ethylene glycol that will yield the same dose as a daily oral administration of 0.2 g/kg can be calculated by assuming that a 70-kg person breathes 20 m^3/d (NRC, 1992a). Such calculations require a knowledge of the fraction absorbed via ingestion versus that via inhalation. Lenk et al. (1989) showed that rats given ethylene glycol orally at 3 or 5 mL/kg excreted 90-95% of the dose as ethylene glycol and glycolate in the urine in 48 h. According to McChesney et al. (1971), rats are known to excrete 2.5% of an oral dose of ethylene glycol in the urine as oxalic acid (Reif, 1950). The excretion data of Lenk et al. (1989) and McChesney et al. (1971) together indicate that orally administered ethylene glycol is essentially completely absorbed in rats. However, Marshall and Cheng (1983) showed that rats absorbed only 60% of inhaled ethylene glycol. As a result, the calculated airborne exposure concentration of ethylene glycol equivalent to an oral dose needs to be adjusted for the incomplete inhalation absorption.

Theoretical 7-d, 30-d, and 180-d ACs for renal toxicity
= 2-y NOAEL × body weight × 1/species factor
× 1/daily respiratory volume × 1/absorption fraction
= 0.2 g/kg/d × 70 kg × 1/10 × 1/20 m^3/d × 1/0.6
= 110 mg/m^3.

The theoretical long-term ACs derived from the animal data, which were based on histopathological results, are much higher than those derived from the inhalation results in men gathered by Wills et al. (1974).

Therefore, the long-term ACs derived from the human inhalation data offer sufficient protection against renal toxicity, notwithstanding the relatively nonsensitive detection methods used by Wills et al. (1974) for renal toxicity.

To derive the 24-h ACs for renal toxicity, the 30-d NOAEL of Wills et al. is not used. It is not used because, theoretically, the 24-h AC should be higher than the 30-d NOAEL determined in a human study, but how much higher is unknown. As a result, the data of Robinson et al. (1990) are used. They reported that male rats given ethylene glycol in drinking water at a concentration of 2620 mg/kg/d for 10 d had no changes in blood urea nitrogen and serum creatinine levels, but the exposure significantly increased the following renal histological changes: mild tubular dilatation, minimal tubular necrosis, intratubular birefringent crystals, and acute inflammation. A similar exposure at 1340 mg/kg/d produced no change in the blood urea nitrogen level, but it increased the serum creatinine level. It did not produce significant increases in histological renal injuries, but birefringent crystals were seen in the renal pelvis of 4 of 10 animals (vs. 0/10 in the control group). However, ethylene glycol given to 10 male rats in a continuous exposure at 650 mg/kg/d for 10 d did not cause any change in the blood urea nitrogen and serum creatinine levels. It did not cause any significant increase in renal histological changes. There were also no birefringent crystals in the kidney in the 650-mg/kg group (Robinson et al., 1990). Therefore, a dose of 650 mg/kg given in 24 h should be devoid of any renal toxicity. To calculate a 24-h AC for renal toxicity from the 1-d NOAEL of 650 mg/kg, an inhalation exposure concentration yielding a dose of 650 mg/kg is calculated as follows.

24-h exposure concentration to yield a NOAEL of 650 mg/kg
= 1-d NOAEL × body weight × 1/daily respiratory volume × 1/absorption fraction
= 650 mg/kg × 70 kg × 1/20 m^3 × 1/0.60
= 3800 mg/m^3.

24-h AC for renal toxicity
= 3800 mg/m^3 × 1/species factor
= 3800 mg/m^3 × 1/10
= 380 mg/m^3.

There are no data on the NOAEL for renal toxicity based on acute experiments. Because it is difficult to estimate a 1-h AC from a NOAEL derived from a 10-d drinking study, no 1-h AC is set for renal toxicity.

As indicated above, the 7-d AC is derived from the 30-d NOAEL based on a human study of Wills et al. (1974). Due to the smaller difference in the lengths of exposure, it can be argued that the 7-d AC should be derived from the 10-d NOAEL based on the animal data of Robinson et al. (1990) instead of the 30-d NOAEL of Wills et al. (1974). However, the 30-d NOAEL of Wills et al. is a better starting point for two reasons. First, the 30-d NOAEL was derived from human subjects, and the 10-d NOAEL was from a study in rats. Second, if the 10-d NOAEL is used as the starting point, the 7-d AC would be 230 mg/m^3, which is too close to the concentration of 256 mg/m^3 that Felts (1969) found to be toxic to the kidney in two chimpanzees after a 28-d inhalation exposure. Although no evidence was found that continuous exposure of the two chimpanzees to ethylene glycol at 256 mg/m^3 was toxic to the kidney in 7 d, 230 mg/m^3 does not provide a sufficient safety margin for a 7-d AC.

The mechanism of ethylene glycol's renal toxicity is unclear. On the one hand, calcium oxalate crystals, formed from the oxalic acid metabolite, have been postulated to cause ethylene glycol's renal toxicity (Levy, 1960). On the other hand, some evidence indicates that ethylene glycol injures the kidney via mechanisms other than calcium oxalate crystallization (Wiley et al., 1938; Frommer and Ayus, 1982; Tyl et al., 1993).

If ethylene glycol does cause renal injuries via formation of calcium oxalate crystals, it can be argued that, because microgravity is known to increase the renal excretion of calcium (Whedon et al., 1977), an uncertainty factor for the calcium effect during spaceflight might be needed for an extra safety margin in deriving ACs for ethylene glycol's renal toxicity. However, such an uncertainty factor is not necessary considering the insignificant load of urinary oxalic acid potentially contributed by a daily exposure to the AC of 13 mg/m^3 estimated below.

Rats absorb about 60% of the inhaled ethylene glycol (Marshall and Cheng, 1983). Assuming that human subjects behave like rats in absorbing 60% of the amount of ethylene glycol inhaled and assuming that astronauts inhale 20 m^3/d (NRC, 1992a), the daily dose of ethylene glycol can be calculated as follows:

Daily dose of ethylene glycol to astronauts
= 13 mg/m^3 × daily respiratory volume × absorption fraction
= 13 mg/m^3 × 20 m^3/d × 0.60
= 156 mg/d.

Based on the findings of Reif (1950), humans convert 2.3% of the dose of ethylene glycol to oxalic acid in urine.

Urinary output of oxalate due to ethylene glycol inhalation
= daily dose of ethylene glycol × conversion factor
= 156 mg/d × 0.023
= 4 mg/d.

According to Pak et al. (1985), the daily urinary excretion of oxalate in normal human subjects is about 23-24 mg/d. They estimated that the daily urinary excretion of oxalate has to increase beyond 45 mg/d to increase the risk of oxalate crystallization in the kidney. The contribution of 4 mg/d to urinary oxalate excretion by ethylene glycol inhalation at the long-term ACs of 13 mg/m^3 is too small to bring the daily urinary oxalate excretion above the threshold of 45 mg/d. Therefore, if ethylene glycol's renal toxicity is due to calcium oxalate crystallization, the ACs of 13 mg/m^3 need not be adjusted downward for microgravity-induced increases in urinary calcium excretion. Finally, it should be noted that, because ethylene glycol might cause renal injuries via a mechanism other than calcium oxalate crystallization, one should not set an AC for renal toxicity by calculating the ethylene glycol exposure concentration required to bring the daily urinary oxalate excretion to the threshold of 45 mg/d.

Establishment of SMAC Values

The ACs for all the important toxic end points are tabulated below. The lowest AC for an exposure duration is selected to be the SMAC for that duration. As a result, 64 mg/m^3 is chosen to be the 1-h and 24-h SMACs, and the 7-d, 30-d, and 180-d SMACs are set at 13 mg/m^3. These SMACs are set with consideration of all spaceflight-induced physiological changes, so no further adjustments are necessary.

RECOMMENDATIONS

Although renal toxicity has been emphasized as ethylene glycol's major toxicity for a long time, the derivation of ACs for its renal toxicity, mucosal irritation, and CNS depression shows that mucosal irritation and CNS depression are more important than renal toxicity for the establishment of exposure limits. The reason is that it takes a lower AC to prevent mucosal irritation and CNS depression than renal toxicity. Comparing the data of mucosal irritation with those of CNS depression, the conclusion is that the data gap on ethylene glycol's propensity to irritate mucous membranes is larger than that on CNS depression. Currently, the ACs for mucosal irritation in short-term exposures are set using the data of Wills' study (Wills et al., 1974; Harris, 1969). Wills and co-workers exposed 20 men to ethylene glycol almost continuously for 30 d, during which the exposure concentration was periodically raised for about 15 min to test ethylene glycol's irritancy. A study is needed in testing its irritancy by exposing human subjects to it for 1 to 2 h.

TABLE 9-9 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors: To NOAEL	Uncertainty Factors: Species	Uncertainty Factors: Small n[a]	Conversion Factor[b]	Absorption Factor[c]	Acceptable Concentrations, mg/m^3: 1 h	24 h	7 d	30 d	180 d
Mucosal irritation											
NOAEL, 64 mg/m^3, 15 min (Wills et al., 1974; Harris, 1969)	Human (n = 20)	—	—	—	—	—	64	64	—	—	—
NOAEL, 64 mg/m^3, 15 min (Wills et al., 1974; Harris, 1969)	Human (n = 20)	—	—	20	—	—	—	—	28	28	28
CNS depression											
LOAEL, 1.1 g/kg, iv (Felts, 1969)	Chimpanzee, monkey	10	10	—	20/24	0.6	1500	—	—	—	—
LOAEL, 1.1 g/kg, iv (Felts, 1969)	Chimpanzee, monkey	10	10	—	20	0.6	—	24	—	—	—
NOAEL, 30 mg/m^3, 24/d, 30 d (Wills et al., 1974)	Human (n = 20)	—	—	20	—	—	—	—	13	13	13
Renal toxicity											
NOAEL, 0.65 g/kg in drinking water (Robinson et al., 1990)	Rat	—	10	—	20	0.6	—	380	—	—	—

TABLE 9-9 *(Continued)*

Effect, Data, Reference	Species	Uncertainty Factors: To NOAEL	Uncertainty Factors: Species	Uncertainty Factors: Small n[a]	Conversion Factor[b]	Absorption Factor[c]	Acceptable Concentrations, mg/m^3: 1 h	24 h	7 d	30 d	180 d
Renal toxicity (cont.)											
NOAEL, 30 mg/m^3, 24/d, 30 d (Wills et al., 1974)	Human	—	—	20	—	—	—	—	13	13	13
SMACs							**64**	**64**	**13**	**13**	**13**

[a]To correct for small number of human test subjects, the factor $1\sqrt{(n/100)}$.

[b]To convert an oral dose to an inhalation exposure concentration. It is usually equal to the respiratory volume.

[c]To correct for the incomplete respiratory absorption of ethylene glycol.

—, Data not considered applicable to the exposure time; HR, Haber's rule.

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B10 Glutaraldehyde

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PHYSICAL AND CHEMICAL PROPERTIES

Glutaraldehyde, an aliphatic dialdehyde, is a highly reactive compound that has been isolated as a water-soluble oil and usually is stored as an aqueous solution to inhibit polymerization (Sax, 1984). Unbuffered aqueous solutions of glutaraldehyde are stable for long periods of time, have a mildly acid pH, a negligible odor, and are not potently antimicrobial. When buffered to an alkaline pH of 7.5 to 8.0 with sodium bicarbonate, the glutaraldehyde is activated; it has a strong pungent odor, and its antimicrobial activity is greatly enhanced for periods of up to 14 days (Stonehill et al., 1963).

Synonyms:	1,5-Pentanedial, glutardialdehyde, glutaric dialdehyde, NCI C55425, 1,5-pentanedione
Formula:	$C_5H_8O_2$; $CHO(CH_2)_3CHO$
CAS number:	111-308
Molecular weight:	100.13
Boiling point:	187-189°C
Melting point:	−14°C
Solubility:	Soluble in all proportions in water, ethanol, and benzene
Conversion factors at 25°C, 1 atm:	1 ppm = 4.09 mg/m^3 1 mg/m^3 = 0.244 ppm

OCCURRENCE AND USE

Glutaraldehyde is widely used in embalming; in the manufacture of adhesives, sealants, and electrical products; as a cross-linking agent for proteins and polyhydroxy compounds; in microcapsules containing flavoring agents; and as a tissue fixative in electron microscopy, the paper and leather tanning industries, and x-ray film developing solutions. It is also used as a sterilizing agent for plastics, rubber, thermometers, lenses, and other surgical, dental, and hospital equipment (Stonehill et al., 1963; Lahav, 1977; Biophysics Research and Consulting Corporation, 1980; Hemminki et al., 1982; Wiggins et al., 1989). Glutaraldehyde is an effective sporicidal agent, requiring about 3 h for an almost complete kill of spores as well as gram-negative and gram-positive bacteria, fungi, and viruses. Glutaraldehyde has been used in surgical procedures including colonic anastomoses (D'Ovidio et al., 1981) and dental pulpotomies (Ranly et al., 1989; Feigal and Messer, 1990; Ketley and Goodman, 1991). Glutaraldehyde solutions (5-25%) are used clinically to treat skin disorders, including warts, hyperhidrosis (excessive sweating of the hands or soles of the feet), herpes simplex, and herpes zoster, and in the preparation of grafts and bioprostheses (Beauchamp et al., 1992). The preservative and antimicrobial properties of glutaraldehyde have found broad application in cosmetic, toiletry, and chemical specialty products because of glutaraldehyde's water solubility and usefulness in systems containing secondary or tertiary amines, quaternary ammonium compounds, or protonated amines (Beauchamp et al., 1992).

Glutaraldehyde solutions often are used as cell and tissue fixatives in biochemical experiments during space-shuttle flights.

TOXICOKINETICS AND METABOLISM

No data were found on the uptake, distribution, metabolism, or elimination of inhaled glutaraldehyde vapors. The discussion below relates to percutaneous or intravenous routes of exposure.

The extent of systemic distribution of glutaraldehyde that was applied to a pulpotomized tooth of a rat was estimated to be 40 nmoles, or 25% of the applied dose. Metabolic studies using that preparation showed that glutaraldehyde was eliminated in the urine (the urinary metabolites

have not been characterized) and in expired gases (as CO_2); 90% was cleared from the rats' body tissues in 3 d (Ranly et al., 1989).

Percutaneous penetration of topically applied glutaraldehyde solutions was studied in vitro using human stratum corneum from the chest, abdomen, and sole, and epidermis from the abdomen (Reifenrath et al., 1985). The results showed that glutaraldehyde does not penetrate the thick stratum corneum of the sole, but that 3.3% to 13.8% of the applied dose penetrated the thin stratum corneum of the chest and abdomen, and 2.8% to 4.4% of the applied dose penetrated the isolated epidermis. More recent studies reported $<1\%$ penetration by glutaraldehyde through the skin of rats, mice, rabbits, guinea pigs, and humans (Tallant et al., 1990).

In studies of rats and rabbits given 0.075% or 0.75% [1,5-^{14}C]-glutaraldehyde intravenously, the majority of the radioactivity was excreted as $^{14}CO_2$; approximately 80% was exhaled in the first 4 h. The fate of the unlabeled carbon atoms has not been directly established, and direct identification of metabolites has not been carried out (Beauchamp et al., 1992). Determination of the fate of glutaraldehyde in vivo is complicated by the fact that $^{14}CO_2$ produced during metabolism of [1,5- ^{14}C]-glutaraldehyde could be recycled and incorporated into the normal monomers required for synthesis of macromolecules. Thus, deposition of radioactivity at the site of administration does not necessarily represent only covalent binding of glutaraldehyde to macromolecules.

TOXICITY SUMMARY

Background

Relatively few toxicity studies have been done on glutaraldehyde, considering its widespread use. Many of the studies that have been done test the toxicity of glutaraldehyde solutions rather than glutaraldehyde vapors. A study comparing the acute oral toxicities versus the acute inhalation toxicities of 108 chemicals concluded that, although a positive correlation of 0.53 ($p = 0.001$) was demonstrated for toxicities via the two routes of exposure, the magnitude of the correlation was so low that the predictive value of one for the other was limited (Kennedy et al., 1991). Thus, even though mention will occasionally be made

below of toxicities due to noninhalation exposures, these will not be used to set acceptable concentrations for inhalation exposures.

Acute Exposures

Irritation and Depressed Weight Gain

Glutaraldehyde is a relatively strong irritant to the nose and a less strong irritant to the eyes and skin (Anonymous, 1976). The odor threshold is 0.04 ppm (Beauchamp et al., 1992). Sensory irritation and inflammation of the nasal mucosa, as well as depressed weight gain, were noted in rats exposed to glutaraldehyde via inhalation for 6 h/d for 9 d at 2.1 or 3.1 ppm; significant mortality was seen at 3.1 ppm (Ballantyne et al., 1985). Fischer 344 (F344) rats exposed to glutaraldehyde for 6 h/d, 5 d/w, for 3 mo at 0.049 or 0.194 ppm showed perinasal wetness and significantly decreased weight gain but no damage to the nasal mucosa and no histopathological lesions in any organ, although the activities of several serum enzymes (phosphokinase, lactate dehydrogenase, and hydroxybutyric dehydrogenase) were increased (Greenspan et al., 1985).

A solution concentration of 1% was reported to be the threshold for glutaraldehyde-induced erythema in rabbit skin (Ballantyne et al., 1985). Instillation of 20-40 mM glutaraldehyde into the nasal cavities of rats caused epithelial changes characteristic of inhalation exposure to a number of irritating gases (St. Clair et al., 1990).

Respiratory Distress

A single 8-h inhalation exposure of rats to saturated glutaraldehyde vapors (concentration not measured) produced excess lacrimation and salivation, audible breathing, and mouth breathing (Ballantyne, 1986). Single exposures (6-8 h) of rats to statically generated steady-state vapor atmospheres produced only signs of sensory irritation to the eyes and respiratory tract (Ballantyne et al., 1985; Ballantyne, 1986). Measurements indicated an initial glutaraldehyde concentration of 11 ppm,

decreasing to 2 ppm at 6 h, and an average of 4.3 ± 3.4 (standard error) ppm (Ballantyne, 1986).

In 2-w inhalation exposures of rats and mice to glutaraldehyde at 0, 0.16, 0.5, 1.6, 5, and 16 ppm for 6 h/d, 5 d/w, all rats and mice exposed to glutaraldehyde at 5 or 16 ppm died before the end of the studies, as did all mice exposed at 1.6 ppm (Kiri, 1992). The deaths were attributed to severe respiratory distress. Mice appeared to be more sensitive than rats because the small airways of the nasal passage were more easily blocked by cell debris and keratin. Lesions noted in the nasal passage and larynx of rats and mice included necrosis, inflammation, and squamous metaplasia. At higher concentrations, similar lesions were present in the trachea of rats and mice and in the lung and tongue of rats (Kiri, 1992).

Similar lesions in the respiratory tract were seen in 13-w studies of rats and mice exposed to glutaraldehyde at 0, 0.0625, 0.125, 0.25, 0.50 and 1.0 ppm, but the evidence of systemic toxicity was unclear following histopathological or clinical pathology assessments (Kiri, 1992). Lesions of the nasal passages in rats were seen at concentrations as low as 0.125 ppm; the severity increased with increasing concentrations. In mice, a no-observed-adverse-effect level (NOAEL) was not reached, because inflammation was found in the anterior nasal passage at concentrations as low as 0.0625 ppm (Kiri, 1992).

Hepatitis

A single 24-h inhalation exposure of NMRI mice to glutaraldehyde vapor at 33 ppm induced toxic hepatitis in 9 of 10 mice, and 8 ppm caused local inflammation of the liver, possibly not of toxic origin, in 1 of 10 mice (Varpela et al., 1971).

Lethality

In rats, the 4-h LC_{50} values for dynamically generated glutaraldehyde vapor were 24 (17-33) ppm for males, and 40 (15-106) ppm for females (Ballantyne et al., 1985). As noted above, however, single exposures (6-8 h) of rats to statically generated, saturated glutaraldehyde-vapor

atmospheres up to 11 ppm produced only signs of sensory irritation to the eyes and respiratory tract (Ballantyne et al., 1985; Ballantyne, 1986). The LC_{50} value (5000 ppm) listed in the National Institute for Occupational Safety and Health's Registry of Toxic Effects of Chemical Substances (RTECS) (NIOSH, 1987) is from a 1972 Czecholovakian publication (Marhold, 1972) and is given little credence because it is so much higher than the values reported by others in more recent studies. The large discrepancy between the LC_{50} values of Ballantyne et al. (1985) and RTECS could be explained if the concentrations reported in the Czech article on which the RTECS value is based were nominal rather than analytical. The concentrations reported by Ballantyne et. al. (1985) were analytical concentrations. The high reactivity of glutaraldehyde makes it very difficult to maintain atmospheric concentrations at nominal values.

Genotoxicity

Glutaraldehyde was found to be negative for genotoxicity in some in vitro and in vivo genotoxicity assays. Negative results were observed at concentrations spanning cytotoxic to noncytotoxic doses in the following test systems: mutagenicity in *Salmonella* strains TA 98 and TA 100 with and without microsomes (Hemminki et al., 1980; Slesinski et al., 1983), the Chinese hamster ovary cell and hypoxanthine guanine phosphoribosyl transferase (CHO/HGPRT) gene-mutation system (Slesinski et al., 1983), the sister-chromatid-exchange (SCE) test with CHO cells (Slesinski et al., 1983), and the primary rat hepatocyte unscheduled DNA synthesis (UDS) system (Slesinski et al., 1983). No increase was seen in the dominant lethal index in mice exposed orally to glutaraldehyde at 30-60 mg/kg (Tamada et al., 1978).

Glutaraldehyde was positive for genotoxicity in other in vitro assays (Sasaki and Endo, 1978; McGregor et al., 1988; St. Clair et al., 1991; Jung et al., 1992). They include induction of DNA-protein cross-linking in human TK6 lymphoblast cells and dose-related increases in mutations at the thymidine kinase locus in the same cells (St. Clair et al., 1991). In primary rat hepatocytes, glutaraldehyde induced a marginal increase in UDS (St. Clair et al., 1991). In a collaborative study by three laboratories, glutaraldehyde was found to be mutagenic in *Salmonella typhimurium* TA 102 by all three laboratories (Jung et al., 1992).

Subchronic Exposures

Lethality

Of 12 rats per group exposed 6 h/d for 9 d to glutaraldehyde at 3.1 ppm, seven males and six females died between the sixth and ninth exposure days (Ballantyne, 1986). Nine of 10 male rats and 7 of 10 female rats died after exposure to glutaraldehyde at 2.1 ppm for 6 h/d for 9 d, as did 1 of 10 male rats exposed at 0.63 ppm (B.J. Greenspan, Pacific Northwest Laboratory, Richland, Wash., personal commun., 1992).

Irritation, Hematological Effects, and Decreased Organ Weights

Rats exposed 6 h/d for 9 d to glutaraldehyde at 3.1 ppm showed signs of irritation; the signs were audible breathing and periocular and perinasal encrustation; increases in neutrophil count, erythrocyte count, hematocrit, and hemoglobin concentration; and decreased organ weights for liver, kidney, lung, heart, and testes (Ballantyne, 1986). At 1.1 ppm, half of the animals demonstrated mouth and abdominal breathing and the same hematological and organ-weight effects as seen at 3.1 ppm except that lung weights were not decreased. At 0.3 ppm, there were no signs of irritation, no significant hematological effects, and only a slight increase in lung weight. (Ballantyne, 1986). Histological examination of tissues from survivors sacrificed the day after the final exposure at 3.1 ppm revealed hepatocellular atrophy, rhinitis, and mild atrophy of the olfactory mucosa. Rhinitis and squamous metaplasia of the nasal mucosa occurred at 1.1 ppm. No histological abnormalities were seen in animals exposed at 0.3 ppm (Ballantyne, 1986).

Contact Dermatitis

There are numerous reports of mild-to-severe contact dermatitis in humans and animals resulting from occasional or incidental contact with glutaraldehyde solutions or vapors. Contact dermatitis, particularly in medical personnel who use 2% glutaraldehyde solutions to sterilize

equipment, has been reported (Jordan et al., 1972; Bardazzi et al., 1986; Di Prima et al., 1988; Nethercott et al., 1988a,b; Stern et al., 1989; Jachuck et al., 1989; Charney, 1990). There appears to be substantial variation in individual susceptibility to glutaraldehyde-induced dermatitis, which, in a few individuals, appears to be due to allergic reactions developing after repeated contact with glutaraldehyde.

Respiratory Difficulty, Rhinitis, Headache, Watering of the Eyes

Numerous reports describe the risks of occupational exposure to glutaraldehyde vapors in hospital staff involved in cold sterilization of endoscopes and other medical equipment. In a study by Jachuck et al. (1989), exposed hospital personnel (eight of nine exposed) complained of headache, respiratory difficulty, rhinitis, and watering of the eyes. Measurements of the glutaraldehyde vapor concentration in a nurse's breathing zone showed 0.12 ppm (Jachuck et al., 1989). A single individual also reported nausea, but this symptom was not reported in any of the other literature found. A case study by Charney (1990) reported hives, chest tightness, and watery eyes in 19 workers in an area where glutaraldehyde was used in counter-top baths for bronchoscope disinfection and where ambient and breathing-zone samples yielded concentrations up to 0.25 ppm. In other similar studies, personal breathing-zone samples in two hospitals ranged up to 6.1 and 8.1 ppm and an area sampling ranged up to 3 ppm (Charney, 1990). A case report by Benson (1984) of a nurse who worked in an endoscopy unit and who complained of constant eye irritation indicated that lung function was reduced from about 400 L/min to about 300 L/min during the work week but fully recovered on the weekends when no exposure occurred. No measurement of vapor concentrations was made, and other workers doing the same job did not have similar complaints. In a survey on routine exposures of English hospital workers, Leinster et al. (1993) collected 77 samples (39 personal and 38 static) from 14 locations in six hospitals. Measured concentrations ranged up to 0.70 ppm for a 15-min sample during the cleaning of suction bottles with Cidex (2% glutaraldehyde); more typical values for other operations were 0.04 ppm to 0.22 ppm (Leinster et al., 1993). The typical pattern of exposure was contact for 4 to 5 min every 30 min throughout the shift when endo-

scopes were put into or removed from cleaning units (Leinster et al., 1993). Spontaneous complaints of rhinitis during this survey imply that some persons can experience adverse effects at concentrations below the current occupational exposure standard (Leinster et al., 1993).

Rats exposed 6 h/d, 5 d/w, for 14 w to glutaraldehyde at concentrations of 0.194, 0.049, or 0.021 ppm showed signs of irritation consisting of periocular and perinasal encrustation at 0.194 and 0.049 ppm, but showed no significant hematological, organ-weight, histological, urinary, or gross pathological effects (Ballantyne, 1986). Thus, subchronic exposures at low concentrations ranging from 49 to 194 ppb produce signs of sensory irritation, and exposures at 20 ppb produce transient reduced body-weight gain, indicating mild sensory irritation but not toxicity (Ballantyne, 1986).

Reproductive and Developmental Toxicity

No significant increases in the risk of spontaneous abortions and fetal malformations were found in retrospective epidemiological studies of Finnish hospital staff performing sterilization or nurses exposed to glutaraldehyde or formaldehyde (Hemminki et al., 1982, 1985). The same studies found that exposure to ethylene oxide was associated with an increased frequency of spontaneous abortions, and maternal use of cytostatic drugs was associated with fetal malformations.

Studies with rodents also have indicated a low teratogenicity due to glutaraldehyde exposure during pregnancy. Glutaraldehyde given by gavage to pregnant albino mice on d 6-15 of gestation was judged not to be teratogenic at doses up to 100 mg/kg/d, which killed 19 of 35 dams and produced a significant increase in the number of stunted fetuses (Marks et al., 1980).

No rodent studies were found that assessed the effects of inhalation of glutaraldehyde vapors on fetal development, but given the known data on glutaraldehyde's high chemical reactivity, metabolism, and toxicokinetics, it seems unlikely that significant systemic absorption and distribution would occur.

No studies were found that assessed the effects of glutaraldehyde on female reproductive function or fertility. Glutaraldehyde did not reduce the fertility of exposed male mice given a single oral dose of glutaraldehyde at 30 or 60 mg/kg, and there were no significant effects on

embryo-fetal viability in a dominant lethal assay (Tamada et al., 1978).

Carcinogenicity

A Union Carbide Corporation study of rats given glutaraldehyde in drinking water at concentrations of 0, 50, 250, or 1000 ppm for 2 y showed a significantly increased incidence of large-granular-cell leukemia at 50, 250, and 1000 ppm in females only (Behen, 1991). Because this tumor type occurs spontaneously (23% to 24%) in females of the strain of rats used, the results might represent a modulating effect on this spontaneously occurring tumor rather than a direct chemical carcinogenic effect.

Although related aldehydes (formaldehyde, acetaldehyde, and malonaldehyde) have been shown to be carcinogenic in laboratory animals, the National Institute of Occupational Safety and Health has stated that the data are insufficient to allow conclusions about the carcinogenicity of glutaraldehyde (NIOSH, 1991). It is not known if that opinion took into account the results of the 2-y Union Carbide study described in the preceding paragraph.

Interactions with Other Chemicals

No reports of interactions or synergistic effects with other chemicals were found.

TABLE 10-1 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
0.25	Occupational (hospital)	Human	Hives, chest tightness, watery eyes	Charney, 1990
0.12	Occupational (hospital)	Human (n = 9)	Headache, rhinitis, watering of eyes, dermatitis, respiratory difficulty	Jachuck et al., 1989
0.12	Occupational (hospital)	Human	NOAEL for embryo toxicity and teratogenicity	Hemminki et al., 1982, 1985
8.1	Occupational (hospital)	Human	Hives, chest tightness, watery eyes	Charney, 1990
0.021	6 h/d, 5 d/w, 14 w	Rat	Transient reduced body-weight gain	Ballantyne, 1986
0.049	6 h/d, 5 d/w, 14 w	Rat	Periocular and perinasal encrustation	Ballantyne, 1986
0.0625	6 h/d, 5 d/w, 13 w	Mouse	Mild-to-moderate inflammation of anterior nasal passages in female mice	Kiri, 1992
0.194	6 h/d, 5 d/w, 14 w	Rat	Periocular and perinasal encrustation	Ballantyne, 1986
0.63	6 h/d, 9 d	Rat	1 of 10 exposed males died	B.J. Greenspan, personal commun., 1992
1.6	6 h/d, 5 d/w, 2 w	Mouse	LC_{100} (necrosis, inflammation, and squamous metaplasia of nasal passages and larynx)	Kiri, 1992
2.1	6 h/d, 9 d	Rat	9 of 10 exposed males died	B.J. Greenspan, personal commun., 1992
2.1	6 h/d, 9 d	Rat	7 of 10 exposed females died	B.J. Greenspan, personal commun., 1992

TABLE 10-1 *(Continued)*

Concentration, ppm	Exposure Duration	Species	Effects	Reference
24	4 h	Rat	LC_{50} for males	Ballantyne et al., 1985
33	24 h	Mouse	Toxic hepatitis	Varpela et al., 1971
40	4 h	Rat	LC_{50} for females	Ballantyne et al., 1985
2 to 11	6-8 h	Rat	Sensory irritation of eyes and respiratory tract	Ballantyne et al., 1985
5000	4 h	Rat	LC_{50}	NIOSH, 1975

TABLE 10-2 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's STEL	0.2 (ceiling)	ACGIH, 1991
OSHA's PEL	0.2 (ceiling)	ACGIH, 1991
NIOSH's REL	0.2	ACGIH, 1991
Germany's MAK	0.2	ACGIH, 1991

STEL, short-term exposure limit; PEL, permissible exposure limit; REL, recommended exposure limit; MAK, maximum allowable concentration.

TABLE 10-3 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	0.12	0.49	Eye irritation, rhinitis, respiratory difficulty, headache
24 h	0.04	0.08	Eye irritation, rhinitis, respiratory difficulty, headache
7 d[a]	0.006	0.024	Lesions of the respiratory tract
30 d	0.003	0.012	Lesions of the respiratory tract
180 d	0.0006	0.002	Lesions of the respiratory tract

[a]Previous 7-d temporary group SMAC for aliphatic aldehydes is 5 ppm (1.2 mg/m^3). Previous 7-d temporary SMAC for glutaraldehyde is 0.1 ppm (0.4 mg/m^3).

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

To set SMACs for glutaraldehyde, acceptable concentrations (ACs) are set for each adverse effect or group of effects for each desired exposure duration, i.e. 1 h, 24 h, 7 d, 30 d, and 180 d. The ACs for the most sensitive adverse effects are set as the SMAC values for those exposure durations. Because of recent data that showed nasal lesions in rodents at very low glutaraldehyde concentrations, the ACs for respiratory tract lesions dominate the resulting SMACs, which are presented in Table 10-3.

Respiratory Difficulty, Rhinitis, Headache, Watering of the Eyes

Since about 1962, when 2% glutaraldehyde was introduced as a sterilizing solution (Stonehill et al., 1963), until about 1990, when the toxicity of glutaraldehyde was recognized, medical personnel had routinely worked with 2% glutaraldehyde solutions in open trays (Benson, 1984; Corrado et al., 1986; Burge, 1989). In a study by Jachuck et al. (1989), the atmospheric concentrations in the breathing zones of the workers measured 0.12 ppm (1-h sample). Such exposures produce some watering of the eyes, rhinitis, headache, and tightness of the chest. Thus, the 1-h AC for these end points was set equal to the lowest reported occupational exposure concentration that produced irritation (assumed to be for 8 h/d):

1-h AC = 0.12 ppm.

For the 24-h AC, the 0.12-ppm AC was reduced (divided by 3), because the degree of irritation tolerable for 8 h might become intolerable for 24 h. Thus,

24 h AC = 0.12 ppm/3 = 0.04 ppm.

For exposures lasting longer than 24 h, no irritation is acceptable; therefore, the 0.12-ppm lowest-observed-adverse-effect level (LOAEL) must be extrapolated to a NOAEL for irritation. To estimate a NOAEL, the ACs for the end points were set equal to one-tenth of the 0.12-ppm effect concentration. Experience has shown that these effects are threshold effects, rather than cumulative effects, such that below a certain concentration, no effects are seen, independent of the length of exposure.

7-d, 30-d, 180-d ACs = 0.12 ppm/10 = 0.012 ppm.

Lesions of the Respiratory Tract

In a recently published 13-w intermittent (6 h/d, 5 d/w) inhalation study in mice, 0.0625 ppm was a LOAEL for inflammation of the ante-

rior nasal passage from exposure to glutaraldehyde (Kiri, 1992). That exposure corresponds to 390 total hours of exposure or about 16 d of continuous exposure. To calculate a concentration that will protect against respiratory-tract lesions during a 7-d exposure, the 16-d LOAEL is divided by 10 to estimate a NOAEL. This 16-d NOAEL is not increased in extrapolating to an AC for 7 d. No adjustment is made for interspecies differences in susceptibility, because experience with formaldehyde has demonstrated that rodents are no less sensitive (Kerns et al., 1983) than humans and probably are more sensitive than humans to aldehyde-induced nasal lesions:

7-d AC = 0.0625 ppm/10 (LOAEL to NOAEL) = 0.006 ppm.

To calculate a concentration that will protect against respiratory-tract lesions during a 30-d exposure, the 16-d LOAEL is divided by 10 to estimate a NOAEL and multiplied by (16/30) to adjust for the longer exposure time:

30-d AC = 0.0625 ppm/10 (LOAEL to NOAEL) × (16 d/30 d)
= 0.003 ppm.

To calculate a concentration that will protect against respiratory-tract lesions during a 180-d exposure, the 16-d LOAEL is divided by 10 to estimate a NOAEL and multiplied by (16/180) to adjust for the longer exposure time:

180-d AC = 0.0625 ppm/10 (LOAEL to NOAEL) × (16 d/180 d)
= 0.0006 ppm.

Hepatitis

Using the 32-ppm 24-h exposure of mice, a 24-h AC for hepatitis can be set by applying factors of 10 for species differences and 10 to estimate a NOAEL from a LOAEL. Thus,

24-h AC = 32 ppm/10 (species)/10 (to NOAEL) = 0.32 ppm.

A 7-d AC can be calculated by dividing the 24-h AC by 7:

7-d AC = 0.32 ppm/7 = 0.05 ppm.

SPACEFLIGHT CONSIDERATIONS

Of the end points induced by exposure to glutaraldehyde, none would be affected by launch, microgravity, or re-entry. Thus, no spaceflight factor was used in calculating any ACs.

TABLE 10-4 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors: To NOAEL	Species	Time	Space-flight	Acceptable Concentrations, ppm: 1 h	24 h	7 d	30 d	180 d
Eye irritation, rhinitis, respiratory difficulty, headache; occupational exposure, 0.12 ppm (Jachuck et al., 1989)	Human	3	1	1	1	0.12	0.04	0.012	0.012	0.012
Respiratory-tract lesions, LOAEL, 0.0625 ppm (Jachuck et al., 1989)	Mouse	10	1	HR	1	—	—	0.006	0.003	0.0006
Hepatitis, LOAEL, 32 ppm, 24 h (Jachuck et al., 1989)	Mouse	10	10	HR	1	—	0.32	0.05	—	—
SMACs						**0.12**	**0.04**	**0.006**	**0.003**	**0.0006**

—, Data not considered applicable to the exposure time; HR, Haber's rule.

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B11 Trichloroethylene

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PHYSICAL AND CHEMICAL PROPERTIES

Trichloroethylene (TCE) is a colorless, nonflammable, volatile liquid, with a sweetish odor resembling chloroform (ACGIH, 1986).

Synonyms:	Ethylene trichloride, trilene
Formula:	$CHCl=CCl_2$
CAS number:	79-01-6
Molecular weight:	131.4
Boiling point:	87°C
Melting point:	−87°C
Conversion factors at 25°C, 1 atm:	1 ppm = 5.38 mg/m^3 1 mg/m^3 = 0.19 ppm

OCCURRENCE AND USE

TCE is widely used as an industrial solvent, particularly in metal degreasing and extraction processes (Torkelson and Rowe, 1981). Other less toxic chemicals have replaced it in some of its former uses, including that as an anesthetic. Although TCE is not used in the spacecraft, it has been found in numerous atmospheric samples collected from the cabin of the space shuttle (Coleman, 1984). In contact with alkaline materials, especially at high temperatures, TCE can be convert-

ed into more toxic compounds. Dichloroacetylene (DCA) is the major product formed from TCE in carbon dioxide scrubbers containing alkaline materials (Saunders, 1967). In an experiment by the Toxicology Laboratory at the National Aeronautics and Space Administration, all the TCE disappeared upon passage over heated alkaline adsorbent, with at least 75% conversion to DCA (Rippstein, 1980). The SMAC limits developed here are only applicable to a spacecraft environment in which an alkaline air scrubber is not present. If an alkaline air scrubber is present, the SMAC values for DCA are applicable to TCE (see Chapter B5).

TOXICOKINETICS AND METABOLISM

Absorption and Distribution

TCE is readily absorbed from the lungs of humans and distributed throughout the body (Waters et al., 1977). Most blood-borne TCE reaches the liver where the majority of its metabolism occurs (Steinberg and DeSesso, 1993).

Elimination

Approximately 20% to 30% of the absorbed chemical is excreted unchanged in the expired air, mostly during the first 24 h, with the rest metabolized and excreted in the urine (Ogata and Bodner, 1971). Because of its high lipid solubility, a portion of the absorbed TCE is stored in tissues, principally fatty tissues, from which it is slowly released and then metabolized and excreted (Müller et al., 1974). After a 4-h exposure of human volunteers to TCE at 100 ppm, trichloroacetic acid (TCA) and trichloroethanol glucuronide accounted for about 20% and 80%, respectively, of the total urinary trichloro compounds (Sato et al., 1977). In contrast to the rapid excretion of trichloroethanol glucuronide by the kidneys, renal clearance of TCA is delayed because of its high degree of protein-binding (Müller et al., 1974).

Metabolism

TCE that is not excreted from the lungs is converted enzymatically in several steps to the principal urinary metabolites, trichloroethanol, trichloroethanol glucuronide, and trichloroacetic acid (Waters et al., 1977), and a minor metabolite, dichloroacetic acid (DCA) (Hathway, 1980). Cytochrome P-450 systems are the primary metabolizing systems in the liver (Steinberg and DeSesso, 1993). In the first metabolic step, TCE is oxidized through intermediates to chloral hydrate, which undergoes reduction to trichloroethanol as well as further oxidation to TCA (Byington and Leibman, 1965; Sellers et al., 1972). Most of the trichloroethanol is conjugated with glucuronic acid in the liver before being excreted in the urine (Waters et al., 1977). Other pathways for trichloroethanol are oxidation to TCA and excretion of unchanged trichloroethanol in the urine. The proportion of urinary metabolites excreted as TCA was predicted to increase in a chronic exposure. TCA and DCA have recently been shown to be complete hepatocarcinogens in male $B6C3F_1$ mice (Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo et al., 1991). Thus, these metabolites might be responsible for hepatic tumors produced in $B6C3F_1$ mice treated with TCE (NCI, 1976). In rodents, DCA can be converted to *S*-(1,2-dichlorvinyl)-L-cysteine and subsequently by β-lyase to the reactive mercaptan (Steinberg and DeSesso, 1993). The pathway to DCA is much more heavily used in rodents than in humans. The biological half-life of trichloroethanol in humans is relatively short compared with that of TCA. In humans exposed to TCE at 50 ppm for 6 h/d for 5 d, the half-lives of trichloroethanol and TCA were approximately 12 h and 99 h, respectively (Müller et al., 1974). For exposures at 100 ppm for 6 h/d for 10 d, the half-lives were 13 h and 86 h, respectively. Physiologically based pharmacokinetic (PB-PK) models of the toxicokinetics and metabolism of TCE in humans (Sato et al., 1977; Fernandez et al., 1977) and in animals (Fernandez et al., 1977; Andersen et al., 1987; Fisher et al., 1989, 1990, 1991; Dallas et al., 1991) have been developed by several groups of investigators. These models have enabled a better understanding of the uptake, distribution, and metabolism of TCE as well as of the kinetics of formation, distribution, and excretion of its metabolites TCA and trichloroethanol.

The metabolism and elimination pattern for TCE is conducive to exposure monitoring with the use of biological markers. The parent com-

pound can be monitored in the breath, blood, or urine (Stewart et al., 1970; Kimmerle and Eden, 1973; Monster et al., 1979), or the metabolites trichloroacetic acid and trichloroethanol can be monitored in the urine (Inoue et al., 1989). Use of these markers might be confounded by interindividual variation and by the presence of other chlorinated hydrocarbons (Inoue et al., 1989). Air monitoring near the breathing zone of exposed persons is still the best predictor of inhalation exposure to TCE. Biological markers of TCE toxicity have focused on nervous system injury or kidney injury (Feldman et al., 1988; Nagaya et al., 1989); however, those markers do not appear to be widely used and are not specific for TCE exposure.

TOXICITY SUMMARY

Acute Exposures

Many cases of accidental acute poisoning by TCE are described in the literature (Cotter, 1950). The predominant physiological effect is depression of the central nervous system (CNS), with reported symptoms of inebriation, loss of coordination, dizziness, visual disturbances, mental confusion, headache, nausea, vomiting, and loss of consciousness (Waters et al., 1977; Cotter, 1950). In one TCE exposure accident, two workmen rapidly lost consciousness upon re-entry into an atmosphere containing TCE at an estimated 3000 ppm after an earlier, less severe exposure (Longley and Jones, 1963). In a controlled laboratory study with a human volunteer, a 2.75-h exposure to TCE at 100 ppm did not cause any significant effects on psychomotor performance (Stopps and McLaughlin, 1967). At 200 ppm, there was a slight decline in performance, which became more pronounced at 300 and 500 ppm. In another study, a 2-h exposure at 100 or 300 ppm did not affect visual-motor performance, but 1000 ppm significantly impaired performance and resulted in subjective responses of lightheadedness and dizziness or lethargy (Vernon and Ferguson, 1969). A longer exposure of 8 h at 110 ppm resulted in a significant decrease in the performance of volunteers in various psychophysiological tests, the greatest decrease being in the more complex tests (Salvini et al., 1971). However, performance decrements were not found in a repeat of this study with an additional concentration of 50 ppm and more end points (Stewart et al.,

1974). It has been suggested that drowsiness can occur at 27 ppm during acute exposures and headaches can occur at 81 ppm (Nomiyama and Nomiyama, 1977). Those conclusions were based on subjective reports of three volunteers; however, because no dose-response relationship was shown for drowsiness (i.e., no drowsiness after 3 h in persons exposed at 200 ppm), the observation is suspect. Even though the prevalence of headache shows a dose response, many other symptoms do not show a dose response, so that the findings must be questioned.

There are reports that cardiac arrhythmias induced by inhaled TCE have resulted in human deaths (Kleinfeld and Tabershaw, 1954; Bell, 1951). TCE has been shown to be have the capability to cause cardiac sensitization to epinephrine in the dog. After a 10-min exposure to 5000 or 10,000 ppm, a challenge injection of epinephrine produced ventricular fibrillation in 1 of 12 and 7 of 12 dogs, respectively (Reinhardt et al., 1973). Those findings in animals are particularly significant in view of the cardiac dysrhythmias seen periodically in crew members of U.S. spaceflights as well as in at least one Soviet cosmonaut (Bungo, 1989; NASA, 1991). Whether spaceflight-associated conditions, such as gravitational stress, thermal load, electrolyte changes, fluid shifts, or catecholamine alterations, caused those cardiac rhythm irregularities is unknown at this time (NASA, 1991).

Short-Term and Subchronic Exposures

Repeated exposures for 7 h/d for 5 consecutive days to TCE at 200 ppm did not adversely affect performance or neurological or biochemical tests in human volunteers, but they elicited a consistent subjective response of a sensation of mild fatigue and sleepiness during the fourth and fifth days (Stewart et al., 1970). In subsequent better-controlled studies, the same investigators did not find objective or subjective adverse effects after repeated 7.5-h daily exposures at 100 or 200 ppm and concluded that 100 ppm probably has a threefold to fourfold margin of safety for most individuals (ACGIH, 1986). According to studies cited by the American Conference of Governmental Industrial Hygienists, daily exposures to TCE at 100 ppm caused no impairment in mental or psychological capabilities in one European study (Triebig et al., 1976), but in a similar study, it caused fatigue, lassitude, and headaches (Ertle et al., 1972).

Several studies of worker complaints have been published involving industrial exposure to TCE; however, the findings are often uncertain and do not agree with results of volunteer exposures. The prevalence of symptoms (e.g., sleepiness, fatigue, nausea, and irritation) was reduced in nine workers after TCE exposures were reduced from 38 ppm TWA (average of 200 ppm for short-term exposure) to 16 ppm TWA (average of 74 ppm for short-term exposures) by improving ventilation and work practices (Landrigan et al., 1987). The reported symptoms probably were elicited by the high-concentration short-term exposures rather than the low-concentration sustained exposures. The National Institute for Occupational Safety and Health (NIOSH) has reviewed a number of work sites because of worker complaints of excess chemical exposure. In one investigation, the TCE exposures were confounded by the presence of other chemicals, and the magnitude of worker complaints did not compare well with the exposure concentrations (Bloom et al., 1974). Three workers reported occasional lightheadedness and headache in a degreasing operation, which had a TWA TCE concentration at 47 ppm, with 1-h maximum exposures up to 94 ppm (Hervin et al., 1974). In a study of printed circuit-board processors, average breathing-zone concentrations of TCE ranged from 29 to 62 ppm, and symptoms reported were nausea (71%), headache (54%), and fatigue and drowsiness (25%) (Okawa and Bodner, 1973). The authors concluded that the symptoms were due to toxic exposures to TCE in the workplace.

The difficulty of interpreting workplace results is indicated by subjective responses reported by volunteers even when not exposed to TCE. For example, two of two test subjects reported headaches; irritation of the eyes, nose, and throat; and odor when no exposures to TCE had occurred (Stewart et al., 1974). In another group of test subjects, only odor was reported, even at exposure concentrations of 50 and 110 ppm (Stewart et al., 1974). Objectively measurable performance decrements were absent.

Chronic Exposures

Noncarcinogenicity

Neurological symptoms, including vertigo, fatigue, insomnia, and

memory loss, have been reported in epidemiological studies of workers chronically exposed to TCE in industry (Grandjean et al., 1955; Bardodej and Vyskocil, 1956). The incidence of these symptoms correlated with the duration of exposure of the workers. Although biochemical tests have also suggested possible hepatic and renal effects in workers chronically exposed to TCE, the evidence is not conclusive (Waters et al., 1977; NIOSH, 1978). In chronic exposure studies with animals, TCE appears to be a weak hepatotoxin and renal toxin because high doses produced mild effects in the liver and kidney. Exposure for 8 h/d, 5 d/w, for 6 w at 730 ppm or for 24 h/d for 90 d at 35 ppm did not result in any evidence of injury to the liver or kidneys of rats, guinea pigs, rabbits, dogs, and monkeys (Prendergast et al., 1967). Exposure of rats, guinea pigs, rabbits, and monkeys for 7 h/d, 5 d/w, for 148 to 178 d at 200 ppm also caused no adverse effects, except decreased growth and body weights in guinea pigs (Adams et al., 1951). Exposure of these species for 7 h/d, 5 d/w, for 161 to 175 d at 400 ppm caused increased liver and kidney weights in rats, increased liver weights in male and female guinea pigs, depressed growth in male guinea pigs, and a slight increase in liver weights of rabbits, but no adverse effects in monkeys (Adams et al., 1951).

Carcinogenicity

The results of most of the carcinogenicity studies with animals show that TCE is a potential carcinogen. TCE produced an increased incidence of hepatocellular carcinomas in B6C3F_1 mice subjected daily for their lifetime to high oral doses of the chemical (NCI, 1976; NTP, 1988, 1990). An increased incidence of these tumors was not detected in rats, but the results indicated the possibility of renal tumorigenic effects in rats. Studies also showed that inhalation exposure to TCE can be carcinogenic in animals (Fukuda et al., 1983; Maltoni et al., 1988). In a recently completed European bioassay, exposure of Swiss and B6C3F_1 mice to TCE for 7 h/d, 5 d/w, for 78 w at 100, 300, or 600 ppm resulted in a significant increase in the incidence of pulmonary tumors (from 11.1% in controls to 25.5% in males exposed at 300 ppm and 30.0% in mice exposed at 600 ppm) and hepatomas (from 4.4% in controls to 14.4% in males exposed at 600 ppm) in male Swiss mice (Maltoni et al., 1988). In female B6C3F_1 mice, there was a significant

increase in the number of total malignant tumors per 100 animals (from 54.4 in controls to 70.0 in mice exposed at 100 ppm, 68.9 at 300 ppm, and 77.7 at 600 ppm) and in the incidence of pulmonary tumors (from 4.4% in controls to 16.7% in mice exposed at 600 ppm). In male and female $B6C3F_1$ mice combined, the increase in incidence of hepatomas from 2.2% in controls to 8.3% in those exposed at 600 ppm was significant. It should be noted that the time of sacrifice of these animals was not specified; however, the reported incidence of hepatomas at 600 ppm appears low compared with the historical lifetime incidences of 20% to 30% and 5%, respectively, in male and female $B6C3F_1$ mice.

In rats similarly exposed for 104 w, there were significant dose-related increases in the incidence of Leydig-cell tumors of the testis from 4.4% in controls to 12.3%, 23.1%, and 23.8% in rats exposed at 100, 300, and 600 ppm, respectively (Maltoni et al., 1988). There was also a non-dose-related increase in the incidence of hemolymphoreticular neoplasias as well as a low incidence of renal adenocarcinomas at the highest dose in male rats.

In humans, two Scandinavian cohort studies did not find an increase in cancer-related mortality in workers exposed to TCE for up to 13 and 20 y (Axelson et al., 1978; Tola et al., 1980).

Genotoxicity

TCE was weakly positive or negative in numerous mutagenicity bioassays (Stott et al., 1982). Those results and a low level of in vivo TCE-DNA binding observed in $B6C3F_1$ mice indicate a weak genotoxic potential of TCE (Stott et al., 1982). Many of the studies did not report purity of test material; hence, it is possible that mutagenic epoxide stabilizers caused false positives (Brown et al., 1990).

Reproductive and Developmental Effects

TCE was found not to be a developmental toxicant in mice or rats exposed to TCE by inhalation at 300 ppm (Schwetz et al., 1975). In more recent studies, administration of TCE to the developing rat fetus in utero and injection into the air sacs of fertilized chick eggs resulted in cardiac teratogenic effects (Dawson et al., 1990; Loeber et al.,

1988). Additional evidence of the possible cardiac teratogenicity of TCE was provided by recent epidemiological studies showing a greater-than-expected number of pediatric patients with congenital heart disease in areas where the drinking water of their parents near the time of conception was contaminated by TCE and other halogenated aliphatic hydrocarbons (Goldberg et al., 1990). The authors noted important limitations to their study that preclude the conclusion of a cause-and-effect relationship.

Interactions with Other Chemicals

Biological interactions between TCE and other chemicals and drugs, such as ethyl alcohol and phenobarbital, have been reported in humans and animals. Degreaser's flush, a transient vasodilation of the skin, occurs in some TCE-exposed workers or subjects after ingestion of even small quantities of ethanol (Müller et al., 1975). Small quantities of ethanol also might increase the concentration of TCE in the blood, suggesting a lower rate of metabolism of TCE in the presence of alcohol (Müller et al., 1975). In Wistar rats, pre-exposure with ethanol or phenobarbital can enhance hepatic damage induced by exposure to TCE vapor (Okino et al., 1991).

TABLE 11-1 Toxicity Summary[a]

Concentration, ppm	Exposure Duration	Species	Effects	Reference
NS	Up to 13 y	Human (workers)	No increase in mortality or cancer-related mortality	Tola et al., 1980
NS	Up to 20 y	Human (workers)	No increase in cancer-related mortality	Axelson et al., 1978
1-335	Up to 15 y	Human (workers)	Nervous disorders increased with exposure duration and levels greater than 40 ppm	Grandjean et al., 1955
5-630	0.5-25 y	Human (workers)	Various symptoms; some correlated with exposure duration	Bardodej and Vyskocil, 1956
29-62 (averages)	Work site	Workers	Nausea, headache, dizziness	Okawa and Bodner, 1973
20, 100, or 200	7.5 h/d, 5 d	Human	No adverse subjective or objective behavioral effects	ACGIH, 1986
50 or 110	8 h	Human	No impairment of performance	Stewart et al., 1974
100	h/d NS, 5 d	Human	No impairment in mental performance	ACGIH, 1986
100, 200, 300, or 500	165 min	Human	Slight decrease in psycho- motor performance at 200 ppm, more pronounced at 300 and 500 ppm	Stopps and McLaughlin, 1967
100, 300, or 1000	2 h	Human	Impairment of visual-motor performance, dizziness, light-headedness at 1000 ppm	Vernon and Ferguson, 1969
110 average (90-130)	8 h	Human	Significant decrease in psychomotor performance; slight dizziness at 130 ppm	Salvini et al., 1971
200	7 h/d, 5 d	Human (n = 5)	No adverse effects on performance, neurological or clinical chemistry tests	Stewart et al., 1970

TABLE 11-1 *(Continued)*[a]

Concentration, ppm	Exposure Duration	Species	Effects	Reference
1000	2 h	Human	CNS effects indicated by optokinetic nystagmus test	Kylin et al., 1967
3000	10 min	Human (workers)	Unconsciousness in two workers exposed previously	Longley and Jones, 1963
10 or 600	6 h	Rat (O-M), mouse ($B6C3F_1$)	Reactive metabolite formation and hepatic macromolecular binding greater in mouse than rat	Stott et al., 1982
35	24 h/d, 90 d continuous	Monkey, dog, rabbit, guinea pig, rat	No deaths, hematological changes, or toxic signs except depressed body-weight gain in rabbits	Prendergast et al., 1967
100	7 h/d, 5 d/w, 132 d	Guinea pig	No adverse effects	Adams et al., 1951
100, 300, or 600	7 h/d, 5 d/w, 78 w	Mouse (Swiss, $B6C3F_1$)	In Swiss males, significant increase in incidence of pulmonary tumors from 11.1% (controls) to 25.5% (300 ppm) and 30.0% (600 ppm), and of hepatomas from 4.4% (controls) to 14.4% (600 ppm); in $B6C3F_1$ females significant increase in total number of malignant tumors per 100 animals from 54.4 (controls) to 70.0 (100 ppm), 68.9 (300 ppm) and 77.7 (600 ppm) and in incidence of pulmonary tumors from 4.4% (controls) to 16.7% (600 ppm); significant increase in hepatomas at 600 ppm in male plus female mice	Maltoni et al., 1988

100, 300, or 600	7 h/d, 5 d/w, 104 w	Rat (S-D)	Significant dose-response related increase in incidence of Leydig cell tumors of testis from 4.4% (controls) to 12.3% (100 ppm), 23.1% (300 ppm), and 23.8% (600 ppm)	Maltoni et al., 1988
100 or 500	6 h/d, 5 d/w, 18 mo	Rat, hamster, mouse	Dose-dependent decrease in survival rate of mouse	Henschler et al., 1980
100 or 500	6 h/d, 5 d/w, 18 mo	Rat, hamster, mouse	No tumorigenic effects except for significant increase in incidence of malignant lymphomas in female mice, from 9/29 (controls) to 17/30 (100 ppm) and 18/28 (500 ppm)	Henschler et al., 1980
100 or 600	7 h/d, 5 d/w, 8 w	Rat (S-D), mouse (Swiss)	No tumorigenic effects	Maltoni et al., 1988
200	7 h/d, 5 d/w, 148-178 d	Monkey, rabbit, guinea pig, rat	No adverse effects on appearance, behavior, hematology, clinical chemistry, histology, or organ/body weights except decreased growth and body weights of guinea pigs	Adams et al., 1951
400	8 h/d, 5 d/w, 10 mo	Rat	No effects on general condition, weight, mortality; swimming speed decreased, exploratory behavior increased	Battig and Grandjean, 1963
400	7 h/d, 5 d/w, 161 d	Monkey	No adverse effects on appearance, behavior, hematology, clinical chemistry, or histology	Adams et al., 1951
400	7 h/d, 5 d/w, 161 d	Rabbit	Slight increase in liver weight	Adams et al., 1951
400	7 h/d, 5 d/w, 167 d	Guinea pig	Increased liver weight, depressed growth in males	Adams et al., 1951
400	7 h/d, 5 d/w, 173 d	Rat	Increased liver and kidney weights	Adams et al., 1951
730	8 h/d, 5 d/w, 6 w	Monkey, dog, rabbit, guinea pig	No effects except on liver enzymes and cofactors in rats and growth depression in dogs	Prendergast et al., 1967

TABLE 11-1 *(Continued)*[a]

Concentration, ppm	Exposure Duration	Species	Effects	Reference
1250 or 2500	Variable	Mouse	Motor activity increased with rapid increase in TCE concentration	Kjellstrand et al., 1990
3000	7 h/d, 5 d/w, 27 d	Rabbit, rat	Mild impairment of equilibrium and coordination; increased liver and kidney weights	Adams et al., 1951
5000	10 min	Dog	Ventricular fibrillation on epinephrine challenge in 1/12	Reinhardt et al., 1973
9000	15 min	Rat (W-M)	Slight difficulty in locomotion	Utesch et al., 1981
10,000	10 min	Dog	Ventricular fibrillation on epinephrine challenge in 7/12	Reinhardt et al., 1973
12,000	10 min	Rat (W-M)	2/6 lost righting reflex	Utesch et al., 1981
14,000	5 min	Rat (W-M)	6/6 lost righting reflex	Utesch et al., 1981
15,000	Until loss of righting reflex	Rat (W-M)	Loss of reflex 3.5 min; recovery 1.25 min	Utesch et al., 1981

[a]Only results of inhalation studies are included.
Strains: S-D (Sprague-Dawley); O-M (Osborne Mendel); W-M (Wistar-Munich); NS, not specified.

TABLE 11-2 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	50 (TWA) 100 (STEL)	ACGIH, 1995
OSHA's PEL	50 (TWA) 200 (STEL)	U.S. Dept. of Labor, 1995
NIOSH's REL	25 (TWA)	ACGIH, 1991
NRC's EEGL	200 (1 h) 10 (24 h)	NRC, 1988

TLV, Threshold Limit Value; TWA, time-weighted average; STEL, short-term exposure limit; PEL, permissible exposure limit; REL, recommended exposure limit; EEGL, emergency exposure guidance level.

TABLE 11-3 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	50	270	Cardiac arrhythmias, CNS effects
24 h	11	60	CNS effects
7 d	9	50	Liver and kidney effects
30 d	4	20	Liver and kidney effects
180 d	2	10	Liver and kidney effects, cancer

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

CNS Effects

Five available human inhalation studies could potentially provide data pertinent to setting short-term SMACs for TCE. Each of the human inhalation studies was reviewed for a number of quality factors as follows: purity of test material, method of exposure-concentration measurement, appropriateness of toxic end point, number of subjects, and statistical methods. The study by Stopps and McLaughlin (1967) is flawed because only one test subject was exposed, the end points were

subjective, and chamber concentrations were nominal. The study by Stewart el al. (1962) is somewhat better but is of limited value because the exposure concentrations were ramped, subjective end points were used, and no statistical analyses were conducted. The study by Vernon and Ferguson (1969) was found to be of high quality and is used as the basis for setting the 1-h AC (CNS effects). The TCE used for exposure was pharmaceutical grade, the exposure concentrations were measured analytically (halide meter), the end points were thorough and objective, there were eight subjects, and a suitable statistical analysis (ANOVA/ Dunnett's test) was done. Human subjects were exposed to 0, 100, 300 or 1000 ppm for 2 h, and six visual-motor performance tests were administered. No statistically significant effects ($p < 0.05$) were reported at concentrations below 1000 ppm; however, one subject with a preexisting visual perception deficit was found to be more susceptible to TCE as measured in a Howard-Dolman depth perception test. Because 1000 ppm caused significant effects in visual perception, steadiness, and coordination, 300 ppm was concluded to be a no-observed-adverse-effect level (NOAEL) for TCE-induced performance decrements for normal individuals in 2-h exposures. To set the 1-h AC (CNS effects), no factor was applied for the greater length of the 2-h exposure to give additional safety below the NOAEL of 300 ppm. Because only eight human subjects were evaluated, the 300-ppm NOAEL was reduced by a factor of 3.5 (approximately $10/\sqrt{n}$) to give a 1-h AC (CNS effects) of 90 ppm.

The 24-h AC (CNS effects) is based on studies in which performance of mental and motor tests was measured in human volunteers exposed to TCE for approximately 8 h. In an early study, exposure of volunteers for 7 h to 100 or 200 ppm did not result in performance or behavioral effects (Stewart et al., 1970). The study was slightly flawed for our purposes in having few objective end points for measuring performance and no statistical analysis. Concentrations were measured analytically (IR spectroscopy), and an adequate number of subjects (five) were studied. In contrast, a statistically significant reduction in efficiency of performing psychophysiological tasks was reported in humans exposed at 110 ppm for 8 h (24-h exposures separated by a 1.5-h break) (Salvini et al., 1971). The concentrations were measured analytically (GC), the number of subjects (12) was adequate, the end points were objective, and the data were subjected to statistical analysis (ANOVA). However, a well-controlled repeat of this study with nine

subjects, an additional TCE concentration (50 ppm), and two additional performance tests did not confirm the results (Stewart et al., 1974). Questionable statistical analysis of the data, inaccurate TCE vapor exposure, or poor experimental execution was suggested as responsible for the positive effects in the original study (Stewart et al., 1974). Other investigators have also been unable to confirm decremental performance effects at 100 to 300 ppm (Annau, 1981). Because the findings of decremental performances at 110 ppm are suspect (Stewart et al., 1974), 110 ppm is concluded to be the NOAEL for TCE-induced performance decrements in 8-h exposures. The 24-h AC (CNS effects) was calculated by dividing the NOAEL of 110 ppm for 8 h by 3 to extrapolate from 8 to 24 h in accordance with Haber's rule and by 3.3 ($10/\sqrt{n}$, where $n = 9$) (Stewart et al., 1974). Consideration was given to combining earlier data on nine subjects exposed at 100 ppm (Stewart et al., 1970) with these data (Stewart et al., 1974); however, the end points measured were more extensive in the later study, and a statistical analysis was not given in the earlier paper. The 24-h AC (CNS effect) was calculated as follows:

$$C = 110 \text{ ppm (NOAEL)} \times 8 \text{ h}/24 \text{ h (Haber's rule)} \; 1/3.3 = 11 \text{ ppm}.$$

Cardiac Sensitization to Arrhythmia

In addition to the CNS effects of TCE, the cardiac sensitizing properties of TCE must be considered for short-term SMACs. A lowest-observed-adverse-effect level (LOAEL) for cardiac sensitization to epinephrine injection in dogs was 5000 ppm (10-min exposure) because 1 of 12 animals exhibited serious arrhythmias (Reinhardt et al., 1973) defined in the study as multiple consecutive ventricular beats or, more seriously, ventricular fibrillation. It was assumed that the potential for cardiac sensitization depended on blood concentrations that would be near equilibrium after only 10 min of exposure on the basis of data from rats exposed at 50 ppm (Dallas et al., 1991). Hence, length of exposure is not a major factor unless the concentration of TCE is high. The NOAEL was estimated to be a factor of 2 below 5000 ppm on the basis of the observation that a doubling of the dose to 10,000 ppm caused the frequency of serious arrhythmias to increase to 7 of 12. A halving of the concentration to 2500 ppm should be a NOAEL for this

end point. The 1-h AC (cardiac effects) was calculated using a species extrapolation factor of 10 and a spaceflight factor of 5 because of the reports of cardiac arrhythmias during both Soviet and U.S. missions (Bungo, 1989; NASA, 1991). The value was calculated as follows:

$$C = 5000 \text{ ppm} \times ½ \times 1/10 \times 1/5 = 50 \text{ ppm}.$$

The U.S. data come primarily from experience in Skylab where all crew members exhibited some form of rhythm disturbance. Most were premature ventricular contractions; however, one crew member had a five-beat run of ventricular tachycardia and another had occasional wandering supraventricular pacemaker (Bungo, 1989). One cosmonaut has experienced more serious cardiac arrhythmias, including atrial extrasystoles, episodes of trigeminy, and a "significant number" of supraventricular extrasystoles (associated with exercise) (NASA, 1991). While such rhythm disturbances are not serious themselves, they should be considered warning signals that whatever activity elicits the disturbance should be stopped as soon as possible.

Because the AC for cardiac effects was below the AC for CNS effects, the 1-h SMAC was set at 50 ppm to protect against cardiac effects. The 24-h to 180-d ACs for cardiac effects are the same as the 1-h AC for cardiac effects (i.e., 50 ppm) because the extended length of exposure is not a major factor in the cardiac sensitizing potential of TCE.

Hepatotoxicity and Nephrotoxicity

Setting longer-term SMACs is complicated by the fact that the only long-term continuous inhalation exposure study is of limited quality. The study by Prendergast et al. (1967) involved exposures of rats, guinea pigs, squirrel monkeys, rabbits, and dogs to TCE at 0 or 35 ppm for 90 d. Chamber concentrations were monitored analytically (GC) and compared with nominal values (data not given). The shortcomings of the study are as follows: the number of animals of each species was extremely small (e.g., three rabbits and two dogs), the end points measured were of limited value (body weights, few biochemical and histological measurements, and gross necropsy), and statistical techniques were not described. Even with those few measurements, the data were

not reported consistently. The body of the paper asserts that the body-weight gain was lower in exposed rabbits when compared with controls, whereas the discussion asserts that a slight growth depression occurred in all species except the dog. Because slight growth depression in the absence of other effects is not considered an adverse effect, a 35-ppm exposure for 90 d was considered a NOAEL. To protect against expected liver and kidney effects, which were reported in intermittent, long-term exposures to TCE (Adams et al., 1951), the 30-d AC was set at 4 ppm by starting with the 35-ppm NOAEL and adjusting it by a factor of 10 for species extrapolation.

The 180-d AC was derived in the same way as the 30-d value except that a time factor of ½ was used to estimate a 180-d NOAEL from the 90 d NOAEL observed at 35 ppm. The 180-d AC was set at 2 ppm to protect against liver and kidney effects.

The 7-d AC was based on a human NOAEL observed using clinical laboratory methods to detect liver or kidney injury during exposure of five subjects exposed to TCE at 200 ppm 35 h (7 h/d, 5 d) (Stewart et al., 1970).

The 7-d AC (for liver and kidney) was calculated as follows:

$$C = 200 \text{ ppm} \times \frac{\sqrt{5}}{10} \times 35/168 = 9 \text{ ppm}.$$

Carcinogenesis

The potential for TCE to cause cancer in humans is a controversial issue, because cohort and case-control epidemiological studies are generally negative for cancer; however, a few studies suggest an association (Brown et al., 1990). In the largest of the studies with negative results, a cohort of almost 7000 workers exposed for many years to TCE exhibited no "significant or persuasive" associations between exposure and excess cancer (Spirtas et al., 1991). Unfortunately, the magnitude of the TCE exposures could be estimated only in relative terms (Stewart et al., 1991). The studies with positive results are of limited value because of poorly characterized exposure history or small sample sizes. Animal data do little to resolve the issue. A number of studies in rodents show a potential for TCE to induce cancer; however, applying those data to human risk is difficult for the following reasons:

1. Some studies used mutagenic epoxide stabilizers in the TCE test material.
2. Some studies used unconventional protocols and incomplete reporting methods or did not comply (apparently) with good laboratory practices.
3. Some tumors appear to be due to metabolic pathways in the test species that differ from those in human beings.
4. Some tumors might involve cytotoxic mechanisms and are not relevant to risk at much lower human exposures.

Despite those uncertainties, we have chosen to calculate a cancer risk based on an estimate by the U.S. Environmental Protection Agency (EPA, 1987). A continuous lifetime exposure to TCE at 1 $\mu g/m^3$ (0.00019 ppm) was estimated to yield an excess tumor risk of 1.7×10^{-6} in humans. Using the approach of the National Research Council (NRC, 1992) and setting $k = 3$ (stages in process), $t = 25{,}550$ d (70-y lifetime), and $s_1 = 10{,}950$ d (earliest exposure, 30 y of age), the adjustment factor was calculated to be 26,082 for a near instantaneous exposure concentration that would yield the same excess tumor risk as a continuous lifetime exposure. The 24-h TCE exposure concentration that would yield an excess tumor risk of 10^{-4} was equal to the following:

$$1.9 \times 10^{-4}\,\text{ppm} \times 26082 \times 10^{-4} \div (1.7 \times 10^{-6}) \text{ or } 290 \text{ ppm}.$$

For the 7-d, 30-d, and 180-d SMACs, adjustment factors were calculated on the basis of the NRC (1992) approach and setting $k = 3$, $t = 25{,}550$ d, and $s_1 = 10{,}950$ d. The adjustment factors are 3728, 871, and 146.7 for continuous 7-d, 30-d, and 180-d exposures, respectively, that would yield the same excess tumor risk of 1.7×10^{-6} as a continuous lifetime exposure. The 7-d, 30-d, and 180-d exposure concentrations that would yield an excess tumor risk of 10^{-4} are equal to the following:

$$1.9 \times 10^{-5} \text{ ppm} \times 3728 \times 10^{-4} \div (1.7 \times 10^{-6}) = 42 \text{ ppm (7 d)}.$$

$$1.9 \times 10^{-5} \text{ ppm} \times 871 \times 10^{-4} \div (1.7 \times 10^{-6}) = 9.7 \text{ ppm (30 d)}.$$

$$1.9 \times 10^{-5} \text{ ppm} \times 146.7 \times 10^{-4} \div (1.7 \times 10^{-6}) = 1.6 \text{ ppm (180 d)}.$$

It must be pointed out that the original EPA estimate was withdrawn, and a revised estimate has not been determined as of this writing (EPA, 1993). Some authors suggest that a threshold model might be more appropriate than a linear extrapolation to low doses to set exposure limits (Steinberg and DeSesso, 1993).

Summary

The 1-h SMAC of 50 ppm was based on cardiac sensitization in dogs and the occurrence of arrhythmias in some crew members during missions. The 24-h SMAC of 12 ppm was based on CNS and neurobehavioral effects in humans rather than on cardiac sensitization in dogs.

The 7-d and 30-d SMACs of 9 and 4 ppm, respectively, were set to protect against liver and kidney injury. Those concentrations protect against cancer at a risk predicted to be below 0.01%. The 180-d SMAC of 2 ppm protects against liver and kidney injury and against cancer at the 95% limit of 0.01% risk per mission.

TABLE 11-4 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors: Small *n*	To NOAEL	Species	Time	Space-flight	Acceptable Concentrations, ppm: 1 h	24 h	7 d	30 d	180 d
CNS effects											
NOAEL = 300 ppm, 2 h (Vernon and Ferguson, 1969)	Human	3.5	1	1	—	1	90	—	—	—	—
NOAEL = 100 ppm, 8 h (Stewart et al., 1974)	Human	3.0	1	1	3 (HR)	1	—	11	—	—	—
Cardiac arrhythmia											
1/12 at 5000 ppm, 10 min (Reinhardt et al., 1973)	Dog	—	2	10	1	5	50	50	50	50	50
Hepatotoxicity and nephrotoxicity											
NOAEL = 35 ppm, 7 h/d, 5 d (Stewart et al., 1970)	Rat, guinea pig, monkey, rabbit, dog	—	1	10	—	1	—	—	—	4	2
NOAEL = 200 ppm, 7 h/d, 5 d (Stewart et al., 1970)	Human	4.5	1	1	5 (HR)	1	—	—	9	—	—
Carcinogenesis											
1.7×10^{-6} [a] at 0.00019 ppm, life continuous (EPA, 1987)	Human	—	NA	1	NA	1	—	300	40	10	2
SMACs							**50**	**11**	**9**	**4**	**2**

[a]Excess tumor risk of 1.7×10^{-6}.

—, Data not considered applicable to the exposure time; HR, Haber's rule.

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B12 Xylene

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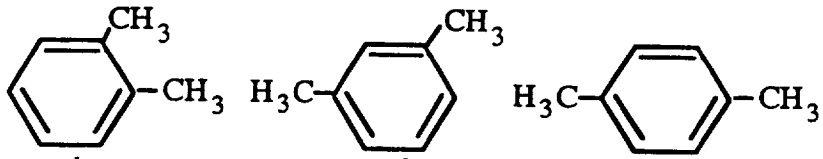
PHYSICAL AND CHEMICAL PROPERTIES

Xylene is a clear, colorless, flammable liquid at room temperature with an "aromatic" odor similar to that of benzene and toluene (Sax, 1984). The three isomers of xylene are *ortho*-(1,2-dimethylbenzene), *meta*-(1,3-dimethylbenzene), and *para*-(1,4-dimethylbenzene). Commercial xylene is a mixture of the three forms, *meta*- (*m*-) being the major component and *ortho*- (*o*-) and *para*- (*p*-) present at minor concentrations. The commercial mixture often contains substantial amounts of ethylbenzene. The three isomers of xylene are very similar in physical, chemical, and toxicological properties. The odor threshold for vapors of mixed xylenes was determined by a panel of six people to be on the order of 1 ppm (Carpenter et al., 1975).



o-xylene *m*-xylene *p*-xylene

Synonyms:	Dimethylbenzene, xylol			
Formula:	C_8H_{10}; $C_6H_4(CH_3)_2$			
	ortho-	*meta-*	*para-*	Mixture
CAS number:	95476	108383	106423	1330207
Molecular weight:	106.18	106.18	106.18	106.18
Boiling point:	144.4°C	139°C	138.3°C	138.5°C
Melting point:	−25.2°C	−47.9°C	13–14°C	
Lower explosive limit:	1.0%	1.1%	1.1%	
Upper explosive limit:	6.0%	7.0%	7.0%	
Autoignition temperature:		563°C	530°C	
Flash point (closed cup):	17°C	25°C	25°C	37.8°C
Specific gravity (20/4°C):	0.880	0.864	0.8611	0.864
Vapor pressure in mmHg at 28°C:	10	10	6.72	
Vapor density:	3.66	3.66		
Solubility:	Insoluble in water; miscible in alcohol, ether, and some organic solvents			
Conversion factors at 25°C, 1 atm:	1 ppm = 4.34 mg/m^3 1 mg/m^3 = 0.23 ppm			

Commercial xylene is synthesized from petroleum and obtained from the destructive distillation of coal tar. Both products are mixtures of three isomers. Commercial xylene frequently contains ethylbenzene and traces of toluene, phenol, thiophene, pyridine, and small amounts of nonaromatic hydrocarbons.

Xylene is used as a solvent, especially in the paint, printing, rubber, and leather industries, and in the manufacture of mirrors. It is used as a cleaning agent and degreaser and as a constituent of aviation and automotive fuels (gasoline). It is used in the chemical industry as a starting material and intermediate for the manufacture of phthalic and terephthalic acids. Those acids are used in the manufacture of plastics and resins, including textile fabrics. Xylenes are used in the coating and impregnation of fabric and paper. Other uses for xylene include dyes, inks, adhesives, cements, and thinners.

Xylene is not used in spacecraft during flight. Xylenes are occasion-

ally found, however, in the spacecraft atmosphere at concentrations up to 0.48 ppm, probably due to off-gassing from nonmetallic materials (Liebich et al., 1975; Huntoon, 1987).

TOXICOKINETICS AND METABOLISM

Humans retain an average of 63.6 ± 4.2% of inhaled xylene, independent of the atmospheric concentration within the range of 46 to 200 ppm (Sedivec and Flek, 1976). Retention is also independent of the duration of exposure for exposure times of 15 min to 8 h in a single day (Sedivec and Flek, 1976) or after 5 d of 6 h/d exposure (Riihimäki et al., 1979a,b). The percentage of retention varies only slightly between individuals and between the three isomers of xylene (Sedivec and Flek, 1976). The values for retention after 15 min of exposure were practically the same as the values between the first and third hours and those between the fifth and seventh hours. Even in repeated experiments performed after a few days or even weeks, retention in a given individual was similar to retention seen at 15 min (Sedivec and Flek, 1976; Riihimäki et al., 1979a,b). Retention might decrease somewhat as ventilation rates increase (Sedivec and Flek, 1976; Riihimäki et al., 1979a,b).

Measurement of xylene in exhaled air after exposure to 46 or 92 ppm for 8 h continuously revealed that a total of about 4% to 6% of the retained xylene is eventually excreted unchanged through the lungs with three-phase elimination kinetics (Sedivec and Flek, 1976; Riihimäki et al., 1979a,b). A trace amount of xylene can be observed in the exhaled air even 48 h after exposure to xylene at 46 or 92 ppm (Sedivec and Flek, 1976). In humans exposed at 100 ppm, the half-lives for elimination of xylene were 0.8 h for the initial phase, 7.7 h for the intermediate phase, and 17.7 h for the slowest phase (Low et al., 1989).

Only trace amounts (0.0047% of body burden) of unchanged xylene appear in the urine after exposure at concentrations of 46 or 92 ppm (Sedivec and Flek, 1976). At retained body burdens of 0.019 g/kg in humans, essentially all the conjugated xylene in the urine was in the form of glycine-bound toluic acid (methylhippuric acid) (Sedivec and Flek, 1976; Riihimäki et al., 1979a,b). In rabbits exposed at doses of

up to 0.6 g/kg of body weight, a portion of the xylene in the urine was conjugated to glucuronic acid, presumably because of overload of the glycine-conjugating system (Bray et al., 1949).

In another study in which 10 human volunteers were exposed to *m*-xylene at 23, 69, and 138 ppm, results contradicted those reported above. In that study, *m*-xylene vapor retained in the lungs tended to decrease at the end of the 8 h exposure, and the amount of the decrease was dependent on the inhaled concentration (Senczuk and Orlowski, 1978). At 139 ppm, the initial retention was 78%, and the retention at the end of the exposure was 65%; at 69 ppm, the values were 83% and 67%; and at 23 ppm, the values were 87% and 84% (Senczuk and Orlowski, 1978). The 8-h exposures were interrupted by two 0.5-h breaks—at 2.5 h and 5.5 h. Metabolism of *m*-xylene to *m*-methylhippuric acid was found to be very rapid compared with the rate of excretion of the *m*-methylhippuric acid into the urine (Senczuk and Orlowski, 1978).

Under steady-state conditions and conditions of increased xylene absorption (physical exercise), the blood xylene concentration in humans was found to be related directly to the rate of xylene uptake (Riihimäki and Savolainen, 1980). Acute effects on the central nervous system (CNS) were correlated with blood xylene concentrations and with a rapid rise in blood xylene concentrations. Thus, exposure to a fluctuating xylene concentration with high uptake rates during the peak concentration caused more pronounced effects than a corresponding exposure to a constant concentration. Physical exercise markedly raised the uptake rates and enhanced the effects (Riihimäki et al., 1979a; Riihimäki and Savolainen, 1980). Kinetic data in humans showed that well-perfused tissues, such as the brain, should reach xylene equilibrium within minutes and muscles within a few hours. Calculation of partition coefficients suggests that brain xylene concentrations should closely follow blood xylene concentrations (Riihimäki and Savolainen, 1980). In adipose tissue, repeated daily exposure results in xylene accumulation over a period of a few weeks; with continuous exposure, steady-state concentrations are reached after a few days (Riihimäki and Savolainen, 1980). Concentrations in gluteal subcutaneous fat reached 10-fold higher concentrations than those in blood after 6 exposure days (5-d exposure, weekend with no exposure, and 1-d exposure). Postexposure excretion of xylene from most tissues initially takes place rapidly. Elimination half-times were reported to be about 0.5 to 1.0 h during the first 2 to 3

h after exposure (Riihimäki and Savolainen, 1980). Consequently, acute xylene effects are probably short-lived after the end of the exposure. Because xylene stored in adipose tissue has an elimination half-life of about 58 h for subcutaneous fat, xylene mobilization results in only low blood concentrations (Riihimäki and Savolainen, 1980).

In mice, whole-body autoradiography reveals that metabolites of inhaled xylene (methylhippuric acid) accumulate in the nasal mucosa. These metabolites also are transported, probably by axonal flow, into the olfactory bulb of the brain (Ghantous et al., 1990). These localizations are interesting in view of case reports of a deteriorated sense of smell after occupational exposure to solvents (Emmet, 1976).

No reports have been found that document synergistic effects between xylene and any other agents.

TOXICITY SUMMARY

Early reports on the toxicity of xylene in humans are often difficult to assess because of impurities (including benzene) present in commercial xylene. In fact, the term "xylene" was often used as a general term for various solvents, including "pure" toluene and "pure" benzene as well as mixtures of various solvents.

Liquid xylene is a skin irritant causing erythema and dryness due to its fat solubilizing action. Xylene vapor is anaesthetic in atmospheric concentrations exceeding about 5000 ppm (Carpenter et al., 1975). At lower concentrations, probably in the range of a few hundred parts per million, workers sometimes have experienced a variety of subjective symptoms. Fatigue, drowsiness, headache, dizziness, dyspepsia, and nausea have been reported during repetitive daily exposure to unmeasured atmospheric concentrations (Browning, 1965).

Acute Exposures

Seven human volunteers reported minimal degrees of subjective responses (eye, nose, or throat irritation; dizziness or lightheadedness; and tearing) to a 15-min inhalation exposure to mixed xylenes at 230 ppm (Carpenter et al., 1975). One of the seven experienced mild throat discomfort during the first minute and again during the seventh minute

of inhalation at 106 ppm, as well as during the first minute of inhalation at 230 ppm, but no discomfort at 460 ppm. Four of the seven volunteers reported either intermittent or continuous mild eye irritation while inhaling 690 ppm (Carpenter et al., 1975).

The cardiac toxicity of a 2-min inhalation of *m*-xylene vapors was examined in dogs. A concentration of *m*-xylene at 0.35% (3500 ppm) was the threshold required to induce a decrease in peak *dp/dt* (the first derivative of left-ventricular pressure) (Kobayashi et al., 1989).

In mice, tests of operant and motor performance were performed after 30-min exposures to xylenes at 500 to 7000 ppm. The results suggested a CNS stimulant action at concentrations of 1400 to 2400 ppm, 1400 ppm being the lowest significantly effective concentration for operant performance (lever pressing) for each xylene isomer (Moser et al., 1985). Minor differences between isomers were seen, however, in the concentrations that produced half-maximal decreases in the response rate (*m*-xylene, 6176 ppm; *p*-xylene, 5611 ppm; *o*-xylene, 5179 ppm) (Moser et al., 1985). Differences between isomers also were seen in their minimally effective concentrations (*m*-xylene at 3000 ppm, *p*-xylene at 2000 ppm, and *o*-xylene at 3000 ppm) and in the concentrations that produced half maximal decreases in motor performance (*m*-xylene at 3790 ppm, *p*-xylene at 2676 ppm, and *o*-xylene at 3640 ppm) (Moser et al., 1985). Thus, the differences between isomers were less than twofold in all cases.

In male rats, slightly increased group motility (movement about the exposure chamber) was observed during a 4-h exposure to *m*-xylene at concentrations between 130 and 1500 ppm; narcosis was observed at 2100 ppm (Molnár et al., 1986).

The LC_{50} for rats in a 4-h inhalation period was 29 mg/L (6700 ppm) (Carpenter et al., 1975). Cats succumbed within 2 h at 41 mg/L (9500 ppm) with signs suggesting CNS effects: the sequential development of salivation, ataxia, tonic and clonic spasms, anesthesia, and death (Carpenter et al., 1975). The only lesions reported that were considered significant in any of the acute inhalation studies were atelectasis, hemorrhage, and interlobular edema of the lung in rats that died after inhaling mixed xylenes at 9900 ppm for several hours (Carpenter et al., 1975). In rats, the oral LD_{50} was 4.0 g/kg.

In humans, a single report (Morley et al., 1970) of a lethality was found that appeared to be attributable to xylene exposure. Three painters who were working in a confined space on a ship were found uncon-

scious after at least 15 h of exposure to paint fumes consisting of >90% xylene with traces of toluene. One painter died; the other two recovered. Lack of oxygen was not considered a factor in the death. Histological examination of the dead man's lungs showed severe congestion with focal intra-alveolar hemorrhage and acute pulmonary edema. The liver was congested with swelling and vacuolation of many cells. The brain showed microscopic petechial hemorrhages in both gray and white matter and hemorrhages in Virchow-Robin spaces. There was evidence of neuronal damage with swelling and loss of Nissl substance. The probable xylene concentration in the confined space in which they were working was estimated to be 10,000 ppm. One of the survivors remembered nothing after beginning work about 10:30 a.m. The other survivor remembered walking home (about 3 miles) for lunch and returning to work about 1:15 p.m., but he remembered nothing after returning until recovering consciousness in the hospital. The three men were discovered by rescuers at 5:00 a.m. the next morning. The two survivors completely recovered within a few days, except for persistent amnesia for events that occurred during the 24 h before their collapse.

Changes in human visual- and auditory-evoked potentials were seen in nine male volunteers exposed by inhalation to *m*-xylene at either a stable 200 ppm or fluctuating 135 to 400 ppm for 3 h in the morning and 40 min in the afternoon with a 40-min lunch break (Seppäläinen et al., 1989). The subjects were either sedentary or exercised at 100 watts for 10 min at the beginning of the exposure. The results suggest some activation of arousal level (decrease in the latency of the evoked potentials) of the subjects after the most intensive exposure situations (i.e., with physical exercise during exposure) (Seppäläinen et al., 1989). The magnitude of these changes was minor compared with the effects seen in general anesthesia or in long-term occupational exposure to hexane (Seppäläinen et al., 1989).

In a complicated study, inhalation of *m*-xylene at 100 to 400 ppm was reported to cause impairment of body balance and increase of reaction times in eight human volunteers (Riihimäki and Savolainen, 1980). Testing was done with constant or fluctuating concentrations of xylene and with subjects either sedentary or exercising on a bicycle ergometer. It appears that no impairment of reaction times was seen at 140 and 280 ppm in this study. With repetitive exposures, tolerance for most of these effects developed within a few days (Riihimäki and Savolainen,

1980). This study was very difficult to evaluate because of the lack of details about the methods used and the results obtained and thus was not used as a basis for setting acceptable exposures for xylene.

This same group examined the effect on sense of balance in humans of inhalation of *m*-xylene either at a fixed concentration of 200 ppm or at fluctuating concentrations of 135 to 400 ppm with or without exercise (Savolainen et al., 1985a,b). Sense of balance was measured by a strain gauge platform three times a day (before entering the chamber and 15 to 20 min into the morning and afternoon exposures). Two reports published within 1 mo of each other in different journals reached contradicting conclusions. The first paper reported a negative correlation between atmospheric *m*-xylene concentration and changes in body sway and a lack of correlation between *m*-xylene blood concentrations and changes in body sway. The second paper reported a positive correlation between *m*-xylene blood concentrations and changes in the eyes-closed-to-eyes-open ratios of both average and maximal body sway. In the first study, Savolainen et al. (1985b) reported that "the increase of body sway was always most pronounced during the sham exposure (control) at rest, declining with the increased atmospheric air *m*-xylene concentration." They also reported that *m*-xylene did not have any effects on manual or pedal reaction times to visual stimuli. The effect of *m*-xylene is to improve the overall human sense of balance, although the ratio of eyes closed to eyes open might suggest a slight impairment in balance with eyes closed. Physical exercise during exposure to *m*-xylene improved rather than impaired body sway (Savolainen et al., 1985b). This study also was not used as a basis for setting acceptable exposure concentrations for xylene because of inconsistencies in the reported results and the difficulty in interpreting them.

Subchronic Exposures

The no-observed-adverse-effect level (NOAEL) for rats and dogs exposed by inhalation to mixed xylenes 6 h/d, 5 d/w, for 13 w was at least 810 ppm. The criteria for a toxic response in this study were changes in body weight, urine chemistry, or blood analyses (Carpenter et al., 1975). Exposure at 1800 ppm caused a slight loss of coordination in rats by 2 h and definite lacrimation in the dog by 1 h and persisted throughout a 4-h exposure (Carpenter et al., 1975).

In general, most subchronic studies have focused on occupational intermittent exposure schedules. Jenkins et al. (1970), however, exposed rats, guinea pigs, monkeys, and dogs continuously for 90 d to *o*-xylene at 78 ppm. They reported that 1 of 15 rats died on d 56 (Jenkins et al., 1970). In the same study, intermittent exposures (8 h/d, 5 d/w, 30 exposures) to *o*-xylene at 780 ppm resulted in mortality in 3 of 15 rats, 0 of 15 guinea pigs, 0 of 2 dogs, and 1 of 4 monkeys (Jenkins et al., 1970). One of the two dogs was reported to have tremors of varying severity throughout the exposure. Histopathological examination of tissue sections of heart, lung, liver, spleen, and kidney of all species and brain and spinal cord of dogs and monkeys were essentially negative. Although this study appeared to involve considerable effort, the quality of the study cannot be established easily. The published report was very brief, and the two-paragraph methods section and the single-paragraph results section did not provide enough information to evaluate the quality of the study adequately.

No credible data were found implicating xylenes as reproductive toxins. Xylene has been reported to inhibit development in the in vitro hydra assay, but only at doses close to those that were toxic to adults (Johnson et al., 1986). In mice, *m*-xylene given by gavage at 2000 mg/kg (toxic to dam) was classified as negative in the Chernoff–Kavlock developmental toxicity screen (Seidenberg and Becker, 1987). In a Hungarian study using rats, however, exposure to *o*-, *m*-, or *p*-xylene at 35, 350, or 700 ppm for 24 h/d from d 7 to 14 of pregnancy was reported to produce the following effects for the three isomers: toxic effects in the dams exposed at 700 ppm and maternal deaths of 4 of 30 rats seen only for *m*-xylene at 700 ppm; reduced fetal weight at 700 ppm for all isomers and at 350 ppm for *o*-xylene; increased incidence of skeletal retardation for *o*-xylene at 700 ppm, for *p*-xylene at all three concentrations, but not for *m*-xylene; and decreased activity of several liver and thymus enzymes at the highest dose for all the isomers (Ungváry et al., 1980). Preimplantation fetal death was reported to be increased 31% by exposure to *m*-xylene at 700 ppm but not by exposure to *o*- or *p*-xylene. Postimplantation fetal death was increased by exposure to *p*-xylene at 700 ppm but not by exposure to *o*- or *m*-xylene. However, because the developmental toxicity of xylene is seen only at doses that are overtly toxic to the dams, xylene is not classified as a developmental toxin. In addition, the reported differences between isomers in their developmental toxicity is suspect because the xylene tested

was described to be from the U.S.S.R. and have "analytical purity"; the studies cited to support the assertion that differences exist between isomers did not provide the support. Thus, no credible evidence was found to support the existence of toxicity differences between xylene isomers.

Several groups of investigators have examined the neurochemical effects of xylene exposure. Male rats exposed to *m*-xylene vapor at concentrations of 49, 393, and 735 ppm for 6 h/d, 5 d/w, for 2 w showed evidence of accumulation of *m*-xylene in some tissues. Xylene concentrations in brain and perirenal fat increased in proportion to the exposure concentrations between w 1 and 2 of exposure (Savolainen and Pfäffli, 1980). An increase in brain NADPH-diaphorase and azoreductase activities was seen after 2 w at the two highest exposure concentrations, and superoxide dismutase activity decreased in a dose-related manner. Analyses 2 w after exposure ended indicated that the biochemical effects were largely abolished within that time. Cerebral RNA, however, remained above the control value at the two highest concentrations. Mice exposed to *m*-xylene at 1600 ppm for 4 h/d, 5 d/w, for 7 w had decreased concentrations of binding of ^{3}H-clonidine to alpha-adrenergic receptors in the hypothalamus region of the brain (Rank, 1985). During exposure at 1600 ppm, the mice ate and drank more than the control group and gained more weight than the controls (Rank, 1985). Increases in food and water consumption had been noted in preliminary experiments at xylene concentrations as low as 100 ppm (Rank, 1985).

Chronic Exposures

Carcinogenicity and Genotoxicity

The National Toxicology Program found no evidence of carcinogenicity in studies of mixed xylenes (commercial mixture containing 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene, and 17% ethylbenzene) in male and female F344/N rats given 250 or 500 mg/kg and male and female mice given 500 or 1000 mg/kg; doses were administered by oral gavage with corn oil 5 d/w for 103 w (NTP, 1986).

Gastrointestinal Effects in Humans

Workers exposed to commercial xylene vapors in concentrations above 200 ppm have complained of nausea, vomiting, heartburn, and loss of appetite (Browning, 1965).

TABLE 12-1 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
200	3.7 h	Human	Increased sensory arousal (evoked potential)	Seppäläinen et al., 1989
200	3-7 h	Human	NOAEL for reaction time and flicker fusion[a]	Ogata et al., 1971
200	4 h	Human	LOAEL for impaired sense of balance	Savolainen et al., 1985a
230	15 min	Human	NOAEL for eye, nose, or throat discomfort	Carpenter et al., 1975
10,000	~16 h	Human	Death in one of three painters; narcosis in other two with amnesia for events during exposure period	Morley et al., 1970
78[b]	90 d, continuous	Monkey	NOAEL for hematological, biochemical, and histopathological effects	Jenkins et al., 1970
780[b]	8 h/d, 5 d/w, 30 exposures	Monkey	Death in one of four monkeys	Jenkins et al., 1970
780[b]	8 h/d, 5 d/w, 30 exposures	Dog, guinea pig, monkey	NOAEL for lethality in 0 of 2 dogs, 0 of 15 guinea pigs, 0 of 3 monkeys	Jenkins et al., 1970
49	6 h/d, 5 d/w, 2 w	Rat	NOAEL for changes in brain enzyme activities	Johnson et al., 1986
78[b]	90 d, continuous	Dog	NOAEL for hematological, biochemical, and histopathological effects	Jenkins et al., 1970
78[b]	90 d, continuous	Rat	Death in 1 of 15 rats	Jenkins et al., 1970
78[b]	90 d, continuous	Guinea pig	NOAEL for hematological, biochemical, and histopathological effects	Jenkins et al., 1970
130	4 h	Rat	LOAEL for slightly increased motility	Molnár et al., 1986

780[b]	8 h/d, 5 d/w, 30 exposures	Rat	Death in 3 of 15 rats	Jenkins et al., 1970
780[b]	8 h/d, 5 d/w, 30 exposures	Dog	Tremors in one of two dogs	Jenkins et al., 1970
810	6 h/d, 5 d/w, 2 w	Rat, dog	NOAEL	Carpenter et al., 1975
1400	30 min	Mouse	LOAEL CNS operant performance	Moser et al., 1985
1600	4 h/d, 5 d/w 7 w	Mouse	Increased feeding and drinking; decreased alpha adrenergic receptor binding to hypothalamus	Rank, 1985
100	Not stated	Mouse	Increased feeding and drinking	Rank, 1985
2100	4 h	Rat	LOAEL narcosis	Molnár et al., 1986
3500	2 min	Dog	LOAEL for decrease in left ventricular *dp/dt*	Kobayashi et al., 1989
5984	6 h	Rat	LC_{50}	Bonnet et al., 1982
6700 (mixed xylenes)	4 h	Rat	LC_{50}	Carpenter et al., 1975
9500 (mixed xylenes)	2 h	Cat	Salivation, ataxia, tonic and clonic spasms, and anesthesia followed by death in four of four cats	Carpenter et al., 1975

[a]Flicker fusion refers to the minimum frequency at which a flickering light no longer appears to flicker.

[b]Exposure was to *o*-xylene.

TABLE 12-2 Exposure Limits Set or Recommended by Other Organizations

Agency or Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	100	ACGIH, 1991
ACGIH's STEL	150	ACGIH, 1991
OSHA's PEL	100	ACGIH, 1991
NIOSH's REL	100	ACGIH, 1991
NRC's 1-h EEGL	200	NRC, 1984
NRC's 24-h EEGL	100	NRC, 1984
NRC's 90-d CEGL	50	NRC, 1984

TLV, Threshold Limit Value; STEL, short-term exposure limit; PEL, permissible exposure limit; REL, recommended exposure limit; EEGL, emergency exposure guidance level; CEGL, continuous exposure guidance limit.

TABLE 12-3 Spacecraft Maximum Allowable Concentrations

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	100	435	Throat irritation, narcosis
24 h	100	435	Throat irritation, narcosis
7 d[a]	50	217	Throat irritation
30 d	50	217	Throat irritation
180 d	50	217	Throat irritation

[a]Previous 7-d SMAC = 20 ppm (86.8 mg/m^3).

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Calculation of the highest acceptable concentration (AC) for each major end point and exposure duration is documented below. The guidelines established by the National Research Council (NRC, 1992) were used to compare the resulting ACs for the various end points, and the lowest AC at each exposure duration was selected as the SMAC.

Lethality

1-h and 24-h ACs in Humans

Three workers painting in a confined space on a ship were found unconscious and taken to a hospital One was dead on arrival. The men were discovered unconscious 15.75 h after their lunch. The atmospheric concentration of xylene in the confined space was estimated to be 10,000 ppm on the basis of the volume of paint applied to the walls, the volume of the confined space, and the poor ventilation. Despite the poor ventilation, Morley et al. (1970) believed that oxygen was adequately diffused into the area through the open access hole. ACs for 1-h and 24-h exposures are based on 10,000 ppm as the lowest-observed-adverse-effect level (LOAEL) for lethality for a 15.75-h exposure. Therefore,

$$\text{1-h AC} = 10{,}000 \text{ ppm}/10 \text{ (to NOAEL)} = 1000 \text{ ppm};$$

and applying Haber's rule for a 24-h exposure,

$$\text{24-h AC} = 10{,}000 \text{ ppm}/10 \text{ (to NOAEL)} \times (16 \text{ h}/24 \text{ h}) = 670 \text{ ppm}.$$

1-h and 24-h ACs in Rats

The LC_{50} in rats for a 4-h exposure to xylene is approximately 6700 ppm (Carpenter et al., 1975). Comparing the data for rats with the data above for humans shows that rats have no less sensitivity to the lethal effects of xylene than do humans; thus, the use of a 10-fold interspecies safety factor is inappropriate in this case. The data used to calculate the LC_{50} comprise two concentrations: one yielded no deaths and the other higher concentration yielded 9 deaths of 10 exposed rats. Therefore, the data do not permit the use of the "benchmark dose" methodology for calculating the lower 95% bound on the dose that gives a 0.1% risk of death. To calculate 1-h and 24-h ACs, the 6700-ppm LC_{50} was divided by 10 to extrapolate to a NOAEL for lethality and adjusted for exposure duration using Haber's rule. For the 1-h ex-

posure, the 6700-ppm 4-h LC_{50} was not increased. Extrapolations beyond 24 h are not appropriate. Thus, on the basis of rat lethality, the 1-h and 24-h ACs are

1-h AC = 6700 ppm/10 (to NOAEL) = 670 ppm.
24-h AC = 6700 ppm/10 (to NOAEL)/(24 h/4 h) = 110 ppm.

Long-term continuous exposure data are not available for *m*-xylene. Jenkins et al. (1970), however, studied the effects of continuous exposure to *o*-xylene for 90 d in several animal species. Of 15 rats exposed continuously to *o*-xylene at 78 ppm, one died on d 56 of a 90-d exposure. It was not stated whether the death was believed to be exposure related; however, because no rats died after 56 d and no guinea pigs, monkeys, or dogs died that were similarly exposed, the one rat death is considered spurious and not related to xylene exposure. Thus, ACs for lethality were not calculated for exposure times greater than 24 h.

Throat Irritation

One of seven human volunteers exposed for 15 min to *m*-xylene reported mild throat discomfort during the first minute and again during the seventh minute of inhalation at 106 ppm and during the first minute of inhalation at 230 ppm, but none reported discomfort while inhaling 460 ppm (Carpenter et al., 1975).

1-h and 24-h ACs

In general, irritation is highly dependent on atmospheric concentration and much less dependent on exposure duration. The degree of irritation reported in this study was minor and was inversely dose related. Such mild irritation would be acceptable for a brief contingency exposure. Thus, in calculating the ACs for 1-h and 24-h exposures, the 106-ppm value was not adjusted to a NOAEL value and was not adjusted for exposure duration. The value was rounded to 100 ppm.

1-h and 24-h AC = 106 ppm, rounded to 100 ppm.

7-d 30-d, and 180-d ACs

Although the irritation reported in the study was mild and intermittent, the 7-d, 30-d, and 180-d ACs were set to prevent even that degree of discomfort. Thus, the 1-h AC, based on the LOAEL, was divided by 2 to estimate a NOAEL:

7-d, 30-d, 180-d AC = 106 ppm/2 = 53 ppm, rounded to 50 ppm.

Eye Irritation

One of seven volunteers exposed for 15 min to *m*-xylene reported intermittent mild eye irritation at 230 ppm (Carpenter et al., 1975). The degree of irritation reported in this study was minor but dose related. Such mild irritation would be acceptable for a brief contingency exposure. Thus, in calculating the ACs for 1-h and 24-h exposures, the 230-ppm LOAEL value was not adjusted to a NOAEL value and was not adjusted for exposure duration.

For a 1-h or 24-h exposure,

AC = 230 ppm, rounded to 250 ppm.

Again, although the irritation reported in the study was mild and intermittent, the 7-d, 30-d, and 180-d ACs were set to prevent even that degree of discomfort. Thus, the 230-ppm LOAEL was divided by 2 to estimate the NOAEL and rounded to a value of 100 ppm.

For 7-d, 30-d, or 180-d exposures,

AC = 230 ppm/2 = 115 ppm, rounded to 100 ppm.

Histopathological Changes

A well-designed and executed subchronic intermittent inhalation study of mixed xylenes was reported by Carpenter et al. (1975). Nine-

teen rats and four dogs exposed for 6 h/d, 5 d/w, for 65 d to mixed xylenes (65% *m*-xylene, 19.3% ethylbenzene, 7.8% *p*-xylene, 7.6% *o*-xylene) at 810, 460, or 180 ppm were monitored for exposure-related changes (Carpenter et al., 1975). Examination revealed no differences at any dose from air-exposed controls in body weight, blood and urine analyses, and histopathological changes of tissues from the adrenal, brain, pituitary, trachea, thyroid, parathyroid, lung, heart, liver, kidney, spleen, stomach, duodenum, pancreas, ileum, jejunum, colon, skeletal muscle, sciatic nerve, and bone-marrow impression smear (Carpenter et al., 1975). To calculate the ACs for 7 d and 30 d, the 810-ppm value was divided by 10 for species extrapolation, multiplied by 16.25 (= 6 h/d × 65 d ÷ 24 h/d), and divided by the exposure duration in days to apply Haber's rule. Use of Haber's rule in extrapolation to shorter times (24 h and 1 h) was not justified because of possible threshold effects in which the body's defense mechanisms (e.g., metabolism) could be overwhelmed. Extrapolation to 180 d was also inappropriate from a total exposure of only 390 h (6 h/d × 65 d). Therefore,

For a 7-d exposure,

$$AC = 810 \text{ ppm}/10 \times 16.25/7 = 188 \text{ ppm, rounded to } 200 \text{ ppm.}$$

For a 30-d exposure,

$$AC = 810 \text{ ppm}/10 \times 16.25/30 = 44 \text{ ppm, rounded to } 50 \text{ ppm.}$$

Narcosis

Groups of eight rats were exposed to *m*-xylene for 4 h at concentrations up to 2100 ppm (Molnár et al., 1986). Narcosis occurred only at the highest concentration (2100 ppm). The next lower concentration tested was 1000 ppm. This was considered the NOAEL for narcosis. In deriving an AC, the NOAEL was not adjusted for the exposure duration because, as was mentioned above, effects on the CNS depend on concentrations of xylene in the blood but do not increase with exposure duration (Savolainen et al., 1985a). Thus, the ACs for narcosis for all

exposure durations were calculated from the NOAEL using only a species extrapolation factor of 10:

AC (1 h through 180 d) = 1000 ppm/10 = 100 ppm.

Reduced Cardiac Output

A threshold value of 0.35% (3500 ppm) *m*-xylene was required to produce a statistically significant decrease in the peak left-ventricular *dp/dt* in 25 dogs anesthetized with sodium pentobarbital and exposed for 2 min to xylene-vapor concentrations up to 2.0% (20,000 ppm) (Kobayashi et al., 1989). A dose response was observed at higher concentrations. A decrease of 10% or more in the *dp/dt* would be of clinical concern due to reduced cardiac output (R. Billica, chief, Medical Operations, NASA Johnson Space Center, Houston, Tex., personal commun., 1992). Although there was considerable scatter in the dose-response data, Kobayashi et al. (1989) calculated a regression line that indicates that a 10% reduction in the *dp/dt* would occur at a concentration of about 1.4% (14,000 ppm) *m*-xylene. Cardiac effects have been shown to depend on the concentration of an organic solvent in the blood and to be independent of the exposure duration (G.D. Whedon, consultant, Shriners Hospital Headquarters, Tampa, Fla., personal commun., 1982); therefore, a calculated AC value should apply for all exposure times from 1 h to 180 d. The assumption must be made that the blood concentrations of xylene achieved in a 2-min exposure approach the concentrations that would be achieved in exposures of 1 h or longer. To calculate an AC based on reduced cardiac output, the 14,000-ppm LOAEL is divided by 10 for interspecies extrapolation and again by 10 to estimate the NOAEL from the LOAEL. Thus, the AC for all exposure durations is

14,000 ppm/10/10 = 140 ppm.

Although spaceflight has been suspected as a factor in increasing the risk of cardiac arrhythmias, a reduction in cardiac output, such as that potentially induced by xylene exposure, would reduce the risk of cardiac arrhythmia (G.D. Whedon, consultant, Shriners Hospital Headquarters, Tampa, Fla., personal commun., 1982).

SPACEFLIGHT CONSIDERATIONS

Of the end points induced by exposure to xylenes, only cardiac output possibly would be affected by launch, microgravity, or re-entry. The decrease in the force of contraction of the left ventricle would tend to counteract the increased susceptibility to arrhythmia induced by spaceflight. Thus, no spaceflight factor was used in calculating the AC for cardiac depression.

TABLE 12-4 Acceptable Concentrations

Effect, Data, Reference	Species	Uncertainty Factors: To NOAEL	Species	Time	Space-flight	Acceptable Concentrations, ppm: 1 h	24 h	7 d	30 d	180 d
Lethality, LC_{50}, 6700 ppm (Carpenter et al., 1975)	Rat	10	1	HR	1	670	110	—	—	—
Lethality, LOAEL, 10,000 ppm (Morley et al., 1970)	Human	10	1	HR	1	1000	670	—	—	—
Mild throat irritation, LOAEL, 10,000 ppm (Carpenter et al., 1975)	Human	1, 2	1	1	1	100	100	50	50	50
Eye irritation, LOAEL, 230 ppm (Carpenter et al., 1975)	Human	1, 2	1	1	1	250	250	100	100	100
Tissue histology, clinical chemistry, hematology, urine analysis, body and organ weights, EKGs, food consumption; NOAEL, 810 ppm, 6 h/d, 5 d/w, 65 d (Carpenter et al., 1975)	Rat, mouse	1	10	HR	1	—	—	200	50	—
Narcosis, NOAEL, 1000 ppm (Molnár et al., 1986)	Rat	1	10	HR	1	100	100	100	100	100
Cardiac depression, LOAEL, 14,000 ppm (Kobayashi et al., 1989)	Dog	10	10	1	1	140	140	140	140	140
SMACs						**100**	**100**	**50**	**50**	**50**

—, Data not considered applicable to the exposure time; HR, Haber's rule.

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