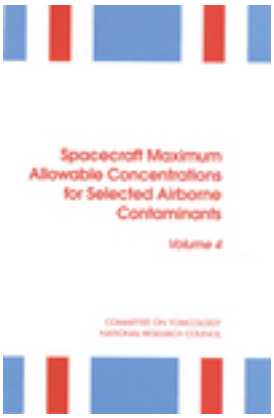

Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants: Volume 4

Subcommittee on Spacecraft Maximum Allowable Concentrations National Research Council

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Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants

Volume 4

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 crew members 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 NRC's Subcommittee on Spacecraft Maximum Allowable Concentrations has been reviewing NASA's documentation of chemical-specific SMACs. This report is the fourth volume in the series *Spacecraft Maximum Allowable Concentrations for Space Station Contaminants*. The first volume was published in 1994 and the second and third in 1996.

This report has been reviewed in draft form by individuals chosen for their technical expertise and diverse perspectives in accordance with procedures approved by the NRC's Report Review Committee for reviewing NRC and Institute of Medicine reports. The purpose of that independent review was to provide candid and critical comments to assist the NRC in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to

protect the integrity of the deliberative process. We wish to thank the following individuals, who are neither officials nor employees of the NRC, for their participation in the review of this report: Rogene Henderson, Lovelace Respiratory Research Institute; Loren Koller, Oregon State University; and George Rusch, AlliedSignal, Inc.

The individuals listed above have provided many constructive comments and suggestions. It must be emphasized, however, that responsibility for the final content of this report rests entirely with the authoring committee and the NRC.

The subcommittee gratefully acknowledges the valuable assistance provided by the following personnel from NASA and its contractors: John James, Martin Coleman, Jay Perry, Kenneth Mitchell (all from NASA), King Lit Wong (U.S. Department of Commerce, Patent and Trademark Office), Hector Garcia, Chiu Wing Lam, and Ragupathy Ramanathan (all from Wyle Laboratories). Lucy Fusco was the senior project assistant. Ruth Crossgrove edited the report. The subcommittee particularly acknowledges Lee Paulson, project director for the subcommittee, and Susan Pang, program officer, for bringing the report to completion.

Donald E. Gardner, *Chair*
Subcommittee on Spacecraft Maximum Allowable Concentrations
Bailus Walker, *Chair*
Committee on Toxicology

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Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants

Volume 4

SPACECRAFT MAXIMUM ALLOWABLE CONCENTRATIONS FOR SELECTED AIRBORNE CONTAMINANTS: INTRODUCTION

Construction of the International Space Station (ISS)—a multinational effort—began in 1999. In its present configuration, the ISS 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 duration 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. COT has since 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, the 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 convened 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. 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. This report, Volume 4, covers 15 compounds: acetone, C3 to C8 aliphatic saturated aldehydes, hydrogen chloride, isoprene, methylhyrazine, perfluoropropane and other aliphatic perfluoroalkanes, polydimethylcyclosiloxanes, dichlorofluoromethane (Freon 21), chlorodifluoromethane (Freon 22), trichlorofluoromethane (Freon 11), dichlorodifluoromethane (Freon 12), 4-methyl-2-pentanone, chloroform, furan, and hydrogen cyanide.

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 concludes that the SMAC reports on 15 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 judgment 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 carcinogenesis 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 ACETONE

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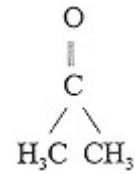
PHYSICAL AND CHEMICAL PROPERTIES

Acetone is a clear, colorless, highly volatile, flammable liquid with a sweet, fruity aroma (odor threshold, 13 ppm) and excellent solvent properties. It forms explosive mixtures with air or oxygen.

Formula:	C_3H_6O
Chemical name:	Acetone
Synonyms:	Propanone, 2-propanone, dimethyl ketone, dimethylformaldehyde, dimethylketal, ketone propane, β-ketopropane, methyl ketone, pyroacetic acid, pyroacetic ether
CAS no.:	67641
Molecular weight:	58.09
Boiling point:	56.48°C
Melting point:	–94.6°C
Lower explosive limit:	2.6% (26,000 ppm)
Upper explosive limit:	12.8% (128,000 ppm)
Autoignition temperature:	465°C
Flash point (closed cup):	–17.8°C
Density (15°C):	0.7972
Vapor pressure at 39.5°C:	400 mm
Vapor pressure at 25°C:	200 mm
Vapor density:	2.00



Solubility:	Infinitely soluble in water; miscible with alcohol, dimethylformamide, chloroform, most oils, and ether
Conversion factors at 25° C, 1 atm:	1 ppm = 2.37 mg/m^3;
	1 mg/m^3 = 0.421 ppm

OCCURRENCE AND USE

Acetone is emitted into the air from plants and trees, volcanic eruptions, forest fires, automobile exhaust, chemical manufacturing, tobacco smoke, wood burning, petroleum storage facilities, landfill sites, and solvent use (ATSDR 1994).

Commercial acetone is obtained by a variety of manufacturing processes, such as fermentation (by-product of butyl alcohol manufacture) and chemical synthesis from isopropanol, cumene (by-product in phenol manufacture), and propane (by-product of oxidation cracking).

Acetone is widely used as a general laboratory solvent; as an industrial solvent for fats, oils, waxes, resins, rubber, plastics, lacquers, varnishes, and rubber cements; and as a solvent to extract various materials from plant and animal substances. It is used in the manufacture of methyl isobutyl ketone, mesityl oxide, acetic acid (ketene process), diacetone alcohol, chloroform, iodoform, bromoform, explosives, airplane dopes, rayon, photographic films, and isoprene (Dietz 1991). Acetone is used to store acetylene, because it takes up 24 times its volume of the gas. It also is used in paint and varnish removers, in nail polish removers, in the purification of paraffin, and in hardening and dehydrating tissues.

Acetone is a product of normal metabolism in humans and animals. It is produced during the breakdown of fat and is used in the synthesis of glucose and fat. Trace amounts are detectable in normal human blood (7 μmol/L) and urine (4-35 μmol/L) (Dietz 1991). Data from a NIOSH report (Stewart et al. 1975) on acetone suggests that normal blood acetone concentrations in women (1.8-4.2 mg%) are 2-3 times higher than those in men (0.5-1.4 mg%), but no other reports could be found to confirm that finding. Raised acetone concentrations in serum and breath are often indicative of altered metabolic states, such as diabetes, vitamin E deficiency, and fasting (Dietz 1991).

Acetone is not routinely used in spacecraft during flight but might be used in payload experiments. Acetone is found in the spacecraft atmosphere on almost every mission at concentrations up to 8 ppm on Skylab (Liebich et al. 1975) and up to 1.2 ppm on shorter shuttle missions, probably because of crew metabolism and off-gassing.

TOXICOKINETICS AND METABOLISM

Absorption

DiVincenzo et al. (1973) reported that volunteers exposed to acetone at 100 or 500 ppm absorbed approximately 75% of the inhaled vapor into the blood-stream. Uptake of acetone was about 45% (39-52%) in eight men exposed for 2 h at 300 or 550 ppm (Wigaeus et al. 1981). The endogenous concentration of acetone in alveolar air was about 0.4 ppm, and the concentration of acetone in alveolar air during exposure at 300 or 550 ppm was 30-40% of the concentration in inspiratory air. Exhaled-breath concentrations of acetone rise during exposure and reach steady state in humans within 2 h during exposure (DiVincenzo et al. 1973). In rats, equilibrium between atmospheric acetone and absorbed acetone was approached after 3-4 h of exposure (Hallier et al. 1981). Breath concentrations of acetone are directly proportional to the concentration and duration of exposure and increase with physical activity because of increased pulmonary ventilation (DiVincenzo et al. 1973; Wigaeus et al. 1981). Vangala et al. (1991) found that a plateau of maximal alveolar-air concentration was reached after exposure to acetone at 990 ppm for 2 h by 16 male volunteers. The concentration of acetone in arterial and venous blood was found to increase linearly for up to 4 h of exposure with no indication that steady state was reached (DiVincenzo et al. 1973; Wigaeus et al. 1981; Brown et al. 1987). No significant difference in uptake or retention was found between men and women (Brown et al. 1987). Endogenous concentrations of acetone up to 10 µg/mL in blood were reported, and concentrations during diabetic ketoacidosis ranged from 100 to 700 μg/mL (Gamis and Wasserman 1988).

Distribution

Mice exposed to 2-[^{14}C]-acetone vapor (500 ppm) for periods of 1 h to 5 d were examined for tissue distribution of radioactivity (Löf et al. 1980). The amount of radioactivity in tissues increased as the exposure time increased from 1 to 6 h but did not increase in most tissues at exposure times greater than 6 h (12 h, 24 h) (Löf et al. 1980). Liver and pancreas showed the highest concentration of radioactivity; the lowest concentrations were in muscles and white adipose tissue. After 3 or 5 d of exposure (6 h/d), the radioactive concentration was highest in brown adipose tissue, followed by liver and pancreas.

In studies of the inhalation toxicokinetics of acetone in rats, Hallier et al. (1981) found that acetone is mainly, but not exclusively, distributed within the body water compartment under conditions of negligible metabolism (saturation of metabolizing enzymes). The kinetics of exhalation of acetone were strictly

mono-exponential, indicating that it does not distribute into a deep compartment.

Excretion

Hallier et al. (1981) studied the toxicokinetics of inhaled acetone in rats and found that metabolic elimination of acetone followed strict Michaelis-Menton saturation kinetics. The K_m corresponded to 160 ppm in the atmosphere. The half-life of the concentration of acetone in exhaled breath of rats after removal from exposure was 2.1 h. Thus, acetone should not accumulate (in the rat) under conditions of intermittent daily exposure at low concentrations ($\leq$ 250 ppm). That conclusion is supported by observations in workers occupationally exposed to acetone for 8 h daily; acetone concentrations in blood and urine showed no signs of accumulation (Baumann and Angerer 1979). Excretion of formate in the urine of rats after a single exposure to acetone was enhanced for 7 d, suggesting the existence of a formate pool in the body from which it is released, after a delay, in limited amounts.

The main route of excretion is via the lungs, regardless of the route of exposure, with very little excreted in the urine. About half of the acetone is exhaled unchanged in humans and the other half is exhaled as carbon dioxide produced from metabolism of acetone. DiVincenzo et al. (1973) reported that the half-life was about 3 h for the elimination of acetone in expired air of volunteers exposed to acetone at 100 or 500 ppm. In human adult acute intoxications, the half-life of acetone in plasma was estimated at approximately 31 h (Ramu et al. 1978).

Parmeggiani and Sassi (1954) exposed volunteers to acetone vapor at 833 ppm for 3 h twice daily with a 1-h break between exposures. They found concentrations of acetone at 190 μg/L in expired air at the end of the day. Sixteen hours later, the concentration of acetone in expired air had decreased to 32 μg/L. The concentration of acetone returned to background concentrations over the weekend. Those results suggest that repeated exposure to high concentrations of acetone might lead to slight accumulation during the workweek (Krasavage et al. 1982).

Stewart et al. (1975) exposed 7 male volunteers to acetone at concentrations of 0, 200, 1000, or 1250 ppm and 10 female volunteers to acetone at 0 or 1000 ppm in a complex exposure schedule. Overall breath-decay curves obtained 1 h after exposure accurately reflect the time-weighted-average vapor concentration, whether the exposure is to a constant or varying concentration, and samples collected within a few minutes after exposure reflect the most recent

vapor concentration. A slight but significant accumulation of acetone occurred in the body following exposure at 1000 ppm, but that small amount did not affect the breath-decay curves.

Metabolism

Ramu et al. (1978) estimated that humans can metabolize acetone at a rate not exceeding 1 g/h. Using that estimate, a person inhaling 0.83 m^3 of air per hour (20 m^3 of air per 24 h) could metabolize in 1 h all the acetone absorbed during inhalation—assuming about 50% uptake of inhaled vapor into the bloodstream—if the concentration of acetone was no more than 2 g/0.83 m^3 of air (i.e., 2.4 g/m^3 or 1000 ppm).

The expired air of mice exposed to 2-(^{14}C)-acetone vapor (500 ppm) for periods of 1 h to 5 d (6 h/d) was analyzed for radioactive acetone, carbon dioxide, and carbon monoxide (Löf et al. 1980; Wigaeus et al. 1982). Carbon dioxide was a major constituent, and no detectable amounts of carbon monoxide were found in the expired air (Löf et al. 1980; Wigaeus et al. 1982). The amount of reduced hepatic glutathione was significantly lowered (percent reduction not stated) after 6 h of exposure at 500 ppm (Löf et al. 1980).

No studies on the metabolism of acetone in humans were found, but several studies in rats showed that acetone can be metabolized via three gluconeogenic pathways, the first step being the hydroxylation of one of the methyl groups by acetone mono-oxygenase to form acetol (Dietz 1991). In hamsters, acetone is considered a nonmetabolizable endogenous and exogenous compound (Morris and Cavanagh 1987).

TOXICITY SUMMARY

Human exposure to acetone at atmospheric concentrations lower than 500 ppm is not commonly associated with any health hazards (Krasavage et al. 1982). Exceptions include slight irritant effects noted in unacclimatized subjects after short exposures (3-5 min) (Rowe and Wolf 1963) and possibly subtle physiological effects on the autonomic nervous system at 250 ppm (Matsushita et al. 1969a,b; Dick et al. 1988, 1989).

At higher concentrations, manifestations of acute acetone toxicity are primarily a result of central-nervous-system (CNS) depression that ranges from lethargy, slurred speech, and ataxia to stupor, coma, and respiratory depression (Gamis and Wasserman 1988). Other adverse effects of high acetone concentrations

include topical irritation and erythema (skin and mucosa), vomiting, hematemesis, polydipsia, polyuria, hyperglycemia, and occasionally metabolic acidosis (Gamis and Wasserman 1988). One Soviet investigator reported that four individuals acutely exposed (one by inhalation and three orally) to unspecified concentrations of acetone developed liver lesions, and two of the orally intoxicated individuals developed mild renal lesions (Mirchev 1977). The quality of this case-study report could not be evaluated because it was written in a non-Russian, Slavic language, probably Bulgarian, and only the abstract was translated to English. Thus, it could not be determined whether the four patients had also been exposed to other agents, such as alcohol, or might have had pre-existing lesions of the liver or kidneys. No other reports of acetone-related liver or renal lesions in humans or animals were found.

Acute and Short-Term Exposures

General

Inhalation exposure to high concentrations of acetone (>1000 ppm) affects the nervous system, gastrointestinal tract, kidney, mucous membranes, and eyes. The following symptoms have been reported: CNS depression indicated by an initial stimulatory or excitatory restlessness phase followed by euphoria and hallucinations, narcosis, anesthesia, dyspnea, headache, vertigo, general muscular weakness including dysarthria and ataxia, and coma; nausea, vomiting, inflammation, and hematemesis; albuminuria, hematuria, and leukocyturia; irritation of the nose, throat, and bronchi and dry throat; irritation of the eyes and transient corneal and conjunctival injury; hyperglycemia and increases in bilirubin and urine urobilin (Rowe and Wolf 1963; Mirchev 1977; Nelson and Webb 1978; Geller et al. 1979a,b; Baselt 1982; Krasavage et al. 1982; Finkel 1983; Inoue 1983; Windholz 1983; Arena and Drew 1986; Grant 1986; ACGIH 1986).

The toxic range of acetone was estimated to be 200 to 300 μg/mL in blood, with lethal concentrations estimated to be greater than 550 μg/mL (Gamis and Wasserman 1988). Intoxication was not observed, however, in volunteers with blood acetone concentrations up to 33 μg/mL from exposure by either ingestion or inhalation (Gamis and Wasserman 1988). The highest blood concentration of acetone reported (445 μg/mL) produced stupor, respiratory depression, and convulsions in a 30-mo-old who ingested nail-polish remover. Its anesthetic potency is greater than that of ethanol at equivalent blood concentrations (Gosselin et al. 1984). No permanent toxic sequelae have been reported (Gamis and Wasserman 1988).

Irritation

Raleigh and McGee (1972) surveyed workers exposed for 8 h/d at an average atmospheric concentration of about 1000 ppm. Eye irritation was transient and generally occurred at atmospheric concentrations greater than 1000 ppm. There was no indication of CNS effects. The authors concluded that 1000 ppm produced no adverse effects except for slight, transient irritation of the eyes, nose, and throat.

Matsushita et al. (1969a) exposed groups of five 22-y-old male students to acetone vapor at 0, 100, 250, 500, or 1000 ppm for 6 h, with a 45-min intermission after the third hour. Slight, transient irritation of the eyes, nose, and throat was noted at 100, 250, 500, and 1000 ppm. The odor of acetone was detected at 100 ppm, but acclimatization occurred rapidly.

Nelson et al. (1943) reported that 3-5 min exposures at 200 ppm caused no nose and throat irritation in humans, and subjects estimated that the concentration could be tolerated for 8 h; 500 ppm, however, did cause nose and throat irritation (Nelson et al. 1943).

Stewart et al. (1975) exposed 7 male volunteers to acetone at concentrations of 0, 200, 1000, or 1250 ppm and 10 female volunteers to acetone at 0 or 1000 ppm in a complex exposure schedule. During three control days, two subjects recorded slight eye irritation, one recorded throat irritation, and one developed a headache while in the chamber. During exposure at 200 ppm, two subjects reported eye irritation on d 1, two reported transient dizziness, one had a headache after 3 h of exposure, and two complained of tiredness. Odor intensity during the first week of exposure at 1000 ppm was reported to be stronger than during the previous week and was present throughout the exposure period. There were three complaints of eye and throat irritation and three complaints of tiredness. During the week of exposure at 1250 ppm, odor intensity and the number of complaints of eye and throat irritation remained the same as those at 1000 ppm (Stewart et al. 1975). Those findings are consistent with the findings of Matsushita et al. (1969a,b), who reported slight irritation, heavy-headedness, and lack of energy at 250 ppm, and Dick et al. (1988, 1989), who reported subtle neurological effects at 250 ppm.

Immunological and Hematological Effects

Matsushita et al. (1969a,b) reported statistically significant increases in white-blood-cell counts, increased eosinophil counts, and decreased phagocytic activity of neutrophils, compared with controls, in volunteers after a 6-h exposure or repeated 6-h/d exposures for 6 d to acetone at 500 ppm. No

significant differences in hematological measurements were seen in the volunteers exposed at 250 ppm compared with controls. According to a review by Koller (1993): "Alteration of cell numbers (peripheral leukocyte and eosinophil counts) does not correlate with immune function. Severe changes in leukocyte counts can affect immune responses but need to be defined by immune function procedures." He concluded that "it would be premature to change existing permissible exposure standards based on these data in the absence of conducting a more thorough immunotoxicological assessment of acetone to determine if the immune system is a primary target organ" (Koller 1993). In another study, hematological findings were within normal limits in volunteers exposed to acetone at 500 ppm for 2 h (DiVincenzo et al. 1973) or at ≤ 1250 ppm intermittently for various durations in a study with a complex protocol (Stewart et al. 1975).

Hematological effects observed in animals include bone-marrow hypoplasia in male rats, but not in female rats, exposed to acetone in drinking water for 14 d at a dose of 6942 mg/kg/d (Dietz 1991); macrocytic anemia in male rats exposed at ≥ 200 mg/kg/d; nonspecific hematological effects not indicative of anemia in female rats exposed at 3100 mg/kg/d via drinking water for 13 w (Dietz 1991); increased hemoglobin, hematocrit, and mean cell volume in male rats, but not in female rats, exposed by gavage at 2500 mg/kg/d for 46 d and in male and female rats exposed at that dose for 13 w (American Biogenics Corp. 1986, unpublished study, cited in ATSDR 1994, p. 99). In mice exposed at ≤ 12,725 mg/kg/d for 14 d, it was not clear whether bone marrow was examined, but in the 13-w study, no hematological effects or histologically observable lesions in hematopoietic tissues were found (Dietz 1991). Thus, species and gender differences are apparent in the hematological effects of acetone in animals.

CNS Toxicity

Stewart et al. (1975) exposed 7 male volunteers to acetone at concentrations of 0, 200, 1000, or 1250 ppm and 10 female volunteers to acetone at 0 or 1000 ppm in a complex exposure schedule. The following neurological tests were performed daily on each subject: a modified Romberg and heel-to-toe equilibrium test (twice daily), spontaneous electroencephalogram (EEG) and visual-evoked responses (VER) (four times per day on Monday, Wednesday, and Friday), coordination test, arithmetic test, and detection of the number 3 in rows of random numbers. No alteration in the VER was observed at 200 or 1000 ppm, but male subjects exposed at 1250 ppm showed an increase in total VER amplitude. The spontaneous EEG was unaltered by any exposure condition. No decrements in cognitive test performance were seen during exposures. One

subject (an author) experienced an episode of vertigo after 40 min of exposure at 1000 ppm. He had previously been diagnosed with Bárány's paroxsymal vertigo and had experienced two similar episodes, which were also associated with exposure to high concentrations of a ketone. Two other subjects reported transient dizziness.

When compared with values obtained after ≤48 h of no acetone exposure, simple reaction times were significantly prolonged (average, 16%) in five workers exposed during their 8-h shifts to acetone vapors from glue at concentrations of about 200 ppm (Israeli et al. 1977).

Matsushita et al. (1969a,b) reported neurological effects, such as a feeling of tension, heavy eyes, heavy head, lack of energy, general weakness, and headache, in 22-y-old male volunteers the morning after exposure to acetone at 500 ppm for 6 h or repeatedly for 6 h/d for 6 d. Those complaints were rare after exposure at 250 ppm and were not seen at 100 ppm.

Matsushita et al. (1969b) also reported 5-10% delayed visual reaction time in subjects exposed to acetone at 250 or 500 ppm for 6 h/d for 6 d. Examination of their data on the reaction time, however, reveals that "the values for the individuals within even the same group were scattered and a significant difference of $p<0.05$ could not be found between any of the acetone exposed groups and the control group" unless "all the numerical values obtained on the various measurement days were averaged and compared with the values on the first day of exposure" (Matsushita et al. 1969b) (i.e., the results during the first day of exposure were normalized to 100% and results from subsequent days were compared with those from the first day). Thus, no biologically significant effect on visual reaction time was seen in the 6 d of exposure.

Dick et al. (1988, 1989) exposed 22 volunteers to acetone at 250 ppm for 4 h and found small but statistically significant differences between the volunteers and the controls in two measures of the auditory tone-discrimination task (a 7-14% increase in response time to detect a 760-Hz tone in a series of 750-Hz tones and a 25% increase in false alarms but no difference in the percent of correct hits) and on the anger-hostility scale (males only) of a psychological test, the profile of mood states (POMS). Although those effects are statistically significant, the small magnitude of the effects and the uncertain biological relevance of the end points argue against using them for the purpose of setting SMACs. No significant effects were seen in three other psychomotor tests (choice reaction time, visual vigilance, and memory scanning), one sensorimotor test (postural sway), and five of six scales of the POMS psychological test. A NOAEL of 125 ppm was reported for all measured effects when the subjects were simultaneously exposed to acetone at 125 ppm and to methyl ethyl ketone at 100 ppm. Measurement of acetone concentrations in venous blood indicated that the concentrations at 2 h were about 60% of those at 4 h. (Dick et al. 1988).

Eight men cleaning an indoor pit were exposed to acetone vapor at >12,000 ppm (Draeger tube measurements) for durations ranging from 2 min to 4 h. Seven of the eight experienced dizziness, a feeling of inebriation, throat and eye irritation, and weakness of the legs. After three 2-min exposures, one man complained of tightness of the chest lasting for about 4 h. Two of the four men who were exposed for >4 h lost consciousness (Ross 1973). One of the two men who lost consciousness was hospitalized for 4 d, but both returned to work 6 d after exposure.

The short-term operant behavior of rats exposed to acetone by inhalation was examined by Goldberg et al. (1964). Female rats were trained according to an avoidance-escape paradigm. Groups were exposed at 3000, 6000, 12,000, or 16,000 ppm for 4 h/d, 5 d/w for 10 d. Body weight and growth were not affected at any dose, but escape behavior was suppressed, and ataxia was noted on d 1 in the 12,000- and 16,000-ppm groups. Avoidance behavior was inhibited in groups exposed at 6000 ppm, 12,000 ppm, and 16,000 ppm. Tolerance developed for all the reported neurobehavioral effects.

Ataxia and Narcosis

Bruckner and Peterson (1981) found that 4-w-old rats and mice exposed to acetone at 12,600 to 50,600 ppm for up to 3 h were slightly more sensitive to its narcotic effects than were 8- to 12-w-old animals. The animals were scored for ataxia (seen at 12,600 ppm), immobility in the absence of stimulation (seen at 19,000 ppm), hypnosis with arousal difficult (seen at 25,300 ppm), and unconsciousness (seen at 50,600 ppm with lethality after 2 h). The degree of CNS depression was linearly related to the exposure duration for a given concentration, and both the degree of CNS depression and the rapidity of its induction were dependent on the concentration of inhaled acetone. The time required for complete recovery from acetone's CNS effects was also dependent on the concentration inhaled: 9 h were required to recover from the effects of a 3-h exposure at 19,000 ppm, and 21 h were required to recover from a 3-h exposure at 25,300 ppm (Bruckner and Peterson 1981).

Pulmonary Function and Cardiotoxicity

The study of Stewart et al. (1975), described in the section on CNS toxicity, monitored a large number of end points. Acetone concentrations in the chamber air were monitored by spectrophotometry every 30 s and gas chromatography every 170 s. Acetone concentrations were measured in exhaled breath, blood, and urine. No significant changes from control EKGs were seen during exposures. No abnormalities were observed in pulmonary-function studies.

Subchronic and Chronic Exposures

No subchronic or chronic studies of acetone exposure were found other than a retrospective occupational epidemiological study of cancer incidence.

Carcinogenicity

There is no evidence that occupational exposure to acetone is associated with an increased incidence of cancer in humans. Oglesby et al. (1949) saw no increased incidence of cancer or any other harmful effects in their review of the accumulated medical and acetone-exposure (200-3000 ppm) records in a production facility of cellulose acetate yarn over 18 y.

Genotoxicity

In unpublished studies by the National Toxicology Program (NTP, Research Triangle Park, NC, 1991), acetone was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, or TA1537 with or without metabolic activation. Acetone did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells at doses up to 5 mg/mL with or without S9, and it did not induce micronuclei or polychromatic erythrocytes in the peripheral blood of mice ingesting acetone at 5000-20,000 ppm in drinking water for 13 w.

Reproductive Effects in Humans

Stewart et al. (1975) exposed 10 female volunteers to acetone at 0 or 1000 ppm for 1 h, 3 h, or 7.5 h/d for 1 w. Three of the four female subjects exposed for 7.5 h/d experienced early menstrual periods after 4 d of exposure at 1000 ppm.

Reproductive and Developmental Toxicity in Animals

Mast et al. (1989) reported mild developmental toxicity and mild maternal toxicity in rats exposed to acetone at 11,000 ppm for 6 h/d, 7 d/w during d 6-19 of gestation, but no effects were seen at 2200 ppm. In the same study, mice exposed at 6600 ppm for 6 h/d, 7 d/w during d 6-17 of gestation had significant increases in resorptions and significant decreases in fetal weights. The effects on maternal weight were weak. At 2200 ppm, no effects were seen in mice.

In the frog embryo teratogenesis assay–*Xenopus* (FETAX), acetone solutions increased the lethality of methylmercury chloride and trichloroethylene but increased the rate of malformations in a greater-than-additive fashion only for methylmercury chloride. Acetone solutions by themselves produced effects (96-h EC_{25}) at 1.0% but not at 0.9%.

Interaction with Other Chemicals

Hepatotoxicity in mice or rats that is induced by chloroform is potentiated by previous administration of ketonic solvents, including acetone, (Hewitt et al. 1980). Acetone induces hepatic cytochrome P-450IIE1, which potentiates the hepatotoxicity of acetaminophen (Moldeus and Gergely 1980; Liu et al. 1991), *N*-nitrosodimethylamine and *N*-nitrosodiethylamine (Sipes et al. 1978; Lorr et al. 1984), thiobenzamide (Chieli et al. 1990), oxygen (Tindberg and Ingelman-Sundberg 1989), and chromate (Cr[VI]) (Mikalsen et al. 1991); the genotoxicity of *N*-nitrosodimethylamine (Glatt et al. 1981; Yoo et al. 1990); the hematotoxicity of benzene (Johansson and Ingelman-Sundberg 1988); the lethality of acetonitrile (Freeman and Hayes 1985; Freeman and Hayes 1988); and the renal toxicity of *N*-(3,5-dichlorophenyl)succinamide (a fungicide) (Lo et al. 1987).

Acetone exposure reduces the rate of elimination of ethanol in mice and prolongs the ethanol-induced loss of righting reflex (Cunningham et al. 1989).

A summary of the toxicity data on acetone is presented in Table 1-1.

TABLE 1-1 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
100	1.5 - 6-h	Human (1-2 of 5 exposed)	Very slight irritation of nose, eyes, and throat	Matsushita et al. 1969a
100	6 h	Human (n = 5)	NOAEL for dullness, weakness, lethargy	Matsushita et al. 1969a
200	7.5 h/d, 4 d	Human	Eye irritation (n = 2/4), transient dizziness (n = 2/4), headache (n = 1/4), tiredness (n = 2/4)	Stewart et al. 1975
200	3.5 min	Human	Nose and throat irritation	Nelson et al. 1943
250	6 h	Human	LOAEL for dullness, weakness, lethargy (1-2 of 5 exposed)	Matsushita et al. 1969a
250	6 h	Human	NOAEL for hematological effects (0 of 5 exposed)	Matsushita et al. 1969a
250	4 h	Human	Slight decrease in auditory tone discrimination; slight increase in anger-hostility level	Dick et al. 1988, 1989
250	6 h	Human	LOAEL for decrease phagocytic activity of neutrophils	Matsushita et al. 1969a,b
500	2 h	Human	NOAEL for hematological effects	DiVincenzo et al. 1973
760-920	6 h/d, 0.5-13 y	Human (n = 7)	Sleepiness, confusion, irritation of eyes and throat, gastritis, and gastroduodenitis	Parmeggiani and Sassi 1954
1000	8 h/d	Human	Transient eye irritation	Raleigh and McGee 1972
1000	7.5 h/d, 4 d	Human	Eye irritation (n = 3/7), Tiredness (n = 3/7)	Stewart 1975
≤1250	Various	Human	NOAEL for hematological effects	Stewart 1975
12,000	2-3 min	Human (n = 4)	Light-headedness, throat irritation, leg weakness	Ross 1973
12,000	4 h	Human (n = 2)	Throat irritation, leg weakness, headache, feeling of inebriation, and in 1 of 2, unconsciousness	Ross 1973

Concentration, ppm	Exposure Duration	Species	Effects	Reference
2200	6 h/d, 7 d/w, d 6-17 of gestation	Mouse	NOAEL for reproductive and developmental effects	Mast et al. 1989
3000	4 h/d, 5 d/w, 10 d	Rat	NOAEL for neurobehavioral effects	Goldberg et al. 1964
6000	4 h/d, 5 d/w, 10 d	Rat	Inhibition of avoidance behavior	Goldberg et al. 1964
12,000	4 h/d, 5 d/w, 10 d	Rat	Ataxia and suppressed escape behavior on d 1	Goldberg et al. 1964
12,600	3 h	Rat, mouse	Ataxia	Bruckner and Peterson 1981
19,000	3 h	Rat, mouse	Immobility in the absence of stimulation	Bruckner and Peterson 1981
25,300	3 h	Rat, mouse	Hypnosis with arousal difficult	Bruckner and Peterson 1981
50,600	2 h	Rat, mouse	Death	Bruckner and Peterson 1981

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 1-2 presents exposure limits set by other organizations and Table 3-1 presents the SMACs established by NASA.

Of the effects of acetone exposure discussed above, irritation, explosion, and dizziness and fatigue are the only ones for which ACs will be set. Effects such

TABLE 1-2 Exposure Limits Set by Other Organizations

Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	500 (TWA)	ACGIH 1997
ACGIH's STEL	750 (ceiling)	ACGIH 1997
OSHA's PEL	750 (TWA)	ACGIH 1991
OSHA's STEL	1000	ACGIH 1991
ATSDR's MRL	26 (acute, 4 h)	ATSDR 1994
	13 (intermediate and chronic)	ATSDR 1994
NIOSH's REL	250	ACGIH 1991
NRC's 1-h EEGL	8500	NRC 1984
NRC's 24-h EEGL	1000	NRC 1984
NRC's 90-d CEGL	200	NRC 1984

TLV, Threshold Limit Value; TWA, time-weighted average; STEL, short-term exposure limit; PEL, permissible exposure limit; MRL, minimal risk level; REL, recommended exposure limit; EEGL, emergency exposure guidance level; CEGL, continuous exposure guidance level.

TABLE 1-3 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	500	210	Fatigue
24 h	200	84	Fatigue
7 d[a]	22	52	Fatigue, headache
30 d	22	52	Fatigue, headache
180 d	22	52	Fatigue, headache

[a] Previous 7-d SMAC = 300 ppm (713 mg/m^3).

as ataxia, immediate CNS depression, and death will be protected against by ACs that protect against more sensitive effects such as irritation or lethargy. Calculation using the guidelines established by the NRC (1992) to determine the highest acceptable concentration (AC) for each major end point and exposure duration is documented below. The resulting ACs for the various end points are complied in Table 1-4 and compared. SMAC values are set at each duration on the basis of the end point that yielded the lowest AC at that exposure duration.

Irritation

Mild, transitory irritation of the eyes, nose, and throat was reported in workers exposed for 8 h/d to atmospheric concentrations of acetone greater than 1000 ppm (Raleigh and McGee 1972). Such irritation would be acceptable for exposures of 1 h or 24 h. Thus,

1-h and 24-h ACs = 1000 ppm.

For longer exposure times, no irritation is acceptable. Because irritation does not increase with longer exposure times, the ACs for exposures >24 h were based on Nelson's et al. (1943) 6-h NOAEL of 200 ppm.

7-d, 30-d, 180-d ACs = 200 ppm.

Explosion

Acetone is highly volatile, flammable, and explosive; therefore, care must be taken to prevent the formation of local high concentrations (26,000-128,000 ppm) of acetone vapor. With nominal shuttle air circulation and mixing, an AC of 0.1 times the lower explosive limit should protect against hazardous accumulation of pockets of concentrated acetone vapor. Thus, the AC for explosion is set at 2600 ppm.

CNS Effects

It is clear from Matsushita et al. (1969a) that acetone at 100 ppm induces no detectable effects in humans exposed for 6 h. For 1-h and 24-h SMACs, a

slight degree of adverse effects is acceptable as long as the effects do not limit an astronaut's ability to perform during an emergency. The slight adverse effects at 200 ppm reported by Stewart et al. (1975) and those at 250 ppm reported by Matsushita et al. (1969a) are acceptable for short-term exposures (24 h), and on the basis of the 1000-ppm results in the Stewart study and the 500-ppm results in the Matsushita study, a 1-h exposure at 500 ppm should not affect performance.

Stewart et al. (1975) used cognitive tests (coordination, arithmetic, and inspection), neurological measures (equilibrium and EEG), and pulmonary functions to determine the effect of repeated exposure to acetone. No acetone-induced changes were found in any of those objective measures of adverse effects, even after repeated daily exposures at 1000 ppm. Subjects were also asked to report their subjective responses to their exposure several times during the exposure. The subjective responses were as follows:

Controls, 4-d exposure:	2 subjects reported slight irritation on 3 of 4 d 1 subject reported throat irritation 1 subject reported headache
200-ppm, 4-d exposure:	2 subjects reported eye irritation on d 1 2 subjects reported transient dizziness 1 subject reported a headache 2 subjects complained of tiredness
1000-ppm, 4- to 7-d exposure:	3 subjects complained of eye and throat irritation 3 subjects complained of tiredness

Although dizziness would ordinarily be a concern, the transient nature of the effect and the lack of its appearance during the 1000-ppm exposures provide convincing evidence that the effect is marginal at best. Although tiredness was reported by several subjects, their objectively measured performance was never significantly affected. Based on the extensive exposures done by Stewart and co-workers, a 1-h AC of 1000 ppm appears acceptable; however, the data from Matsushita et al. (1969a,b) need to be considered as well.

The effects reported by Matsushita et al. (1969b) for repeated exposures at 250 ppm might be a concern because they could compromise performance. The morning after each of six repeated exposures, six subjects were asked whether they felt any of the following effects "clearly (2 points) or "a little" (1 point): heavy head, headache, general weakness, lack of energy, or heavy eyes.

Toxicokinetic analysis of the data reported by Matsushita et al. (1969a) indicates that immediate-irritation effects and potential delayed CNS effects must be considered for the 1-h AC. During the first 90 min of the single 6-h exposure to acetone at 500 ppm, the degree of irritation reported was scored at 4-5 out of a possible 10. That means that most of the subjects felt a little irritation, or two of the subjects clearly felt irritation and the rest felt nothing. That degree of irritation is acceptable for a 1-h contingency exposure. The morning after the single 6-h exposure at 500 ppm, Matsushita et al. (1969a) reported that most subjects clearly felt tension, heavy eyes, and a lack of energy. The scores reported by those exposed at 250 ppm could have been attained either by one-half of the subjects having no complaints and the other half feeling a little tension, heavy eyes, and lack of energy or by most feeling nothing and one clearly feeling the symptoms. The symptoms are the result of a cumulative 6-h exposure to acetone. It can be estimated from the Matsushita data that the blood concentration of acetone after 1 h of exposure at 500 ppm would be no more than the blood concentration after 6 h of exposure at 250 ppm. Hence, any delayed systemic symptoms due to a 1-h exposure at 500 ppm are unlikely to be worse than those from a 6-h exposure at 250 ppm. Accordingly,

1-h AC = 500 ppm.

In nonworking subjects exposed at 250 ppm, there was a significant increase the following day in the incidence of lack of energy compared with working controls. (No report was given of nonworking controls.) General weakness was reported with similar incidence in both groups, and it is not clear how that differs from lack of energy. Somewhat similar effects (e.g., tiredness) were reported by Stewart et al. (1975) during the 200-ppm exposures without measurable performance decrements. Because the Matsushita observations are subjective, we conclude that exposures of 200 ppm are very unlikely to impair the crew's ability to perform its tasks for exposure periods up to 24 h. Hence,

24-h AC = 200 ppm.

The long-term ACs (7-180 d) must be set to avoid any adverse effects. Hence, one must begin with the 100-ppm NOAEL reported by Matsushita et al. (1969a) in five test subjects exposed for 6 h. Repeated exposures up to 1000 ppm suggest that adverse effects do not become worse with prolonged exposure (Matsushita et al. (1969b). (The Stewart study suggests a tolerance to acetone.) Furthermore, blood concentrations during exposures at 100 ppm do not increase

after 3 h; hence, we conclude that 100 ppm is below the threshold for effects such as irritation, headache, and loss of energy no matter how long the exposure lasts. The ACs are as follows:

7 -d, 30 -d, 180 -d AC = 100 ppm $\times \frac{\sqrt{5}}{10}$ = 22 ppm.

A safety factor of $\frac{\sqrt{n}}{10}$ was used to account for the uncertainty in the NOAEL because of the small number (n = 5) of test subjects.

Reproductive Effects

After 4 d of exposure to acetone at 1000 ppm for 7 h/d, women volunteers experienced premature menstrual periods. Other than transient irritation, no other adverse effects were reported (Stewart et al. 1975). NASA tests female astronauts for pregnancy before flight and those found to be pregnant are not permitted to fly; therefore, premature menses is not considered an adverse effect and will not be used to set an AC.

Spaceflight Considerations

None of the end points induced by exposure to acetone is expected to be affected by launch, microgravity, or re-entry.

RECOMMENDATIONS

A continuous inhalation exposure study of acetone at concentrations of 100 to 1000 ppm needs to be conducted on 10 or more human volunteers per exposure group. The study should include several objective measures, such as CNS effects (e.g., reaction times, visual acuity, and work capacity) and effects on menstrual cycle and hormonal levels, as well as subjective measures, such as sleepiness and headache.

A chronic exposure, continuous inhalation study of the immunotoxic and hematotoxic effects of acetone exposure on bone-marrow cells in rodents or primates is needed.

TABLE 1-4 Acceptable Concentrations

End Point, Exposure		Uncertainty Factors				Acceptable Concentrations, ppm				
Data, Reference	Species	NOAEL	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d
Irritation	Human	10	1	1	1	1000	1000	200	200	200
LOAEL, 1000 ppm for 8 h; NOAEL, 200 ppm for 6 h (Raleigh and McGee 1972)										
Explosion	—[a]	10	1	—	1	2600	2600	2600	2600	2600
LEL[b], 26,000 ppm (Sax 1984)										
Fatigue, headache	Human	10/`*n*	1	1	1	500	200	22	22	22
NOAEL, 100 ppm for 6 h (Stewart et al. 1975; Matsushita et al. 1969a,b)	(n = 5)									
SMACs						**500**	**200**	**22**	**22**	**22**

[a]—, not applicable.
[b]LEL, lower explosive limit.

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B2 C3 TO C8 ALIPHATIC SATURATED ALDEHYDES

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PHYSICAL AND CHEMICAL PROPERTIES

The chemical and physical properties for C3 to C8, straight-chain, aliphatic aldehydes are shown in Table 2-1. Formaldehyde and acetaldehyde have been previously reviewed, and spacecraft maximum allowable concentrations (SMACs) have been set for those compounds (Wong 1994a,b).

TABLE 2-1 Physical Properties of Selected Aldehydes

Names:	propanal	butanal	pentanal	hexanal	heptanal	octanal
$CH_3(CH_2)_nCHO$:	n =1	n =2	n =3	n = 4	n = 5	n = 6
Synonyms:	propion-aldehyde	*n*-butyr-aldehyde	*n*-valer-aldehyde	capro-aldehyde	*n*-hept-aldehyde	capryl-aldehyde
CAS nos.:	171426-73-6	171339-76-7	110-62-3	66-25-1	111-71-7	124-13-0
Molecular weights:	58.1	72.1	86.1	100.2	114.2	128.2
Boiling points (°C):	49	76	103	128	154	171
Melting points (°C):	−81	−99	−92	−56	−45	N/A
Vapor pressures (mmHg): (at °C):	687 (45)	92 (20)	50 (25)	10 (20)	3 (25)	N/A
Conversion factors:[a] 1 ppm = 1 mg/m³ =	2.3 mg/m³ 0.422 ppm	2.9 0.340	3.5 0.284	4.1 0.245	4.6 0.215	5.2 0.191

[a]1 ppm converted to milligrams per cubic meter, and 1 mg/m³ converted to parts per million.
N/A, not available.

OCCURRENCE AND USE

The major use of saturated aldehydes of higher molecular weight than acetaldehyde is in flavorings, perfumes, and essential oils (Brabec 1993). Many saturated aldehydes also occur naturally in foods. Propanal and butanal are industrially important intermediates with U.S. production of 280 and 3000 million pounds, respectively (Brabec 1993). These aldehydes can enter spacecraft air by incomplete oxidation of alcohols in the air revitalization system, by human metabolism, by materials off-gassing, and during food preparation. The higher-molecular-weight aldehydes have not been seen often on the shuttle; however, each aldehyde (C3-C8) was present in air samples from Mir 18 (March to June 1995) at concentrations typically near or below 0.1 mg/m^3 (J.T. James, Johnson Space Center, Houston, Tex., unpublished data, 1995).

TOXICOKINETICS AND METABOLISM

Absorption

No specific data were found on the absorption of C3-C8 aldehyde vapors in the respiratory tract. Based on data from acetaldehyde exposures in humans, the aliphatic saturated aldehydes should be well adsorbed in the respiratory tract at concentrations of 100 ppm or less (Egle 1970).

Distribution

No specific data were found on the distribution of C3-C8 aliphatic saturated aldehydes absorbed from the respiratory tract.

Excretion

No specific data were found on the excretion of C3-C8 aliphatic saturated aldehydes from living systems.

Metabolism

There are limited specific data on the metabolism of aliphatic saturated aldehydes with three or more carbon atoms. In principle, these aldehydes can be oxidized to their respective acids by aldehyde dehydrogenase (ALDH), aldehyde

oxidase, and xanthine oxidase, or they can be reduced to alcohols by aldehyde reductases (McMahon 1982; Brabec 1993). The major route of oxidation is via ALDH (E.C.1.2.1.3), which is a soluble NAD^+-dependent enzyme found in liver and many other tissues. Purified human-liver ALDH has been shown to facilitate the oxidation of propanal, butanal, and pentanal at relative rates that range from 0.81 to 1.1 when tested at substrate concentrations ranging from 0.05 to 3.0 mM (Blair and Bodley 1969). The rates for these three aldehydes were indexed to a value of 1.0 for acetaldehyde at 3.0 mM; hence, the rate of oxidation of the larger aldehydes is comparable to acetaldehyde.

In addition to ALDH, certain other enzymes might play a role in aldehyde metabolism. Reduction of aldehydes by aldehyde reductases might be a minor pathway in vivo (McMahon 1982). Cytochrome P-450 might also play a minor role in oxidation of aldehydes to carboxylic acids (Parkinson 1996); however, for certain C_5 branched aldehydes, olefinic products and formic acid are produced in reactions catalyzed by any one of five isozymes of cytochrome P-450 from rabbit liver or nasal mucosa (Roberts et al. 1991). Acetaldehyde is a substrate for ethanol-inducible cytochrome P-450 (CYP2E1) (Terelius et al., 1991). In a series of papers, Watanabe et al. (1990, 1992, 1995) showed that microsomal aldehyde oxygenase (CYP2C29) catalyzes the oxidation of tolualdehydes, other substituted cyclic aldehydes, and alpha or beta unsaturated aldehydes. In the 1992 study, saturated aldehydes (C8 to C11) were not readily oxidized by this form of cytochrome P-450.

Hepatic enzymes associated with peroxisomes (catalase and carnitine acetyltransferase) and the number of peroxisomes were induced in F344 rats fed either 2-ethylhexyl aldehyde or hexanal as 2% of their diet for 3 w (Moody and Reddy 1978). The branched aldehyde was a more effective inducer than the straight-chain aldehyde.

TOXICITY SUMMARY

The toxicity data base on aliphatic saturated aldehydes was extensive; however, there are important deficiencies, especially in terms of the effects of acute exposures on humans, the sublethal effects of short-term exposures on animals, and the effects of chronic exposure in animals and humans.

Acute and Short-Term Exposures

The inhalation toxicity data base consists mostly of acute and short-term exposures of animals to various aldehydes. The sensory irritation properties

have been studied in rodents for many of the aliphatic saturated aldehydes, as shown in Table 2-2 (Steinhagen and Barrow 1984; Babiuk et al. 1985).

The RD_{50} data show that sensory irritation properties within this group of aldehydes are roughly comparable, but there is no clear trend of decreased irritancy with increasing molecular weight or chain branching. Human irritation studies have been reported for the first four aldehydes listed in Table 2-2. Acetaldehyde was slightly irritating at 134 ppm in 14 test subjects exposed for 30 min (Sim and Pattle 1957). Irritancy data on three additional aldehydes are given in Table 2-3; however, the original report gave a confusing result on propanal. In their table 2, this aldehyde was listed as tested for 30 min on four subjects and found to be a nonirritant; however, in the text of the original

TABLE 2-2 Concentrations Inducing a 50% Depression in Rate of Breathing (RD50)

Species (Strain)	Aldehyde	RD_{50}, ppm
Rat (Fischer 344)	Acetaldehyde	2990
	Propanal	6790
	Butanal	5570
Mouse ($B6C3F_1$)	Acetaldehyde	2930
	Propanal	2080
	Butanal	1530
	Isobutanal	3020
	Pentanal	1190
	Isopentanal	760
	Hexanal	1120
	2-Ethylbutanal	1340
Mouse (Swiss-Webster)	Acetaldehyde	2840
	Propanal	2050
	Butanal	1010
	Isobutanal	4170
	Pentanal	1120
	Isopentanal	1010
	Hexanal	1030
	2-Ethylbutanal	843

report, propanal was described as tested for 30 min on 12 subjects and found to be "mildly irritating to the mucosal surfaces" (Sim and Pattle 1957). In addition, there is confusion on the duration of exposures to butanal, because the table lists the exposure time as 10 min and the text gives the exposure as 30 min. We chose to use the data reported in the text of Sim and Pattle (1957) in Table 2-3. Data on other effects of aldehyde exposures are also given in the table.

There is limited evidence in animals that C3-C8 aldehydes might affect the liver. Liver-cell vacuolization was noted in rats exposed six times for 6 h to propanal at 1300 ppm; however, 12 exposures for 6 h to either butanal or isobutanal at 1000 ppm did not cause liver-cell vacuolization (Gage 1970). Hypolipidemia was induced in male F344 rats given 2-ethylhexyl aldehyde or hexanal as 2% of their diet for 3 w (Moody and Reddy 1982). Serum cholesterol decreased 20% and 10%, respectively after the two aldehydes were administered. Serum triglycerides decreased 60% after administration of either of the aldehydes. Because hepatic peroxisome proliferators (e.g., 2-ethyl hexyl aldehyde and hexanal) are often promoting agents and can induce hepatic neoplasms in rodents after long-term administration at high doses (Reddy and Lalwani 1983), there is some concern that aldehydes could pose a liver-cancer risk. Peroxisome proliferators have not been shown to cause cancer in humans.

There is one report of an accidental exposure of seven chemists to "2-methylbutanal" in a chemical laboratory (Wilkinson 1940). Based on structures given in the report, the compound involved in the exposures was actually 3-methylbutanal (isovaleraldehyde). The victims experienced tightness of the chest, cough, and marked weakness. Some reported dizziness, headaches, and nausea. All recovered within a few days without special treatment. The duration and concentration of the exposures were unknown.

Subchronic and Chronic Exposures

Originally, the only study that could be placed in this category is that of Gage (1970) in which four male and four female rats were exposed 20 times to propanal at 90 ppm for 6 h. The experimental design called for observation of clinical signs, urine chemistry tests at the end of exposures, hematology, gross pathology, and histopathology of the lungs, liver, kidney, spleen, adrenals, and occasionally the heart, jejunum, ileum, and thymus. No toxic signs were detected, the organs examined at autopsy were normal, and presumably the remainder of the end points were negative. Brabec (1993) commented without citing specific references that "in general, the aldehydes are remarkably free of actions that lead to definite cumulative organ damage to tissues other than those that may be associated with primary irritation or sensitization. However, the

questions of mutagenicity, carcinogenicity, and teratology hang over . . . [the] aldehydes."

A recent 13-w study of the effects of isobutanal vapor inhaled by rodents has added significantly to the subchronic toxicity data base. In preparation for a 2-y study, groups of 10 male and female F344 rats and $B6C3F_1$ mice were exposed to isobutanal at 500, 1000, 2000, 4000, and 8000 ppm, 6 h/d, 5 d/w, for 13 w (Abdo et al. 1998). Depressed weight gains and death were observed at concentrations of 4000 and 8000 ppm. Chemically related lesions were confined to the upper respiratory system of the rodents and consisted of inflammation, olfactory epithelial degeneration, epithelial hyperplasia, squamous metaplasia, and osteodystrophy. Effects on the trachea and larynx were noted only in the highest-concentration group of rats. In general, mice seemed to be more susceptible than rats to the formation of nasal lesions.

Carcinogenicity

Although formaldehyde and acetaldehyde have been found to be carcinogenic to the rodent nasal mucosa, the only substantive test for carcinogenic potential in a larger aldehyde (isobutanal) was negative (Abdo et al. 1998). One reason for doing the cancer bioassay was that some larger saturated aldehydes induce hepatic peroxisome proliferation and could present a cancer risk at high doses administered for prolonged periods. A 2-y bioassay of isobutanal in rats and mice exposed at 500, 1000, and 2000 ppm for 6 h/d, 5 d/w showed only non-neoplastic lesions in the nasal cavities of both species (Abdo et al. 1998). The authors attribute the lack of carcinogenicity of the larger aldehyde to the metabolic decomposition of isobutanal to propylene and formic acid, neither of which seem to be carcinogenic.

There is a report that attempts to associate worker exposure to various aldehydes with a possible increase in cancer incidence (Bittersohl 1975). Approximately 150 workers with >20 y experience participated in the study. They were exposed to concentrations giving a strong and sometimes irritating odor. In one sampling period, butanal concentrations were among the highest of the measured components at 5-70 mg/m^3, but concomitant exposures to other aldehydes, acetaldol, and alcohols took place. Despite the finding of nine malignant neoplasms in a 5-y period among the workers, there were no matched controls, so the findings must remain inconclusive.

Genotoxicity

There is a single report that propanal and heptanal were weakly mutagenic to *Drosophila* (Rapoport 1948, as cited in Auerbach et al. 1977).

Developmental Toxicity

No data were found on the developmental toxicity of the aliphatic saturated aldehydes with three or more carbons.

Reproductive Toxicity

One report suggests that a single intraperitoneal injection of butanal (30 mg/kg) into Q-strain mice interferes with spermatogenesis (Moutschen-Dahmen et al. 1976). Injections resulted in degeneration of spermatogenic cells, polyploidy, and an increased frequency of acrosomeless spermatozoa in the ductus deferens.

Interactions with Other Chemicals

No data were found to suggest that aliphatic saturated aldehydes interact with other compounds to modify toxic effects.

TABLE 2-3 Inhalation Toxicity Summary

Compound and Concentration, ppm	Duration	Species	Effects	Reference
Propanal				
134	0.5 h	Human (n = 12)	Mildly irritating?	Sim and Pattle 1957
90	20 × 6 h	Rat	NOAEL: toxic signs, gross pathology	Gage 1970
1200 (aerosol)	4.6 h	Mice	Mean lethal exposure	Salem and Cullumbine 1960
	>10 h	Guinea pig	3 of 20 died	
	4.3 h	Rabbit	Mean lethal exposure	
1300	6 × 6 h	Rat	No weight gain, liver-cell vacuolization	Gage 1970
8000	4 h	Rat	5 of 6 died	Smyth et al. 1951
26,000	0.5 h	Rat	LC_{50}	Skog 1950
60,000	0.3 h	Rat	3 of 3 died	Fassett 1962
Butanal				
200	0.5 h	Human	Nonirritating	AIHA 1968
230	30 min?	Human (n = 15)	Noniritating	Sim and Pattle 1957
1000	12 × 6 h	Rat	NOAEL: toxic signs, gross pathology	Gage 1970
2700 (aerosol)	5.7 h	Mice	Mean lethal exposure	Salem and Cullumbine 1960
	>10 h	Guinea pig	3 of 20 died	
	5.1 h	Rabbit	Mean lethal exposure	
8000	4 h	Rat	1 of 6 died	Smyth et al. 1951
59,000	0.5 h	Rat	LC_{50}	Skog 1950

TABLE 2-3 *(Continued)*

Compound and Concentration, ppm	Duration	Species	Effects	Reference
Isobutanal				
207	0.5 h	Human (n = 15)	Nonirritating, one subject with nausea and vomiting	Sim and Pattle 1957
500	2 y	Rat	19 of 99 had squamous metaplasia of respiratory epithelium	Abdo et al. 1998
1000	13 w	Mice	11 of 20 had nasal-cavity inflammation	
1000	2 y	Mice	38 of 100 had olfactory epithelial degeneration	
2000	13 w	Rat	10 of 20 had olfactory epithelial degeneration	
1000	12 × 6 h	Rat	Slight nasal irritation, NOAEL for gross pathology	Gage 1970
2500 (aerosol)	2.7 h	Mice	Mean lethal exposure	Salem and Cullumbine 1960
	4.9 h	Guinea pig	Mean lethal exposure	
	4.2 h	Rabbit	Mean lethal exposure	
8000	4 h	Rat	1 of 6 died	Smyth et al. 1954
Pentanal				
670 (aerosol)	>10 h	Mice	4 of 50 died	Salem and Cullumbine 1960
	>10 h	Guinea pig	5 of 20 died	
	>10 h	Rabbit	0 of 5 died	
1400	6 h	Rat	0 of 3 died	Fassett 1962
4000	4 h	Rat	3 of 6 died	Smyth et al. 1969
8000	4 h	Rat	3 of 6 died	Diechmann and Gerarde 1969
48,000	1.2 h	Rat	3 of 3 died	Fassett 1962
Isopentanal				
1500 (aerosol)	>10 h	Mice	3 of 50 died	Salem and Cullumbine 1960

Compound and Concentration, ppm	Duration	Species	Effects	Reference
	>10 h	Guinea pig	5 of 20 died	
	>10 h	Rabbit	0 of 5 died	
2-Methylbutanal				
3800	6 h	Rat	0 of 3 died	Fassett 1962
67,000	0.3 h	Rat	3 of 3 died	Fassett 1962
Hexanal				
2000	4 h	Rat	1 of 6 died	Smyth et al. 1954
2-Ethylbutanal				
8000	4 h	Rat	5 of 6 died	Smyth et al. 1954
4-Methylpentanal				
8000	4 h	Rat	0 of 6 died	Deichmann and Gerarde 1969
2,3-Dimethylpentanal				
6000	4 h	Rat	0 of 6 died	Deichmann and Gerarde 1969
2-Ethylhexanal				
145	6 h	Rat	0 of 3 died	Fassett 1962
2000	23 min	Rat	3 of 3 died	Fassett 1962
4000	4 h	Rat	1 of 6 died	Smyth et al. 1951
25,000	13 min	Rat	3 of 3 died	Fassett 1962

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 2-4 presents exposure limits for aliphatic saturated aldehydes set by other organizations and Table 2-5 presents the SMACs established by NASA.

Group SMACs for aliphatic saturated aldehydes depended on the completeness of the toxicity data base and whether the compounds exhibit similar toxicities. The toxicity of aldehydes has been reviewed previously by the National Research Council (NRC 1981). The toxicity data base is limited in many important respects; however, the toxicities of the C3-C8 aliphatic saturated aldehydes appear to be similar. Acceptable concentrations (ACs) for this group (Table 2-7) were set by selecting the AC for the most active compound for a given toxic effect. Where applicable, the guidelines from the Committee on Toxicology were used to set ACs (NRC 1992). The toxic effects that were considered include the following: mucosal irritation, nasal cavity injury, nausea and vomiting, and possible liver damage. This group of aldehydes appears to be much less toxic than unsaturated aldehydes or those with other functional groups, such as halogens or hydroxyl moieties.

TABLE 2-4 Exposure Limits Set by Other Organizations

Organization	Compound	Exposure Limit, ppm	Reference
ACGIH's TLV-TWA	Pentanal	50	ACGIH 1998
OSHA's PEL-TWA	Pentanal	50	U.S. Dept. of Labor 1989

TLV, Theshold Limit Value; TWA, time-weighted average; PEL, permissible exposure limit.

TABLE 2-5 Spacecraft Maxium Allowable Concentrations

Duration	ppm	mg/m^3	Target Toxicity
1 h	50	125-250[a]	Mucosal irritation
24 h	50	125-250	Mucosal irritation
7 d	6	15-30[b]	Liver injury, mucosal irritation
30 d	1.5	4-8	Liver injury
180 d	1.5	4-8	Liver injury

[a] The value depends on the molecular weight of the aldehyde.
[b] Former 7-d SMACs: propanal, 95 mg/m^3; butanal, 120 mg/m^3; pentanal, 110 mg/m^3.

Mucosal Irritation

Human irritancy data were available for three of the aldehydes in the series (Sim and Pattle 1957). Propanal was mildly irritating at 134 ppm, whereas butanal and isobutanal were not irritating at 230 and 207 ppm, respectively. The ACs for short-term exposure permit a risk of mild irritation; however, the mouse data (Table 2-2) suggest that some aliphatic aldehydes might be 2-3 times more irritating than propanal. Therefore, the short-term ACs for mucosal irritation were set at 50 ppm. For long-duration exposures (more than 24 h), even mild mucosal irritation would not be allowed. Because no concentration-response data were available, the no-observed-adverse-effect level (NOAEL) for irritation was estimated by the default approach of dividing the mildly irritating value of 134 ppm by 10. Hence, the AC (7 d, 30 d, and 180 d) was set at

134 ppm ÷ 10 = 13 ppm.

Another approach to the question of irritation is to directly apply the extensive rodent RD_{50} data shown in Table 2-2. Except for two branched aldehydes, the RD_{50}'s were above 1000 ppm. A NOAEL for mucosal irritation in humans can be estimated from the 1000-ppm RD_{50} by applying a factor of 10 to reach a rodent NOAEL and another factor of 10 for possible species differences. This conservative approach suggests that 10 ppm would be safely below concentrations that could induce irritation in humans even after prolonged exposure. It is also reasonably consistent with 13 ppm estimated above from the human data.

Nasal-Cavity Injury

The 13-w and 2-y studies recently reported by Abdo et al. (1998) showed that the nasal cavities of both rats and mice are the target of isobutanal vapor. In the 13-w subchronic-toxicity study (cumulative exposure, 390 h), the NOAEL for the nasal-cavity effects in both species was 500 ppm. That value can be used to estimate an AC as follows:

7-d AC = 500 ppm × 1/10 (species) = 50 ppm.

(no time extrapolation needed)

30-d AC = 500 ppm × 1/10 (species) × 390 h/720 h = 27 ppm.

From the 2-y study (cumulative exposure, 3120 h), the 180-d AC was estimated

from the lowest-observed-adverse-effect level (LOAEL) of 500 ppm in female rats (500 ppm was a NOAEL in other sexes and species tested) as follows:

180-d AC = 500 ppm × 1/3 (LOAEL to NOAEL) × 1/10 (species) × 3120 h/4320 h = 12 ppm.

The LOAEL-to-NOAEL extrapolation factor of 3 was based on the steep dose response seen in the other species and sexes for nasal lesions (Table 2-6).

TABLE 2-6 Dose Responses for Nasal Lesions in 2-y Study[a]

	Incidences Squamous Metaplasia or Olfactory Degeneration in Groups			
Species (Sex)	0 ppm	500 ppm	1000 ppm	2000 ppm
Rat (M)	1/50	1/49	10/49	44/50
Rat (F)	1/49	11/50	9/49	44/50
Mice (M)	1/50	0/50	11/50	45/50
Mice (F)	1/50	1/50	27/50	49/50

[a] Rats showed squamous metaplasia, whereas mice showed olfactory epithelial degeneration.

Potential Liver Injury

Liver-cell vacuolization was noted in rats exposed 6 times for 6 h to propanal at 1,300 ppm (Gage 1970). Liver-cell vacuolization itself is not an adverse effect; however, it suggests that prolonged exposure to aldehydes could lead to liver injury. To set ACs to prevent liver injury, it was noted that 20 exposures of 6-h durations (120 h total) to propanal at 90 ppm did not give detectable adverse effects in rats (Gage 1970). There are three possible ways to approach the problem of setting ACs for exposures that far exceed the 120-h duration of exposures in the data base. The approachs are as follows: (1) do not set an AC; (2) use the default approach, which calls for using Haber's rule; and (3) use metabolic arguments to support a threshold concentration below which no injury can occur. A combination of the last two approaches was used. The default approach was used to set the 7-d and 30-d ACs; however, the metabolic products of aldehyde metabolism (organic acids) would not be expected to accumulate or be harmful below a certain concentration. The 180-d AC was set

at the same value as the 30-d AC based on this expectation. The calculations were as follows:

7-d AC = 90 ppm × 1/10 (species) × 120/168 (time factor) = 6.4 ppm.

30- or 180-d AC = 90 ppm × 1/10 (species) × 120/720 (time factor) = 1.5 ppm.

A recent report on 2-y isobutanal exposures to rats and mice with no effects on the liver suggests that at least some of the large aldehydes cause no adverse effects on the liver (Abdo et al. 1998). In view of that recent report, the ACs to prevent potential liver injury based on hepatocyte vacuolization in rats by propanal are probably conservative.

TABLE 2-7 Acceptable Concentrations

End Point, Exposure Data, Reference		Uncertainty Factors				Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d	
Mucosal irritation	Human	2-3	1	1	1	50	50	—	—	—
Slight to propanal at 134 ppm for 30 min (Sim and Pattle 1957)	(n = 12)	10				—	—	13	13	13
RD_{50}s > 1000 ppm for saturated aldehydes (Steinhagen and Barrow 1984; Babuik et al. 1985)	Rat, mouse	10	—	10	1	—	—	10	10	10
Nasal-cavity injury										
NOAEL, 500 ppm for 13-w exposure to isobutanal (Abdo et al. 1998)	Rat, mouse	1	HR[a]	10	1	—	—	50	27	—
LOAEL, 500 ppm for 2-y exposure to isobutanal (Abdo et al. 1998)	Rat, mouse	3	HR	10	1	—	—	—	—	12
Potential liver injury	Rat	1	HR threshold	10	1	—	—	6	1.5	1.5
NOAEL, 90 ppm for 20 6-h exposures to propanal (Gage 1970)										
SMACs						**50**	**50**	**6**	**1.5**	**1.5**

[a] HR, Haber's rule.
—, not applicable.

RECOMMENDATIONS

There are major shortcomings in the toxicity data base. Short-term human exposures with measurement of various end points in addition to irritation, exposures to several of the aldehydes, and measurement at several concentrations would be useful. Additional long-term animal inhalation studies would be useful in defining whether any of these aldehydes can induce organ damage outside the respiratory system.

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B3 HYDROGEN CHLORIDE

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PHYSICAL AND CHEMICAL PROPERTIES

Hydrogen chloride is a colorless, nonflammable gas with a pungent odor (ACGIH 1991). It fumes in air and condenses with moisture to form hydrochloric acid (Henderson and Haggard 1943).

Formula:	HCl
CAS no.:	7647-01-1
Synonym:	Muriatic acid
Molecular weight:	36.5
Boiling point:	–85.05°C
Melting point:	–114.22°C
Vapor pressure:	>1 atm
Solubility	67.3 g per 100 g water at 30 °C
Conversion factors	1 ppm = 1.49 mg/m^3;
at 25°C, 1 atm:	1 mg/m^3 = 0.67 ppm

OCCURRENCE AND USE

Anhydrous hydrogen chloride is used in making alkyl chlorides and vinyl chloride from olefins and acetylene, respectively (Sax and Lewis 1987). It is also used in hydrochlorination, alkylation, and polymerization reactions. Hydrochloric acid is the hydrated form of hydrogen chloride. It is one of the most important industrial chemicals.

HCl gas is a potential thermodegradation product of chlorinated polymers, such as polyvinyl chloride (PVC) and chlorinated acrylics (Coleman and

Thomas 1954). When PVC or chlorinated acrylics were heated to about 300, 600, or 900°C in air, more than 99.9% of the chlorine atoms in these polymers were released in the form of HCl, and the remaining chlorine atoms were released as carbonyl chloride; no chlorine gas was formed at all. HCl has been detected in fires involving chlorinated polymers, most commonly PVC (Dyer and Esch 1976; Gold et al. 1978; Jankovic et al. 1991). Jankovic et al. detected HCl (1 to 8.5 ppm) in 2 of 22 fires or firefighters' training fires. A study by Gold et al. showed that Boston firefighters who were at the immediate location of the fire were exposed to HCl at 18, 32, 75, 128, or 150 ppm (time-weighted concentration) in 5 of 90 fires. For two of these five fires, the firefighters specifically identified "plastics" as among the combustibles.

HCl generation was suspected in an industrial incident in which a PVC extruding machine was overheated to 360°C (Froneberg et al. 1982). Sixty-three workers at this PVC plant were exposed to fumes from the overheating machine. They experienced irritation of the upper and lower respiratory tracts, headache, nausea, and fainting. The symptoms were attributed to exposure to HCl and carbon monoxide, which are known to form when PVC is heated to 300°C. During the space-shuttle mission STS-40, the electric motor of a freezer-refrigerator overheated. Postflight chemical analyses of off-gassed compounds from the motor suggested that a low concentration of HCl could have been present in the spacecraft cabin after this incident (Huntoon 1991).

TOXICOKINETICS AND METABOLISM

Absorption

No reports on the upper-respiratory-tract (URT) absorption of HCl have been found. The uptake of two water-soluble gases, hydrogen fluoride and formaldehyde, by the URT of the rat were 100% and 93%, respectively (Morgan and Monticello 1990). Hydrogen fluoride is infinitely soluble in water (Stokinger 1981); the solubility of HCl (67.3 g/100 g at 30°C) is greater than that of formaldehyde (55 g/100 g at 25°C) (Barrow et al. 1984). Morris and Smith (1982) predicted that the URT would remove more than 99% of inhaled HCl in rats.

Stone (1975) conducted a study to simulate human URT absorption of HCl. A 1-m long tube of 4-mm inside diameter (cross-sectional area 0.13 cm^2) was wetted with water so as to mimic the URT. When HCl at 60, 600, or 6000 ppm in room air was introduced into the tube at about 4 L/min for 30 min, it was very well retained by the water film. The corresponding retention efficiencies were 100%, 98%, or 93%.

Because PVC combustion releases both gaseous HCl and HCl adsorbed on soot particles, it is of interest to assess their toxicological implication in the respiratory system. As noted above, pyrolysis of PVC releases essentially all of its chlorine atoms as HCl. Stone et al. (1973) observed that flame pyrolysis of PVC at 1100°C released the bulk of HCl in the gaseous form; less than 2% of the HCl was associated with soot particles. The soot particles generated ranged from 0.03 to 0.11 μm in diameter. If these particles could penetrate deep into the lung, then the HCl in the solid particle matrix also could reach the deep lung. Yu (1978) predicted that 20-40% of the inhaled particles in that size range would be deposited in the alveolar region of the human lung. Assuming that 40% of the soot could reach the alveolar region, these data suggest that only 0.8% of the HCl generated from the PVC combustion could reach the lung. Thus, the toxicological impact of soot-associated HCl is relatively small compared with that of the gaseous HCl.

Metabolism

HCl is not metabolized in the body. Chloride is one of the major extracellular anions in living organisms (White et al. 1978). Chloride ions resulting from HCl adsorption in the URT should be distributed throughout the body.

TOXICITY SUMMARY

HCl primarily causes URT irritation. At moderate exposure concentrations, nasal lesions could also occur. At high concentrations (as in industrial accidents), in addition to causing URT irritation and lesions, HCl can reach the lung, causing pulmonary edema, retrosternal pain, and dyspnea (Ellenhorn and Barceloux 1988). Severe pulmonary injury can result in death. Because chloride ions are normal electrolytes in the body, prolonged exposures to low concentrations or brief exposures to high HCl concentrations will not perturb the electrolyte homeostasis in the body enough to result in any systemic toxicity.

Acute or Short-Term Exposures

Irritation to the Respiratory System

Human Studies

HCl is an irritant to the mucous membranes and eyes; skin irritation could occur at very high exposure concentrations (Elkins 1959; Rom and Barkman

1983). Hydrated HCl is less toxic than the dry gas, because the former does not have the dehydrating action of the latter (Henderson and Haggard 1943). According to a review by Henderson and Haggard (1943), HCl at 1000 to 2000 ppm is dangerous in even short exposures. An exposure to 10-50 ppm was tolerable for several hours; an exposure to 50-100 ppm was tolerable for 1 h. At 35 ppm, HCl caused throat irritation. However, Elkins (1959) reported greater HCl irritancy than that observed by others. Elkins noted that exposures to HCl above 10 ppm were highly irritating. Inhalation of HCl at 5 ppm or more was immediately irritating. Concentrations of HCl at less than 5 ppm "are apparently not harmful, although they possibly promote tooth decay." Workers developed some tolerance toward the irritant effect of HCl (Elkins 1959).

Animal Studies

Kaplan et al. (1986) exposed male juvenile baboons (2-3 y old, one per exposure concentration) to HCl at 190, 810, 890, 940, 2780, 11,400, 16,600, or 17,300 ppm for 5 min. Irritation signs were seen at 810-17,300 ppm but not at 190 ppm. The signs ranged from frothing at the mouth and coughing at the lower concentrations to head shaking, profuse salivation, blinking, and eye rubbing at the higher concentrations. The two baboons exposed to HCl at 16,600 or 17,300 ppm experienced severe and persistent dyspnea; pneumonia, pulmonary edema, and tracheitis were the major pathological findings in those two animals, which died at 18 or 76 d after the exposure. Kaplan et al. also exposed single rats for 5 min to 1 of 12 HCl concentrations ranging from 11,800 to 87,700 ppm. All the exposed rats showed severe irritation of the respiratory tract and eyes. Most of the rats had persistent respiratory symptoms, and some died after the exposure.

The irritancy of HCl to animals exposed to high concentrations also was investigated by Darmer et al. (1974). In this study, rats were exposed to HCl at 30,000-57,000 ppm for 5 min or 2100-6700 ppm for 30 min; mice were exposed at 3200-30,000 ppm for 5 min or 410-5400 ppm for 30 min. HCl was found to be extremely irritating to the mucous membranes and exposed skin. The symptoms included excessive grooming and preening, corneal erosion and cloudiness, and rapid shallow breathing. The toxicity to exposed skin was manifested as scrotal ulceration and greenish discoloration of the fur.

A similar study was conducted in rats and mice exposed for 60 min to 1800-4500 ppm and 560-2500 ppm, respectively (Wohlslagel et al. 1976). The findings were very similar to those of Darmer et al. (1974). Wohlslagel et al. reported eye and mucous membrane irritation, respiratory distress, corneal opacity, and erythema of exposed skin in these rats and mice during the exposure.

Machle et al. (1942) exposed groups of three rabbits and three guinea pigs to various concentrations (34-4360 ppm) of HCl for 5 min, 15 min, 1 h, 2 h, 6 h, 2 d (6 h/d), or 5 d (6 h/d). Irritation to the eyes and mucous membranes was present to various degrees in all the exposure groups. Acute distress was evident in the groups exposed at high concentrations.

The irritating effect of HCl on the URT and the pulmonary region was further investigated in male guinea pigs (Burleigh-Flayer et al. 1985). Sensory irritation, characterized by a decrease in respiratory rate with lengthened expiration, results primarily from irritation of the nasal cavity; pulmonary irritation is characterized by an initial rise followed by a fall in respiratory rate, with a pause after each expiration. The guinea pigs were exposed in head-only chambers to HCl at 320 to 1380 ppm for 30 min, and respiratory patterns were monitored. Both types of irritation were detected; however, sensory irritation was seen before the onset of pulmonary irritation. That is because the majority of inhaled HCl is captured by the URT (the site where sensory irritation originated); eventually, however, enough HCl escapes scrubbing by the nose and reaches the lung to cause pulmonary irritation. Corneal opacities, a direct result of the corrosive property of HCl, were observed in animals exposed at 680 ppm or higher. The results are summarized in Table 3-1.

TABLE 3-1 Time of Onset of Sensory and Pulmonary Irritation Produced by HCl

HCl Exposure Concentration, ppm	Sensory Irritation, Time of Onset, min	Pulmonary Irritation, Time of Onset, min	Animal Corneal Opacity	Mortality
320	6	20	0/4	0/4
680	<1	13	1/4	0/4
1040	<1	9	4/8	2/8
1380	<1	4	5/8	3/8

Morphological Injuries to the Respiratory System

In Vitro Studies

The irritation/toxicity of HCl and several other irritant gases was studied in vitro. Cralley (1942) sought a gaseous concentration that would stop ciliary

activity in 10 min. Such concentrations were found to stop the ciliary activity in rabbit tracheal explants for the following: HCl at 60 ppm, chlorine at 30 ppm, sulfur dioxide at 30 ppm, NO_2 at 60 ppm, formaldehyde at 30-60 ppm, and ammonia at 600 ppm. In reacting with water, 30-ppm chlorine is converted to roughly 60-ppm HCl, 30-ppm SO_2 is converted to 30-ppm H_2SO_3, and 60-ppm NO_2 is converted to 60-ppm HNO_3. Interestingly, all of those acid species, including HCl, at their effective concentrations generated roughly 60-ppm H^+. The data suggest that the toxicity of those acidic species on the ciliary cells in this in vitro system is due primarily to the hydrogen ions formed in the mucosal surface.

Human Data

Doub (1933) reported a case involving a man occupationally exposed to HCl fumes of an unknown concentration for about 10 min. The man started to cough at the end of the exposure. He then coughed up some blood; that continued for a day. On the second day, he was hospitalized and given a chest X-ray, which revealed a dense, hazy mottled shadow spanning both lungs together with areas of consolidation. Coarse, bubbling rales were also heard over both lungs. He was diagnosed with acute bronchitis and bronchopneumonia, and recovered fully 9 d after the exposure.

Inhalation exposures to respiratory irritants, such as HCl, are known to trigger asthmatic attacks in people with asthma (Boulet 1988). A nonatopic, nonsmoking man with a 6-y history of mild asthma developed a rapidly progressive and severe bronchospasm after cleaning a pool for about an hour with a product containing hydrochloric acid. After the incident, his asthma changed from mild to severe. However, it is not known whether the cleaning product contained any other offending ingredients or whether any volatile reaction products were formed during the cleaning.

Animal Data

According to Machle et al. (1942), HCl injures primarily the respiratory tract at concentrations higher than 34 ppm. Repeated exposures to HCl at 67 ppm for 5 d (6 h/d) induced mild bronchitis with some peribronchial fibrosis in guinea pigs but did not cause severe lesions. However, in rabbits, lobular pneumonia and pulmonary abscesses were commonly detected after repetitive exposures at 67 ppm. Machle et al. (1942) concluded that "high concentrations produce necrosis of the tracheal, bronchial and alveolar epithelium, accompanied

by extensive pulmonary edema, atelectasis, and emphysema." However, the exact concentrations that produced those toxicities were not reported. Edema and necrosis of the intima and media of the pulmonary blood vessels, accompanied by thrombi and pulmonary infarcts, were also found. Unfortunately, the reported results of this large-scale study, which consisted of 37 experiments with varying exposure times and concentrations, lacked details.

Morphological changes induced by HCl were investigated in male guinea pigs exposed to the compound at 1040 ppm for 30 min (Burleigh-Flayer et al. 1985). When examined by light microscopy 2 d after the exposure, the larger conducting airways showed squamous metaplasia with a loss of cilia and acute submucosal inflammation. Multifocal acute inflammation with congestion and mild hemorrhage were found in the alveoli. Fifteen days after the HCl exposure, goblet-cell hyperplasia and mild inflammation in the larger conducting airways were observed; mild lymphoid hyperplasia in the parenchyma and interstitial inflammation in the lung also were noted. These data demonstrated that HCl exposures at 1040 ppm led to tissue damage in the airways and the alveolar regions.

Morphological insults from HCl in the respiratory tract were also studied in mice and rats (Darmer et al. 1974). In this study, rats were exposed to HCl at 2100 to 57,000 ppm, and mice were exposed to 410 to 30,000 ppm, for 5 or 30 min. Darmer et al. reported observing badly damaged nasal and tracheal epithelium, moderate-to-severe alveolar emphysema, pulmonary edema, atelectasis, and occasional spotting of the lung; however, the exposure concentrations that produced those toxicities were not specified. The survivors of exposures to high concentrations showed a clicking breathing noise, breathing difficulty, and bloody discharge from the nares. Buckley et al. (1984) reported that 5-d exposures (6 h/d) of mice to HCl at 310 ppm resulted in necrosis, exfoliation, erosion, and ulceration of the respiratory epithelium in the nose, but no histopathological changes in the lung.

Because HCl gas is well absorbed in the nasal cavity, the toxicity of HCl depends in part on whether the exposure is via breathing through the nose or the mouth. Stavert et al. (1991) fitted male rats with mouthpieces coupled with endotracheal tubes to simulate mouth breathing. They exposed the "mouth-breathing" rats and the normal (i.e., nose-breathing) rats to HCl at 1300 ppm for 30 min. About 46% of the mouth-breathing rats died versus only 6% of the nose-breathing rats. The survivors were killed 24 h after the HCl exposure. The mouth-breathing rats had epithelial and submucosal necrosis in the trachea with fibrinous and neutrophilic exudates. The nose-breathing rats developed necrosis of the epithelium, submucosa, and bone, with fibrinous and neutrophilic exudates, but showed no tracheal injury. The dry and wet weights of the lungs of the mouth-breathing rats were increased, compared with controls, but

those weights in the nose-breathing rats were unchanged. In summary, the toxicity of HCl is confined primarily to the nose in normal breathing. Mouth breathing allows HCl to reach deep into the lung to produce injury.

Functional Injuries to the Respiratory System

Hartzell et al. (1985) reported that respiratory minute volume (RMV) decreased by 30% in rats exposed to HCl at 200 or 300 ppm for 30 min. Concentrations of 780 to 1500 ppm reduced the RMV further to about 60%. The logarithm of the exposure concentrations was linearly related to the percentage decrease in RMV or respiratory rate. The drops in RMV paralleled the decreases in respiratory rate; that finding indicates that tidal volume was probably not affected. In rats exposed to HCl at 780 ppm, the decrease in RMV began almost as soon as the HCl exposure started and reached a maximum 3 min into the 30-min exposure. The decreases in the respiratory rate and minute volume of these rats were typical of the respiratory responses to sensory irritants (Alarie 1981).

In contrast to rats, HCl increased the RMV in baboons. A 30-min exposure of baboons of HCl at 500, 5000, or 10,000 ppm increased the respiratory rate in a concentration-dependent fashion, with no significant changes in tidal volume (Kaplan et al. 1988). However, analyses conducted on blood samples collected during the exposure and within 10 min of the exposure showed a drop in arterial pO_2 by about 45% in the baboons exposed at 5000 or 10,000 ppm. Arterial pH and pCO_2 showed no changes. The finding on hypoxemia is not consistent with an increased RMV, which should increase arterial pO_2. Pulmonary edema or small airway constriction was suspected in the exposed baboons. Because chest X-rays taken within 1 h of exposure were negative, the investigators believed that pulmonary edema, even if present, could not have been severe. Blood analyses conducted 3 d and 3 mo after the exposure showed no hypoxemia. Results of pulmonary function tests conducted at those times showed no changes in functional residual capacity, vital capacity, inspiratory capacity, diffusing capacity of the lungs for carbon monoxide, the diffusing capacity per unit lung volume, pulmonary blood flow, and pulmonary static compliance.

Systemic Injuries outside the Respiratory system

Darmer et al. (1974) found that acute HCl exposures of rats and mice failed to produce any gross or histological injuries to tissues other than the respiratory

tract. However, the exposures were short (up to 30 min). HCl can produce systemic toxicity if the exposure period is long and the concentration is high enough. When 111 rabbits and 111 guinea pigs were exposed in 37 experiments to HCl at 34 to 4360 ppm for durations of 5 min to 4 w (6 h/d), 51 rabbits and 57 guinea pigs died shortly or several months after the exposures (Machle et al. 1942). Death attributed to hepatic damage was observed in 12 rabbits and 23 guinea pigs; pathological findings included extensive parenchymal edema, congestion, necrosis, hemorrhage, fatty changes, cirrhotic sclerosis, or other degenerative changes. Liver lesions were also seen in animals that died of other causes. The kidneys in some animals showed hyaline thickening of the glomerular tufts, glomerular sclerosis, tubular atrophy and degeneration, and chronic cellular infiltration of the interstitium. In the heart, HCl exposures produced myocardial degeneration, hyaline necrosis with fibrous replacement, and chronic cellular infiltrations of the myocardial bundles and interstitium.

The comparative toxicity of HCl and hydrogen fluoride (HF) was investigated by Machle's group (1934, 1935, and 1942). Repetitive exposures of guinea pigs and rabbits to HF at 20 ppm for 10 w (6 h/d, 5 d/w) led to injuries in the respiratory tract and liver (Machle and Kitzmiller 1935); the exposed rabbits also showed kidney damage. Comparing those results with the toxicity results for HCl described above, Machle et al. (1942) concluded that the acute irritant effects of HCl and HF were similar. However, HF is more systemically toxic than HCl because the pathological changes were more severe and frequent. Notably, chloride ion is a normal electrolyte in the body, and fluoride ion is not. Machle et al. (1942) further concluded that in prolonged exposures, the safe concentration of HF is lower than that of HCl.

Death

As discussed above, high concentrations of HCl can cause pulmonary injury. Severe pulmonary injury can lead to death. Machle et al. (1942) reported that an acute exposure to HCl at 1000 mg/m^3 (670 ppm) for 2 h killed all three rabbits and all three guinea pigs exposed; an exposure at 6500 mg/m^3 (4400 ppm) took only 30 min to kill all the exposed rabbits and guinea pigs. Guinea pigs tended to succumb faster than rabbits; 30% of the guinea-pig deaths occurred within 48 h of the start of the exposure compared with only 6% of the rabbit deaths. These early deaths were primarily caused by acute respiratory damage. Animals that did not die immediately after exposure succumbed later to pulmonary and nasal infections. Longer exposures to moderately high concentrations can cause death from hepatic damage (Machle et al. 1942). However, some animals survived a single 5-min exposure to 5500 mg/m^3 (3700

ppm). Five days (6 h/d) of exposure to a relatively low concentration of HCl (67 ppm) did not kill any exposed animal (Machle et al. 1942).

The acute toxicity of HCl was also studied in mice and rats (Darmer et al. 1974). Rats were more tolerant of HCl than mice; the 5-min and 30-min LC_{50}s (lethal concentrations for 50% of the animals) for rats were two to three times those for mice. More delayed deaths were noted in mice than in rats. A similar study by Wohlslagel et al. (1976) also showed that rats were more tolerant of HCl than mice. The LC_{50} values reported by Darmer et al. (1974) and Wohlslagel et al. (1976) are listed in Table 3-2.

Using those data to calculate the C × T values for exposures that produced 50% mortality in rats exposed for 5 min, 30 min, or 60 min yielded 205,000, 141,000, and 186,000 ppm-min, respectively. The corresponding values in mice were 70,000, 78,000, and 66,000 ppm-min. Thus, for HCl exposures of 60 min or less, the C × T values that produce 50% mortality are relatively constant for rats and mice. The mortality response curves of HCl in rats and mice are both quite steep. Data from Wohlslagel et al. (1976) showed that to reduce the mortality of a 60-min HCl exposure from 80% to 20%, the exposure concentration would need to be reduced by only 34% for rats and by 70% for mice.

Species sensitivity to the acute toxicity of HCl was further investigated by Kaplan et al. (1988). Three groups of baboons (three per group) were each exposed to HCl at either 500, 5000, or 10,000 ppm for 15 min and were observed for 3 mo afterward. None of the animals died, and all gained weight normally. When six mice were exposed at 2550 ppm for the same length of time, five died. Kaplan et al. (1988) concluded that primates are less sensitive to HCl than rodents, and "baboons can survive exposure to concentrations of HCl that are at least five times greater than those that are lethal to the mouse."

TABLE 3-2 LC50 Values Reported by Darmer et al.(1974) and Wohlslagel et al. (1976)

Species	5-min LC_{50}, ppm	30-min LC_{50}, ppm	60-min LC_{50}, ppm
Rat	41,000[a]	4700	3100
	(35,000-48,000)[b]	(4100-5400)	(2800-3500)
Mouse	14,000	2600	1100
	(10,000-18,000)	(2300-3100)	(870-1400)

[a] Maximum likelihood estimate.
[b] The concentration range predicted with 95% confidence.

The comparative acute toxicity of HCl and HF also was studied by Wohlslaget et al. (1976). On the basis of LC_{50} values, HF was more deadly than HCl in rats and mice. The 60-min LC_{50} values of HF for rats and mice were only 30-45% of the LC_{50} values of HCl, with no overlap of the respective 95% confidence limits.

Effect on Exercise Ability

HCl is generated in household fires that involve burning of chlorinated polymers. In an effort to study the potential escape ability of fire victims who might be exposed to HCl, Malek and Alarie (1989) studied the effect of a 30-min HCl exposure on the ability of guinea pigs to run on a wheel. The guinea pigs were allowed to run on the wheel for 10 min while breathing air before the HCl exposure began. When exposed to HCl at 107 ppm, three guinea pigs were able to run for the entire 30-min exposure. However, at 140, 160, or 590 ppm, all the guinea pigs were incapacitated after 17, 1.3, or 0.7 min (on the average), respectively, into the HCl exposure. When the guinea pigs reached the incapacitation stage, they stopped running abruptly and were "severely compromised." Signs of mild irritation were observed at 107 ppm, and severe irritation was detected at 590 ppm with lacrimation, frothing at the mouth, coughing, and cyanosis. The six guinea pigs in the 107-ppm and 140-ppm groups (three per group) survived the 30-min exposure. The two guinea pigs in the 160-ppm group also survived the 30-min exposure, but all four guinea pigs exposed at 590 ppm died in about 3 min. Those data show that an acute HCl exposure that is only mildly irritating is not incapacitating at least in guinea pigs, but a severely irritating acute HCl exposure can be incapacitating. However, the data are of little value for assessing the ability of HCl to prevent fire victims from running for their lives.

Subchronic and Chronic Exposures

Toxicity of HCl in the Respiratory Tract

Similar to acute and short-term repetitive exposures, subchronic and chronic exposures to HCl produce primarily mucosal irritation and possibly injuries to the upper respiratory system.

Human Studies

One case-control study was conducted with workers exposed to HCl gas in chemical plants (Bond et al. 1991). However, no data were gathered on whether the exposures led to the irritation in the workers, and no data were gathered on the exposure concentrations that might have led to complaints of an irritant. Those data were not collected because this retrospective epidemiological study was carried out after most of the subjects had left their jobs (Bond, G., Dow Chemical Co., personal commun., 1993).

Animal Studies

Ronzani (1909) exposed 15 rabbits and 20 guinea pigs to HCl at 100 ppm for 50 d (6 h/d). The animals showed signs of agitation, nasal discharge, and mild lacrimation in the first hour of each day of exposure. No significant changes were found in red-blood-cell count; hemoglobin concentration; body-weight gain; bactericidal capacity of the lung; or susceptibility to a pulmonary challenge of anthrax, diplococcal bacteria, typhus, and tuberculosis. However, numerous guinea pigs, but not rabbits, developed slight emphysema.

In a study sponsored by the Chemical Industry Institute of Toxicology (CIIT), four groups of rodents were exposed to HCl (Toxigenics 1983). Each group consisted of 52 Fischer 344 (F344) rats, 52 Sprague-Dawley (SD) rats, and 52 B3C3F_1 mice (31 males and 21 females of each strain). The groups were exposed to HCl at 0, 10, 20, or 50 ppm for 90 d (6 h/d, 5 d/w). Interim killings of 10 animals per sex-species-strain-exposure group exposed for 5 d showed that the effects of HCl were confined to the URT. The rats of the 20-ppm group showed minimal-to-mild rhinitis, and rhinitis in the 50-ppm group was mild. Similar results were observed in the rats killed after 90 d of HCl exposure (i.e., both strains of rats exposed to either 20 ppm or 50 ppm showed minimal-to-mild rhinitis). A 90-d exposure to HCl at 10 ppm caused minimal rhinitis in some of the exposed F344 rats and in none of the SD rats. However, rhinitis was not present in all exposed mice. Instead, mice exposed to HCl at 20 or 50 ppm for 90 d showed minimal-to-mild eosinophilic globules in the nose. In addition, the 50-ppm mouse group had varying degrees of cheilitis (inflammation of the lip) characterized by the presence of hemosiderin-laden macrophages (Toxigenics 1983). The incidence of nasal lesions is summarized in Table 3-3.

Sellakumar et al. (1985) found that chronic exposure of rats to HCl at 10 ppm for 128 w (6 h/d, 5 d/w) produced a nonstatistically significant increase

in rhinitis, epithelial hyperplasia, or squamous metaplasia in the nose. They concluded that at 10 ppm "HCl did not induce any serious irritating effects in the nasal epithelium." The HCl exposure, however, increased the incidence of hyperplasia in the laryngeal and tracheal epithelium (21% and 26%, respectively, in the test rats vs. 2% and 6%, respectively, in control rats). The authors did not specify the degree of severity of the hyperplastic change observed in the exposed rats. Because the HCl exposure did not produce any mucosal injury in the nose and because HCl is believed to affect the nasal cavity more than other parts of the respiratory tract, the laryngeal and tracheal hyperplasia seen in 20% of the exposed rats was most likely only mild. Hyperplasia is an increase in the number of normal cells in response to stimuli without any loss of the normal cellular arrangement in a tissue (Robbins et al. 1984; Anderson et al. 1988). Mild laryngeal and tracheal hyperplasia should be viewed as adaptive changes without any significant functional decrement. Moreover, exposure of rats and mice to HCl at 10, 20 or 50 ppm in the CIIT-sponsored study revealed no lesions outside the nasal cavity (Toxigenics 1983). Therefore, laryngeal and tracheal hyperplasia will not be considered in setting the human exposure limits.

TABLE 3-3 Incidence of Nasal Lesions in Male and Female Animals

Exposure Concentration, ppm	F344 Rat Rhinitis Incidence		SD Rat Rhinitis Incidence		$B6C3F_1$ Mouse Eosinophilic Globules	
5 d	90 d	5 d	90 d	5 d	90 d	
0	0/20	0/20	0/20[a]	5/20[a]	0/20	0/20
10	0/20	6/20[a,b]	0/20	8/20[a]	0/20	3/20[c]
20	5,20[a,b]	12/20[b,c]	4/20[a]	13/20[b,c]	0/20	6/20[a,b]
50	13/20[b,c]	5/20[b,c]	9/20[b,c]	13/20[a,b]	0/20	8/20[b,c]

[a] The responders were affected minimally.
[b] Statistically significant compared with the controls according to Fisher's exact probability test ($p < 0.05$).
[c] The responders were affected either minimally or mildly.

Systemic Toxicity of HCl

In the CIIT-sponsored study in which rats and mice were exposed to HCl at 0, 10, 20, or 50 ppm for 90 d (Toxigenics 1983), results of interim killings revealed no systemic macroscopic and microscopic lesions after 5 d of exposure. Clinical observations showed that the males and the females in the 50-

ppm group had depressed body-weight gain starting the third week of exposure. Male rats in the 50-ppm group also had depressed body-weight gain in the third to eighth weeks of exposure. After 90 d of exposure, no changes were found on urinalysis (volume, specific gravity, pH, protein, ketone, glucose, appearance, or presence of blood), hematology (erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, total and differential white-blood-cell counts, or platelet and thrombocyte counts), or serum chemistry (glutamic pyruvic transaminase, urea nitrogen, total bilirubin, glucose, alkaline phosphatase, inorganic phosphorus, or calcium). Histopathological results of the 10 animals killed in each sex-species-strain-exposure group at the end of 90 d revealed no lesions in trachea, lungs, liver, kidneys, or other tissues.

Machle et al. (1942) reported that systemic histopathological changes were not seen in three rabbits and three guinea pigs exposed to HCl at 34 ppm for 4 w (6 h/d, 5 d/w). Sellakumar et al. (1985) also found no systemic toxicity in rats exposed to HCl at 10 ppm for 128 w.

Lack of Carcinogenic Response

Human Studies

Reports in the literature have failed to associate HCl exposure with tumors. Bond et al. (1991) did a case-control study with workers exposed to HCl gas in chemical plants to determine the correlation, if any, between cancer and HCl exposures. The workers were classified on the basis of HCl exposure concentrations (0, 0.25, 1.5, and 3.75 ppm [time-weighted average], as estimated by an industrial hygienist) and length of occupational HCl exposure (less than l y, 1-4.9 y, or 5 y or more). They studied a group of 308 workers who died of cancer of the lungs, bronchus, and trachea. The 95% confidence intervals of adjusted relative risk associated with cumulative exposure among the lung-cancer cases and controls were as follows: cumulative exposure of 0.1-3.0 ppm-y, 0.6-1.3; 4.0-12.4 ppm-y, 0.8-1.9; and at least 12.5 ppm-y, 0.6-1.8. Bond et al. (1991) concluded that there was "no evidence of an association between HCl exposure and lung cancer."

Animal Studies

Albert et al. (1982) exposed rats to HCl at 10 ppm for 84 w (6 h/d, 5 d/w) and found no carcinogenic response. A lifetime cancer bioassay conducted by the

same laboratory on rats exposed to the same concentration of HCl for 128 w showed no increase in tumor incidence in the nasal cavity or any organs (Sellakumar et al. 1985). A reported increase in nasal squamous metaplasia was not statistically significant. A co-exposure of rats to HCl at 10 ppm and formaldehyde at 14 ppm for 128 w did not affect formaldehyde carcinogenesis in the nasal cavity.

Genotoxicity

No genotoxicity data for HCl were found in the literature.

Developmental Toxicity

Only one report was found regarding possible developmental effects of HCl (Pavlova 1976). A 1-h exposure of an unspecified number of female rats to HCl at 300 ppm (450 mg/m^3) on d 9 of gestation resulted in severe dyspnea and cyanosis. One-third of the dams died; autopsy revealed lung congestion, edema, and hemorrhage. Hypoxemia was detected in the surviving dams 5 d after the HCl exposure. These findings indicate that the exposure caused maternal toxicity. The surviving dams were allowed to deliver; more progeny of dams in the exposure group died than in the control group. In utero HCl exposure also might affect renal function. Diuresis was seen in the progeny of the HCl-exposed dams when they reached 2 mo of age, but it disappeared at 3 mo of age. However, it is not known that the renal-function impairment was due to HCl-induced maternal toxicity or the direct action of HCl on the embryoes. The severe maternal toxicity found by Pavlova (1976) renders any findings of the developmental toxicity of HCl questionable.

Interaction with Other Chemicals

No literature reports on the synergistic effects of HCl and other chemicals have been found, but there are reports of HCl interacting with other chemicals. Because the combustion of chlorinated plastics is known to produce both HCl and carbon monoxide (CO) (Coleman and Thomas 1954), the potential interaction of HCl and CO has been of toxicological interest (Hartzell et al. 1985, 1987). These authors showed that a co-exposure of rats to HCl at 400-1000 ppm lengthened the time CO took to incapacitate the rats by means of depressing of respiration. High concentrations of HCl have been shown to cause

pulmonary edema. Edema might slow the diffusion of CO across the pulmonary lining, thereby delaying the toxic action of CO.

Hartzell et al. (1987) also compared the lethality in rats of pure HCl, HCl in smoke generated by flaming thermodegradation of PVC, or HCl in smoke generated by flaming thermodegradation of PVC. The authors observed that HCl in smoke generated by flaming thermodegradation of PVC (30-min LC_{50} = 2141 ppm, 95% confidence limit [CL] = 1584 and 2505 ppm) was slightly more lethal than HCl with smoke generated by nonflaming thermodegradation (LC_{50} = 2924 ppm, 95% CL = 2171 and 3662 ppm), which in turn was slightly more lethal than pure HCl (LC_{50} = 3817 ppm, 95% CL = 3051 and 4830 ppm).

Summary

Summaries of inhalation toxicity for humans and for animals are shown in Tables 3-4 and 3-5, respectively. The data are arranged in ascending order according to inhalation exposure concentrations.

TABLE 3-4 Toxicity Summary of Human Inhalation Exposure

Concentration, ppm	Exposure Duration	Species	Effects	Reference
<5	NS	Human	Apparently not harmful	Elkins 1959
≥5	NS	Human	Immediately irritating	Elkins 1959
10-50	Several h	Human	Tolerable	Henderson and Haggard 1943
>10	NS	Human	Highly irritating	Elkins 1959
35	NS	Human	Throat irritation	Henderson and Haggard 1943
50-100	1 h	Human	Tolerable	Henderson and Haggard 1943
1000-2000	NS	Human	Known to be extremely dangerous for even short exposures	Henderson and Haggard 1943

NS, not specified.

TABLE 3-5 Toxicity Summary of Animal Inhalation Exposure

Concentration, ppm	Exposure Duration	Species	Effects	Reference
10	90 d (6 h/d, 5 d/w)	Mouse	No significant changes in histopathology; no changes in urinalysis, serum chemistry, or hematology	Toxigenics 1983
10	90 d (6 h/d, 5 d/w)	Rat	Significant increase in incidence of minimal rhinitis in F344 rats, but not in Sprague-Dawley rats; no changes in urinalysis, serum chemistry, or hematology	Toxigenics 1983
10	128 w (6 h/d, 5 d/w)	Rat	Incidence of mucosal hyperplasia was increased in the larynx and trachea but not in the nose; no increase in tumor incidence	Sellakumar et al. 1985
20	90 d (6 h/d, 5 d/w)	Mouse	Minimal increase in eosinophilic globules in nose; no histopathology in other tissues; no changes in urinalysis, serum chemistry, or hematology	Toxigenics 1983
20	90 d (6 h/d, 5 d/w)	Rat	Minimal-to-mild rhinitis, but no histopathology in other tissues; no changes in urinalysis, serum chemistry, or hematology	Toxigenics 1983
34	4 w (6 h/d, 5 d/w)	Rabbit, guinea pig	No histopathology	Machle et al. 1942
50	90 d (6 h/d, 5 d/w)	Mice	Pigmented macrophages in lips; minimal ulcerative cheilitis; minimal-to-mild eosinophilic globules in nose; but no changes in urinalysis, serum chemistry, or hematology, and no histopathology in tissues other than the lip or nose; depressed body weight gain	Toxigenics 1983
50	90 d (6 h/d, 5 d/w)	Rat	Depressed body weight gain in the w 3 to 8 of exposure in males; minimal-to-mild rhinitis; no changes in urinalysis, serum chemistry, and hematology, as well as no histopathology in tissues other than the nose	
67	5 d (6 h/d)	Guinea pig	Mild bronchitis with some peribronchial fibrosis; no deaths	Machle et al. 1942
67	5 d (6 h/d)	Rabbit	Lobular pneumonia and lung abscesses; no deaths	Machle et al. 1942

TABLE 3-5 *(Continued)*

Concentration, ppm	Exposure Duration	Species	Effects	Reference
100	50 d (6 h/d)	Rabbit, guinea pig	All animals showed signs of agitation; guinea pigs had nasal discharge and mild lacrimation in the first hour of each day of exposure; no changes in RBC count, hemoglobin concentration, body-weight gain, bactericidal capacity of lungs, or susceptibility to pulmonary challenges with bacteria; guinea pigs developed slight emphysema	Ronzani 1909
107	30 min	Guinea pig	No incapacitation: able to run on a wheel but showed signs of mild sensory irritation	Malek and Alarie 1989
140	30 min	Guinea pig	Unable to run on a wheel by 17 min into exposure	Malek and Alarie 1989
160	30 min	Guinea pig	Unable to run on a wheel by 1.3 min into exposure	Malek and Alarie 1989
190	5 min	Baboon (n=1)	No signs of irritation	Kaplan et al. 1986
200 or 300	30 min	Rat	30% decrease in respiratory rate and minute volume	Hartzell et al. 1985
310	5 d (6 h/d)	Mouse	Necrosis, exfoliation, erosion, and ulceration of respiratory epithelium in the nose; no lung injury	Buckley et al. 1984
320	30 min	Guinea pig	Sensory irritation began in 6 min; lung irritation began in 20 min	Burleigh-Flayer et al. 1985
410-5400	30 min	Mouse	Extreme irritation of mucous membranes and some irritation of exposed skin	Doub 1933
500	30 min	Baboon (n=3)	Increased respiratory rate and minute volume during exposure; no changes in lung function, arterial pH, pO_2, or pCO_2 at 3 d or 3 mo after the exposure	Kaplan et al. 1988
560	60 min	Mouse	2 of 10 mice died	Wohlslagel et al. 1976
560-2500	60 min	Mouse	Eye and mucous membrane irritation, respiratory distress, corneal opacity, and erythema of exposed skin	Wohlslagel et al. 1976

590	30 min	Guinea pig	Incapacitated at 0.7 min into exposure; lacrimation, frothing at the mouth, coughing, cyanosis, and death in about 3 min	Malek and Alarie 1989
670	2-6 h	Rabbit, guinea pig	All died; guinea pigs died faster than rabbits; the early deaths due to respiratory damage; hepatic damage was the most common cause of death in 2 to 7 d after the exposure; lung infection was the most common cause of death after 7 d	Machle et al. 1942
680	30 min	Guinea pig	Sensory irritation began in <1 min; lung irritation began in 13 min; corneal opacities in 1 of 4 guinea pigs	Burleigh-Flayer et al. 1985
780-1500	30 min	Rat	60% reduction in respiratory rate and minute volume	Hartzell et al. 1985
810-940	5 min	Baboon (n=3)	Frothing at the mouth and coughing	Kaplan et al. 1988
1040	30 min	Guinea pig	Sensory irritation began in <1 min; lung irritation began in 9 min; corneal opacities in 4 of 8 guinea pigs; 2 of 8 died; squamous metaplasia with ciliary loss and submucosal inflammation in large airways and multifocal acute alveolitis 2 d after exposure; goblet-cell hyperplasia and mild inflammation in large airways, mild lymphoid hyperplasia and interstitial inflammation in the lung 15 d after exposure	Burleigh-Flayer et al. 1985
1100	60 min	Mouse	Half died	Wohlslagel et al. 1976
1300	30 min	Nose-breathing rat, "mouth-breathing" rat	6% of nose-breathing rats died vs. 46% of "mouth-breathing" rats; necrosis of the mucosa, submucosa, bone, and submucosal gland in the nose-breathing rats; necrosis of the tracheal mucosa and submucosa of the mouth-breathing rats; the dry and wet lung weights were elevated in the mouth-breathing rats but not in normal rats	Stavert et al. 1991
1380	30 min	Guinea pig	Sensory irritation began in <1 min; lung irritation began in 4 min; corneal opacities in 5 of 8 guinea pigs; 3 out of 8 died	Burleigh-Flayer et al. 1985
1800	60 min	Rat	None of the 10 died	Wohlslagel et al. 1976

Concentration, ppm	Exposure Duration	Species	Effects	Reference
1800-4500	60 min	Rat	Eye and mucous-membrane irritation, respiratory distress, corneal opacity, and erythema of exposed skin	Wohlslagel et al. 1976
1900	60 min	Mouse	8 of 10 died	Wohlslagel et al. 1976
2100-6700	30 min	Rat	Extreme irritation of mucous membranes and some irritation to exposed skin	Darmer et al. 1974
2600	60 min	Rat	2 of 10 died	Wohlslagel et al. 1976
2600	30 min	Mouse	Half died	Wohlslagel et al. 1976
3100	60 min	Rat	Half died	Wohlslagel et al. 1976
3200-30,000	5 min	Mouse	Extreme irritation of mucous membranes and some irritation to exposed skin	Darmer et al. 1974
3690	5 min	Rabbit, guinea pig	No deaths	Machle et al. 1942
3900	60 min	Rat	8 of 10 died	Wohlslagel et al. 1976
4360	30 min	Rabbit, guinea pig	All died	Machle et al. 1942
4700	30 min	Rat	Half died	Wohlslagel et al. 1976
5000	30 min	Baboon (n=3)	Increased respiratory rate and minute volume during exposure, hypoxemia; normal chest x-ray 1 h after exposure; normal lung function 3 d or 3 mo after exposure	Kaplan et al. 1988
11,800-18,400	5 min	Rat	Severe irritation of the respiratory tract and eyes	Kaplan et al. 1986
14,000	5 min	Mouse	Half died	Wohlslagel et al. 1976

Concentration, ppm	Exposure	Species	Effects	Reference
16,600-17,300	5 min	Baboon (n=2)	Head shaking, profuse salivation, blinking, and eye rubbing during exposure; severe dyspnea persisted after exposure; died of pneumonia, lung edema with tracheitis 18 or 76 d after exposure	Kaplan et al. 1986
30,000-57,000	5 min	Rat	Extreme irritation to mucous membranes and some irritation to exposed skin	Darmer et al. 1974
41,000	5 min	Rat	Half died	Wohlslagel et al. 1976

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 3-6 presents exposure limits for hydrogen chloride set by other organizations and Table 3-7 presents the SMACs established by NASA.

SMACs are derived in accordance with guidelines developed by the SMAC subcommittee of the Committee on Toxicology (NRC 1992). The SMACs are set by choosing the lowest values among the acceptable concentrations (Acs)

TABLE 3-6 Exposure Limits Set or Recommended by Other Organizations

Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	5 (ceiling)	ACGIH 1991
OSHA's PEL	5 (ceiling)	NIOSH 1990
NIOSH's REL	5 (ceiling)	NIOSH 1990
NIOSH's IDLH	100	NIOSH 1990
NRC's 90-d CEGL	0.5	NRC 1987
NRC's 24-h SPEGL	1	NRC 1987
NRC's 1-h SPEGL	1	NRC 1987
NRC's 24-h EEGL	20	NRC 1987
NRC's 1-h EEGL	20	NRC 1987
NRC's 10-min EEGL	100	NRC 1987

TLV, Theshold Limit Value; PEL, permissible exposure limit; REL, recommended exposure limit; IDLH, immediately dangerous to life and health; CEGL, continuous exposure guidance level; SPEGL, short-term public emergency guidance level; EEGL, emergency exposure guidance level.

TABLE 3-7 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	5	7.5	URT irritation
24 h	2.5	3.8	URT irritation
7 d[a]	1	1.5	URT irritation, lesions
30 d	1	1.5	URT irritation, lesions
180 d	1	1.5	URT irritation, lesions

[a] Previous 7-d SMAC = 1 ppm (1.5 mg/m^3).

(see Table 3-8). HCl primarily produces URT irritation, lesions, or both. The ACs of HCl, therefore, are set on the basis of sensory irritation or pathological changes of the URT found in humans and rodents. Toxicity of HCl in the liver and kidney of rabbits and guinea pigs was reported by Machle et al. (1942). However, these systemic toxicities were observed in animals exposed to high HCl concentrations for prolonged periods. A 90-d exposure to HCl at up to 50 ppm in mice and in two strains of rats produced no systemic toxicity, including hepatotoxicity or renotoxicity (Toxigenics 1983); life-time exposures of rats (128 w) to HCl at 10 ppm also produced no systemic toxicity (Sellakumar et al. 1985). Liver and kidney lesions were found only in animals exposed to conditions that would not be encountered by humans; thus, lesions in these organs are not considered in setting the SMAC values.

1-h and 24-h ACs

Henderson and Haggard (1943), in their review of HCl data gathered from human exposures, stated that exposure to HCl at 10-50 ppm is tolerable for several hours. However, Elkins (1959) noted that exposures at more than 10 ppm are highly irritating in humans, exposures at 5 ppm or more are immediately irritating, and exposures at less than 5 ppm apparently are not harmful. Unfortunately, Elkins did not specify the degree of sensory irritation caused by HCl at 5 ppm. Judging by Henderson and Haggard's finding that HCl at 10-50 ppm is tolerable for several hours and Bond's finding that some workers in chemical plants were routinely exposed to an average HCl concentration of 3.75 ppm (Bond et al. 1991), it seems that HCl at 5 ppm would be likely to cause only mild or, at most, moderate irritation. Therefore, the 1-h AC is set at 5 ppm. Because the possibility of moderate irritation would not be acceptable for a 24-h exposure, the concentration is reduced by a factor of 2 to reach a concentration that would cause only slight-to-mild irritation. The AC of 2.5 ppm for a 24-h exposure is derived as follows:

24-h AC = 5 ppm × ½ = 2.5 ppm.

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

AC Based on Human Exposure Data

Because irritation is not a time-dependent clinical toxic sign, a given HCl concentration will produce the same magnitude of irritation regardless of the duration of exposure. HCl is a very water-soluble compound and is not accumulated

in the URT; thus, an exposure concentration that does not produce irritation in 7 d also will not produce irritation in 180 d. Therefore, the same AC value is set for 7 d, 30 d, and 180 d. Mild irritation is acceptable for up to 24 h of exposure but is not acceptable for longer exposures. The 24-h AC is further reduced from the 1-h AC by a factor of 2 to 1 ppm for the longer exposure periods.

AC Based on Animal Data

The nasal cavity is the primary target of HCl. A CIIT-sponsored study in which rodents were exposed to HCl at 10, 20, or 50 ppm for 5 d or 90 d revealed no histopathological changes in any organs, except for very minimal-to-mild inflammation (rhinitis) of the nasal cavity in the rats but not in the mice (Toxigenics 1983). In the 10-ppm exposed groups, mild rhinitis was seen in 6 of the 20 exposed F344 rats, but the incidence of rhinitis was not statistically increased in SD rats. An increase in mild rhinitis in SD rats exposed to HCl at 10 ppm for 128 w (6 h/d, 5 d/w) in another study also was not statistically significant (Sellakumar et al. 1985). Therefore, 10 ppm was the overall lowest-observed-adverse-effect level (LOAEL) of HCl. Because the rhinitis was mild and was only statistically increased in the F344 rats, but not in mice or SD rats, an extrapolation factor of 3 instead of 10 is applied to the LOAEL to obtain the no-observed-adverse-effect level (NOAEL) of 3 ppm. Rhinitis in these animals was due to superficial irritation by HCl. Tissue responses to irritation would not differ greatly among animal species. Therefore, a species factor of 3 instead of 10 is used for extrapolation from animal to human.

Increasing the exposure time from 5 d to 90 d increased the incidence of minimal or mild rhinitis in rats exposed at 10 or 20 ppm but not at 50 ppm. In fact, for the F344 rats exposed at 50 ppm, the incidence of mild rhinitis was actually lower when the exposure time increased (12 of 20 rats exposed for 5 d vs. 5 of 20 rats exposed for 90 d). Furthermore, exposing SD rats to HCl at 10 ppm for 90 d or 128 w produced an insignificant increase in mild rhinitis. Since no strong correlation was found between rhinitis and exposure length, no time adjustment factor is applied. Therefore, the AC for 5-d, 30-d, or 180-d exposure is derived as follows:

AC = 10 ppm ÷ 3 ÷ 3 = 1 ppm (rounded from 1.3).

AC Summary Table

The ACs derived from various toxicity end points are summarized in Table 3-8. The SMACs are set by choosing the lowest values among these ACs.

TABLE 3-8 Acceptable Concentrations

End point, Exposure Data, Reference		Safety Factors			Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	1 h	24 h	7 d	30 d	180 d	
Nasal irritation	Human	1 to 5	1	1	5	2.5	1	1	1
LOAEL, 5 ppm (Elkin 1959)									
Minimal rhinitis	Rats	3	1	3			1	1	1
LOAEL, 10 ppm for 90 d (Toxigenics 1983)									
SMACs					**5**	**2.5**	**1**	**1**	**1**

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B4 ISOPRENE

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PHYSICAL AND CHEMICAL PROPERTIES

Isoprene is a colorless, highly volatile liquid or gas with a weak aromatic odor (IARC 1994).

Formula:	$H_2C=C(CH_3)-CH=CH_2$
Synonyms:	2-Methyl-1,3-butadiene (IUPAC), isopentadiene
CAS no.:	78-79-5
Molecular weight:	68.12
Boiling point:	34°C
Melting point:	–146°C
Vapor pressure at 20°C:	60.7 kPa
Conversion factors at 25°C:	1 ppm = 2.79 mg/m^3
	1 mg/m^3 = 0.358 ppm

OCCURRENCE AND USE

Isoprene occurs widely in nature and is an important industrial chemical. Isoprene is produced by plants during the photosynthesis process and constitutes a major portion of the nonmethane hydrocarbons released by the biosphere. The concentration of isoprene inside a forest canopy might be 10 times higher than outside the canopy (Khalil and Rasmussen 1992). Isoprene is present in tobacco and wood smoke and can be considered the building block of many natural products including natural rubber, terpenes, vitamins A and K, and the steroid sex hormones (IARC 1994). Isoprene is produced endogeneously

in humans at a rate of about 0.15 to 0.35 μmol/kg•h (Hartmann and Kessler 1990; Filser et al. 1996), and the blood concentration ranges from 10 to 70 nmol/L (Cailleux et al. 1992; Filser et al. 1996). Similarly, isoprene is endogenously produced in rats at a rate of 1.9 μmol/kg•h and in mice at a rate of 0.4 μmol/kg•h (Peter et al. 1987). Ambient air concentrations in cities have been reported as high as 0.04 mg/m^3 (0.014 ppm) (Lonneman et al. 1979), although most reports indicate a much lower concentration (IARC 1994).

Industrially, isoprene is used to make isoprene rubber, which is used in vehicle tires; styrene block polymers, which are useful in adhesives; and butyl rubber, which is useful in lining hoses and tires to limit gas escape (IARC 1994). Isoprene is commercially important in the synthesis of selected terpenes that are used in flavorings and in fragrances (IARC 1994).

Isoprene has been found in about one-third of of the space-shuttle air samples taken with either the grab sample containers (GSCs) or the solid sorbent air sampler (SSAS). Typical concentrations are below 0.1 mg/m^3 (0.036 ppm) (James et al. 1994). Seven air samples obtained with the SSAS during Mir 17 showed a concentration range of 0.17 to 0.35 mg/m^3 (0.061 to 0.12 ppm), whereas 12 samples taken with GSCs during Mir 18 showed concentrations below 0.11 mg/m^3 (0.039 ppm) (J.T. James, T.F. Limero, S.W. Beck, L. Yang, M.P. Martin, M.L. Matney, P.A. Covington, and J.F. Boyd, Johnson Space Center, Houston, Tex., unpublished data, 1995).

TOXICOKINETICS AND METABOLISM

The absorption, distribution, metabolism, and excretion of isoprene has been studied by inhalation exposure and in vitro methods in several species; however, metabolism studies in humans are limited in scope. The differences in susceptibility between rats and mice (see toxicity section) are not clearly explained by known differences in the toxicokinetics of isoprene. Because of structural similarities, the metabolism of isoprene has been often compared to that of 1,3-butadiene; however, that comparison will be avoided here.

Absorption

In human subjects, the pulmonary retention of isoprene from cigarette smoke deeply inhaled by mouth in a single breath was found to be 99% (Dalhamn et al. 1968). The concentration of isoprene in the smoke was not apparent, so it is difficult to relate these data to experimental data on rodents or other species. In anesthesized dogs, percentage retention ranged from 65% to 75% when

evaluated over a ventilatory range of 6 to 30 breaths/min, tidal volumes of 110 to 220 mL, and exposure concentrations of 360 to 720 mg/m^3 (130 to 260 ppm) (Egle and Gochberg 1975). At an exposure concentration of 960 mg/m^3 (344 ppm), the apparent retention was only 40%.

The retention and metabolism of ^{14}C-isoprene was studied in rats exposed for 6 h to concentrations that ranged from 8 to 8200 ppm (Dahl et al. 1987). As the exposure concentrations increased, the percentage of unchanged isoprene exhaled increased from 75% to 96% and the percentage inhaled and metabolized decreased from 25% to 4%. In mice exposed for 6 h to ^{14}C-isoprene at concentrations from 18 to 2000 ppm, the percentage retained decreased from 19% to 6% at the highest concentration (Bond et al. 1991). Rats metabolized a greater fraction of the inhaled dose than did mice at all exposure concentrations.

Distribution

The tissue distribution of inhaled isoprene and its tentatively identified metabolites has been reported in detail in rats exposed to 1480 ppm for 6 h (Dahl et al. 1987). Isoprene and its metabolites were found in nasal tissue, lung, liver, kidney, fat, and blood. Highest amounts were evident in the liver and especially the body fat; several metabolites were also demonstrated in nasal and lung tissue (Dahl et al. 1987). Rats exposed to near-lethal concentrations of isoprene for 3 to 4 h showed 5-10-fold higher concentrations of isoprene in fat tissue than in brain, liver, kidney or spleen (Shugaev 1968).

Metabolism

Metabolic studies have been reported in several species, particularly in rats and mice; however, no studies of the metabolism of isoprene in humans or human tissue could be found. Studies in animals consist of toxicodynamic studies in whole animals and in vitro studies of liver microsomes. Studies of metabolism in nonhepatic tissues were lacking.

The metabolism of isoprene in liver microsomes from rats, mice, rabbits, and hamsters occurs by oxidation of either of the double bonds to form monoepoxides, which in turn can be hydrolized to diols; diepoxides can be formed by oxidation of a second double bond as shown in Figure 4-1 (Gervasi and Longo 1990; Wistuba et al. 1994). The major pathway in all species tested is through the epoxide formed on the methylated double bond. Oxygen and a NADPH-generating system were necessary, and the metabolism could be

inhibited by several known inhibitors of the cytochrome-P-450 system (Gervasi and Longo 1990).

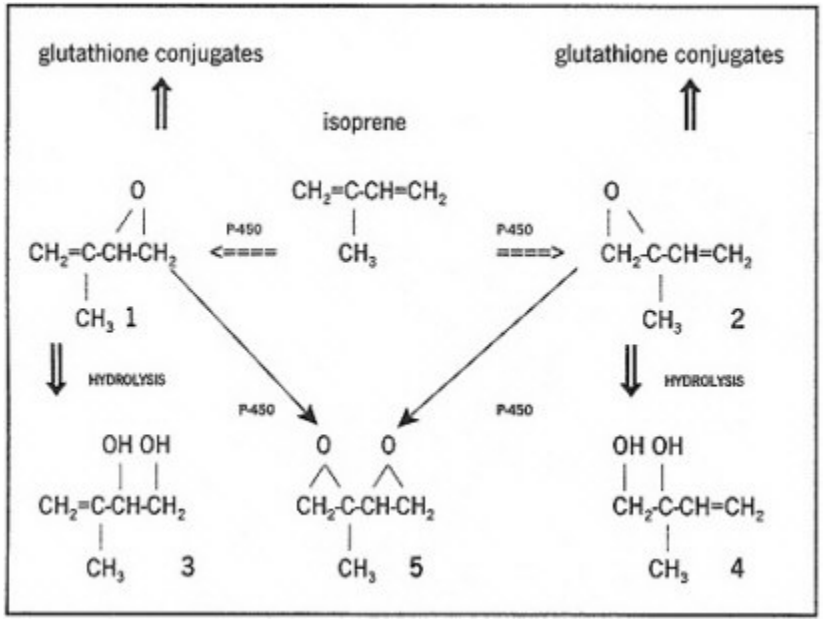

FIGURE 4-1 Metabolism of isoprene in microsomes from several species (Gervasi and Longo 1990; Wistuba et al. 1994).

Isoprene itself was not mutagenic in *Salmonella typhimurium* strains TA98 and TA100, even with metabolic activation, nor were either of the epoxides; however, the diepoxide was mutagenic (Gervasi and Longo 1990).

In male Wistar rats and B6C3F$_1$ mice exposed to concentrations from 5 to about 4000 ppm, the rate of isoprene metabolism was proportional to concentrations below 300 ppm, but saturation was apparent above 1000 and 2000 ppm in rats and mice, respectively (Peter et al. 1987). At moderate concentrations, the half-life of isoprene was 7 min in rats and 4 min in mice, and the fraction exhaled in unchanged form was 15% and 25%, respectively. The maximum velocity for metabolism of isoprene was 3 times higher in mice than in rats (Peter et al. 1987).

There are important species differences in the metabolism of inhaled isoprene in Fischer 344 (F344) rats and B6C3F$_1$ mice (Dahl et al. 1987; Bond et al. 1991). B6C3F$_1$ mice appear to be more sensitive to inhaled isoprene than

rats. The basis for that observation is that rat respiration changes little during high-concentration exposures, whereas the minute volume in mice decreases about 20% during exposures at 2000 ppm (Bond et al. 1991).

As shown in Figure 4-1, the epoxidation of isoprene occurs with preferential oxygen attack at the di-substituted carbon-carbon double bond. In $B6C3F_1$ mouse-liver microsomes, there was no more than slight enantiomer selectivity in either product 1 or 2. In F344 rat-liver microsomes, product 2 was formed with only slight enantiomer selectivity; however, product 1 was formed with a strong selectivity for the (S)-enantiomer (Wistuba et al. 1994). A similar species dependence has been noted for other small aliphatic alkenes.

Isoprene forms hemoglobin adducts in rodents, and the adducts might be a useful marker of repeated exposure to isoprene (Sun et al. 1989). After male $B6C3F_1$ mice and Sprague-Dawley rats were injected intraperitoneally (ip) with ^{14}C-isoprene at 0.3 to 3000 μmol/kg of body weight, the amount of hemoglobin adduct formed after 24 h was proportional to the dose up to 500 μmol/kg (Sun et al. 1989). Repeated administration of 500 μmol/kg for 3 d resulted in linear accumulation of hemoglobin adducts that persisted for approximately 24 or 65 d in mice and rats, respectively. The efficiency of adduct formation, corrected for exhaled isoprene, was twice as high in mice as in rats (Sun et al. 1989).

Excretion

In both F344 rats and $B6C3F_1$ mice, most isoprene metabolites (52-81%) are excreted in the urine, with lesser amounts in the feces and breath (as carbon dioxide) (Bond et al. 1991). As mentioned above, much of an inhaled dose of isoprene is exhaled unchanged from rats and mice. The proportion of metabolites excreted via several routes has been compared in rats and mice exposed for 6 h to concentrations ranging from 8 to 2000 ppm (Bond et al. 1991). The major quantitative differences were the percentage of total metabolites in the feces (mice 6-37%, rats 2-3%) and the percentage of total metabolites present in the bodies 64 h after exposure (mice 3-9% and rats 8-18%).

Comparative Toxicokinetics

In view of the large differences in tumorigenic responses of rats and mice (see below) and the need to extrapolate rodent data to human risks, it is necessary to compare the toxicokinetics of isoprene in these three species. Filser et al. (1996) measured partition coefficients using an in vitro head-space technique and blood from rats, mice, and humans. The tissue/air partition coefficients

were determined for rat tissue. In addition, the uptake of isoprene in intact mice, rats, and humans was measured. They found that a two-compartment model provided a best fit to the data. On the basis of data taken from humans breathing into a respirometer, the endogenous production of isoprene was estimated to be 24 μmol/h for a 70-kg person. Of the endogenous production, 90% underwent metabolism, and the remaining 10% was exhaled. Filser et al. (1996) estimated that 3.4 mg/24 h was exhaled, an estimate that agrees well with earlier estimates of 0.4 to 9.4 mg/24 h and 2 to 4 mg/24 h from Conkle et al. (1975) and Gelmont et al. (1981), respectively. At a concentration of 40 ppm or lower, the rate of isoprene metabolism was 14-fold slower in mice than in humans and 8-fold slower in rats than in humans. Filser et al. (1996) suggest that it will be necessary to measure blood concentrations of isoprene and its metabolites in humans exposed to isoprene to determine whether the toxicokinetic model is correct. The modeling did not address formation of the diepoxide, the putative active metabolite of isoprene. As a result, the model and ensuing risk assessment must be updated when the data become available.

TOXICITY SUMMARY

A reasonably complete toxicity data base is available for short-term and chronic exposures of mice and rats to isoprene by inhalation. Mice exhibit a broad spectrum of lesions, both neoplastic and non-neoplastic, that often does not show an expected dose-response relationship. The data base consistently shows the much greater susceptibility of mice over rats. Data from acute-and human-exposure studies, including epidemiological studies, are sparse.

Acute Exposures

Some data are available on the effects of acute exposure of mice to very high concentrations of isoprene. Citing an earlier study, von Oettingen (1940) stated that 100,000 to 120,000 mg/m^3 (36,000 to 43,000 ppm) caused deep narcosis in mice and that 140,000 mg/m^3 (50,000 ppm) was fatal. Likewise, Gostinskii (1965) reported that 109,000 mg/m^3 (39,000 ppm) resulted in collapse of 50% of the white mice exposed for 2 h. The LC_{50} (lethal concentration for 50% of the animals) for males and females combined was 144,000 mg/m^3 (51,500 ppm) (range 138,000 to 149,000 mg/m^3); females were somewhat more resistant than males. Exposed mice that recovered from narcosis were able to do better than control mice in a swin test administered 24 h later. White mice exposed for 40 min to a mean concentration of 2200 mg/m^3 (790 ppm) exhibited a reduced ability of their central nervous system to "summate subthreshold impulses," and

at half that concentration, there was an increase in motor activity (Gostinskii 1965). Also, exposures of rabbits to concentrations of 1500 to 8000 mg/m^3 (540 to 2900 ppm) "hastened the development of the reflex muscular contraction and weakened the strength of the reflex" during exposures (Gostinskii 1965). In another investigation, the 2-h LC_{50} for mice was 160,000 mg/m^3 (57,000 ppm), and the 4-h LC_{50} in rats was 180,000 mg/m^3 (64,00 ppm) (Shugaev 1968). Mamedov (1979) reported increases in the thymus mitotic index and peripheral lymphocyte count in rats exposed at only 0.8 mg/m^3 (0.3 ppm) for 4 h, but data and statistical analysis to support that observation were not provided.

In one of the few human studies, Gostinskii (1965) reported that 10 subjects could perceive the odor of isoprene at 10 mg/m^3 (3.6 ppm) within a minute. Slight irritation of the mucosa of the nose, pharynx, or larynx was reported when concentrations were raised to 160 mg/m^3 (57 ppm). Increases in the respiratory rate of rabbits (presumably a measure of irritation) were noted at 190 mg/m^3 (68 ppm).

Short-Term Exposures

The species difference in susceptibility was evident in exposures of $B6C3F_1$ mice and F344 rats exposed 6 h/d, 5 d/w for 2 w to isoprene at concentrations of 0, 440, 880, 1750, 3500, or 7000 ppm (Melnick et al. 1990). Animals were evaluated by observation of clinical signs, measurement of body weights, hematology, clinical chemistry, urinalysis (rats only), gross pathology, and histopathology. Histopathology was performed on the liver, 6 points in the respiratory system, heart, brain, thymus, spleen, kidney, and testis. No changes could be detected in any of the rats exposed to isoprene; however, the mice showed changes as summarized in Table 4-1. A NOAEL was not found in mice for red-blood-cell (RBC) changes, decreased relative liver weight, vacuolization of hepatocytes, and epithelial hyperplasia of the forestomach (Melnick et al. 1990).

Male Wistar rats exposed 4 h/d for 30 d to isoprene vapor at a concentration of 0.36 ppm or 0.035 ppm showed variations in thymus cellularity and mitotic index (Mamadov 1979). The changes were not large, and there was not a consistent dose-response relationship. If the thymus is a target organ of isoprene in the rat, then it is surprising that rats exposed to much higher concentrations (Melnick et al. 1990) showed no evidence of injury. Furthermore, endogenous isoprene production in rats has been estimated to be equivalent to inhalation exposures of 0.5 ppm (Melnick et al. 1994); hence, it is unclear how exposures as low as those used by Mamedov (1979) could cause adverse effects.

TABLE 4-1 Incidence of Lesions in Mice Exposed to Isoprene for 2 W in the Melnick et al. (1990) Study

	Exposure Concentration, ppm					
Lesion	0	438	875	1750	3500	7000
Males						
Thymic atrophy	0/10	ne[a]	ne	ne	0/10	7/9
Testes atrophy	0/10	ne	ne	ne	0/10	9/10
Liver vacuolized cytoplasm	0/10	8/10	9/10	10/10	10/10	10/10
Olfactory epithelial degeneration	0/10	ne	0/10	3/10	6/10	9/10
Forestomach epithelial hyperplasia	0/10	3/10	5/10	10/10	8/10	9/10
Females						
Forestomach epithelial hyperplasia	1/10	8/10	7/10	10/10	9/10	9/10

[a] Not evaluated.

Subchronic and Chronic Exposures

The data base consists of Russian epidemiological studies, a variety of animal studies of noncancer end points, a 26-w inhalation carcinogenesis study conducted in rats and mice with an unusually long 26-w recovery period, and a chronic oncogenicity study in mice exposed by inhalation up to 80 w. Despite the apparent high quality of the two latter studies, their conclusions regarding neurotoxicity in mice were found to be difficult to reconcile. The genotoxicity and carcinogenesis data, along with the data showing endogenous production of isoprene, indicate clearly that isoprene is a threshold-type carcinogen. A threshold-type carcinogen does not increase the incidence of tumors if the exposure concentration is below the "threshold" concentration, no matter how long the exposure.

Epidemiological Data

Three Russian epidemiological studies reported effects in workers associated with isoprene exposure in the isoprene rubber industry. Workers exposed to

isoprene, dimethyldioxane, formaldehyde, and methanol (unknown concentrations) appeared to have a reduced immune response to typhoid vaccination compared with controls (Nikul'tseva 1967). The confounding of exposures and the unknown concentrations prevent those data from being useful for setting limits. Similarly, isoprene rubber workers were reported to have excess fatigue, as measured by a photogenic reflex test, at the end of their work shift because of exposure to many organic compounds, including isoprene. Isoprene concentrations were above the Russian maximum allowable concentration (MAC) 16% of the time; however, the experiment was not well controlled in that some of the 300 workers were exposed to excess heat (up to 30 to 35°C), and only 15 control subjects (plant managers) were used (Pigolev 1971). In another study of isoprene rubber workers, Mitin (1969) studied 630 workers for 4 y with 150 control subjects. The exposed workers were divided into three groups based on the length of work service. The author concluded that upper-respiratory-tract injury increased with increasing work time, nose bleeds increased with longer work times, and loss of olfactory sensitivity increased. Those findings cannot be used to set limits for isoprene because other chemicals were present (formaldehyde and dimethyldioxane) at poorly defined concentrations in the production plant, and exposed groups were not controlled for age and smoking habits.

Animal Data on Noncancer End Points

Subchronic and chronic exposures of rats, mice, and rabbits have been reported for isoprene. Rats, mice, and rabbits were exposed for 4-5 mo, 4 h/d to isoprene vapor at a target concentration of 5000 mg/m^3 (1800 ppm) (Gostinskii 1965). Actual concentrations varied between 2200 and 4900 mg/m^3 (790 to 1750 ppm) based on a conductometric analytical method. There were no weight-gain differences as a result of isoprene exposure in any species. Rabbits showed an increase in leukocyte counts, a decrease in erythrocyte count (not quite statistically significant), and histological evidence of irritation in the bronchi, hepatocyte degeneration, myocardium changes, and thyroid irritation (Gostinskii 1965). Mice exposed to isoprene showed a 50% decrease in swim time and increases in the weights of the lungs, brain, and kidney. Rats had histopathological changes similar to those seen in rabbits. Those findings led the author to recommend an exposure limit for workers of 40 mg/m^3 (Gostinskii 1965).

Mamedov (1979) reported a decrease in thymus cellularity, weight, and mitotic index during a 4-mo exposure of rats to isoprene at 0.042 ppm. Apparently, exposures at 0.0039 ppm did not elicit a significant difference from controls. That result is not consistent with results reported in 2-w exposures of

F344 rats to concentrations as high as 7000 ppm; however, a decrease in thymus weight was reported in B6C3F$_1$ mice exposed at 875 ppm and above (Melnick et al. 1990).

The long-term effects of isoprene exposure have been assessed in F344 rats and B6C3F$_1$ mice exposed 6 h/d, 5 d/w for 13 w (males and females) or 26 w (males only) to concentrations ranging from 70 to 7000 ppm (Melnick et al. 1994). After exposure, the animals were evaluated by hematology, clinical chemistry, urinalysis (rats only), sperm motility and vaginal cytology, limb grip strength (mice only), and histopathology. No discernable effects were apparent in rats exposed for 13 w, and the change in rats after 26 w of exposure was limited to interstitial-cell hyperplasia in the testis of the 7000-ppm group. After a 26-w recovery of rats exposed for 26 w, rats in the 700-ppm and higher groups showed an increased incidence of interstitial-cell adenomas, with a very weak dose-response relationship (Melnick et al. 1994).

Mice exhibited a broad range of histological changes in the 13-w study as shown in Table 4-2. A similar pattern of non-neoplastic lesions was reported by Placke et al. (1996) in the same strain of mice, which were exposed up to 80 w to isoprene. One exception was that Placke and co-workers reported myeloid hyperplasia of the bone marrow without significant changes in RBC

TABLE 4-2 Lesions in Mice Exposed to Isoprene for 13 W in the Melnick et al. (1994) Study

	Exposure Concentration, ppm					
Lesion	0	70	220	700	2200	7000
Males						
Forestomach epithelial hyperplasia	0[a]	0	0	9[b]	8[b]	9[b]
Olfactory epithelial degeneration	0	0	0	0	0	10[b]
Liver cytoplasmic vacuolization	0	0	0	0	0	10[b]
Testis atrophy	0	0	0	0	0	2
Females						
Forestomach epithelial hyperplasia	0	0	0	10[b]	9[b]	10[b]

[a] Incidence in 10 mice.
[b] Significantly different from controls ($p < 0.01$).

measures, whereas a mild anemia was reported in males and females exposed for 24 d with a no-observed-adverse-effect level (NOAEL) at 70 ppm (Melnick et al. 1994). Histopathological lesions showed a NOAEL at 220 ppm. Hindlimb grip strength was substantially lower in mice exposed at 220 ppm and above for 26 w; however, 4 w into recovery, the grip strengths had returned to control values. The NOAEL for neoplastic and non-neoplastic lesions in mice exposed for 26 w and recovered for 26 w was 220 ppm except for nasal-turbinate epithelial degeneration and spinal-cord degeneration. The incidences of the lesions are given in Table 4-3.

The dose-response relationship for olfactory epithelial degeneration in "recovered" mice suggests that this lesion is directly related to isoprene exposures; however, the dose-response curve for spinal-cord degeneration in recovered mice is difficult to understand in terms of isoprene exposures. The situation is confounded because loss of hind-limb grip strength *was* present in mice exposed at only 220 ppm immediately after the 26-w exposures were completed; however, the grip strengths apparently returned to control values during the first few weeks of the recovery period, even as spinal-cord degeneration *was* developing.

A similar uncoupling of functional and morphological evidence of neurotoxicity has been recently reported in rats given ip injections of acrylamide (Crofton et al., 1996). Male Long-Evans hooded rats given acrylamide (20 mg/kg/d) for 30 d showed a 50% loss of grip strength, which was recovered to near normal 30-60 d after the exposures ended. Rats given a slightly lower dose (15 mg/kg/d) for 30 d did not show axonal degeneration at the end of exposure; however, 28 d after dosing ended, moderate degeneration (1-5% of the fibers degenerated) was found in the sciatic nerve and spinal cord. The authors attributed that degeneration to a "delay between initiation of fiber breakdown and the histological degeneration at the level of the sciatic nerve."

TABLE 4-3 Incidences of Lesions in Mice Exposed for 26 W in the Melnick et al. (1994) Study

	26-w Recovery	Exposure Concentration, ppm					
Lesion	0	70	220	700	2200	7000	
Olfactory	Yes	1/30	2/30	5/29[a]	11/30[a]	25/30[a]	28/28[a]
epithelial degeneration	No	0/10	1/10	0/10	1/10	1/10	10/10[a]
Spinal-cord	Yes	4/30	20/30[a]	19/29[a]	28/30[a]	17/29[a]	13/28[a]
degeneration	No	0/10	0/10	0/10	0/10	1/10	10/10[a]

[a] Incidence was statistically greater than in controls.

Unfortunately, very recent data on $B6C3F_1$ mice exposed up to 80 w to isoprene at relatively high concentrations did not confirm the findings of neurotoxicity, even after considerable recovery times (Placke et al. 1996). Mice exposed at 2200 ppm (8 h/d, 5 d/w) for 40 or 80 w with recovery periods of 56 w or 16 w, respectively, exhibited no apparent effects on motor function and showed no exposure-related lesions in the spinal cord (Placke et al. 1996).

Carcinogenicity

The IARC evaluation of the carcinogenesis data on isoprene concluded that there was inadequate evidence in humans and sufficient evidence in experimental animals that isoprene is carcinogenic (IARC 1994). The compound was placed in category 2B, meaning that isoprene is possibly carcinogenic to humans. There is weak evidence of carcinogenicity in rats and convincing evidence in mice after 26 w of exposure and a 26-w recovery period. The pertinent data are in Table 4-4 (Melnick et al. 1994).

The IARC noted that the exposure duration of only 26 w was too short to evaluate carcinogenicity adequately in rats; however, interstitial-cell tumors were also noted to have a high spontaneous incidence in F344 rats (IARC 1994). The mouse data clearly show the carcinogenic potential of isoprene, although the dose-response relationship appears flat for liver and harderiangland tumors induced at concentrations above 220 ppm.

The weight of evidence supports the hypothesis that isoprene induces cancer by mechanisms that are threshold in nature. The evidence consists of genotoxicity data, reports that isoprene is endogenously produced, and tumor-incidence data (Table 4-4). The genotoxicity data consist of observations suggesting that isoprene exhibits activity most consistent with aneugenic activity rather than clastogenic activity. Specifically, isoprene induces an increase in micronuclei in polychromatic erythrocytes without altering the frequency of gene mutations (Tice et al. 1988). This observation can be readily explained by postulating that isoprene is an aneugen, which exerts its effect by interfering with microtubule proteins in the spindle formed during cell division so that a whole chromosome is left behind in the cytoplasm to form a micronucleus. A threshold occurs because many microtubles would have to be disrupted before chromosomal separation is disrupted. On the other hand, a leveling off would be expected at higher concentrations as the cells become incapable of division because spindle function is nearly eliminated. The data in Table 4-4 show the kind of leveling off that would be expected from an aneugen. Finally, the endogenous production of isoprene (Hartmann and Kessler 1990) is more consistent with a carcinogenic mechanism based on a threshold, presumably one above the endogenous

TABLE 4-4 Neoplasms Induced in Mice by Exposure to Isoprene for 26 W Followed by 26 W of Recovery

		Exposure Concentration, ppm					
Species	Tumor	0	70	220	700	2200	7000
Male rat	Testis interstitial cell adenoma	3/30	3/30	4/30	7/30	8/30	9/30
Male mouse	Liver adenoma or carcinoma	7/30	3/30	7/29	15/30[a]	18/30	17/28
	Lung adenoma or carcinoma	2/30	2/30	1/29	5/30	10/30[a]	9/28
	Forestomach papilloma or carcinoma	0/30	0/30	0/29	1/30	4/30	6/28[a]
	Harderian-gland adenoma	2/30	6/30	4/29	14/30[a]	13/30	12/28

[a] Statistically significant at and above this concentration.

production rate, than a model that postulates finite risk even with a single molecular "hit."

A recently reported chronic inhalation exposure of male B6C3F$_1$ mice to isoprene strongly supports the hypothesis that isoprene is a threshold-type carcinogen. Groups of 50 male mice were exposed to isoprene for 20, 40 or 80 w (4 or 8 h/d, 5 d/w) at concentrations of 0, 10, 70, 140, 280, 700, and 2200 ppm (Placke et al. 1996). Only selected combinations of the exposure characteristics were tested. Groups of 50 female mice were exposed at 0, 10 or 70 ppm, 8 h/d for 80 w. An increased incidence of tumors was found in various sites; the lowest-observed-adverse-effect levels (LOAELs) were dependent on the site and sex of the group of mice. Exposures at 10 ppm for 80 w were NOAELs in both sexes, but 70 ppm appeared to be a LOAEL for certain tumors (harderian gland in both sexes and possibly pituitary adenomas in females). Placke et al. (1996) found that the traditional multistage risk model does not adequately predict the changes in tumor incidence when concentrations and cumulative exposure times are halved or doubled. For example, the authors concluded that "a threshold effect level and strong nonlinearities with respect to concentration appeared to exist for tumor development." Statistical analysis of the tumor incidence data to test the applicability of the equivalent-dose-metric hypothesis indicated that that concept was not applicable for isoprene carcinogenesis in mice (Cox et al. 1996)

Genotoxicity

The data base of genotoxicity on isoprene includes results of mutagenicity testing in *Salmonella*, demonstration of mutations in the *ras* proto-oncogene from isoprene-induced harderian-gland tumors in mice, and cytogenetic studies in inhalation-exposed mice.

On the basis of results from several laboratories, isoprene does not appear to be mutagenic in *Salmonella* tester strains, even after activation by rat-liver microsomes (de Meester et al. 1981; Gervasi et al. 1985; Kushi et al. 1985; Mortelmans et al. 1986). In testing of isoprene metabolites, the main metabolite of isoprene (compound 2, Figure 4-1) was not mutagenic or alkylating (Gervasi and Longo 1990). Likewise, the minor metabolite (compound 1) was not mutagenic or alkylating; however, the diepoxide (compound 5) was found to be both mutagenic and alkylating (Gervasi and Longo 1990).

Hong et al. (1995) have reported in an abstract that the harderian-gland tumors induced in B6C3F$_1$ mice by isoprene exposures at 2200 and 7000 ppm for 26 w, with a 26-w recovery (Melnick et al. 1994), showed mutations in K- and H-*ras* proto-oncogenes. These mutations were detected at a high frequency

(100%) in the isoprene-induced tumors but were not detected in tumors that appeared in controls. The predominant mutations were A to T transversions at K-*ras* codon 61 (15/30) and C to A transversions at H-*ras* codon 61 (8/30). The authors concluded that activation of *ras* proto-oncogenes contributes to induction of harderian-gland tumors by isoprene (Hong et al. 1995).

The results of cytogenetic studies of inhaled isoprene in mice, completed before any carcinogenesis studies had been started, led to the prediction that isoprene would induce tumors at multiple sites in mice (Tice et al. 1988). Male $B6C3F_1$ mice were exposed to isoprene at concentrations of 0, 438, 1750, and 7000 ppm, 6 h/d for 12 d. Isoprene induced an increase in sister chromatid exchanges (SCE) in bone-marrow cells and in the level of micronucleated polychromatic erythrocytes (PCE) (Tice et al. 1988). The exposure did not alter the frequency of chromosomal aberrations or mitotic index in bonemarrow cells. Shelby and Witt (1995) point out that it is unusual for a compound to be positive in a micronucleus test and negative in a chromosomal aberration test; however, the data used to make their observation (Tice et al., 1988) were obtained before application of the centromere fluorescent-probe technique, which facilitates detection of whole chromosomes in micronuclei caused by aneugens. Recent comparisons of aneugens and clastogens and their ability to produce micronuclei without chromosomal aberrations (Elhajouji et al. 1995) suggest that isoprene could be more of an aneugen than clastogen. Carcinogenic activity caused by an aneugen could have a low-concentration threshold for cancer induction and might also show a plateau at high concentrations where spindle destruction occurs.

Developmental Toxicity

An inhalation developmental study has been reported in Swiss (CD-1) mice and Sprague-Dawley rats exposed to isoprene at 0, 280, 1400, or 7000 ppm for 6 h/d, 7 d/w on gestational days 6-17 (mice) or 6-19 (rats) (Mast et al. 1989). In rats, there were no effects on body-weight gains, reproductive indices, or fetal malformations; however, an increased incidence of reduced vertebral ossifications in the 7000-ppm group suggests that this concentration might be the threshold for developmental toxicity. In mice, the body weights and uterine weights were reduced at 7000 ppm. Fetal weights were subnormal in females from the 280-ppm group and in males from the 1400-ppm group. An increase in supernumerary ribs in mouse fetuses from the 7000-ppm group was reported. The authors concluded that 1400 ppm is a NOAEL for rat maternal and developmental toxicity and mouse maternal toxicity. The NOAEL for mouse developmental toxicity cannot be assigned from the results (Mast et al. 1990).

Reproductive Toxicity

Indirect evidence in rats includes results from exposures up to 2200 ppm for 26 w in which no abnormalities were found (Melnick et al. 1994). At 7000 ppm, interstitial-cell hyperplasia of testis and adenoma were seen in rats after recovery, and testicular atrophy was found in mice after recovery (Melnick et al. 1994). Testicular atrophy was also noted in mice exposed for only 2 w to isoprene at 7000 ppm (Melnick et al. 1990). A functional reproductive study has not been reported.

Interactions with Other Chemicals

No reports of interactions of isoprene with other chemicals were found. A summary of the toxicity data on isoprene is presented in Table 4-5.

TABLE 4-5 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
Unknown	Industrial, 1 y	Human (n = 148)	Immunological response to vaccination was different from control response	Nikul'tseva 1967
Unknown + other compounds	4 y	Human (n = 630)	Inflammation and degeneration of nasal mucosa	Mitin 1969
Up to 26 + other hydrocarb.	8 h	Human (n = 300)	20% slower photogenic reflex response at end of work day; cardiovascular changes	Pigolev 1971
57	Few min	Human (n = 10)	Slight mucosal irritation	Gostinskii 1965
0.035 or 0.36	4 h/d, 30 d	Rat (M)	Variations in thymus cellularity and mitotic index.	Mamedov 1979
0.042	4 h/d, 4 mo	Rat (M)	Reversible variations in thymus weight, cellularity, and mitotic index	Mamedov 1979
0.3 or 0.8	4 h	Rat (M)	Increased thymus mitotic index and peripheral lymphocyte count (1 d post-exposure)	Mamedov 1979
10	8 h/d, 5 d/w, 80 w	Mouse (M, F)	NOAEL for increased incidence of neoplasms	Placke et al. 1996
70	8 h/d, 5 d/w, 80 w	Mouse (M, F)	LOAEL for harderian-gland tumors, NOAEL for lung, liver, and forestomach tumors	Placke et al. 1996
70	6 h/d, 5 d/w, 26 w, and recovery	Mouse (M) Mouse (M)	20 of 30 with spinal-cord degeneration, 4 of 30 in controls NOAEL for neoplastic lesions	Melnick et al. 1994
220	6 h/d, 5 d/w, 26 w, and recovery	Mouse (M)	Olfactory epithelial degeneration, reversible decrease in hind-limb grip strength	Melnick et al. 1994
220	6 h/d, 24 d	Mouse (F) Mouse (M)	Decreased RBC count, hemoglobin, hematocrit Decreased RBC volume	Melnick et al. 1994

TABLE 4-5 *(Continued)*

Concentration, ppm	Exposure Duration	Species	Effects	Reference
438	6 h/d, 5 d/w, 2 w	Mouse (M) Mouse (F)	Vacuolized cytoplasm in hepatocytes, Forestomach epithelial hyperplasia	Melnick et al. 1990
540	40 min	Rabbit	Hastened and weakened flexor reflex contractions	Gostinskii 1965
700	6 h/d, 5 d/w, 26 w, and recovery	Mouse (M)	Liver tumors, forestomach epithelial, hyperplasia, harderian-gland adenoma	Melnick et al. 1994
700	6 h/d, 5 d/w, 13 w	Mouse (M, F)	Forestomach epithelial hyperplasia	Melnick et al. 1994
790	40 min	Mouse	Decreased ability of CNS to summate subthreshold impulses	Gostinskii 1965
790 to 1750	4 h/d, 4-5 mo	Rabbit Rat Mouse	Increased leukocyte count Decreased RBC count, bronchial irritation, hepatocyte degeneration, myocardial changes, thyroid irritation 50% decrease in swim time	Gostinskii 1965
1670	6 h/d, 15 d	Rat	No toxic signs, no gross organ damage	Gage 1970
6000	6 h/d, 6 d	Rat	No toxic signs, lung slightly congested, other normal	Gage 1970
7000	6 h/d, 26 w	Rat (M) Rat (F)	Interstitial cell hyperplasia of testis (no recovery) and adenoma (after 26-w recovery) NOAEL	Melnick et al. 1994
36-43,000	Unknown	Mouse	Deep narcosis	von Oettingen 1940
39,000	2 h +?	Mouse	Collapse in 50	Gostinskii 1965
51,000	2 h +?	Mouse	LC_{50}	Gostinskii 1965
57,000	2 h	Mouse	LC_{50}	Shugaev 1968
64,000	4 h	Rat	LC_{50}	Shugaev 1968

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 4-6 presents exposure limits for isoprene set by other organizations or countries and Table 4-7 presents the SMACs established by NASA.

In many respects, the toxicity data base on isoprene is useful in setting SMACs; however, there are several observations that are perplexing and confound the process of setting SMACs. The most obvious question is why mice are approximately 100-fold more susceptible than rats. Rats show virtually no effects (with the possible exception of testicular adenoma), even after long-term exposures at 7000 ppm, whereas exposures as low as 70 ppm are needed before most adverse effects disappear in mice. There are no data to suggest which species is the best model for humans; hence, the most sensitive species, mice, will be used to set limits (NRC 1992). There are several reports from Russian literature, but the results are difficult to reconcile with more recent U.S. studies and with the levels of endogenous isoprene present in rodents. Finally, the carcinogenesis data are perplexing in that there seems to be a threshold effect rather than an increasing incidence of tumors with increasing doses; therefore, the linearized multistage model, which is ordinarily used to estimate cancer risks, will not be applied.

TABLE 4-6 Exposure Limit Set by Other Organizations or Countries

Organization or Country	Exposure Limit, ppm	Reference
Russia	14 (STEL)	IARC 1994

STEL, short-term exposure limit.

TABLE 4-7 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	50	140	Mucosal irritation
24 h	25	70	Mucosal irritation
7 d[a]	2	6	Anemia, mucosal irritation
30 d	2	6	Anemia, mucosal irritation
180 d	1	3	Anemia, respiratory-system injury, neurotoxicity

[a] Previous 7-d SMAC = 200 ppm (560 mg/m^3).

Perspective Provided by Comparison to 1,3-Butadiene

It is tempting to evoke analogies between isoprene and 1,3-butadiene to explain some of the important aspects of isoprene toxicity. On the other hand, one must remain aware of important differences in the metabolism, genotoxicity, carcinogenicity, and neurotoxicity of the compounds. In addition, isoprene is endogeneously produced in humans and rodents, but butadiene is not endogenously produced. Both compounds are metabolized to mutagenic compounds; however, metabolic saturation of activation pathways might limit the ability of isoprene to achieve the same levels of mutagenic epoxides as butadiene does at lower concentrations (Melnick et al, 1994). Butadiene appears to be a much more potent genotoxin in mice than isoprene (Tice 1988; Shelby 1990). Both induce cancer in multiple organs; however, isoprene does not produce an increased incidence of lymphomas in mice as butadiene does (Melnick et al., 1994). Furthermore, repeated exposure to isoprene is neurotoxic to mice (Melnick et al. 1994), but butadiene is not neurotoxic in similar experiments.

Short-Term Limits (1-h or 24-h Exposure)

Acute Neurotoxicity

The reports of lethality or deep narcosis as a result of exposures near or above 40,000 ppm are not useful for setting short-term limits. Gostinskii (1965) reported that mice exposed for only 40 min to isoprene at 2200 mg/m^3 (790 ppm) had changes in their ability to summate subthreshold impulses, and rabbits, after a 40-min exposure at 4100 mg/m^3 (1500 ppm), had changed flexor reflexes. No details of the test method, control method, or statistical analyses were given, so these reported findings are not suitable for setting limits for acute neurotoxicity.

Mucosal Irritation

In the Gostinskii report, 10 human subjects experienced slight mucosal irritation when exposed to isoprene at 160 mg/m^3 (57 ppm) for an unspecified period of time. That suggests that a 1-h AC for exposure to isoprene should be 50 ppm to produce no more than slight irritation; however, a lower concentration might be necessary for a 24-h exposure. A factor of 2 was applied to give a 24-h AC of 25 ppm to prevent all but minimal irritation.

Lesions of the Forestomach

Additional data come from the 2-w exposure of rats and mice to concentrations ranging from 440 to 7000 ppm (Melnick et al. 1990). The exposures were 6 h/d for 10 d for a total of 60 h. No exposure-related changes were found in rats; however, mice showed forestomach epithelial degeneration. In contrast, mice exposed to isoprene for 13 w at 220 ppm did not show either of these types of lesions (the 700-ppm group did, however), which suggests a thresholdtype effect with a NOAEL at 220 ppm. The AC for a 24-h exposure was calculated as follows:

24-h AC = 220 ppm × 1/10 = 22 ppm.

NOAEL Species

These data are not suitable for estimating a 1-h limit. Neither type of lesion (liver or forestomach) should be increased in risk because of spaceflight factors.

Long-Term Exposure Limits (7- to 180-d Exposures)

Mucosal Irritation

The NOAEL for mucosal irritation can be conservatively estimated to be 10 fold lower than the 57-ppm short-term LOAEL in humans reported by Gostinskii (1965), or 6 ppm. Time factors were not applied, because mucosal irritation generally is experienced only in the first few minutes of exposure until adaptation occurs, and the 10-fold factor applied to the LOAEL is conservative.

Neurotoxicity

Physiological and histopathological data in mice exposed to isoprene for 26 w suggest that the nervous system is a target organ (Melnick et al. 1994). At the end of 26 w of exposure, hind-limb grip strength was reduced in mice exposed at 220 ppm or more when compared with controls; however, within 4 w the hind-limb grip strength in exposed mice recovered to control values. That finding is perplexing in view of the appearance of spinal-cord degeneration (Table 4-3). The degeneration was not observed at the end of the 26-w

exposure except in the 7000-ppm group, whereas it was observed in all exposure groups (including the 70-ppm group) at the end of the 26-w recovery period. In addition, the dose response of the spinal-cord degeneration does not suggest a direct relationship with isoprene exposure. For example, less than half of the 7000-ppm group showed the lesion, whereas two-thirds of the 70-ppm group showed the lesion. Data on acrylamide-exposed rats tend to support the significance of this lesion (Crofton et al. 1996); however, similar lesions were not found in the same strain of mouse exposed to much higher concentrations of isoprene (Placke et al. 1996).

Because there was no logical approach to gleaning a dose-response relationship from the incidence of spinal-cord degeneration (Table 4-3), the lowest concentration of 70 ppm was taken as a NOAEL in mice, because functional deficits were not found at this concentration (Melnick et al. 1994) and because spinal-cord lesions were not reported by Placke et al. (1996) at much higher concentrations. To convert that NOAEL to a NOAEL in humans, a factor of 10 for potential species differences was applied. The human NOAEL was estimated to be 7 ppm or 2 mg/m^3. This result is from mice exposed for a total of 780 h (30 h/w × 26 w), which is slightly longer than 30 d of continuous exposure, so 7 ppm was the AC for 30 d of exposure. There are no data showing that the neurotoxicity would not be more severe with prolonged exposure, so the default approach using Haber's rule was applied for the 180-d AC.

180-d AC = 7 ppm × (30/180) = 1 ppm.

According to the NRC guidelines, the 7-d AC for neurotoxicity should not be increased from the 30-d AC without evidence that the effect would be reduced with shorter exposure. There was no such evidence, so the 7-d AC was set at 7 ppm.

Spaceflight is not known to cause nervous-system effects that could interact with isoprene-induced neurotoxicity to increase the risk of an effect.

Hematological Changes

Chronic exposure of mice to isoprene up to 2200 ppm resulted in chronic myeloid hyperplasia of the bone marrow without any effect on RBC measures (Placke et al. 1996); however, Melnick et al. (1994) described an anemia in mice resulting from isoprene exposures that was initially (3 d) normocytic but converted to macrocytic anemia later (24 d). These anemias were evident not only after 3 or 24 d but also after 13 w or 26 w in exposed mice. On the basis

of the 24-d data, the 70-ppm group was not adversely affected, and the anemia did not appear to become worse with prolonged exposures. The ACs to protect against hematological effects were calculated as follows:

7-d, 30-d, 180-d AC = 70 ppm × 1/10 × 1/3 = 2.3 ppm.

The ACs are independent of time of exposure; however, a spaceflight factor of 3 was applied because of the loss of RBC mass in crew members during spaceflight.

Liver Effects and Tumors

Cytoplasmic vacuolization of hepatocytes was reported in male mice exposed for 2 w to isoprene concentrations as low as 440 ppm (Table 4-1); however, the same investigators reported no such effects in the same strain of male mice exposed at 700 ppm for 13 w (Table 4-2). These effects were not mentioned in male mice exposed for 26 w and recovered for 26 w; however, liver tumors were significantly increased in exposed mice when compared with control mice at exposures of 700 ppm or more (Table 4-4). The NOAEL for such tumors was 220 ppm. The hepatocyte vacuolization was considered an adaptive rather than adverse effect. Liver tumors were no more sensitive as a neoplastic end point than harderian-gland tumors, and the incidence of liver tumors in unexposed mice was so high (11/50) that a separate risk assessment on hepatocellular tumors was not attempted. The discussion below on harderian-gland tumors would apply to liver tumors as well.

The NOAELs for harderian-gland tumors were 220 ppm for 26 w of exposure (Melnick et al. 1994) and 70 ppm for 80 w of exposure (Placke et al. 1996). Because the weight of evidence suggests that isoprene is a threshold-type carcinogen, the ACs for cancer will be estimated on that basis. The mice in the Melnick study were exposed for 30 h/w × 26 w = 780 h, which is approximately equivalent to 30 d of continuous exposure. The mice in the Placke study were exposed for 40 h/w × 80 w = 3200 h, which is roughly 180 d of continuous exposure. Applying a species factor of 10 gives the following estimates:

7 and 30-d AC = 22 ppm.

180-AC = 7 ppm.

Isoprene exposures at those concentrations are not expected to present any risk

of inducing cancer in crew members. Spaceflight would not be expected to increase the risk of isoprene-induced cancer.

Respiratory-System Injury

Olfactory degeneration and lung tumors have been associated with isoprene exposures in male mice (Tables 4-1, 4-2, 4-3, and 4-4). Again, the data are perplexing because the lowest concentration that induced olfactory degeneration for 2-w exposures was between 1750 and 3500 ppm (Table 1-1), whereas for 13-w and 26-w (no recovery) exposures, the lowest effect level was 7000 ppm (Tables 4-2 and 4-3). The incidence of lung tumors was significantly above controls in the mice exposed for 26 w (with recovery) at concentrations of 2200 ppm and 7000 ppm (Table 4-4). The 2-w data, because of the short cummulative exposure time of only 60 h, were not used to set ACs. From the longer-term data, it is apparent that 70 ppm is a NOAEL for respiratory-system injury (olfactory epithelial degeneration, Table 4-3). Using a species factor of 10 for all ACs and a time factor for the 180-d AC, the ACs were as follows:

7-d AC = 70 ppm × 1/10 = 7 ppm.

30-d AC = 70 ppm × 1/10 =7 ppm.

180-d AC =70 ppm × 1/10 × 780 h/4320 h = 1.3 ppm.

The risk of respiratory-system injury should not increase as a result of changes associated with spaceflight.

Forestomach Epithelial Hyperplasia

Although the 2-w exposures (Table 4-1) suggest hyperplasia at concentrations of isoprene as low as 440 ppm, later data (Tables 4-2 and 4-3) suggest that the effect does not occur until mice are exposed at 700 ppm for 13 or 26 w (26 w recovery). Apparently, 220 ppm is a NOAEL, and the effect is independent of time of exposure (threshold-type effect). A significant increase in fore-stomach tumors was noted in mice exposed for 26 w at 7000 ppm; however, setting the AC to protect against hyperplasia will also protect against tumors. Using a species factor of 10 and no time factors, the ACs were as follows:

7-d, 30-d, 180-d ACs = 220 ppm × 1/10 = 22 ppm.

The risk of forestomach lesions should not be affected by spaceflight.

Testicular Injury

Exposures of mice to isoprene for 2 w or 26 w resulted in testicular atrophy at 7000 ppm but not at 3500 ppm (Table 4-1; Melnick et al. 1994). Male rats exposed for 26 w with a 26-w recovery showed a gradually increasing incidence of testicular tumors (Table 4-4) and interstitial-cell hyperplasia after 26-w exposures at 7000 ppm without recovery (Melnick et al. 1994). Apparently, 220 ppm is a NOAEL for these effects; hence, with a species factor of 10 and no time factor, the ACs were as follows:

7-d, 30-d, 180-d ACs = 220 ppm × 1/10 = 22 ppm.

The study from which this calculation was made was not the usual 2-y bioassay; therefore, no-effect concentrations estimated from the findings are subject to the caveat that a longer study could have resulted in lower no-effect concentrations. The 80-w study reported by Placke et al. (1996) reported adverse effects on testes of mice; however, details on the incidence were not provided. For those reasons, the AC analysis is not presented in Table 4-8. The risk of this injury would not be increased as a result of spaceflight.

Endogenous Production of Isoprene

For systemic effects such as neurotoxicity, it would be illogical to set an acceptable exposure concentration for isoprene that would lead to an uptake rate much below the endogenous production rate. According to an abstract, the endogenous production rate for isoprene in humans is 0.15 μmol/(kg•h) or 10 μg/(kg•h) (Hartmann and Kessler 1990). For a 70-kg person, that is equivalent to 0.71 mg/h. This is about half the rate of 24 μmol/h (1.6 mg/h), which was estimated by Filser et al. (1996). The latter rate will be used in the calculations below because it is more reliably reported.

The uptake of a pollutant from air is the product of the uptake percentage, the concentration in the air, and the hourly ventilation. Assuming an uptake of 50% and a ventilation rate of 0.6 M^3/h (15 M^3/d), the airborne concentration to match endogenous production would need to be 1.6/(0.5 × 0.6) = 5 mg/M^3, which is equivalent to 2 ppm. This lower concentration bound for isoprene-induced systemic effects is comparable to the lowest longer-term ACs for isoprene-induced toxic effects (Table 4-8). This comparison suggests that the limits set for isoprene are fully protective.

TABLE 4-8 Acceptable Concentrations

End Point, Exposure Data, Reference		Uncertainty Factors			Acceptable Concentrations, ppm					
Species	NOAEL	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d	
Mucosal irritation	Human	—[a]	1-2	1	1	50	25	—	—	—
Slight irritation at 57 ppm; LOAEL, 57 ppm (Gostinskii 1965)	10	1	1	1	—	—	6	6	6	
Neurotoxicity	Mice	—	1 HR[b]	10	1	—	—	7	7	1
NOAEL, 70 ppm for functional deficit, 26-w exposure (Melnick et al. 1994)										
Hematological changes	Mice	—	1	10	3	—	—	2	2	2
NOAEL, 70 ppm for anemia, 26-w exposure (Melnick et al. 1994)										
Cancer										
NOAEL, 220 ppm 780-h exposure (Melnick et al. 1994)	Mice	—	1	10	1	—	—	22	22	—
NOAEL, 70 ppm; 3200-h exposure (Placke et al. 1996)	Mice	—	1	10	1	—	—	—	—	7
Respiratory system injury	Mice	—	1 HR	10	1	—	—	7	7	1
NOAEL, 70 ppm; olfactory degeneration tumors, 26-w exposure (Melnick et al. 1994)										

End Point, Exposure Data, Reference		Uncertainty Factors			Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d
Forestomach epithelial lesions	Mice	1	10	1	—	—	22	22	22
NOAEL, 220 ppm 26-w exposure (Melnick et al. 1994)									
SMACs					**50**	**25**	**2**	**2**	**1**

[a]—, not applicable.
[b]HR, Haber's rule.

RECOMMENDATIONS

The isoprene exposure limits proposed may be extremely conservative because they were based on the most susceptible species, mice. Rats are approximately 100-fold less susceptible than mice. Human metabolic and tissue studies are needed to determine which of the rodent species is the best model. In particular, these studies should address the formation of the diepoxide in both human tissues and in test subjects. Better data are needed on the effects of acute exposures to moderate concentrations of isoprene.

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B5 METHYLHYDRAZINE

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PHYSICAL AND CHEMICAL PROPERTIES

Methylhydrazine (MH) is a clear, colorless, hygroscopic, flammable liquid with an ammonia-like odor (ACGIH 1991). It is a strong reducing agent; its vapor is extremely reactive and undergoes rapid autoxidation in air (NAS 1974).

Formula:	$CH_3HN\text{-}NH_2$
CAS no.:	60-34-4
RTECS no.:	MV5600000
Synonyms:	Hydrazomethane, 1-Methylhydrazine, Monomethylhydrazine.
Molecular weight:	46.09
Boiling point:	87.8°C
Melting point:	-20.9°C
Liquid density at 25°C:	0.874
Vapor density:	1.6
Vapor pressure:	49.63 mmHg at 25°C (36 torr at 20°C) Slightly soluble in water; soluble in
Solubility:	alcohol, ether and hydrocarbons 1-3 ppm (Jacobson et al. 1955)
Odor threshold:	
Conversion factors	1 ppm = 1.88 mg/m^3 and
at 25°C, 1 atm:	1 mg/m^3 = 0.53 ppm

OCCURRENCE AND USE

MH is used as a solvent, as an organic intermediate, and as a rocket propellant, either singly, or mixed with other hydrazines. It is extremely flammable, but if kept out of contact with air, it is stable up to its boiling point. No MH should be present in the spacecraft cabin atmosphere unless it is introduced as an undetected contaminant on a crew member's spacesuit upon return from extravehicular activity. The amount that may be introduced in that manner is difficult to predict but, given the procedural safeguards currently in use, the amount should be very small. An accident possibly involving entry of MH or nitrogen tetroxide or both into a spacecraft occurred during the descent of an Apollo capsule in 1975 and lasted 8-10 min until the hatch was opened. The crew experienced mild-to-moderate, but spontaneously reversible, pulmonary function deficits (DeJournette 1977).

TOXICOKINETICS

Absorption

No data were found on the toxicokinetics of absorption of inhaled MH vapor by humans or animals. Liquid MH applied to the skin of anesthetized dogs was rapidly absorbed into the bloodstream and could be detected in plasma of blood samples from the femoral artery within 30 sec after application to the shaved chest (Smith and Clark 1969). The plasma level peaked around 60 min (for doses <3 mmol/kg) and decreased slowly thereafter. As the dose increased, the time required to reach peak blood concentrations increased and the rate of subsequent decline decreased until, with doses of 4-6 mmol/kg, no peak was reached in the 6-h observation period. Concentrations of methemoglobin peaked at about 2 h after application of MH to the skin.

Distribution

No data were found on the distribution of inhaled MH vapor by humans or animals. After intraperitoneal (ip) injections of 22 mg/kg of ^{14}C-MH in mice, 15 mg/kg in rats, and 10 mg/kg in monkeys and dogs, the highest concentrations of ^{14}C were found in liver, kidney, bladder, pancreas, and blood serum (Pinkerton et al. 1967).

Metabolism

MH is metabolized by rat liver to CO_2 and to reactive metabolites, including formaldehyde, which bind covalently to nucleic acids and proteins (Hawks and Magee 1974; Diaz Gomez and Castro 1986). In mice, rats, monkeys and dogs injected with ^{14}C-MH, approximately 50% of the total ^{14}C excretion, at all experimental times, appeared to be unchanged MH (Pinkerton et al. 1967). MH vapor is extremely reactive and will auto-oxidize on exposure to air to produce a variety of products, including primarily molecular nitrogen and methane, with traces of carbon monoxide, methanol, acetaldehyde, and various carbon or nitrogen heterocyclic compounds (Vernot et al. 1967; Haun et al. 1969). This oxidation can be catalyzed by a variety of materials including some plastics and formulations of stainless steel (Haun et al. 1969).

Excretion

The mouse, rat, and monkey excreted twice as much as the dog in the first 2 h after ip injection, but all four species excreted 25-40% of the total dose by 24 h after injection (Pinkerton et al. 1967).

TOXICITY SUMMARY

MH vapor is extremely toxic in both acute and chronic exposures. Acute exposures can produce nose and eye irritation, anemia, bilirubinemia, methemoglobinemia, vomiting, neurological effects, damage to lungs, liver, kidney, and brain, convulsions, and death. Chronic exposures can produce anemia, methemoglobinemia, liver, spleen, and kidney damage, and cancer.

Acute and Short-Term Exposures

Noninhalation Exposures

Of the methyl-substituted hydrazines, the most acutely toxic is MH (Jacobson et al. 1955), for which the LC_{50} (lethal concentration for 50% of the animals) by intravenous (iv) injection was reported to be 0.26 mmole/kg for male mongrel dogs observed for a 10-d period (Witkin 1956). Both clinically

and pathologically, the dog was much more susceptible than mice, rats, or monkeys to the toxic effects of ip injected MH, especially to severe kidney damage (Pinkerton et al. 1967). The main targets of MH toxicity are the blood (hemolytic anemia with decreased hemoglobin, red cell count, and hematocrit; reticulocytosis; methemoglobinemia; and Heinz body formation) and central nervous system (hyperactivity, tremors, and severe clonic-tonic convulsions), with kidney toxicity possibly associated with hemolytic anemia. The convulsive, toxic, and lethal effects of MH can be prevented by administration of large doses of the vitamin pyridoxine hydrochloride before or after MH (Toth and Erickson 1977). MH administered iv to dogs acts as a weak diuretic by an unknown mechanism (Coe et al. 1967). Even in anesthetized dogs, MH liquid applied to the skin caused convulsions at plasma concentrations of at least 10 mg/mL, with the time of onset of convulsions varying generally with the dose applied and the plasma concentration of MH (Smith and Clark 1969).

Acute Inhalation Toxicity

MacEwen et al. (1970) exposed human subjects to MH at 90 ppm for 10 min and recorded the subjective irritancy and measurements of clinical chemistry and hematology. The subjects were given pretest physicals, which included nasal and neurological examinations, and were monitored for 60 d post-exposure. To establish a relative irritancy scale, each subject was also exposed to two concentrations of ammonia (30 ppm and 50 ppm), in random order. No changes were seen in any of 14 clinical chemistry tests despite subjective reports of a moderate to strong odor and slight moistening of the eyes and tickling of the nose. No mention was made of any nasal lesions resulting from the MH exposure. Heinz bodies appeared in 3% to 5% of red blood cells (RBCs) by the seventh-day post-exposure, began to decrease after 2 w, and were not detectable 60 d post-exposure. Heinz bodies were not accompanied by any signs of anemia or reticulocytosis. The report did not give any details regarding the method used to establish and check the accuracy of the measurement of the MH concentrations in the exposure chambers except to state that "The [MH] concentrations, which were continuously monitored, were established and stabilized in the chamber and then the subject inserted his head for 10 min and his sensations were recorded." Subsequent papers from that laboratory showed that the investigators had an appreciation for the difficulty of accurately determining MH concentrations due to humidity and reaction with various materials used in the exposure chambers. Nevertheless, it was not stated whether all of the materials used for human exposure conditions (e.g., the plastic sheet and rubber diaphragm through which the subjects inserted their

heads into the Rochester chambers, and the aircraft radio headset worn by the subjects during exposures) had been tested for their compatibility with MH and their effect on MH concentrations. It seems unlikely, however, that these factors would be sufficient to account for the differences between the MacEwen study findings and the subsequent findings of a NASA odor panel described below.

In 1975, NASA conducted an odor panel test at NASA's White Sands Testing Facility. In this study (Hoffman and Schluter 1976), designed to determine if personnel were capable of detecting the odor of MH at the current Theshold Limit Value of 0.2 ppm, 42 NASA and Lockheed Electronics employees sniffed a single 30-cc airborne bolus of MH at 0.2 ppm injected into a face mask. Approximately two-thirds of the subjects were able to detect the odor. The major complaint was a feeling of irritation comparable to inhaling something strong. Two hours after the odor test, the subjects were medically examined for any signs of injury of the nose or throat. Most employees checked showed an increased appearance of dryness, and 75% of the subjects complained of a tingling, irritating sensation of their noses after the test. Twelve of the 42 showed marked injury with clear blisters or cavitation of the mucosa, 4 of those showed signs of slight bleeding, and 3 showed white patches and had sinus congestion. There was no correlation between those signs and symptoms and the subjects' previous or recent routine exposure to MH, nor to the ability to detect the odor of MH in this study.

In evaluating the disparity between the results from the NASA study and those of the MacEwen study, the level of detail reported was a major consideration. The NASA report included a detailed description of the preparation and dilution of the MH samples and the methods used to calculate nominal and determine analytical concentrations, whereas the MacEwen report did not provide that information. A careful examination of the NASA methods revealed no problems or errors that would allow one to disregard the study or consider the results suspect.

Haun et al. (1969) studied the acute inhalation toxicity of MH in rats, mice, beagles, squirrel monkeys, and rhesus monkeys at a range of MH concentrations from 25 ppm to about 500 ppm. Squirrel monkeys were the most sensitive and rats the least sensitive to the lethal effects of MH. The cause of death after exposure to lethal concentrations of MH was attributed to CNS damage, which was commonly accompanied by pulmonary, renal, and hepatic congestion and hemorrhage, and, in dogs, bloodless spleens. The most common and persistent pathological finding, however, was renal damage, which ranged from mild swelling of the tubular epithelium to vacuolization and coagulative necrosis of the epithelial cells. As the MH concentration was increased for each series of experiments, the number and degree of toxic signs in mice and rats

increased from mild to severe, in the following sequence: (1) irritation of nose and eyes; (2) diarrhea, abnormally frequent urination, and more rapid and labored breathing; (3) increased alertness, piloerection, hyperactivity, and interrupted by periods of inactivity characterized by rigid posture and exophthalmos; and (4) tonoclonic convulsions and tremors, mucous discharge from mouth and nose and frequent biting. The last two categories of toxic signs occurred either during exposure or within a few hours post-exposure. The rodents that developed all of the toxic signs except convulsions survived, whereas those that convulsed died during or following exposure. The mice that succumbed to MH usually died immediately after a single convulsive seizure.

The general pattern of signs observed in dogs and monkeys was similar to that seen in rodents. However, some additional signs were noted in the larger animals. In the general order of occurrence during and after exposure, the signs were as follows: (1) eye irritation; (2) salivation and licking; (3) emesis (occurred earliest in dogs, but was more severe and reoccurred frequently in monkeys); (4) diarrhea, frequent urination, pupil dilation, and ataxia in dogs; (5) hyperactivity, tremors and cyanosis (dogs only), and convulsions, which did *not* inevitably lead to death; and (6) prostration and apparent unconsciousness. Convulsions were produced in rhesus and squirrel monkeys as late as 10 and 24 h after exposure, respectively, and in squirrel monkeys at all doses, but the frequency of episodes was greatest at the highest MH doses tested.

Blood was observed in the urine and feces of two dogs, one exposed to MH at 92 ppm for 60 min, the other to MH at 180 ppm for 30 min. RBC hemolysis was induced in dogs and monkeys, with moderate to severe anemia in all surviving dogs and mild to moderate hemolytic effects in all surviving rhesus monkeys. Some evidence for development of tolerance to repeated exposures was seen in rhesus monkeys, but the evidence is inconclusive.

A summary of the LC_{50} values for MH are presented in Table 5-1.

TABLE 5-1 LC50 Values for MH

Species	1-h Exposure, ppm	4-h Exposure, ppm
Squirrel monkeys	82	—
Dogs (beagles)	96	—
Mice	122	56
Rhesus monkeys	162	—
Rats	244	74
Hamsters	—	143

Subchronic and Chronic Exposures

In a study at Wright-Patterson Air Force Base, dogs, monkeys, rats and mice were exposed 6 h/d, 5 d/w for 6 mo to MH vapors at 0, 2, and 5 ppm (Haun 1970). Signs of toxicity observed at 5 ppm included photophobia and prominent nictitating membranes in dogs and rough, yellowed fur coats, lethargy, increased mortality, and enlarged spleens in mice. No signs of ocular effects were seen in dogs at 2 ppm. Rats showed dose related depressed body weight gains and splenic hemosiderosis. Dogs had black livers, dose related decreased hematocrit, hemoglobin, and RBC counts and showed Heinz body formation and abnormal bone marrows. Monkeys had similar, but less severe dose-related hematologic effects.

In another study at Wright-Patterson Air Force Base, dogs, hamsters, rats, and mice were exposed 6 h/d, 5 d/w for 1 y to MH vapors at 0, 0.02, 0.2, 2.0, and 5.0 ppm (Kroe 1971). Non-neoplastic lesions observed at statistically significant frequencies included: at 0.02 ppm, nasal inflammation and plasmacytosis of the mandibular lymph node in mice; at 0.2 ppm, liver cysts in mice and hamsters, kidney cysts and angiectasis in mice, and rhinitis and submucosal cysts of the nares in hamsters; at 2 ppm, nasal polyps, kidney interstitial fibrosis and lung atelectasis in hamsters, and, in mice, bile duct hyperplasia, hepatocyte pleomorphism, gall bladder crystals, and kidney hydronephrosis.

In further studies at Wright-Patterson Air Force Base, Darmer and MacEwen (1973) reported the results of a 90-d continuous exposure of dogs, monkeys, and rats to MH at 0.04 and 0.10 ppm. No significant hematologic or growth rate effects were reported at 0.04 ppm, but at 0.1 ppm, rat growth rate was depressed and, in dogs, increased osmotic fragility of RBCs and increases in serum phosphorus and alkaline phosphatase were noted. No effects were seen in monkeys at either tested dose. Another set of experiments showed that a single continuous 24-h exposure at 1 ppm was a no-observed-effect level (NOEL) and 2 ppm was a lowest-observed-effect level (LOEL) for hemolysis of RBCs (involving methemoglobin formation and Heinz body production) in dogs, monkeys, and rats monitored for 30 d post-exposure.

Carcinogenicity

In an Air Force study not subjected to peer review, chronic inhalation of MH at concentrations of 0, 0.02, 0.2, 2, and 5 ppm by rats, mice, and hamsters, and up to 2 ppm by beagles for 6 h/d, 5 d/w for 1 y produced significant increases in lung tumors, nasal adenomas, nasal polyps, nasal osteomas, hemangiomas,

and liver adenomas and carcinomas in female mice at 2 ppm compared with controls (Kinkead et al. 1985). Also, a significant increase in benign nasal tumors was observed in hamsters at 2 and 5 ppm compared with controls. Those effects follow the administration of toxic doses that are irritating to the sensitive nasal epithelium of the rodent over most of its lifetime. The main target organs are the liver, lungs, and the nasal epithelium (following inhalation). Rats and beagles showed no exposure-related tumors.

CDF1 mice given eight weekly doses of MH at 0.46 mg by gavage or 0.23 mg by ip injection, one dose per week, and observed for 20-24 w had no increase in the incidence of tumors compared with controls (Kelly et al. 1969).

Genotoxicity

MH does not appear to be strongly genotoxic (Brusick and Matheson 1976; Rogers and Back 1981; Rogan et al. 1982). It is not mutagenic to cultured mouse cells (Brusick and Matheson 1976; Rogers and Back 1981), nor does it cause unscheduled DNA synthesis in cultured human cells (Brusick and Matheson 1976), although it is mutagenic in the Ames bacterial assay if performed in suspension rather than in plates (Brusick and Matheson 1976; Rogan et al. 1982).

Reproductive Toxicity

Aqueous MH solutions injected ip daily for 5 d at 0.26, 0.86, or 2.6 mg/kg in 7- to 8-w-old male ICR mice and 0.215, 0.72, and 2.15 mg/kg in 10- to 12-w-old male Sprague-Dawley rats 2 d before mating with virgin females did not induce significant dominant lethality in either rats or mice (Brusick and Matheson 1976).

Developmental Toxicity

MH administered ip on d 6-15 of pregnancy was not selectively embryotoxic or teratogenic in Fischer 344 rats at doses that induced a dose-related decrease in maternal weight gain. (Keller et al. 1984).

Interaction with Other Chemicals

MH is believed to react with pyridoxal in rodent brain, producing the convulsant agent pyridoxal methylhydrazone and inhibiting the activity of pyridoxal

dependent enzymes such as DOPA decarboxylase and glutamic acid decarboxylase (Furst et al. 1969). Those enzymes are essential for the formation and metabolism of a number of brain biogenic amines like seritonin, norepinephrine, dopamine and GABA (Furst et al. 1969). The development of convulsions after exposure to MH can be reduced or prevented by ip administration of large doses of vitamin B6 (pyridoxine hydrochloride) (Toth and Erickson 1977).

A summary of the toxicity data on MH is presented in Table 5-2.

TABLE 5-2 Inhalation Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
0.2	30 cc, 1 sniff	Human	Nasal injury	Hoffman et al. 1976
90	10 min	Human	Heinz bodies	MacEwen et al. 1970
0.02	6 h/d, 5 d/w, 1 y	Mouse	Nasal inflammation and plasmacytosis of the mandibular lymph node	Kroe 1971
0.04	90 d, continuous	Rat, dog, monkey	NOAEL for all signs	Darmer and MacEwen 1973
0.1	90 d, continuous	Dog	Hemolytic effects; liver pathology	Darmer and MacEwen 1973
0.1	90 d, continuous	Monkey	NOAEL	Darmer and MacEwen 1973
0.1	90 d, continuous	Rat	Depressed growth rate	Darmer and MacEwen 1973
0.2	6 h/d, 5 d/w, 1 y	Mouse	Kidney cysts; angiectasis; liver cysts	Kroe 1971
0.2	6 h/d, 5 d/w, 1 y	Hamster	Submucosal cysts in nares; liver cysts	Kroe 1971
1	24 h	Dog, monkey, rat	NOEL for methemoglobin and Heinz body formation	Darmer and MacEwen 1973
2	24 h	Dog, monkey, rat	LOEL for methemoglobin and Heinz body formation	Darmer and MacEwen 1973
2	6 h/d, 5 d/w, 6 mo	Dog	NOAEL for ocular effects	Haun 1970
2	6 h/d, 5 d/w, 1 y	Mouse	Lung tumors, hemangiomas	Kinkead et al. 1985
2	6 h/d, 5 d/w, 1 y	Mouse	Bile duct hyperplasia, hydronephrosis; hepatocyte pleomorphism; gall bladder crystals	Kroe 1971
2	6 h/d, 5 d/w, 1 y	Hamster	Nasal polyps, kidney interstitial fibrosis; lung atelectasis	Kroe 1971

Concentration, ppm	Exposure Duration	Species	Effects	Reference
5	6 h/d, 5 d/w, 6 mo	Dog	Black livers, photophobia; prominent nictitating membranes; dose-related decreased hematocrit, hemoglobin, and RBC counts; Heinz bodies; abnormal bone marrows	Haun 1970
5	6 h/d, 5 d/w, 6 mo	Monkey	Decreased hematocrit, hemoglobin, and RBC counts; Heinz bodies; abnormal bone marrows (all less severe than for dogs)	Haun 1970
5	6 h/d, 5 d/w, 6 mo	Mouse	Rough, yellowed fur coats; lethargy; increased mortality; enlarged spleens	Haun 1970
5	6 h/d, 5 d/w, 6 mo	Rat	Splenic hemosiderosis; dose-related depressed body weight gains	Haun 1970
85	1 h	Monkey	Death in 2 of 4 exposed	Haun et al. 1969
130	30 min	Monkey	No deaths in 3 exposed	Haun et al. 1969

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 5-3 presents exposure limits for MH set by other organizations and Table 5-4 presents the SMACs established by NASA.

MH can induce a variety of toxic effects. The initial overt signs of acute MH toxicity in mice, rats, dogs, and monkeys include irritation of nose and eyes and methemoglobiniemia (seen as hemolytic anemia and Heinz bodies in humans, monkeys and dogs and black or mottled liver in dogs). In addition, chronic exposure to low concentrations of MH has been shown to induce methemoglobiniemia in dogs and cancer in mice and hamsters. Exposure to higher doses produces salivation, emesis, diarrhea, hyperactivity, tremors and severe tonicclonic convulsions, which precede death. To set SMAC values for MH, acceptable concentrations (ACs) were calculated for the induction of each adverse

TABLE 5-3 Exposure Limits Set by Other Organizations

Organization	Exposure Limit, ppm	Reference
ACGIH's TLV-TWA	0.01 (skin)	ACGIH 1998
NIOSH's 2-h STEL	0.04 (ceiling) (lowest detectable level for 2 h air sample)	ACGIH 1991
OSHA's PEL	0.2 (ceiling) (skin)	ACGIH 1991
NRC's 1-h SPEGL	0.24	NRC 1985
NRC's 24-h SPEGL	0.01	NRC 1985

TLV, Theshold Limit Value; TWA, time-weighted average; STEL, short-term exposure limit; PEL, permissible exposure limit; SPEGL, short-term public emergency guidance level.

TABLE 5-4 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	0.002	0.004	Nasal injury
24 h	0.002	0.004	Nasal injury
7 d[a]	0.002	0.004	Nasal injury
30 d	0.002	0.004	Nasal injury
180 d	0.002	0.004	Nasal injury

[a] Previous 7-d SMAC = 0.04 ppm (0.08 mg/m^3).

effect (nasal irritation and injury, nasal tumors, and methemoglobinemia) using the guidelines established by the NRC (1992). No ACs were calculated for effects seen only at high doses, because such effects would be prevented by protecting against the effects seen at lower doses. For each exposure time (1 h, 24 h, 7 d, 30 d, and 180 d), the lowest AC was selected as the SMAC value (Table 5-5).

Nasal Injury

The most sensitive end point for toxicity at concentrations greater than or equal to the odor threshold is nasal injury. Because the data for injury were obtained from human subjects, no species conversion is required. Nevertheless, because 75% of the subjects complained of irritating odor and 28% developed significant nasal pathology under the test conditions, the tested concentration of 0.2 ppm must be lowered to a concentration that would be anticipated to produce no adverse effects. Because there are no dose-response data for nasal injury, a safety factor of 10 was used to estimate the NOAEL. An additional safety factor of 10 is warranted to account for the fact that a single sniff caused the observed effects, whereas the AC must be set to protect during continuous, much longer term potential exposures. The resulting concentration of 0.002 ppm is less than or equal to even the 180-d ACs for all other end points. Because the end point is injury of the nasal mucosa and because no epidemiological data are available to indicate that long term exposure to sub-irritating (short-term) concentrations of MH would lead to cumulative effects, the AC for MH-induced nasal injury is set at 0.002 ppm for all exposure durations.

Carcinogenesis

The NRC (1985) used the data of Kinkead et al. (1985) as input to the multistage model of Crump and Howe (1984) to obtain a 95% lower confidence limit of 0.116 ppm for a lung tumor risk in mice of 0.01, based on a work-week exposure schedule. Using Haber's rule to convert to a continuous lifetime exposure yielded the value of 0.01 ppm corresponding to a lifetime tumor risk of 0.01.

In another document, the NRC (1992) used the model of Crump and Howe (1984) to derive an equation, simplified here for $k = 3$ stages, for estimating the constant exposure concentration, D, during the time interval between t_0 and t_1,

which gives the lifetime (t hours) excess risk equivalent to the constant lifetime dose rate, d, for a model having k stages:

$$D = \frac{dt^k}{(t-t_0)^k - (t-t_1)^k}.$$

That model assumes that the carcinogen affects only the first stage of a process having three to six stages and that the risk of carcinogenesis is a function of the age at exposure. The NRC (1992) stated that for a 3-6 stage process (with only the first stage dose-related), the worst case (highest risk) would occur with a 3-stage process, with exposure at the earliest age possible. To calculate MH AC values, k in the equation above was set to 3 and a minimum astronaut age of 30 y was assumed. ACs were calculated using a continuous lifetime exposure at 0.01 ppm for which the NRC Committee on Toxicology calculated an upper 95% risk of 0.01. Because the model is conservative, no safety factor was used to convert animal test data to human exposure limits.

Therefore, the following equation, based on Crump and Howe's multistage model with only the first stage dose-related, was used to calculate the exposure concentrations, D, which would yield a tumor risk of 10^{-4} for exposure durations of 24 h, 7 d, 30 d, and 180 d:

$$D = \frac{d \bullet (25{,}6000)^k \bullet (10^{-4} / \text{risk})}{(25{,}600 - (365 \bullet \text{age}))^k - (25{,}600 - (365 \bullet \text{age}) - t)^k},$$

where

d	is the concentration during a lifetime exposure (0.01 ppm in this case)
25,600	is the number of days in a 70-y human lifetime
k	is the number of stages in the model (three in this case)
10^{-4}	is the acceptable risk level
age	is the minimum age of an astronaut, in years (30 in this case)
t	is the exposure duration, in days (1, 7, 30, or 180)
risk	is the risk of tumor for lifetime exposure to d (10^{-2} in this case)

That equation yields values of

D_{24h}	= 2.6 ppm.
D_{7d}	= 0.37 ppm.
D_{30d}	= 0.087 ppm.
D_{180d}	= 0.015 ppm.

Microgravity-induced physiological changes are not anticipated to affect the carcinogenic potency of concentration.

Effects on Red Blood Cells

Humans exposed for 10 min to MH at 90 ppm showed no increase in methemoglobin but did have a delayed appearance of Heinz bodies in 3-5% of RBCs with no signs of anemia or reticulocytosis (Darmer and MacEwen 1973). Starting with that as a LOAEL, an AC for a 1-h exposure was calculated by applying a factor of 10 to estimate a NOAEL, using Haber's rule to extrapolate to a 1-h exposure, and applying a spaceflight factor of 3 for blood toxicants. Thus,

1-h AC = 90 ppm (LOAEL) ÷ 6 (exposure duration) ÷ 3 (spaceflight) = 5 ppm.

In continuous, 90-d low-dose inhalation exposures of rats, dogs and monkeys at 0.04 pm and 0.1 ppm, dogs showed significant decreases in hematocrit, hemoglobin levels and RBC count at 0.1 ppm but no effects at 0.04 ppm (Darmer and MacEwen 1973). According to the authors, methemoglobin formed during the exposures is rapidly converted back to oxyhemoglobin (mainly by methemoglobin reductase) during chronic exposure conditions. The methemoglobinemia reaches an equilibrium level unique to the exposure concentration and is accompanied by Heinz body formation (Darmer and MacEwen 1973). Thus, the AC value, based on the 0.04-ppm NOAEL, is not adjusted for exposure duration for durations of at least 24 h. Because dogs and humans have equivalent levels of methemoglobin reductase activity (Smith 1996), the default species extrapolation factor of 10 was not applied. A spaceflight factor of 3 for blood toxicants was applied. Thus, the calculated ACs are

24-h, 7-d, 30-d, 180-d AC = 0.04 ppm (NOAEL) ÷ 3 (spaceflight)
= 0.01 ppm.

TABLE 5-5 Acceptable Concentrations

End Point, Exposure		Uncertainty Factors				Acceptable Concentrations, ppm				
Data, Reference	Species	NOAEL	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d
Nasal injury	Human	10	10	1	1	0.002	0.002	0.002	0.002	0.002
12/42 exposed to 0.2 ppm, 30 cc, single sniff (Hoffman and Schluter 1976)										
Carcinogenesis	Mice	LMS	LMS	1	1	NS	2.6	0.37	0.087	0.015
2 ppm, 1 y, intermittent (Kinkead et al. 1985)										
Effects on RBCs (5% of red cells)	Human	—	1	1	3	5	NS	NS	NS	NS
LOAEL, 90 ppm for 10 min (MacEwen et al. 1970)										
Effects on RBCs	Dogs	1	1	1	3	NS	0.01	0.01	0.01	0.01
NOAEL, 0.04 ppm for 90 d, continuous (Darmer and MacEwen 1973)										
SMACs						**0.002**	**0.002**	**0.002**	**0.002**	**0.002**

—, not applicable.
LMS, linearized multistage model.
NS, not set.

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B6 PERFLUOROPROPANE AND OTHER ALIPHATIC PERFLUOROALKANES

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PHYSICAL AND CHEMICAL PROPERTIES

Perfluoropropane (PFA3, octafluoropropane) is gaseous at room temperature. It is colorless and odorless. Some physical characteristics are as follows:

Formula:	$CF_3CF_2CF_3$
CAS no.:	76-19-7
Synonyms:	Freon 218, FC-218, PF 5030 3M Performance Fluid
Molecular weight:	188.08
Boiling point:	–37°C
Vapor pressure:	114.8 psia at 21°C (3M 1995a)
Solubility in water:	Extremely low
Conversion factors:	1 ppm = 7.68 mg/m^3
	1 mg/m^3 = 0.13 ppm (at 25°C)

OCCURRENCE AND USE

When compressed, gaseous PFA3 is easily condensed into liquid. PFA3 is currently used as a secondary coolant in refrigerators aboard the Russian space-station Mir. According to Russian toxicologists, if all the PFA3 were to escape from the cooling system into the Mir cabin, the cabin concentration could reach 5000 mg/m^3 (G.I.Solomina and L.N.Mouakhamedieva, Institute of Biomedical Problems, Moscow, personal commun., 1996). PFA3 is not used in the U.S. space program, and to our knowledge, astronauts have not been exposed to

PFA3 in U.S. spacecraft. However, NASA has joined the Russian Space Agency in using the Mir space station. Mir-18 was the first mission that involved a U.S. astronaut living onboard the Russian spacecraft. For this mission, the cabin air samples showed that PFA3 was the trace contaminant present in the highest concentration; its concentrations in Mir ranged from 20 to 48 mg/m^3 (Limero 1995).

TOXICOKINETICS AND METABOLISM

Toxicokinetics

PFA3 is practically insoluble in water (3M 1995a). The air/blood/liver/fat partition coefficients (PCs) of PFA3 were 1/0.25/0.07/0.04 (Creech et al. 1995), as determined by the vial equilibration method of Gargas et al. (1986). For solubility comparison, the corresponding PCs of chloroform, determined by the same method, were 1/20.8/21.1/203 (Gargas et al. 1989). Those data suggest that at the same airbrone exposure concentration, the blood, liver, and fat would take up, respectively, 83, 300, and 5000 times more chloroform than PFA3 when the equilibrium is reached. Theoretical predictions showed that concentrations of very low water-soluble, volatile compounds in body water would approach steady state within 1 hof exposure (Goldstein 1974).

Metabolism

Perfluoroalkanes (PFAs) are very stable. They are not oxidized even by ozone to any appreciable extent; their atmospheric half-life greater than 5000 y (R.G. Perkins, 3M Company, personal commun., 1995). Creech et al. (1995) detected no increases in fluoride in urine of rats exposed to 1% PFA3 for 4 h.

TOXICITY SUMMARY

PFA3 is a low-molecular-weight PFA. PFAs are chemically inert; included in this family is Teflon (a polymeric, high-molecular-weight PFA). The major concern from exposure to high concentrations of gaseous PFAs is their potential for cardiac toxicity. Cardiac effects are known to occur when humans or animals are exposed to high concentrations of other fluorinated hydrocarbons (FCs), including Freons (Table 6-1). FCs, such as chlorofluorocarbons, could induce cardiac arrhythmias by sensitizing the heart to epinephrine (Aviado and

Micozzi 1981; Hanig and Herman 1991). The inertness of PFAs has attracted few attempts to investigate the toxic properties of these compounds. Only a few unpublished toxicological studies on PFA3 and other PFAs were found; none of them were conducted with human subjects. These studies revealed that PFAs are very low in toxicity (McHale 1972; 3M 1993a,b, 1995a,b,c) (Table 6-2). At very high concentrations, PFA3 could indeed induce cardiac effects (3M 1993a). However, the concentrations of PFA3 that produce cardiac effects are substantially higher than those of other halogenated compounds that are not fully fluorinated. A survey of the literature on fluorine-containing alkanes reveals that substituting fluorine for chlorine or hydrogen atoms in an FC decreases the compound's toxicity, including cardiac toxicity (Table 6-1).

CNS toxicity that could impair cognitive performance is another concern associated with exposures to high concentrations of relatively biologically inert FCs (e.g., bromotrifluoromethane (NRC 1984a)). The extremely low solubility of PFA3 in water, blood, and tissues would imply that the PFA3 concentration in the brain would be low. Any CNS toxicity in humans due to PFA3, if it occurs, would likely manifest at only very high concentrations. That is likely to be true for other PFAs also. These speculations are indeed supported by the findings that rats exposed to 80% PFA1, PFA2, or PFA3 experienced only minimal effects (initial hyperactivity followed by hypoactivity (see Table 6-2)).

TABLE 6-1 Acute Inhalation Toxicity of Selected Fluorinated Alkanes

Compound	Name[a]	Exposure, ppm × time	Toxicity in Rats	Epinephrine[b] + Exposure (ppm)	Cardiac Toxicity in Dogs	References
$CFCl_3$	FC 11	26,220 × 4 h	LC	5000	Lowest concentration that elicited a marked response	NRC 1984b
$CFCl_2H$	FC 21	50,000 × 4 h	LC	10,000	Affected 2/12 dogs	NRC 1984c
CF_2ClH	FC 22	300,000 × 2 h	LC	50,000	Effects observed	ACGIH 1991a
CF_2Cl_2	FC 12	620,000 × 3 h	LC	80,000	EC50	NRC 1984d
CF_3Br	FC1301	560,000 × 1 h	Mild-to-moderate CNS effects	200,000	EC50	McHale 1972
CF_4	FC 14 (PFA1)	780,000 × 1 h	Mild effects	600,000	Very mild effect	McHale 1972
CF_3CCl_3	FC 113	50,000 × 4 h	LC	5000	EC: 20-35%	NRC 1984e
CF_3CFCl_2	FC 114	600,000 × 2 h	LC	45,000	EC_{50}	NRC 1984f
CF_3CF_2Cl	FC 115	800,000 × 4 h	No clinic signs	150,000	Affected 1/13 dog	ACGIH 1991b
CF_3CF_3	FC 116 (PFA2)	800,000 × 1 h	Very mild CNS effects, no deaths	—	—	McHale 1972
$CF_3CF_2CF_3$	FC 218 (PFA3)	800,000 × 1 h	Very mild CNS effects, no deaths	400,000	1/8 dog: definite positive response; 1/8 dog: weak response	McHale 1972 3M 1993a
$CF_3(CF_2)_2CF_3$	(PFA4)	800,000 × 1 h	No toxic signs, no deaths	400,000	No effects	3M 1995a
$CF_3(CF_2)_3CF_3$	(PFA5)	280,000 × 4 h	No effects, no deaths	—	—	3M 1993a
$CF_3(CF_2)_4CF_3$	(PFA6)	381,000 × 1 h	No clinical signs, no pathological changes or deaths	170,000	No or very mild effects	3M 1995b

[a]PFAs are abbreviations for perfluoroalkanes used in this document.
[b]Pretreated with epinephrine before inhalation exposure to the compound.
LC, lethal concentration; EC, effect concentration; EC_{50} = concentration that produces an effect on 50% of exposed animals.

TABLE 6-2 Toxicity Summary of Perfluoroalkanes

PFAs	Exposure Concentration	Exposure Length	Animals	Effects	Reference
PFA1	80%	1 h	10 rats	Initial hyperactivity followed by hypoactivity, hyperemia	McHale 1972
	22.6%	10 d (24 h/d)	20 rats, 20 guinea pigs	No clinical signs, no macroscopic or microscopic lesions	McHale 1972
	20% or 60%	—	6 dogs/group (epinephrine-sensitized)	Occasional preventricular contractions in three of the six dogs exposed to 60%	McHale 1972
PFA2	80%	1 h	10 rats	Initial hyperactivity followed by hypoactivity, hyperemia	McHale 1972
	12.1%	10 d (24 h/d)	20 rats, 20 guinea pigs	No clinical signs, no macroscopic or microscopic lesions	McHale 1972
PFA3	80%	1 h	10 rats	Initial hyperactivity followed by hypoactivity, hyperemia	McHale 1972
	11%	4 h	10 rats	No clinical signs, no macroscopic and microscopic lesions	3M 1993a
	11.3%	10 d (24 h/d)	20 rats, 20 guinea pigs	No clinic signs, no microscopic lesions	McHale 1972
	5%, 10%, 20%, 30%, and 40%	—	6 dogs/group (epinephrine-sensitized)	1 positive cardiac response (multiple and multi-focal ectopic beats), 1 weak positive response in the eight 40%-exposed dogs; no effects at ≤30%	3M 1993a
PFA4	9.8% and 79%	4 h	10 rats/group	No clinical signs, no macroscopic or microscopic lesions	3M 1993b
	5%, 10%, 20%, 30%, or 40%	—	6-8 dogs/group (epinephrine-sensitized)	No cardiac effects	3M 1993b
PFA6	38% (saturated vapor)	1 h	Rats	No clinical signs, no deaths; necropsy showed no gross pathological changes	3M 1995b

PFAs	Exposure Concentration	Exposure Length	Animals	Effects	Reference
PFA6	30.5%	30 exposures, 7 h/d, 5 d/w	26 rats	Some clinical chemistry and hematological variables differed slightly from those of control rats, but were within biologically acceptable ranges; no macroscopic or microscopic lesions	3M 1995a
	5%	2 w (6 h/d, 5 d/w)	20 rats	No clinical signs; no macroscopic lesions	3M 1995a
	0.5%, 1.5%, or 5%	90 d (6 h/d, 5 d/w)	10 rats/group	No clinical signs; some clinical chemistry and hematological variables differed slightly from those of control rats, but were within biologically acceptable ranges; no macroscopic or microscopic lesions	3M 1995a
	5%, 10%, or 17.5%	—	6 dogs/group (epinephrine-sensitized)	No cardiac toxicity	3M 1995a

Acute Exposures

General Toxicity

McHale (1972) evaluated the acute toxicity of inhaled perfluoromethane (PFA1), perfluoroethane (PFA2), and PFA3. Three groups of male rats (10 per group) were exposed for 1 h to 80% (target concentration) of these PFAs (with 20% oxygen) (Table 6-2). Two additional groups of rats were exposed to either air (control) or 56% bromotrifluoromethane (also with 20% oxygen). Bromotrifluoromethane, a compound of known toxicity, was used for comparison. During the exposures, all animals exposed to the PFAs exhibited initial hyperactivity and subsequent hypoactivity, hyperemia (redness of skin), and closed eyes. Rats exposed to bromotrifluoromethane also exhibited initial hyperactivity and subsequent hypoactivity, but also showed increases in respiration rate, abdominal breathing, slight-to-moderate ataxia (incoordination), and a slight bluish tint to the skin. All animals seemed normal during the 14-d post-exposure observation period, and none died. A study by the 3M Company (3M 1993a) on 10 rats (5 males, 5 females) exposed to 11% PFA3 for 4 h showed neither deaths nor clinical signs. Necropsy of these animals after a 15-d observation period revealed some lung congestion in one rat. Microscopic pathological examination of lungs, liver, and kidneys showed no abnormalities in any of the PFA-exposed rats.

The higher molecular-weight PFAs also showed little or no biological activity even at high exposure concentrations. Groups of 10 rats (5 males, 5 females) were exposed to 9.8% or 79% perfluorobutane (PFA4) for 4 h or to 38.1% perfluorohexane (PFA6) for 1 h; neither deaths nor pharmacotoxic signs were observed during the exposure or during the 14-d post-exposure period. Necropsy revealed no gross pathological changes (3M 1993a, 1995c). Microscopic findings on animals exposed to PFA4 showed no abnormalities. No information was provided regarding whether tissues of rats exposed to PFA6 were examined microscopically.

Cardiac Effects

Cardiac sensitization was assessed in a study in which dogs (six to eight per group) were pretreated with epinephrine and exposed either to 5%, 10%, 20%, 30%, or 40% PFA3. One definite positive cardiac response (multiple and multifocal ectopic beats) and one questionable (weak) response were observed among the eight dogs exposed to 40% PFA3 (3M 1993a). However, exposures to PFA4 at the same concentrations produced no cardiac abnormalities (3M

1993b). No cardiac effects were observed in any of the dogs exposed to 5%, 10%, or 17.5% PFA6 after being injected with epinephrine (3M 1995c). Trichlorofluoromethane at 2%, used as a positive control, elicited 100% cardiac response in the dogs in all three 3M cardiac-sensitization studies. McHale (1972) also assessed cardiac sensitization in beagles injected intravenously with epinephrine (8 μg/kg) and exposed to 20% PFA1 (80% air) or 60% PFA1 (40% O_2). No cardiac arrhythmias were observed; the only responses were occasional preventricular contractions in three of the six dogs exposed to 60% PFA1. These results showed that PFAs have very low cardiac-sensitizing activity.

Short-Term and Subchronic Exposures

McHale (1972) conducted a 10-d continuous (24 h/d) exposure study with PFA1, PFA2, PFA3, or bromotrifluoromethane. Each exposure group consisted of 10 male and 10 female rats, and 10 male and 10 female guinea pigs. The average analytical concentrations were 20.6% (PFA1), 12.1% (PFA2), 11.3% (PFA3), and 5.1% (bromotrifluoromethane). Parameters studied included toxicity signs, clinical chemistry, hematology, gross pathology of all organs, microscopic histopathology of selected organs (lungs, liver, heart, kidneys, and spleen), organ weights, and organ-to-body-weight ratios of these organs and adrenals.

No overt signs of toxicity were present during the exposures. Clinical chemistry data were unremarkable. Hematological examinations of rats and guinea pigs revealed elevation of total leukocyte counts in some PFA-exposed groups (Table 6-3). However, all groups had pneumonitis and associated inflammation that could easily account for the mild elevation in leukocyte counts. Moreover, the elevation of leukocyte counts was not statistically significant for all three PFAs, and not considered an adverse effect of these compounds. Gross pathology showed no lesions associated with any particular group. Histopathological findings also revealed no differences between the FC-exposed animals and the controls.

Another inhalation study was conducted with two groups of rats (10 males and 10 females per group) exposed to either air or 10% PFA4 for 2 w (6 h/d; 5 d/w) (3M 1993b). A similar study was also conducted with 5% PFA6 (3M 1995c). Clinical observations, body and organ weight measurements, and gross pathological examination were conducted. No deaths or exposure-related effects were observed for either compound except for a small increase in liver weight of the female rats and in kidney weight of the male rats in the PFA6-exposed group. Microscopic examination showed no difference between the exposed and control groups. 3M also conducted an inhalation study with 26 rats (16 males, 10 females) given 30 exposures (7h/d, 5 d/w) to "near-saturated"

(30.5%) PFA6 vapor (3M 1995c). Mortality, abnormal weight patterns, and gross pathological changes were not observed. Microscopic examination of the lung and liver showed no significant histopathological changes. Blood chemistry revealed that some of the parameters in exposed animals were different from those of control animals. However, according to 3M, all of these blood results were within biologically acceptable ranges. A 90-d inhalation study (6 h/d, 5 d/w) conducted with groups of 10 rats (5 males, 5 females) exposed to 0, 5000, 15,000, or 50,000 ppm PFA6 produced no exposure-related deaths. Clinical signs were normal. Minor differences in several hematological and clinical-chemistry variables were observed, but according to 3M (1995b), the values from the exposed animals were within normal limits and were not considered toxicologically significant. Histopathological examination showed no exposure-related histological changes.

Genotoxicity

Vapors of PFA3, PFA4, PFA5, or PFA6 were tested for their potential mutagenic activity on *Salmonella typhimurium* (strains TA1535, TA1537, TA1538, TA98, and TA100) in the presence or absence of liver enzymes. The concentrations tested were 80% for PFA3 and PF4 and near-saturated vapors (> 10%) for PFA5 and PFA6. No mutagenic activity was observed (3M 1993a, b, 1995b, c). These results are not surprising given the extremely low water solubility and chemical inertness of these compounds.

EXPOSURE LIMITS

Exposure Limits Set by Other Organizations

No exposure limits have been established for any PFAs by any organization in the United States, including 3M, the manufacturer of the products. The Russian Space Agency has set a maximum allowable concentration for PFA3 of 150 mg/m^3 (G. I. Solomina and L. N. Mouakhamedieva, Institute of Biomedical Problems, Moscow, personal commun., 1996).

Nasa Spacecraft Maximum Allowable Concentrations (SMACs)

SMACs are derived in accordance with guidelines developed by the SMACs subcommittee of the Committee on Toxicology (NRC 1992). The SMACs (Table 6-3) are set by choosing the lowest values among the ACs (see Table 6-4).

TABLE 6-3 Spacecraft Maximum Allowable Concentrations (SMACs) of Perfluoropropane

Exposure Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	11,000	85,000	CNS effects
24 h	11,000	85,000	CNS effects
7 d	11,000	85,000	CNS effects
30 d	11,000	85,000	CNS effects
180 d	11,000	85,000	CNS effects

RATIONALE FOR ACCEPTABLE CONCENTRATIONS (ACS) FOR EXPOSURES

ACs Based on the CNS Effects of the Acute Exposure Studies

PFA3 is not metabolized. The brain is a richly and fast-perfused organ. Thus, the CNS effects of PFA3 would be due solely to PFA3 concentration in the brain. For a given exposure concentration, the blood concentration of PFA3 would likely approach a steady state within 60 min, and the concentration in the brain will follow the blood in a comparable time. Thus, the possible CNS effects induced by PFA3 would be independent of exposure length of ≤ 60 min. Thus, one AC value is set for all exposure durations.

McHale (1972) reported that a 1-h exposure of rats to 80% PFA3 resulted in only mild CNS responses. 3M reported that a 4-h exposure of rats to 11% PFA3 caused no clinical signs (3M 1993a). An AC of 1.1% is obtained by applying an animal-to-human extrapolation safety factor of 10 to the NOAEL of 11%.

ACs Based on Cardiac Effects

The heart, like the brain, is also a richly and fast-perfused organ. For the reasons presented above, one AC value could be set for all exposure durations. 3M (1993a) has reported that no cardiac effects were observed in epinephrine-treated dogs exposed to 30% PFA3. Therefore, using an uncertainty factor of 10 to account for interspecies variability, the AC is set at 3% (30% ÷ 10). The space factor of 5 is not used here because the epinephrine-treated dog model is a conservative test, and epinephrine is probably associated with cardiac arrhythmias observed in humans.

ACs Based on Subchronic Animal Exposure Data

McHale (1972) showed that continuous inhalation exposure of rats to 11.3% PFA3 for 10 d (24 h/d) produced neither clinical signs nor microscopic lesions. Using an uncertainty factor of 10 for interspecies variability, ACs for 7 d, 30 d, and 180 d of exposure are set at 1.1% (11.3% ÷ 10). The AC derived from various toxicity end points are summarized in Table 6-4. The SMACs are set by choosing the lowest values among these ACs.

TABLE 6-4 Acceptable Concentrations of PFA3

End Point, Exposure Data, Reference		Uncertainty Factors			Acceptable Concentrations, ppm				
Species	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d	
CNS effects	Rat	1	10	—	11,000	11,000	11,000	11,000	11,000
NOAEL, 11% (3M 1993a)									
Cardiotoxicity	Dog	1	10	1[a]	30,000	30,000	30,000	30,000	30,000
NOAEL, 30% (3M 1993a)									
Subchronic toxicity	Rat	1	10	—	NS	NS	11,000	11,000	11,000
NOAEL, 11.3% (McHale 1972)									
SMACs					**11,000**	**11,000**	**11,000**	**11,000**	**11,000**

—, not applicable.
NS, not set.
[a]See text for explanation of not using the spaceflight factor 5.

SMACs for Other Volatile Perfluoroalkanes

As discussed above, PFAs have extremely low solubility in water and are biologically inert and extremely low in toxicity. No evidence exists to suggest that increasing or decreasing the molecular weight of these compounds drastically changes their toxicity. Therefore, the SMACs for the other straight-chain PFAs are set at the same values as those of PFA3. A survey of perfluorocyclobutane toxicity indicates that this cyclic PFA is more cardiotoxic than the aliphatic PFAs discussed in this document. The generalization about the toxicity of aliphatic PFAs would not be applicable to the cyclic PFAs. Therefore, the SMACs set for PFAs in this document would not be applicable to cyclic PFAs.

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B7 POLYDIMETHYLCYCLOSILOXANES

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PHYSICAL AND CHEMICAL PROPERTIES

Name:	Hexamethylcyclo trisiloxane (HMCTS) D_3	Octamethylcyclo tetrasiloxane (OMCTS) D_4	Decamethylcyclo pentasiloxane (DMCPS) D_5
Formula:	$((CH_3)_2SiO)_3$	$((CH_3)_2SiO)_4$	$((CH_3)_2SiO)_5$
Form:	white solid	oily liquid	oily liquid
CAS no.:	541-05-9	556-67-2	541-02-6
Molecular weight:	222.46	296.64	370.80
Boiling point:	135°C	175°C	210°C
Melting point:	65°C	17.5°C	7.5°C
Spec. Gravity:	1.10	0.954 at 25°C	0.959 at 25°C
Vapor pressure:	8 mmHg at 20°C	1 mmHg at 22°C	0.2 mmHg at 25°C
Solubility	insoluble	0.05 mg/L at 25°C	0.24 mg/L at 25°C
Conversion factors:	1 ppm = 9.1 mg/M^3	12.0 mg/M^3	15.1 mg/M^3
	1 mg/M^3 = 0.110 ppm	0.0835 ppm	0.0662 ppm

OCCURRENCE AND USE

Because siloxane fluids are thought to be relatively nontoxic and are water repellent, they have found widespread use in cosmetics, creams, and antiperspirants. Concentrations of OMCTS in the sub-parts-per-billion range have been reported in indoor air (Shah and Singh 1988). During the 6 min after use of

antiperspirants the combined concentration of polydimethylcyclosiloxanes in the breathing zone of a person wearing the antiperspirant ranged from 0.3 to 2.9 ppm (Dow Corning Corporation 1989). One or more of the polydimethylcyclosiloxanes is found in most samples of space shuttle air. The combined concentrations are typically in the 0.1 to 1 mg/M^3 range; however, occasionally the concentration exceeds 1 mg/M^3 (James et al. 1994).

TOXICOKINETICS AND METABOLISM

Absorption

No data could be found on the absorption of any of the three specific siloxanes under review. In testing of three other silicone oils on five human subjects, Hobbs et al. (1972) found that dermal application of 50 mg/kg/d for 10 d did not significantly increase the blood or urinary silicon concentrations. The detection limit was 5 μg/mL.

Distribution

No data were found on the distribution of these siloxanes after administration by any route of administration.

Elimination

The elimination of OMCTS metabolites from male and female Fischer 344 (F344) rats exposed by nose-only inhalation at 700 ppm (8.4 mg/L) was reported in an abstract (Plotzke et al. 1996). Rats were exposed for 14 d (presumably 6 h/d) to the siloxane and then given a ^{14}C-OMCTS exposure on the 15th day. Immediately after exposure, the animals were placed in metabolism cages and excreta collected for 168 h. The radioactivity was recovered as follows: urine, 38%; expired volatiles, 36% (non-CO_2); feces, 18%; and CO_2, 4.5%. There was no apparent difference between the sexes of rat. Data on HMCTS and DMCPS were not found.

Metabolism

There were no peer-reviewed studies found on the metabolism of the three siloxanes under consideration; however, minimal information was found on

structurally related siloxanes. Apparently, liver enzymes can be induced in mice by oral administration of phenylheptamethylcyclotetrasiloxane (PHMCTS), suggesting that the compound might be metabolized there. Mice given PHMCTS at 100 mg/kg for 4 d showed a hexabarbital sleep time of only 8 min on d 5, compared with 34 min in controls (Bennett et al. 1972). Rats given the same dosage of PHMCTS did not show the reduced sleep time after administration of hexabarbital.

Recent investigations reported in abstract form have begun to address the metabolism of OMCTS and DMCPS. In particular, there seems to be much interest in the ability of these siloxanes to induce various enzymes in the liver as an explanation of the hepatomegaly which is seen after prolonged exposure. F344 rats exposed for 28 d (6 h/d, 5 d/w) to 8.4 mg/L OMCTS showed 14% to 20% increases in liver mass and 43% to 54% increases in total cytochrome P-450 activity (McKim et al. 1995). The activity of ethoxycoumarin-*O*-deethylase increased by over 100% in the same animals. A later abstract indicated that the types of cytochrome P-450 induced were the 2B1 and 1A1 forms (Salyers et al. 1996). That report also indicated that six metabolites of OMCTS were found in urine samples. Similarly, an abstract report described the induction of hepatic cytochrome P-450 2B1 and 1A1 in female F344 rats inhaling DMCPS for 28 d at a concentration of 160 ppm (2.4 mg/L) (McKim et al. 1996).

TOXICITY SUMMARY

The toxicity data base consists mostly of studies conducted on OMCTS because it is used often in cosmetics and other products that contact human skin. The data base demonstrates that this siloxane is of low toxicity by inhalation and other routes of administration. Data on HMCTS and DMCPS were not available in peer-reviewed reports; however, available information from abstract reports and summary documents will be given.

Acute and Short-Term Exposures

Hexamethylcyclotrisiloxane

A "toxicity profile" and a summary of a 4-w inhalation study were available for review (GE Silicones and LPT Report No. 6529/91 (Summary) provided by K. West, General Electric, unpublished information, August 8, 1996).[1] According

[1] Referred to as LPT Report throughout Chapter B7.

to these documents, HMCTS has been studied by the oral and inhalation routes. Gavage studies of rats given up to 1600 mg/kg/d for 28 d showed increased liver weights at concentrations as low as 400 mg/kg/d; however, none of the rats showed histopathological lesions associated with the hepatomegaly. In the LPT inhalation study, Sprague-Dawley rats of both sexes were exposed to actual concentrations of 0.08, 0.94, and 9.0 mg/L (nominal concentrations were 1.1, 6.9, and 34.7 mg/L), 6 h/d (7 d/w?) for 4 w. The concentrations were generated by heating HMCTS to about 85°C before mixing with air entering the nose-only chambers. The large differences in analytical and nominal concentrations were not explained in the abstract report. Between test days 13 and 16, 3/10 males and 1/10 females in the high-concentration group (9.0 mg/L) were found dead. These animals showed congestion of the kidneys, liver, and lungs (with perivascular edema). Rats in the high-concentration group showed slight inflammatory changes in the nasal cavity (microscopically) and exhibited slight narcosis after exposure. The pulmonary effects were not fully reversible during a 4-w recovery period. None of the animals showed changes in body-weight gain, clinical laboratory values, organ weights, and histopathology (adrenals, heart, kidneys, lacriminal gland, liver, pharynx, and spleen). The only effect noted in the intermediate group was slight red encrustation around the nares; however, the authors concluded that the no-effect concentration was the lowest concentration (0.08 mg/L).

Octamethylcyclotetrasiloxane

The acute effects of OMCTS have been studied by the dermal, oral, and inhalation routes of exposure. In 200 test subjects, 0.9 mL of OMCTS was applied to the upper arm on a disc for 24 h with occluded dressing. After removal of the dressing, the application site was examined, rested for 24 h (or 48 h on weekends), and then OMCTS was reapplied for a total of 15 applications (Shelanski 1974). Two of the 200 subjects showed skin changes signifying injury during applications 2 to 15. After a 2-w rest, the material was again applied to test for sensitization. There was no evidence of sensitization in the test population. Albino rats given 64 mL/kg by stomach tube showed sluggishness and labored breathing but did not die as a result of the dosage (Weil 1972). Mild growth retardation was induced in female rats given OMCTS at 2 g/kg/d for 28 d by oral gavage; however, there was no clear effect on growth in males (Manston 1988). Neither sex showed treatment-related effects during gross necropsy.

The results of several acute inhalation tests are shown in the toxicity summary table. The tests consist mostly of screening tests conducted by laboratories

supporting companies with a commercial interest in OMCTS. One human study has been reported by abstract. Twelve subjects were exposed for 1 h to OMCTS at 10 ppm (0.12 mg/L) and evaluated for immune function using a variety of end points (Looney et al. 1996). These initial studies indicated that exposure to OMCTS did not adversely affect the human immune system. In a screening test, concentrations near saturation were required to kill two of six rats during exposures of 8 h (Weil 1972). The two rats died 6 and 7 h into the exposure and showed breathing difficulties and spasms before dying. Exposures to OMCTS at near-saturation concentrations for 6 h failed to kill any of the 10 test rats, and gross pathology showed "nothing remarkable" (Myers et al. 1982). Exposures of 8 h to 42 mg/L did not cause death in any of the six test animals; however, loss of coordination and slow, irregular breathing were noted 7 h into the exposure (Clayton 1974). Although those tests are useful for screening purposes, they lack the detailed assessment of toxic effects and dose-response information necessary to facilitate their use in setting human exposure limits.

A 4-w inhalation study using Wistar rats revealed that repeated exposures to OMCTS cause few, if any, adverse effects even at high concentrations (Appleman et al. 1984). Male and female rats (10 each per group) were exposed at 0, 0.2, 1.0, and 5.1 mg/L, 6 h/d, 5 d/w for 4 w. The control and high-concentration groups were supplemented with five rats per group and sex to assess the reversibility of any adverse effects. The animals were killed 14 d after exposure ended. Observations included clinical signs, body weights, food consumption, clinical chemistry, hematology, urinalysis, gross pathology, and histopathology. The statistically significant findings were as follows:

5.1 mg/L in females:	3% reduction in weight gain after 28 d (reversible)
	16% increase in relative liver weights after 28 d
5.1 mg/L in males:	7% increase in relative liver weight after 28 d

In addition, there was a "very slight" increase in mitotic figures in the liver across several of the exposure groups as shown in Table 7-1.

After the 14-d recovery period, the increase in mitotic figures had disappeared. The authors concluded that the test material, under the conditions of the study, caused slight, reversible effects on the liver. In our opinion, the reported effects do not fit the criteria for adverse effects. A recent abstract suggests liver-weight increases in rats exposed to OMCTS (6 h/d, 5 d/w, 28 d) can occur at concentrations as low as 0.84 mg/L (McKim et al. 1995).

A second inhalation study, in which male and female mice, guinea pigs, rabbits, and hamsters were exposed to OMCTS at 8.3 mg/L, 6 h/d for 28 consecutive days has been reported (Siddiqui et al. 1989a). There were 10

animals per group and sex, except rabbits, where only 5 animals were used for each sex and group. The observations included body weights, food consumption, clinical signs, gross pathology, selected organ weights, and selected histopathology. Clinical chemistry and hematology were not reported and the chamber temperatures reported (26-27°C) were somewhat high for some species involved. The only change linked to OMCTS exposure was hepatomegaly. Relative liver weights were statistically increased as follows: male mice, 16%; female mice, 30%; and female hamsters, 16%. There were no statistically increased liver weights in the other exposure groups, nor could any histopathological changes be correlated with those groups that showed the hepatomegaly. In our opinion, the reported changes do not reflect adverse effects.

TABLE 7-1 Incidence of "Very Slight" Increases in Hepatocyte Mitotic Figures

Group	Incidence in Males	Incidence in Females
Control	0/10	0/10
0.2 mg/L	2/10	0/10
1.0 mg/L	3/10	3/10
5.1 mg/L	4/10	2/10

Decamethylcyclopentasiloxane

The acute inhalation toxicity has been evaluated in F344 rats (five males and five females per group) exposed for 4 h to DMCPS at 4.6, 6.7, 9.8, and 15.4 mg/L (aerosol and vapor) and held for 15 d after exposure. No animals died at the lowest concentration, but four of five animals of each sex died in the upper-intermediate group, and all animals died in the highest-concentration group. The summary report is not clear about how many animals died in the lower-intermediate group. The description indicates that four of five died in each sex in the group, but the LC_{50} (lethal concentration for 50% of the animals) could not be 8.7 mg/L (as reported) if that were the case (Dow Corning 1995a).

An inhalation study of F344 rats exposed 6 h/d, 5 d/w, by nose only to DMCPS at 0.44, 0.65, 1.50, and 3.06 mg/L has been reported by abstract (Kelly et al. 1995). Animals in the two highest-concentration groups had increased mean corpuscular volumes (MCVs) and triglycerides. Females in the highest-

concentration group showed increased liver weights, and morphological evidence of irritation in their nasal cavities.

In another study reported in abstract form, F344 rats were exposed whole body, 6 h/d, 7 d/w for 28 d to DMCPS at concentrations of 10, 25, 75, or 160 ppm (0.15, 0.38, 1.1, and 2.4 mg/L, respectively) (Burns et al., 1996). A satellite group was held for 14 d after exposures ended to assess reversibility of effects. Animals were evaluated for clinical signs, body weights, organ weights, gross pathology, histopathology, and clinical laboratory (including immune function) changes. At the end of exposures, the only effects reported were in the high-concentration group, and these included the following: slight anemia in females, increases in liver weight (both sexes), and increased thymus weight (males only). All of these were reversible after 14 d.

Subchronic and Chronic Exposures

Hexamethylcyclotrisiloxane

No inhalation studies longer than 4 w could be found on this compound; however, dietary administration of HMCTS to rats for 1 y at 1% did not produce evidence of adverse effects using standard end points (toxicity profile document 1965-10065-1179-01 from GE Silicones, provided by K. West, unpublished information, July 17, 1996).

Octamethylcyclotetrasiloxane

A subchronic inhalation toxicity study of OMCTS has been reported in Sprague-Dawley rats exposed to vapor concentrations of 0, 0.61, 3.6, and 8.4 mg/L, 6 h/d for 90 d (Siddiqui et al. 1989b). There were 10 rats per sex and exposure concentration as well as satellite groups (control and high concentration). The satellite groups were killed 28 d after the end of exposures to assess the reversibility of any lesions present immediately after exposures ended. Observations included the following: clinical signs, body weights, food consumption, hematology, clinical chemistry, urinalysis, gross pathology, organ weights, and histopathology. At the terminal sacrifice, females in the 8.4-mg/L group showed statistically increased red-blood-cell (RBC) count, hematocrit, and hemoglobin compared with controls. The magnitude of the increase was 4-6% and had disappeared when the satellite females were evaluated 28 d after the end of exposure. The changes were not considered biologically significant

by the authors. At the terminal sacrifice, all groups of exposed males showed statistically increased relative liver weights (9-22%), but there was no apparent dose-response relationship. Relative liver weights were also increased in the two highest-exposure female groups at sacrifice; the increase was 30% in the highest-exposure group. The hepatomegaly had disappeared in satellite males and was reduced to 10% in satellite females. Ovarian weights were statistically decreased (28%) in the satellite group. The authors felt that that was not a direct effect of OMCTS exposure; however, structurally related compounds are known to interfere with female reproduction (see below). There were no histopathological changes associated with exposure to OMCTS.

The effects in Sprague-Dawley rats of 13 w of exposure to OMCTS vapor at concentrations of 0, 0.06, 0.12, and 3.6 mg/L have been reported (Ulrich 1991). The exposure groups consisted of 10 rats per sex and concentration, with additional animals in the control and high-concentration groups for recovery studies. The end points included in this study were as follows: clinical signs, body weights, food consumption, ophthalmological examination, clinical chemistry, hematology, urinalysis, gross pathology, organ weights, and histopathology. There were a number of statistically significant differences between controls and exposed groups; however, the differences typically did not show a dose-response relationship, occurred randomly throughout the exposed groups, and had disappeared in the 4-w recovery group. The authors concluded that the apparent differences were not toxicologically significant and were within expected normal ranges for animals of the age and strain used. Only a 21% increase in liver weights in females at the terminal sacrifice was attributed to the test compound. This conclusion was partly based on results of previous studies. In contrast to the results above, ovarian weights at terminal sacrifice were increased relative to controls. Significant histopathological changes were not reported in the exposed animals.

In a study reported by abstract, F344 rats were exposed nose-only for 90 d to 0.3, 1.2, 5.0, and 12.0 mg/L OMCTS for 6 h/d, 5 d/w (Mast et al. 1996). A satellite group was retained for 28 d after the last exposure to assess reversibility of lesions. Animals were evaluated for body weight changes, clinical signs, clinical laboratory parameters, gross pathology, organ weights, and histopathology. The major findings were as follows:

high dose:	reduced weight gain, moderate increase in serum gammaglutamyl transferase, minimal goblet cell proliferation in nasopharyngeal duct, ovarian atrophy, and vaginal mucification
second dose:	minimal goblet cell proliferation in nasopharyngeal duct, ovarian atrophy.

In addition, there was a dose-dependent, reversible increase in liver weights; the concentration at which this effect disappeared was not specified. During the recovery period, essentially all the histopathological changes disappeared.

Decamethylcyclopentasiloxane

Two 13-w inhalation studies were available in summary form (Dow Corning 1995a). Since the second of these studies involved more test animals and a more thorough assessment of end points, it will be summarized here. Five groups of F344 rats were exposed 6 h/d, 5 d/w for 13 w to concentrations of 0, 0.44, 0.75, 1.33, and 3.5 mg/L. Each group consisted of at least 20 rats per sex. There were no clinical signs of toxicity during the exposures in any group. Liver weights were decreased in male rats (group 5) and female rats (groups 3,4 and 5) after exposures. There was a dose-related increase in serum gammaglutamyltransferase in females and lung weights remained elevated in female group 5 even after the 28-d recovery period. The NOAEL was 0.44 mg/L for both sexes.

Carcinogenicity

No studies were found on the carcinogenicity potential of any of the siloxanes.

Genotoxicity

The dimethylcyclosiloxanes have generally shown little genotoxicity when evaluated in batteries of in vitro and in vivo tests. All three siloxanes were negative in the following tests with and without S9 activation: *Salmonella typhimurium* and *Saccharomyces cerevisiae* reverse mutation assays, *E. coli* repair test for DNA damage, and the mouse lymphoma cell mutation test. There was an increase in the sister chromatid exchanges in mouse lymphoma cells when HMCTS was tested with and without the S9 fraction present (Isquith et al. 1988a). In vivo testing of HMCTS in the bone marrow cytogenic assay and rat dominant lethal test indicated no clastogenic activity (Isquith et al. 1988b). OMCTS was tested in cultured mouse lymphoma cells for induction of SCEs, point mutations, chromosomal aberrations, and primary DNA damage, with and without microsomal activation (Dow Corning 1995b). All results were negative except that with the activation system the indices of SCE frequency were increased. A dominant lethal assay in Sprague-Dawley rats given OMCTS by

gavage up to 1000 mg/kg/d, 5 d/w, for 8 w was negative for chromosomal damage in germinal cells of males, and for fertility, number of implants, number of dead implants in females mated to dosed males (Dow Corning 1995b).

Reproductive Toxicology

Few studies of the reproductive toxicity of dimethylcyclosiloxanes could be found; however, structurally related organosiloxanes produce adverse reproductive effects on male and female rats. Bennett et al. (1972) found that 7 d of oral administration of PHMCTC was "quite active" in producing adverse effects on the male reproductive systems of rats. The effect seemed to be specific to the route of administration because subcutaneous and intraperitoneal administrations caused much reduced effects on the male rats. In a study of the effects of 32 organosiloxanes on the weight of uteri from ovariectomized immature rats, PHMCTS was found to be the most estrogenic (+4) (Hayden and Barlow 1972). Three days of oral administration of this compound resulted in a 300% increase in uterine weight compared to controls. In the same study, OMCTS was found to have slight (+1) estrogenic activity and phenylpentamethylcyclotrisiloxane had moderate (+2) activity.

Developmental Toxicity

No developmental toxicity data could be found on the specific siloxanes of interest here; however, some data were found on structurally related compounds. PHMCTS administered at a dose of 220 mg/kg/d on gestational days 16 to 21 did not cause urogenital malformations in female rat pups; however, diphenylhexamethylcyclotetrasiloxane did elicit such malformations (Le Fevre et al. 1972). Neither compound caused abnormalities in male pups; however, either compound administered earlier in gestation caused adverse effects on the pregnancy.

Interactions with Other Chemicals

None of these siloxanes have been evaluated for their ability to alter the toxicity of other compounds; however, they have been shown to induce a number of hepatic enzymes (see "Metabolism" section).

A summary of the toxicity data on the polydimethylcyclosiloxanes are presented in Table 7-2.

TABLE 7-2 Toxicity Summary

Concentration or Dosage	Exposure Duration	Species	Effects	Reference
0.12 mg/L OMCTS	1 h, inhalation	Human	No immunological effects	Looney et al. 1996
0.9 mL OMCTS	Skin patch, 15 applications	Human (n = 200)	2/200 showed skin reaction, no sensitization found 2 w after end of regular applications	Shelanski 1974
0.06, 0.12, 3.6 mg/L OMCTS	6 h/d, 5 d/w, 13 w, inhalation	Rat (M, F)	28% increased liver weight in high exposure females (reversible), no other effects	Ulrich 1991
0.44, 0.75, 1.3, 3.5 mg/L DMCPS	6 h/d, 5 d/w, 13 w, inhalation	Rat (M, F)	Increased liver weight at 0.75 mg/L and above (F), dose related increase in GGT; high group had increased lung weights and possible histological effects on ovaries and testis; NOAEL for all effects was 0.44 mg/L	Dow Corning 1995a
0.08, 0.94 mg/L HMCTS	6 h/d, 7 d/w (?), 4 w, inhalation	Rat (M, F)	NOAEL for all end points measured, higher dose rats showed some blood encrustation of nares	LPT Report
0.2, 1.0, 5.1 mg/L OMCTS	6 h/d, 5 d/w, 4 w, inhalation	Rat (M, F)	Slight growth retardation in high exposure females, increase in relative liver weights (high exposure, both sexes, reversible)	Appleton et al. 1984
0.3, 1.2 mg/L OMCTS	6 h/d, 5 d/w, 13 w, inhalation	Rat (M, F)	NOAEL for histopathology	Mast et al. 1996
0.84, 8.4 mg/L OMCTS	6 h/d, 5 d/w, 28 d, inhalation	Rat (M, F)	Increased liver size	McKim et al. 1995
1.1 mg/L DMCPS	6 h/d, 7 d/w, 28 d, inhalation	Rat (M, F)	NOAEL clinical pathology, immunotoxicity, organ weights	Burns et al. 1996
2.4 mg/L DMCPS	6 h/d, 7 d/w, 28 d, inhalation	Rat (F)	17% increase in liver weight (reversible)	McKim et al. 1996

TABLE 7-2 *(Continued)*

Concentration or Dosage	Exposure Duration	Species	Effects	Reference
2.4 mg/L DMCPS	6 h/d, 7 d/w, 28 d, inhalation	Rat (F)	Slight anemia (reversible)	Burns et al. 1996
	14 d recovery	Rat (M)	5% increase in liver:body weight ratio, 14% increase in thymus:body weight ratio (both reversible)	
2.3, 3.1 mg/L DMCPS	6 h/d, 5 d/w, 4 w, inhalation	Rat (M, F)	Increased mean corpuscular volume and triglycerides, morphological evidence of nasal and pulmonary irritation in highest group	Kelly et al. 1995
2.8, 5.1, 8.6, 13 mg/L OMCTS	6 h/d, 5 d/w, 20-21 d, inhalation	Rat (M, F)	Hunched posture, abnormal gait, head tilt in 3 highest groups, sedation in highest group	Dow Corning 1995b
3.1 m/L DMCPS	6 h/d, 5 d/w, 4 w, inhalation	Rat (F)	Hepatocellular hypertrophy	Kelly et al. 1995
5.0, 12.0 mg/L OMCTS	6 h/d, 5 d/w, 13 w, inhalation, 28 d recovery	Rat (M, F)	Reversible hepatomegaly, minimal goblet cell proliferation in nasopharynx, macrophage influx in lungs, ovarian atrophy (slight), and vaginal mucification (all reversible)	Mast et al. 1996
6.5 mg/L OMCTS	4 h, inhalation	Rat (M, F)	0/10 died, no gross pathological changes	Appleman 1984
8.3 mg/L OMCTS	6 h/d, 7 d/w, 4 w, inhalation	Mouse (M, F) hamster (F)	10 to 30% increase in relative liver weights, but no histopathological changes	Siddiqui et al. 1989a
8.3 mg/L OMCTS	6 h/d, 7 d/w, 4 w, inhalation	Guinea pig (M, F) hamster (M) Rabbit (M,F)	No significant changes in liver weights; no histopathological changes	Siddiqui et al. 1989a

Concentration or Dosage	Exposure Duration	Species	Effects	Reference
0.61, 3.6, 8.4 mg/L OMCTS	6 h/d, 90 d, inhalation	Rat (M, F)	No histopathological changes, high-exposure females showed 30% increase in liver weights and 5% increase in RBC parameters	Siddiqui et al. 1989b
8.7 mg/L DMCPS	4 h, inhalation	Rat (M, F)	LC_{50}	Dow Corning 1995a
9.0 mg/L HMCTS	6 h/d, 7 d/w (?), 4 w, inhalation	Rat (M, F)	3/10 males and 1/10 females died, survivors showed inflammation of nasal cavity, slight narcosis, no effects reported on liver	LPT Report
20 mg/L OMCTS	4 h, inhalation	Rat (M, F)	Hunched posture, stiff gait, rales, head drop	Dow Corning 1995b
25 mg/L OMCTS	4 h, aerosol inhalation	Rat	0/6 died	Clayton 1974
36 mg/L OMCTS	4 h, inhalation	Rat	LC_{50}	Dow Corning 1995b
42 mg/L (est) OMCTS	8 h, vapor inhalation	Rat	0/6 died, CNS effects, slow breathing after 7 h	Clayton 1974
Near saturation OMCTS	1 h, inhalation	Rat	0/6 died	Weil 1972
Near saturation OMCTS	6 h, inhalation	Rat	0/10 died	Myers et al. 1982
Near saturation OMCTS	8 h, inhalation	Rat	2/6 died	Weil 1972
48 mg/L OMCTS	4 h, aerosol inhalation	Rat	1/6 died	Clayton 1974

Concentration or Dosage	Exposure Duration	Species	Effects	Reference
52 mg/L OMCTS	8 h, aerosol inhalation	Rat	5/6 died	Clayton 1974
2 g/kg/d OMCTS	28 d, oral gavage	Rat (M, F)	Up to 7% growth retardation in females, no treatment effects by gross necropsy	Manston 1988
>16 mL/kg OMCTS	Skin patch	Rabbit	No deaths, erythema at site	Weil 1972
>64 mL/kg OMCTS	Oral gavage	Rat	No deaths, animals sluggish	Weil 1972

No exposure limits have been set by other organizations.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

The toxicological data base, much of it not peer reviewed, on these three siloxanes suggests that they are relatively nontoxic compounds. Clinical signs indicating CNS depression have been noted in high-concentration exposures and only hepatomegaly, without histopathological change, respiratory irritation, and possibly injury to the ovaries or testis have been noted after prolonged exposures to high concentrations. With some studies showing decreased RBC measurements and others showing increased RBC measurements, the findings on anemia are inconsistent. The guidelines developed by the Committee on Toxicology have been used to derive SMACs (NRC 1992). Although the compounds have toxicological similarities, significant differences in the toxicological data bases of the compounds led the NRC to recommend that each compound be treated individually, rather than as members of a group with similar structural and toxicological properties.

The most serious limitation of the toxicological data bases is the lack of good acute data. Most acute toxicity studies focus on lethality as an end point, so it is difficult to know if injury to organ systems has occurred as a result of a brief exposure. Fortunately, there is no need for short-term SMACs for these compounds because there is no known mechanism by which large amounts could be released into the cabin. For that reason, short-term SMACs were not set. To the extent possible, SMACs were based on studies that have been reported in detail, rather than by abstract. In addition, toxic effects that were superficially reported (e.g., ovarian effects and CNS depression) for two or more of the compounds were considered for purposes of setting SMACs.

Possible Liver Injury

Several studies indicate that both OMCTS and DMCPS induce a reversible hepatomegaly in rats. OMCPS concentrations as low as 0.84 mg/L have been shown to induce increased liver weight during 4-w exposures (McKim et al., 1995). A reversible increase in hepatocyte mitotic figures was noted in rats exposed to OMCTS at 1.0 or 5.1 mg/L for 4 w (Appleton et al., 1984). DMCPS exposures of 1.1 mg/L did not induce hepatomegaly in rats in 4 w; however, 2.4 mg/L induced a 17% weight increase in the livers of female rats (McKim et al. 1996). In contrast, HMCTS was not reported to induce hepatomegaly in rats exposed at 9.0 mg/L for 4 w (LPT Report). Reversible hepatomegaly without

histological evidence of injury is considered an adaptive rather than an adverse effect.

Hexamethylcyclotrisiloxane

The best data available on the inhalation toxicity of this compound come from rats exposed to analytical concentrations of 0.08. 0.94, and 9.0 mg/L (9,100, and 1000 ppm) 6 h/d, 7 d/w, for 4 w (LPT Report). The summary report describes microscopically observed inflammatory changes in the nasal cavities and an increased incidence of pulmonary perivascular infiltration in the high-exposure rats. The NOAEL for respiratory system injury was 100 ppm for a cumulative exposure of 168 h. Applying a species factor of 10, the 7-d AC to protect from respiratory-system injury was calculated to be 10 ppm (Table 7-3). That AC was extended to 30 d by dividing by a factor of 2 (30-d AC = 5 ppm), because 4-w and 13-w data on the other two siloxanes suggest that a factor of 2 (or less) is appropriate (see Tables 7-4 and 7-5). Because no data beyond 13 w were available, the default approach (Haber's rule) was used to extrapolate from the 30-d AC to the 180-d AC. The 180-d AC was set at 1 ppm.

In the LPT Report, signs of slight temporary sedation were noted in the highest-exposure rats immediately after exposure. Because no signs of CNS depression were reported in the 100-ppm (middle) group, that concentration was selected as the NOAEL. Applying a species factor of 10 and no time factor gave long-term SMACs of 10 ppm. A time factor was not applied because CNS depression of this sort is a threshold-type effect.

Octamethylcyclotetrasiloxane

There was minimal evidence of respiratory-system effects caused by exposure to OMCTS; however, ACs were set for this possible effect. The evidence consisted of minimal goblet-cell proliferation in the nasopharyngeal duct and minor influx of alveolar macrophages in the lungs of rats exposed to concentrations as low as 5 mg/L for 6 h/d, 5 d/w, for 13 w (Mast et al. 1996). NOAELs for this effect came from a 4-w study in which rats were exposed to OMCTS at 5.1 mg/L (425 ppm), 6 h/d, 5 d/w (Appleman et al. 1984), and a 13-w study in which rats were exposed to OMCTS at 3.6 mg/L (300 ppm), 6 h/d, 5 d/w for 13 w (Ulrich et al. 1991). The 7-d AC was calculated from the 4-w data as follows:

7-d AC = 425 ppm × 120 h/168 h × 1/10 (species) = 32 ppm.

Similarly, the 30-d AC was calculated as follows:

30-d AC = 300 ppm × 390 h/720 h × 1/10 (species) = 16 ppm.

In rats, the ACs only differ by a factor of 2, even though the exposure times are more than 4-fold different. With no data beyond 13 w, the default approach (Haber's rule) was used to extrapolate from the 30-d AC to the 180-d AC. The 180-d AC was set at 3 ppm.

Indications of CNS effects were reported in rats exposed to OMCTS for 6 h/d, 5 d/w for 4 w (Dow Corning 1995b). Abnormal gait was reported in rats exposed at 5.1 mg/L and above, but 2.8 mg/L (230 ppm) was a NOAEL for all clinical signs. Applying a species factor of 10 gave ACs for protection from CNS effects of 23 ppm.

Ovarian atrophy was reported in rats exposed to OMCTS at 5.0 mg/L by nose only for 6 h/d, 5 d/w for 13 w but not to in the next lower group exposed at 1.2 mg/L (100 ppm) (Mast et al. 1996). The atrophy was apparently observed microscopically but disappeared during the 28-d recovery period. The 30-d AC was calculated as follows:

30-d AC = 100 ppm × 390 h/ 720 h × 1/10 (species) = 5 ppm.

The 180-d AC was calculated using Haber's rule to give 1 ppm. The 7-d AC for ovarian effects was calculated to be 32 ppm from the 4-w NOAEL of 425 ppm (Appleton et al. 1984).

Decamethylcyclopentasiloxane

Rats exposed to DMCPS at 2.3 to 3.1 mg/L (200 ppm), 6 h/d, 5 d/w (120 h total) for 4 w showed morphological changes in their nasal cavities as a result of irritation and slight inflammation in the lung paranchyma (Kelly et al. 1995, Dow Corning 1995a); however, the next lower group, 1.5 mg/L (100 ppm), did not show these changes. The 7-d AC was calculated as follows:

7-d AC = 100 ppm × 120 h/168 h × 1/10 (species) = 7 ppm.

Increased lung weights were reported in rats exposed to DMCPS at 3.5 mg/L (230 ppm), 6 h/d, 5 d/w for 13 w but not in rats exposed at 1.3 mg/L (90 ppm) (Dow Corning 1995a). The 30-d AC was calculated as follows:

30-d AC = 90 ppm × 390 h/720 h × 1/10 (species) = 5 ppm.

The 30-d AC is less than a factor of 2 below the 7-d AC.

In the same 13-w study used for the respiratory-system injury (Dow Corning 1995a), possible ovarian and testicular effects were reported in the highest-exposure group (230 ppm) but not in the next lower group (90 ppm), nor were they reported in rats exposed for a shorter time (Kelly et al. 1995). The 7-d AC for gonad effects was based on the lack of effects reported in rats exposed at 200 ppm 6 h/d, 5 d/w for 4 w (Kelly et al. 1995). The calculation was as follows:

7-d AC = 200 ppm × 120/168 × 1/10 (species) = 14 ppm.

The 30-d AC to avoid injury to the gonads was set at 5 ppm by using the same calculation as that for the respiratory effects. With no data beyond 13 w, the default approach (Haber's rule) was used to extrapolate from the 30-d AC to the 180-d AC. The 180-d AC was set at 1 ppm.

TABLE 7-3 Acceptable Concentrations for Hexamethylcyclostrisiloxane

End Point, Exposure Data, Reference		Uncertainty Factors			Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	1 h	24 h	7 d	30 d	180 d	
Respiratory system injury	Rat	1	HR[a]	10	—	—	10	5	1
NOAEL, 100 ppm for 6 h/d, 7 d/w, 4 w (LPT Report)									
CNS depression	Rat	1	1	10	—	—	10	10	10
NOAEL, 100 ppm for 6 h/d, 7 d/w, 4 w (LPT Report)									
SMACs (ppm)					**NS[b]**	**NS[b]**	**10**	**5**	**1**
(mg/m^3)							**90**	**45**	**9**

[a] Limits for 30 d and 180 d were set by analogy with OMCTS and DMCPS injury to respiratory system of rats.
[b] Short-term limits were not set because of the lack of data and because there is no need for these values in the spacecraft.
HR, Haber's rule.
—, not applicable.
NS, not set.

TABLE 7-4 Acceptable Concentrations for Octamethylcyclotetrasiloxane

End Point, Exposure Data, Reference		Uncertainty Factors			Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	1 h	24 h	7 d	30 d	180 d	
Respiratory system injury	Rat	1	HR	10	—	—	32	—	—
NOAEL, 425 ppm for 6 h/d, 5 d/w, 4 w (Appleman et al. 1984)									
NOAEL, 300 ppm for 6 h/d, 5 d/w, 13 w (Ulrich et al. 1991)	Rat	1	HR	10	—	—	—	16	3
CNS depression	Rat	1	1	10	—	—	23	23	23
NOAEL, 230 ppm for 6 h/d, 5 d/w, 20 d (Dow Corning 1995b)									
Ovarian atrophy	Rat	1	HR	10	—	—	32	—	—
NOAEL, 425 ppm for 6 h/d, 5 d/w, 4 w (Appleman et al. 1984)									
NOAEL, 100 ppm for 6 h/d, 5 d/w, 13 w (Mast et al. 1996)	Rat	1	HR	10	—	—	—	5	1
SMACs (ppm)					**NS[a]**	**NS[a]**	**23**	**5**	**1**
(mg/m^3)							**280**	**60**	**12**

[a] Short-term limits were not set because of the lack of data and because there is no need for these values in the spacecraft.
HR, Haber's rule.
—, not applicable.
NS, not set.

TABLE 7-5 Acceptable Concentrations for Decamethylcyclopentasiloxane

End Point, Exposure Data, Reference		Uncertainty Factors			Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	1 h	24 h	7 d	30 d	180 d	
Respiratory system injury	Rat	1	HR	10	—	—	7	—	—
NOAEL, 100 ppm for 6 h/d, 5 d/w, 4 w (Kelly et al. 1995; Dow Corning 1995a)									
NOAEL, 90 ppm for 6 h/d, 5 d/w, 13 w (Dow Corning 1995a)	Rat	1	HR	10	—	—	—	5	1
Ovarian/testicular histopathology	Rat	1	HR	10	—	—	—	5	1
NOAEL, 90 ppm for 6 h/d, 5 d/w, 13 w (Dow Corning 1995a)									
NOAEL, 200 ppm for 6 h/d, 5 d/w, 4 w (Kelly et al. 1995)	Rat	1	HR	10	—	—	14	—	—
SMACs (ppm)					**NS[a]**	**NS[a]**	**7**	**5**	**1**
(mg/m^3)							**100**	**75**	**15**

[a] Short-term limits were not set because of the lack of data and because there is no need for these values in the spacecraft.
HR, Haber's rule.
—, not applicable.
NS, not set.

RECOMMENDATIONS

The descriptive toxicity data base for OMCTS is fairly complete, with the possible exception of short-term exposure effects. It is difficult to imagine a large accidental release of OMCTS that would require a better understanding of the acute effects of this silicone oil. Our understanding of the mechanisms of toxicity of OMCTS would be improved by toxicokinetics studies and further evaluation of the biochemical changes associated with the induction of hepatomegaly. Descriptive toxicity studies of HMCTS and DMCPS would seem to show that their toxicity is comparable to that of OMCTS, but these studies need peer review and publication.

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B8 DICHLOROFLUOROMETHANE (FREON 21)

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PHYSICAL AND CHEMICAL PROPERTIES

Dichlorofluoromethane is a colorless, nonflammable gas at room temperature with a slight etherlike odor (ACGIH 1991).

Formula:	CCl_2FH
CAS no.:	75-43-4
Chemical name:	Dichlorofluoromethane
Synonyms:	FC-21; fluorocarbon 21; Genetron 21; CFC-21; HCFC-21; refrigerant 21; dichloromonofluoromethane, fluorodichloromethane.
Molecular weight:	102.92
Boiling point:	8.9°C, 48°F
Melting point:	– 135°C
Specific gravity:	1.405 g/L (9°C)
Vapor density:	3.82 (air is 1.0)
Solubility:	Insoluble in water
	Soluble in alcohol and ether
Conversion factors:	1 ppm = 4.2 mg/m^3
	1 mg/m^3 = 0.24 ppm

OCCURRENCE AND USE

HCFC-21 does not occur naturally. It has been manufactured for use principally as a specialty refrigerant, a solvent, an aerosol propellant, and a heat-

exchange fluid in geothermal-energy applications. The shuttle orbiter carries about 650 pounds of HCFC-21, which is used in a heat exchanger. Toxicological reports for the 28 missions from STS-26 to STS-55 show that HCFC-21 has been detected in shuttle cabin air at concentrations of 0.1 to 1 mg/m^3 in one mission and 0.01 to 0.1 mg/m^3 in three missions (James et al. 1994).

UPTAKE, METABOLISM, AND TOXICOKINETICS

Limited data are available on the toxicokinetics of HCFC-21 in animals, and no data were found for human exposures. Trochimowicz et al. (1977) showed that HCFC-21 is partially metabolized in rats and dogs, probably by a cytochrome-P-450-dependent pathway. Peter et al. (1986) injected HCFC-21 into rats intraperitoneally (ip) and showed that it was only partially exhaled, indicating some metabolism, in contrast to CFC-22, which is completely exhaled and not metabolized (Peter et al. 1986). Rats exposed to HCFC-21 at 150 ppm for 6 h/d, 5 d/w for 46 d showed increased fluoride concentrations in urine (Industrial Bio-Test Labs 1979), as did rats exposed at 1000 and 5000 ppm for 6 h/d, 5 d/w for 13 w (E.I. du Pont de Nemours 1977). Although no reports were found that examined the metabolic products of HCFC-21, its hepatotoxicity suggests that its metabolism might be similar to that of chloroform, presumably yielding highly reactive species, such as phosgene.

TOXICITY SUMMARY

The only studies found of the effects of HCFC-21 exposure on humans were case reports of bronchoconstriction in some patients inhaling bronchodilator aerosols in which the propellant contained a mixture of HCFC-21 and CFC-11 and one German study of arterial hypoxic patients inhaling propellants from pressurized aerosols. In animals, the toxicity of HCFC-21 appears to be more similar to chloroform than to other chlorofluorocarbons, particularly in its ability to induce liver toxicity from subchronic or chronic exposures. HCFC-21 exhibits the cardiotoxicity (sensitization to arrhythmia) common to many chlorofluorocarbons.

Acute and Short-Term Exposures

In general, toxicity due to acute exposures to HCFC-21 is low, but HCFC-21 is appreciably more toxic than related difluorinated methanes. Short-term exposures to high concentrations of HCFC-21 can produce adverse effects on

weight gain, the central nervous system (CNS), liver, heart, and respiratory system. Very high concentrations can cause unconsciousness or death.

CNS Effects

A 1935 report states that a 1-h exposure of guinea pigs to HCFC-21 at 12,000 ppm induced slight stupor (Dufour 1935). In a more recent study, groups of 10 Charles River-CD male rats exposed to HCFC-21 at 10,000 ppm for 6 h/d, 5 d/w for 2 w showed no appreciable change in behavior as measured by motor coordination and response to noise (Kelly and Trochimowicz 1976). Unconsciousness was produced in guinea pigs after 30-min exposures at 50,000 ppm (Nuckolls 1959).

Lethality

The LC_{50} (lethal concentration for 50% of the animals) value in rats for a 4-h exposure is 49,900 ppm (C.H. Tappan and R.S. Waritz, E.I. du Pont de Nemours, unpublished data, 1964). Exposure to HCFC-21 at 100,000 ppm killed rats and guinea pigs in 1 h (Weigand 1971).

Respiratory and Bronchopulmonary Effects

In a series of papers from the laboratory of Domingo Aviado, HCFC-21 was shown in rats to be the fastest-acting apnea inducer of a series of aerosol propellants, including FC-11, FC-114, FC-12, FC-115, and FC C-318, and was shown to be a potent depressant for respiratory minute volume in monkeys (Belej and Aviado 1973; Friedman et al. 1973; Belej et al. 1974; Aviado 1975). HCFC-21 did not, however, increase bronchoconstriction as indicated by pulmonary resistance in anesthetized rats (Friedman et al. 1973), and in monkeys, HCFC-21 acted as a bronchodilator (Belej and Aviado 1973).

Bronchoconstriction was reported in a small percentage (4.4%) of asthmatic patients after inhaling a metered dose (90 μg) of the bronchodilator, salmetrol, which contained a mixture of HCFC-21 and trichlorofluoromethane as propellant (Wilkinson et al. 1992).

Heart and Circulatory System Effects

The cardiac effects of inhaling pressurized aerosols were examined in 10 patients with marked arterial hypoxia resulting from bronchial pulmonary

disease and 10 subjects without lung disease. Diseased subjects were administered 10 12.6-mL puffs (1 puff per breath for 10 consecutive breaths) of propellant, consisting of 60% HCFC-21 and 40% CFC-11. EKGs were recorded immediately after administration and at 10 min following administration. Normal subjects were administered 16 puffs, according to the following schedule: 1 puff + 1 puff + 2 puffs + 4 puffs + 4 puffs + 4 puffs, with 25 min between each administration. EKGs were recorded at 2, 5, 10, and 20 min following each administration. No statistically significant changes in the EKG (conduction disturbances or contour changes) and no bradycardia or tachycardia were seen in either set of subjects (Fabel et al. 1972).

Life-threatening arrhythmias were seen in 2 of 12 beagle dogs exposed for 10 min to HCFC-21 at 10,000 ppm with intravenous epinephrine and in one of one beagle dog exposed to HCFC-21 at 25,000 ppm with intravenous epinephrine (E.I. du Pont de Nemours 1989). Although HCFC-21 at a concentration of 2.5% in air was shown to induce arrhythmia in monkeys and to sensitize the mouse heart to epinephrine, it did not induce bradycardia in rats, in contrast to several other halocarbons (Friedman et al. 1973; Belej et al. 1974). HCFC-21 induced tachycardia and hypotension (Aviado 1975), reduced the pulmonary arterial blood pressure, and depressed the myocardial contractility in monkeys at 2.5% (Belej and Aviado 1973); in dogs, 2.5% induced tachycardia, but hypotension was not seen at concentrations of <10% HCFC-21 (Belej and Aviado 1975).

Subchronic and Chronic Exposures

Longer term exposures to HCFC-21, primarily in rats, result in decreased weight gain, hair loss, respiratory, hepatic and other systemic effects. Reports of carcinogenicity and testicular toxicity appear to be unfounded.

Respiratory Toxicity

A case report from Italy implicates chronic occupational exposure to HCFC-21 in the development of chronic nasopharyngeal irritation and edema (Tanturri et al. 1988). Unfortunately, however, no measurement or estimate was made of the concentration of Freon in the work atmosphere. That was because Freon was determined to be the cause of the patient's complaints only when his symptoms cleared up after he quit working in the Freon-containing environment. The report did not give any indication of whether any other similarly exposed workers suffered similar complaints.

Hepatotoxicity

Rats exposed to HCFC-21 at 500 ppm for 6 h/d, 5 d/w for 51 d developed portal cirrhosis of the liver at 100% incidence, whereas rats similarly exposed to 150 ppm for up to 131 d had no incidence of cirrhosis (Table 8-1) (Industrial Bio-Test Laboratories 1979). The data reported by Industrial Bio-Test had not been reviewed internally by their Quality Assurance department because the department had been closed down. Results of other tests run by that testing laboratory were found questionable by the scientific community during this time period. Nevertheless, the findings of hepatotoxicity for HCFC-21 were confirmed by tests done at E.I. du Pont de Nemours.

Testing by DuPont's Haskell Laboratory found liver damage in rats exposed to HCFC-21 at 10,000 ppm, 6 h/d, 5 d/w for 2 w, and in rats exposed at 1,000 ppm, 6 h/d, 5 d/w for 13 w (E.I. du Pont de Nemours 1977). A no-effect level was not determined in rats; dogs exposed for 6 h/d, 5 d/w for 13 w showed liver damage at 5000 ppm but not at 1000 ppm.

TABLE 8-1 Incidence of Cirrhosis in Rats (Industrial Bio-Test Laboratories 1979)

	Exposure Duration	Concentration, ppm			
Gender	0	50	150	500	
Male	51 d	0/5	0/5	0/5	5/5
Female	51 d	0.5	0/5	0/5	5/5
Male	99-101 d	0/20	0/20	0/20	13/13
Female	99-101 d	0/18	0/20	0/20	20/20
Male	130-131 d	0/10	0/10	0/9	5/5
Female	130-131 d	0/9	0/10	0/10	8/8

Pancreatic Effects

Two of 10 rats exposed to HCFC-21 at 50 ppm, 6 h/d, 5 d/w for 51 d (the lowest tested dose) developed interstitial edema of the pancreas (Industrial Bio-Test Laboratories 1979). That is not considered an adverse effect, but rather a normal, reversible, physiological response.

Other Systemic Effects

Testing by DuPont's Haskell Laboratory found heavy spleens, enlarged

lymph nodes, decreased plasma proteins, increased plasma enzyme activities, increased urine volume, and increased urine fluoride in rats exposed to HCFC-21 at 1000 and 5000 ppm for 6 h/d, 5 d/w for 13 w (E.I. du Pont de Nemours 1977).

Carcinogenicity

Of 40 rats (20 male and 20 female) examined after exposure to HCFC-21 at 500 ppm, 6 h/d, 5 d/w for 99 d, 3 males developed "leukemia," which was described as being localized in the lung in two animals and the liver in a third (Industrial Bio-Test Laboratories 1979). None of 40 rats examined after exposure to HCFC-21 at 0, 50, or 150 ppm, 6 h/d, 5 d/w for 99 d had developed "leukemia" (Industrial Bio-Test Laboratories 1979). The strain of rat tested was not given, other than "albino rat." From the description given and the failure to report large increases in the absolute number of peripheral lymphocytes, it is unlikely that these were leukemias, but rather, according to B. Wagner (Wagner Associates, Inc., Millburn, NJ, personal commun., 1995), were most likely circumscribed lymphomas, which are common in some strains of rats and are not indications of exposure to carcinogens.

Lethality

Testing by DuPont's Haskell Laboratory found increased mortality in both male and female rats exposed to HCFC-21 at 1000 and 5000 ppm for 6 h/d, 5 d/w for 13 w (E.I. du Pont de Nemours 1977). In another subchronic toxicity study (Industrial Bio-Test Laboratories 1979), rats (35 males and 35 females per group) were exposed 6 h/d, 5 d/w for 99 days to HCFC-21 at 0, 50, 150, or 500 ppm and followed for 30 d after exposure; 15 exposure-related deaths were reported. One male rat in the 150-ppm group was sacrificed on study day 107 in extremis with back-leg paralysis. Thirteen males and one female in the 500 ppm group were either found dead or sacrificed in extremis on study days 71 through 128.

Genotoxicity

HCFC-21 did not induce mutations in bacterial tester strains TA1535, TA1538, TA98, and TA100 exposed for 24 h in the Ames *Salmonella* bacterial reversion assay, nor did it induce cell transformation in BHK21 cells (+S9) in vitro (Longstaff et al. 1984).

Reproductive and Developmental Toxicity

Exposure of pregnant rats to HCFC-21 at 8800 ppm for 6 h/d on d 6 through 15 of gestation adversely affected maternal weight gain and appeared to cause a total preimplantation loss of embryos in 15 of 25 dams, but was not teratogenic (Culik and Kelly 1976; Kelly et al. 1978).

Testicular toxicity was reported by Industrial Bio-Test Laboratories in rats exposed to HCFC-21 at 500 ppm for 6 h/d, 5 d/w for 130 d, but the report was internally inconsistent. Different sections of the report gave contradictory statements as to how many animals were affected, at which doses, for which exposure durations, and whether the results were considered exposure-related and significant. A cover letter to the report stated that it had not been reviewed by IBT's quality assurance unit, because the unit had been discontinued due to loss of personnel. No other laboratories have reported testicular toxicity even for exposures to much higher concentrations; therefore, it must be assumed that this effect was not related to exposure to HCFC-21.

Spaceflight Effects

Spaceflight, on rare occasions, has been accompanied by non-life-threatening cardiac dysrhythmias at a higher frequency than observed for the affected individuals in tests on earth. Such a putative spaceflight-induced predisposition to cardiac dysrhythmias might enhance the arrhythmogenic effects of HCFC-21 in a manner similar to the sensitization seen in animals upon injection of epinephrine.

Synergistic Effects

Moderate-to-heavy exercise or the use of epinephrine or other heart stimulants should be avoided during exposure to HCFC-21 because of HCFC-21's ability to sensitize the heart to arrhythmias. Concomitant exposure to HCFC-21 and other chlorocarbons, such as chloroform, might produce liver toxicity, which would not be seen during exposure to either compound alone at low concentrations.

Table 8-2 presents a summary of the toxicity data on HCFC-21.

TABLE 8-2 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
50	6 h/d, 5 d/w, 130-131 d[a]	Rat	Pancreatic interstitial edema in 18 of 20	Industrial Bio-Test Labs 1979
150	6 h/d, 5 d/w, 51 d[a]	Rat	Increased urine fluoride	Industrial Bio-Test Labs 1979
150	6 h/d, 5 d/w, 99-101 d[a]	Rat	Alopecia; LOAEL for atrophy of seminiferous epithelium; NOAEL for "leukemia" and cirrhosis of liver	Industrial Bio-Test Labs 1979
500	6 h/d, 5 d/w, 51 d[a]	Rat	Increased 24 h urine volume, elevated SAP values, increased leukocytes, elevated SGPT values, cirrhosis of the liver	Industrial Bio-Test Labs 1979
500	6 h/d, 5 d/w, 99-101 d[a]	Rat	Abdominal swelling, diarrhea, liver cirrhosis, "leukemia" in 3 males	Industrial Bio-Test Labs 1979
500	6 h/d, 5 d/w, 130-131 d[a]	Rat	Hypospermatogenesis, cirrhosis; interstitial pancreatitis; "leukemia" in 3 males	Industrial Bio-Test Labs 1979
1000	6 h/d, 5 d/w, 13 w	Rat	Alopecia, increased urine volume, increased urine fluoride, decreased plasma protein, increased plasma enzyme activities, liver cirrhosis, heavy spleens, enlarged lymph nodes, increased mortality (20/54)	Trochimowicz et al. 1977; E.I.du Pont de Nemours 1977
1000	6 h/d, 5 d/w, 13 w	Dog	NOAEL for histopathology, hematopathology, and clinical signs	Trochimowicz et al. 1977; E.I. du Pont de Nemours 1977
5000	6 h/d, 5 d/w, 13 w	Rat	Liver cirrhosis, increased hematopoesis, kidney nephrosis and hemorrhage, hemosiderin, deposits, edema, and lymphocytic depletion in lymph nodes	Trochimowicz et al. 1977; E.I. du Pont de Nemours 1977
5000	6 h/d, 5 d/w, 13 w	Dog	Increased plasma enzyme activity, increased urine fluoride, slight hepatotoxicity	Trochimowicz et al. 1977; E.I. du Pont de Nemours 1977

Concentration, ppm	Exposure Duration	Species	Effects	Reference
12,000	1 h	Guinea pig	Slight stupor, irregular breathing, rubbing noses	Dufour 1935
25,000	5 min	Dog	Tachycardia, increased pulmonary resistance, decreased pulmonary compliance	Belej and Aviado 1975
25,000	5 min	Monkey	Tachycardia, hypotension	Belej et al. 1974
25,000	5 min	Monkey	Decreased myocardial force, decreased aortic blood pressure, decreased pulmonary arterial pressure	Belej et al. 1974
49,900	4 h	Rat	LC_{50}	Tappan and Waritz 1964
50,000	5 min	Monkey	Increased left atrial pressure, tachycardia	Belej et al. 1974
50,000	30 min	Guinea pig	LC_0, unconsciousness, convulsive tremors	Dufour 1935
100,000	1 h	Rat, guinea pig	Death	Weigand 1971
100,000	30 min	Guinea pig	LC_{100}	Dufour 1935
100,000	5 min	Dog	Hypotension	Belej and Aviado 1975

[a] Includes two nonexposure days per week and holidays.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 8-3 presents exposure limits for HCFC-21 set by other organizations and Table 8-4 presents the SMACs established by NASA.

To set SMAC values for HCFC-21, acceptable concentrations (ACs) are calculated for the induction of each adverse effect (CNS depression, cardiac effects, and hepatotoxicity) using the guidelines established by the National

TABLE 8-3 Exposure Limits Set by Other Organizations

Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	10 (TWA)	ACGIH 1997
ACGIH's TLV	Not set (STEL)	ACGIH 1997
OSHA's PEL	10	ACGIH 1991
OSHA's STEL	Not set (ceiling)	ACGIH 1991
NIOSH's REL	10	ACGIH 1991
NIOSH's STEL	Not set (ceiling)	ACGIH 1991
NRC's 1-h EEL	100	NRC 1984
NRC's 24-h EEL	3	NRC 1984
NRC's 90-d CEL	1	NRC 1984

TLV, Theshold Limit Value; TWA, time-weighted average; STEL, short-term exposure limit; PEL, permissible exposure limit; REL, recommended exposure limit; EEL, emergency exposure limit; CEL, continuous exposure limit.

TABLE 8-4 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	50	210	Cardiovascular toxicity
24 h	50	210	Cardiovascular toxicity
7 d[a]	15	63	Liver cirrhosis
30 d	15	50	Liver cirrhosis
180 d	2	8	Liver cirrhosis

[a] Previous 7-d SMAC = 10 ppm (42 mg/m^3).

Research Council's Committee on Toxicology (NRC, 1992). ACs are not set for the following end points: leukemia and testicular toxicity (because the data are not convincing), heavy spleens, enlarged lymph nodes, decreased plasma proteins, increased plasma enzyme activities, increased urine volume, increased urine fluoride, and interstitial edema of the pancreas. None of those effects is considered an adverse effect per se. For each exposure time (1 h, 24 h, 7 d, 30 d, and 180 d), the lowest AC is selected as the SMAC value (Table 8-5).

CNS Depression

An AC for CNS depression is set based on a report that a 1-h exposure of guinea pigs to HCFC-21 at 12,000 ppm induced slight stupor (Dufour 1935). The 12,000-ppm value is divided by 10 to estimate a no-observed-adverse-effect level (NOAEL). The resultant value is further divided by 10 for species extrapolation.

AC = 12,000 ppm ÷ 10 (to NOAEL) ÷ 10 (species) = 120 ppm.

Because CNS effects are generally independent of exposure duration, once blood concentrations have achieved steady state, as would be expected for a 1-h exposure to a chlorofluorocarbon, the resultant value of 120 ppm is set as the AC for all exposure durations—1 h, 24 h, 7 d, 30 d, and 180 d.

Cardiovascular Effects

An AC for cardiovascular effects (tachycardia and hypotension) is based on the reports by Belej and co-workers of effects in dogs (Belej and Aviado 1975) and similar findings in monkeys (Belej et al. 1974) exposed to HCFC-21 at 25,000 ppm for 5 min. The 25,000-ppm value is divided by 10 to estimate a NOAEL, again by 10 for potential species differences in response, and by 5 for potential spaceflight effects.

AC = 25,000 ppm ÷ 10 (to NOAEL) ÷ 10 (species) ÷ 5 (spaceflight)
= 50 ppm.

No adjustment is made for exposure durations beyond 1 h, because the cardiovascular effects are generally dependent only on blood concentrations.

The ACs for all exposure durations from 1 h to 180 d are therefore set at 50 ppm.

Hepatotoxicity

An AC for toxicity to the liver is based on the 1979 report by Industrial Bio-Test Laboratories that subchronic (6 h/d, 5 d/w, 131 d; comparable to 23 d of continuous exposure) exposure of rats to HCFC-21 at 150 ppm was a NOAEL for cirrhosis of the liver. The reliability of the data can be questioned on the basis of allegations that Industrial Bio-Test Laboratories falsified test results in the past, and the fact that the data for HCFC-21 were not reviewed by its quality-assurance department or published in peer-reviewed literature and were only in a report to Allied Chemical Corporation. Nevertheless, the results are consistent with the findings of Trochimowicz et al. (1977), from DuPont's Haskell Laboratories, who, in a 90-d study on ChR-CD rats and beagle dogs exposed to HCFC-21 at 1000 or 5000 ppm for 6 h/d, 5 d/w, found extensive liver damage in rats at either test concentration, minimal morphological change in the liver of dogs exposed at 5000 ppm, and a NOAEL for liver toxicity in dogs exposed at 1000 ppm. Thus, the 150-ppm NOAEL is used as a starting point and divided by 10 for potential species differences in response. The resulting value is adjusted for the duration of potential exposure on the basis of 6 h/d × 131 d, approximating 23 d continuous exposure, except that the value is not increased for exposure durations shorter than 23 d. ACs are not calculated for 1 h and 24 h, because that would require an extrapolation of greater than 10-fold.

7-d AC = 150 ppm ÷ 10 (species) = 15 ppm.

30-d AC = 150 ppm ÷ 10 (species) × 23 d/30 d = 12 ppm.

180-d AC = 150 ppm ÷ 10 (species) × 23 d/180 d = 2 ppm.

TABLE 8-5 Acceptable Concentrations

End Point, Exposure Data, Reference		Uncertainty Factors				Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d	
CNS Depression	Guinea pig	10	1	10	1	120	120	120	120	120
LOAEL, 12,000 ppm for 1 h (Dufour 1935)										
Tachycardia, hypotension	Monkey, mouse	10	1	10	5	50	50	50	50	50
LOAEL, 25,000 ppm for 5 min (Aviado 1975; Belej and Aviado 1973)										
Liver cirrhosis	Rat	1	HR	10	1	NS	NS	15	12	2
NOAEL, 150 ppm for 6 h/d, 5 d/w, 131 d (Industrial Bio-Test Laboratories 1979)										
SMACs						**50**	**50**	**15**	**12**	**2**

HR, Haber's rule.
NS, not set.

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B9 CHLORODIFLUOROMETHANE (FREON 22)

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PHYSICAL AND CHEMICAL PROPERTIES

Chlorodifluoromethane is a colorless, nearly odorless nonflammable gas at room temperature.

Formula:	CIF_2CH
CAS no.:	75-45-6
Chemical name:	Chlorodifluoromethane
Synonyms:	Algeon 22, algofrene type 6, algofrene 22, arcton 4, chlorofluorocarbon 22, CFC-22, eskimon 22, electro-CF 22, FC-22, flugene 22, fluorocarbon 22, forane 22, Freon 22, frigen 22, Genetron 22, HCFC 22, HSDB 143, hydrochlorodifluorocarbon 22, isceon 22, isotron 22, kaltron 22, khladon 22, monochlorodifluoromethane, propellant 22, R 22, refrigerant 22, ucon 22
Molecular weight:	86.46
Boiling point:	-40.8°C
Melting point:	-146°C to -147°C
Specific gravity:	1.4909 g/mL (-69°C)
Solubility:	Soluble in water (0.30% wt/wt at 25°C)
	Soluble in acetone, chloroform, and ether
Conversion Factors at 25°C, 1 atm:	1 ppm = 3.54 mg/m^3
	1 mg/m^3 = 0.282 ppm

OCCURRENCE AND USE

Chlorodifluoromethane (HCFC-22) is manufactured for use as an aerosol propellant, a refrigerant, and a low-temperature solvent. The shuttle orbiter uses HCFC-22 in an azeotropic 49:51 mixture of HCFC-22 and Freon 115, called Freon 502, as a refrigerant in refrigerators and freezers used in the mid-deck and the modules. Up to four refrigerator-freezers could be flown on any one mission, each of which could be charged with up to 50 g of FC-502 for a total of up to 100 g of HCFC-22 on any given mission. Low concentrations ($\leq$ 0.03 ppm) of HCFC-22 are seen in the spacecraft atmosphere in about one-fourth of shuttle missions (James et al. 1994).

UPTAKE, METABOLISM, AND TOXICOKINETICS

Absorption

The solubility of inhaled HCFC-22 in blood is greater than that of nitrous oxide but less than ether or acetone (Varene et al. 1989). It has an average blood/gas partition coefficient in humans of 0.77 (Woolen et al. 1992) and a mean Bunsen coefficient of solubility of 0.804 cm^3 of blood per atmosphere at 37°C (Varene et al. 1989). It is absorbed from the blood by fatty tissues, where it can persist for up to 1 d. In rabbits exposed by inhalation, blood concentrations of HCFC-22 increased very rapidly, steady state being approached in about 1 min, after which a plateau phase continued throughout a 30-min exposure (Sakata et al. 1981). In male volunteers inhaling HCFC-22 at either 92 or 518 ppm for 4 h, breath concentrations during exposure were similar to exposure concentrations from 0.5 h onward. Within the first hour of exposure, blood concentrations approached a plateau, which was proportional to the dose (Woolen et al. 1992).

Distribution

No data were found on the distribution of inhaled HCFC-22 in humans or animals except that its fat/blood partition coefficient was estimated to be about 10 (Woolen et al. 1992).

Excretion

Following inhalation by male volunteers, there was a rapid initial decline in blood concentrations followed by a slower decline (Woolen et al. 1992). Breath concentrations declined steadily, following a three-compartment model. Physiologically based pharmacokinetic modeling indicated that >90% of the HCFC-22 absorbed is eliminated within 10 h. Some HCFC-22 was detectable in the urine for up to 22 h in the high-dose exposure group. Woolen et al. (1992) estimated half-lives for three elimination phases in humans to be 0.005, 0.2, and 2.6 h, but stated that some might persist in fatty tissues for up to 1 d. The authors' estimated half-life value of 0.005 h, which equals 18 s, would allow only enough time for about 30% of the blood in the body to pass through the lung. Thus, it cannot represent a true half-life for elimination from the blood, but probably represents clearance of residual unabsorbed compound from within the lung.

Following cessation of exposure to 100,000 ppm or 200,000 ppm, blood concentrations of HCFC-22 in rabbits decreased rapidly—up to 50% within 1 min—and after 15-20 min, blood concentrations became 8-9 μL/g in all cases, irrespective of inhaled concentration (Sakata et al. 1981).

Metabolism

Following inhalation by male volunteers, urinary fluoride concentrations were all within the normal range for unexposed controls, indicating that HCFC-22 is not metabolized to fluoride to any significant extent (Woolen et al. 1992). Measurement of urinary fluoride concentrations, however, would not detect metabolic removal of chlorine atoms, which would occur before removal of fluorine atoms. Thus, extensive metabolism could have occurred undetected.

Peter et al. (1986) showed that HCFC-22 administered to rats by either intraperitoneal (ip) injection of 3.08 mL/kg or inhalation at 160 ppm (initial concentration) in a 6.38-L dessicator for 7 h was exhaled almost completely unchanged, even when rats were pretreated with phenobarbital or DDT to enhance liver metabolism.

TOXICITY SUMMARY

The toxicity of HCFC-22 is reported to be low compared with many other chlorofluorocarbons. Demonstrated effects include sensitization to cardiac arrhythmia, central-nervous-system (CNS) effects at high doses, and minimal genotoxicity. There were reports of small or missing eyes in offspring of rats

(at a low incidence), but not of rabbits, exposed at 50,000 ppm during gestation. For exposures of at least 4 mo at 14,000 ppm, pathological changes in the blood and internal organs were also reported (Karpov 1963a). These toxic effects are described in detail below.

Acute and Short-Term Exposures

Studies of the effects of brief exposures to HCFC-22 have demonstrated that high concentrations affect the nervous system and sensitize the heart to arrhythmias. CNS disturbances have been observed in both human "sniffing" cases and animals exposed to high doses. Although a number of human "sniffing" deaths have been blamed on HCFC-22-induced arrhythmias, the most reliable data come from animal studies.

Lethality

Karpov (1963b) reported that the 2-h LC_{50} (lethal concentration for 50% of the animals), lowest-observed-adverse-effect level (LOAEL), and no-observed-adverse-effect level (NOAEL) for lethality of HCFC-22 in mice were 390,000 ppm, 367,000 ppm, and 316,000 ppm, respectively.

Autopsies were performed on two of six fishermen who died from exposure to HCFC-22 in a confined space in a commercial fishing boat accident. The results revealed fine lipid droplets in the cytoplasm of hepatocytes, mainly in the peripheral zone of hepatic lobules, in addition to findings expected for suffocation from oxygen deficiency (i.e., lung congestion and edema) (Morita et al. 1977). Similar fine granular fat droplets were seen in hepatocytes of mice exposed to HCFC-22 at 250,000 ppm (16% oxygen, 59% nitrogen) for 60 min, but not in hepatocytes of control mice (Morita et al. 1977).

Cardiac Arrhythmia

One study reported HCFC-22-induced cardiac arrhythmias in humans, but its results were questionable due to methodological flaws. In that epidemiological study of medical students during their pathology residency in a Boston hospital, half of those exposed to HCFC-22 during preparation of frozen sections reported heart palpitations. These consisted of mostly premature atrial contractions and episodes of paroxysmal atrial fibrillation, as confirmed by electrocardiography (Speizer et al. 1975). A dose response was found when the frequency of reported episodes of palpitation was compared with the number

of frozen sections performed per week. The data and interpretation are not compelling, however. An average exposure of 300 ppm was reported, but much higher peak exposures were likely to have occurred due to the measurement technique used: a 2-min sample was collected from the breathing zone while two 10-s blasts of HCFC-22 were expelled into an open cryostat.

In another epidemiological study, no increase was found in HCFC-22-related mortality due to heart or circulatory disorders (ACGIH 1991a).

In beagle dogs, cardiac sensitization to induction of arrhythmias by epinephrine was seen experimentally during 5-min exposures to HCFC-22 at 50,000 ppm (2 of 12 exposed dogs) but not at 25,000 ppm (0 of 12 exposed dogs) (Reinhardt et al. 1971).

Cardiac sensitization to arrhythmias by all chlorofluorocarbons tested to date appears to be dependent on blood concentrations but independent of exposure duration, once steady state has been achieved. That was demonstrated by Reinhardt using a different Freon, CFC-12 (dichlorodifluoromethane). Exposure for up to 1 h to CFC-12 at 25,000 ppm did not induce arrhythmias, whereas a 5-min exposure at 50,000 ppm produced marked responses in 5 of 12 exposed dogs, and a 0.5-min exposure to 135,000 ppm produced marked responses in 2 of 7 exposed dogs (Reinhardt et al. 1971).

FC-22 induced cardiac arrhythmias in anesthetized Swiss mice (three of five exposures) exposed for 6 min at 400,000 ppm only if they received intravenous injections of epinephrine at 6 μg/kg for 2 min after the start of inhalation (Aviado and Belej 1974). The arrhythmias were characterized as second-degree atrioventricular block, ventricular extrasystoles, and ventricular fibrillation. The mice showed no arrhythmias without epinephrine. No sensitization to epinephrine-induced arrhythmias was seen in mice exposed to HCFC-22 at 200,000 ppm (Aviado and Belej 1974) or in monkeys exposed at 200,000 ppm (Belej et al. 1974), although that concentration caused depressed cardiac contractility with a fall in aortic blood pressure in monkeys.

One of 14 rabbits exposed to HCFC-22 at 60,000 ppm for 5 h/d, 5 d/w for 8 w, and receiving sodium phenobarbital at 0.5 g/L of drinking water to stimulate drug-metabolizing enzyme systems, developed "well-defined cardiac arrhythmias of probable supraventricular or ventricular origin" (Van Stee and McConnell 1977a).

CNS Effects

CNS effects (excitation or equilibrium disturbances) were reported in rats and guinea pigs exposed to HCFC-22 for 2 h at 75,000 to 100,000 ppm (Weigland 1971). Narcosis occurred in this study at 200,000 ppm.

The following sequence of effects were observed in rabbits exposed to

HCFC-22 at concentrations that increased continuously from 0% to about 40% (vol/vol) at various rates, with effects beginning at about 8% HCFC-22: (1) reeling, (2) weakness of the forelegs, (3) falling down, (4) flow of mucous fluid from mouth and nose, dilation of the pupils, and lacrymation, (5) violent movement of body and extremities, i.e., running, (6) cyanosis, and (7) death (Sakata et al. 1981). Death occurred at exposure concentrations of 29% to 42% (vol/vol), depending on the rate of increase of the concentration of HCFC-22 (0.5%/min and 2.0%/min, respectively). At fixed concentrations for 30-min exposures, symptoms appeared in the same order, 5% HCFC-22 being a NOAEL and death occurring in 15 min at 30% and in 10 min at 40% (Sakata et al. 1981).

In guinea pigs exposed for 5 min, 30 min, 1 h, or 2 h to HCFC-22, 10,000 ppm was found to be a NOAEL for all exposure times (Nuckolls 1940). Exposure at 24,000-27,000 ppm induced somewhat rapid breathing at 5 min and slight lacrymation with partially closed eyes at 2 h. Exposure at 51,000-54,000 ppm induced deep breathing and slight lacrymation at 5 min and irregular breathing, rubbing of their noses, and shaking their heads at 2 h. Exposure at 95,000-117,000 ppm induced rapid breathing, slight lacrymation, head shaking, nose rubbing, and sniffing at 5 min, labored breathing, slight lacrymation, weakness of the rear legs and occasional tremors at 30 min, stupor, occasional gross tremors, loss of equilibrium, weakness of rear legs, and difficulty standing at 60 min, and at 2 h, stupor, difficulty standing, weakness of rear legs and partially closed eyes. Recovery was rapid after all these exposures up to 117,000 ppm. Exposure at 180,000-226,000 ppm for 5 min caused rapid breathing, convulsive tremors, inability to stand or walk, lacrymation and nasal discharge. Exposure for 30 min induced stupor, inability to stand, convulsive leg movements and tremors, labored breathing, and nasal discharge. Exposure for 60 or 120 min induced audible breathing, lacrymation, nasal discharge, convulsive leg movements, inability to stand, and the animals lay on their backs. Complete recovery after exposures at 180,000-226,000 ppm took 1 d (Nuckolls 1940).

Karpov (1963b) reported that 40,000 ppm was a 40-min LOAEL for an increased reflex response time and a decreased reflex response strength in rabbits.

Subchronic and Chronic Exposures

Subchronic or chronic studies of the effect of HCFC-22 exposure on animals were reported by Karpov (1963a), Tinston et al. (1981 a,b), and Maltoni (1988). The Tinston and Maltoni studies focused on the carcinogenic potential of HCFC-22. The Karpov study examined the systemic toxicity of HCFC-22,

reporting that some effects began to be noticeable only around the fourth to sixth month of exposure. In a 1977 study by Van Stee, liver histopathology and cardiac arrhythmias were seen in rabbits exposed to HCFC-22 at 60,000 ppm beginning at 4 w of intermittent exposure and increasing until deaths were seen at 9 w (Van Stee et al. 1977b). In another study, no adverse effects were seen in rats after 2 mo of exposures to HCFC-22 for 5 h/d at 50,000 ppm (Lee and Suzuki 1981).

Endurance and Oxygen Consumption

Karpov (1963a) reported that mice exposed to HCFC-22 at 14,000 ppm for 6 h/d, 6 d/w. for 10 mo could swim for only 25-110 min compared with control mice, which swam for 55-170 min. Starting with the fourth through sixth month of exposure, Karpov found a progressive decrease in oxygen consumption in rats exposed at 14,000 ppm (6 h/d, 6 d/w for 10 mo) compared to control rats. The swim times of mice and oxygen consumption of rats exposed similarly at 2000 ppm were not different from controls (Karpov 1963a).

Decreased Weight Gain

Karpov (1963a) reported that mice exposed to HCFC-22 at 14,000 ppm for 6 h/d, 6 d/w for 10 mo weighed 26 ± 1.5 g compared with controls, which weighed 34 ± 1.8 g, with decreased weight gains noticeable beginning around the fourth to sixth month of exposure. Mice exposed similarly to HCFC-22 at 2000 ppm were unaffected (Karpov 1963a)

Histopathological Effects

An NIEHS study (Van Stee and McConnell 1977a) found that the livers of New Zealand White rabbits exposed 5 h/d, 5 d/w for 10 w to HCFC-22 at 60,000 ppm were pale, and there was a modest increase in the activities of some serum enzymes beginning the 4th w of exposure. Effects were more pronounced in females and in animals treated concomitantly with sodium phenobarbital (NaPB) at 0.5 g/L of drinking water. One female rabbit on NaPB became clinically ill by the end of the 9th w of exposure. This rabbit also developed cardiac arrhythmias during w 6-8.

In another NIEHS study, Lee and Suzuki (1981) found no signs of histopathology, hematological toxicity, decreased fertility, dominant lethality, teratogenicity,

or exposure-related changes in organ weights in Sprague-Dawley rats exposed to HCFC-22 at 50,000 ppm for 5 h/d for 8 w except for biologically insignificant reductions in the weights of the prostatic and coagulating glands.

Karpov (1963a) reported pathological changes upon histopathological examination of the internal organs of rabbits, rats, and mice exposed at 14,000 ppm (6 h/d, 6 d/w, 10 mo). These changes included dilation of blood-vessel walls; emphysema, atelectasis, and enlarged and torn alveolae in lungs; edema and nerve-cell death in the brain and spinal cord and macrophage proliferation in liver and kidneys; and degeneration of the spleen, beginning anywhere from the first months to the eighth month of exposure. Mice and rats exposed similarly at 2000 ppm were unaffected (Karpov 1963a). Karpov's descriptions of histopathology are often vague and nonquantitative, making it difficult to judge the true significance of the reported effects.

In a study by Tinston et al. (1981 a), no exposure-related non-neoplastic organ pathology was observed in rats and mice exposed to HCFC-22 for 5 h/d, 5 d/w at 0, 1000, 10,000, or 50,000 ppm for the rodents' lifetimes except for the following mild effects seen at the highest dose: a decrease in body-weight gain in male rats up to w 80; increased liver, kidney, adrenal, and pituitary absolute weights in female rats; and hyperactivity in mice. A clear no-effect level was seen at 10,000 ppm.

Effects on Reflexes

Karpov (1963a) reported that mice exposed to HCFC-22 at 14,000 ppm for 6 h/d, 6 d/w for 10 mo were slower in establishing conditioned reflexes than control mice. Beginning around the fourth to sixth month of exposure, rats exposed at 14,000 ppm for 6 h/d, 6 d/w showed a decreased ability to sum subthreshold electrical stimuli for reflex activity (Karpov 1963a). Mice and rats exposed similarly at 2000 ppm were unaffected.

Carcinogenicity

Tinston et al. (1981a) reported no differences in the total number of animals bearing tumors (without regard to site or type of tumor) in male and female Alderly Park (Wistar-derived) rats exposed to HCFC-22 for a lifetime (5 h/d, 5 d/w) at 0, 1000, 10,000, or 50,000 ppm. Of the tumor-bearing animals, however, an increase in the incidence of subcutaneous fibrosarcomas (most consistently at the salivary gland) was seen only in male rats exposed at 50,000 ppm (18 of 80), compared with the incidence in two rat control groups (5 of 80

and 7 of 80), in rats exposed at 1000 ppm (8 of 80), and in rats exposed at 10,000 ppm (5 of 80). In a review of the studies, Litchfield and Longstaff (1984) discounted the biological significance of the salivary-gland tumors, because they were increased only in males and only at 50,000 ppm. Because spontaneously occurring fibrosarcomas are not uncommon in aging male rats, they concluded that the apparent increase in the spontaneous-tumor incidence was possibly due to a nonspecific action, such as stress at the high exposure concentration or a weak promotional effect (Litchfield and Longstaff 1984). There was also a slightly increased incidence of Zymbal-gland carcinomas in the 50,000-ppm male group (4 of 80 vs. 0 of 80 in all other exposure groups) (Tinston et al. 1981a). Again, Litchfield and Longstaff discounted the biological significance of these tumors. Effects similar to those seen in the male rats were not observed in the female rats, nor were they seen in male or female mice in a parallel study (Tinston et al. 1981b) or in Alderly Park rats receiving HCFC-22 orally in corn oil at 300 mg/kg/d, 5 d/w for 52 w (Longstaff et al. 1984). Litchfield and Longstaff's conclusion that the observed tumors were not exposure related is based in part on their finding that no non-neoplastic histopathological changes were seen in either salivary glands or Zymbal glands (i.e., the exposure did not produce any organ-specific non-neoplastic toxicity (H. Trochimowicz, Haskell Laboratory, E.I. du Pont de Nemours, Wilmington, Del., personal commun., March 25, 1998).

In another study, Sprague-Dawley rats exposed to HCFC-22 at 5000 or 1000 ppm for 4 h/d, 5 d/w for 104 w and Swiss mice exposed for 4 h/d, 5 d/w for 78 w had no increase in tumor incidence compared with controls (Maltoni et al. 1988).

Epidemiological studies (e.g., studies of refrigeration workers) have been inconclusive because of the difficulty of finding sufficiently large study populations with well-defined exposures to HCFC-22 and without confounding exposures (Axelson 1985).

Genotoxicity

HCFC-22 was found to be weakly to moderately mutagenic only at high levels when tested at concentrations up to 50% in the Ames *Salmonella* bacterial mutation assay using tester strains TA1535 and TA100 for exposures lasting 24 h to 3 d (Longstaff et al. 1984). HCFC-22 was negative for mutagenicity in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* and in a host-mediated assay with those two yeast strains (Loprieno and Abbondandolo 1980). It did not induce cell transformation when tested as a gas or liquid in BHK21 cells in vitro in the presence of S-9 mix (Longstaff et al. 1984).

Loprieno and Abbondandolo (1980) found HCFC-22 to be negative in unscheduled DNA synthesis assays in the human heteroploid EUE cell line and in V-79 and CHO Chinese hamster cells.

Reproductive and Developmental Toxicity

Exposure of rats to HCFC-22 at 50,000 ppm for 5 h/d for 8 w did not affect male fertility or induce dominant dominant lethality (Lee and Suzuki 1981). Female rats exposed at 50,000 ppm for 6 h/d on d 6 to 15 of pregnancy produced a low incidence of micropthalmia (small eyes) and anopthalmia (missing eyes) in the offspring (Palmer et al. 1978a); those effects were not seen in rabbits (Palmer et al. 1978b).

Spaceflight Effects

Spaceflight, on rare occasions, has been accompanied by non-life-threatening cardiac dysrhythmias (but no life-threatening arrhythmias) at a higher frequency than observed for the affected individuals in tests on earth (Charles et al. 1994). Such a putative spaceflight-induced predisposition to cardiac dysrhythmias might enhance the arrhythmogenic effects of HCFC-22 in a manner similar to the sensitization seen in animals upon injection of epinephrine.

Synergistic Effects

Although its effects might not be strictly termed synergistic, epinephrine predisposes the heart to arrhythmia. Dogs (Reinhardt et al. 1971) and mice (Aviado and Belej 1974) administered epinephrine developed cardiac arrhythmia when exposed to HCFC-22 at 50,000 and 400,000 ppm, respectively. That effect was not seen at concentrations of HCFC-22 near 25,000 ppm in dogs and 200,000 ppm in mice, even with injection of epinephrine. More data would be required to establish whether those concentrations could be considered thresholds for cardiac arrhythmia.

A summary of the toxicity data on HCFC-22 is presented in Table 9-1.

TABLE 9-1 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
300	Occupational	Human	Cardiac arrhythmias	Speizer et al. 1975
Unknown, high	A few min (industrial accident)	Human (n = 2)	Fine lipid droplets in the cytoplasm of hepatocytes; death due to suffocation	Morita et al. 1977
2000	6 h/d, 6 d/w, 10 mo	Rat, mouse, rabbit	NOAEL for decreased swim time, decreased weight gain, decreased oxygen consumption, toxicity and histopathological changes in internal organs, slowed formation of conditioned reflexes, and decreased ability to sum sub-threshold stimuli	Karpov 1963a
5000	4 h/d, 5 d/w, 104 w	Rat	NOAEL for tumors	Maltoni et al. 1988
5000	4 h/d, 5 d/w, 78 w	Mouse	NOAEL for tumors	Maltoni et al. 1988
10,000	Lifetime	Alderly Park rat	NOAEL for subcutaneous fibrosarcomas and increased hepatomas in males	Tinston et al. 1981a
10,000	2 h	Guinea pig (n = 12)	NOAEL (occasional chewing motions; recovered immediately)	Nuckolls 1940
14,000	6 h/d, 6 d/w, 10 mo	Mouse	Decreased swim time; decreased weight gain and decreased oxygen consumption beginning 4th to 6th mo	Karpov 1963a
14,000	6 h/d, 6 d/w, 10 mo	Rabbit, mouse, rat	Hematological toxicity and histopathology of internal organs (blood vessels, lungs, CNS, heart, liver, kidneys, spleen) beginning 4th to 6th mo., slowed formation of conditioned reflexes and decreased ability to sum subthreshold stimuli	Karpov 1963a

24,000-27,000	2 h	Guinea pig (n = 12)	Slight lacrymation, eyes partially closed; occasional chewing motions; recovered quickly	Nuckolls 1940
25,000	A few min	Beagle dog	NOAEL for sensitization to induction of cardiac arrhythmia by epinephrine	Reinhardt et al. 1971
40,000	40 min	Rabbit	Increased reflex reaction time; decreased reflex strength	Karpov 1963b
50,000	5 h/d, 8 w	Sprague-Dawley rat	NOAEL for hematotoxicity, histopathology, dominant lethality and reduced male fertility	Lee and Suzuki 1981
50,000	A few min.	Beagle dog	Sensitization to induction of cardiac arrhythmia by epinephrine	Reinhardt et al. 1971
50,000	Males: 131 w females: 118 w	Alderly Park rat	Increased incidence of sub-cutaneous fibrosarcomas and Zymbal gland carcinomas in males; decreased body-weight gain up to week 80 in males; and increased liver, kidney, adrenal, and pituitary weights in females	Tinston et al. 1981a
50,000	Males: 83 w females: 94 w	Alderly Park mouse	Limited evidence of increased hepatomas in males Hyperactivity	Tinston et al. 1981b
50,000	6 h/d, d 6-15	Pregnant CD rat	Low incidence of micropthalmia and anopthalmia in offspring	Palmer et al. 1978a
50,000	6 h/d, d 6-15	Pregnant New Zealand rabbit	NOAEL for micropthalmia and anopthalmia in offspring	Palmer et al. 1978b
51,000-54,000	5 min	Guinea pig (n = 12)	Slight lacrymation; deep breathing; occasional chewing motions; recovered quickly	Nuckolls 1940
51,000-54,000	30 min	Guinea pig (n = 12)	Slight lacrymation; fast and deep breathing; occasional chewing motions; occasionally rubbed their noses and shook their heads; recovered quickly	Nuckolls 1940
51,000-54,000	60 min	Guinea pig (n = 12)	Slight lacrymation; fast and shallow breathing; eyes partially closed; slight clear nasal discharge; recovered quickly	Nuckolls 1940

Concentration, ppm	Exposure Duration	Species	Effects	Reference
51,000-54,000	2 h	Guinea pig (n = 12)	Slight lacrymation; irregular breathing; eyes partially closed; occasionally rubbed their noses and shook their heads; recovered quickly	Nuckolls 1940
60,000	5 h/d, 5 d/w, 8-12 w	Rabbit	LOAEL for cardiac arrhythmia in 1 of 14 exposed rabbits	Van Stee and McConnell 1977a
60,000	5 h/d, 5 d/w, 4-12 w	Rabbit	LOAEL for pale livers, modestly elevated serum enzymes	Van Stee and McConnell 1977a
75,000	2 h	Rat, guinea pig	CNS effects (excitation and/or equilibrium disturbances)	Weigland 1971
95,000-117,000	5 min	Guinea pig (n = 12)	Slight lacrymation; fast breathing; eyes partially closed; occasionally rubbed their noses, sniffed, shook their heads, and made chewing motions; recovered quickly	Nuckolls 1940
95,000-117,000	30 min	Guinea pig (n = 12)	Slight lacrymation; labored breathing; eyes partially closed; occasional chewing motions and tremors; weakness in rear legs; recovered quickly	Nuckolls 1940
95,000-117,000	60 min	Guinea pig (n = 12)	Occasional gross tremors and loss of equilibrium; difficulty standing; eyes partially closed; weakness in rear legs; stupor; recovered quickly	Nuckolls 1940
95,000-117,000	2 h	Guinea pig (n = 12)	Stupor; eyes partially closed; difficulty standing; weakness in rear legs; recovered quickly	Nuckolls 1940
180,000-226,000	5 min	Guinea pig (n = 12)	Stupor; eyes partially closed; unable to stand and walk; weakness in rear legs; slight nasal discharge and lacrymation; apparently recovered in 1 d.	Nuckolls 1940
180,000-226,000	30 min	Guinea pig (n = 12)	Stupor; eyes partially closed; unable to stand and walk; convulsive leg movements and tremors; nasal discharge and lacrymation; labored breathing; apparently recovered in 1 d	Nuckolls 1940

180,000-226,000	60 min	Guinea pig (n = 12)	Animals lying on backs, unable to stand; convulsive leg movements; nasal discharge and lacrymation; eyes partially closed; audible breathing; apparently recovered in 1 d	Nuckolls 1940
180,000-226,000	2 h	Guinea pig (n = 12)	Unable to stand; convulsive leg movements and tremors; nasal discharge and lacrymation; audible breathing; apparently recovered in 1 d	Nuckolls 1940
200,000	2 h	Rat, guinea pig	Narcosis	Weigland 1971
200,000 (epinephrine treated)	6 min	Male Swiss mouse	NOAEL for cardiac arrhythmia	Aviado and Belej 1974
200,000	5 min	Monkey	Depressed cardiac contractility, decreased aortic blood pressure, NOAEL for cardiac arrhythmia	Belej et al. 1974
250,000	60 min	Mice	Fine granular fat droplets in the cytoplasm of hepatocytes	Morita et al. 1977
300,000	30-92 min	Rabbit	Reeling, weakness of forelegs, ataxia, flow of mucous fluid from mouth and nose, mydriasis, lacrymation, violent movement of body and extremities, cyanosis, death	Sakata et al. 1981
316,000	2 h	Mouse (n = 20)	LC_0	Karpov 1963b
367,000	2 h	Mouse (n = 20)	LC_{low}	Karpov 1963b
390,000	2 h	Mouse (n = 20)	LC_{50} with deaths both during exposure and on subsequent days	Karpov 1963b
400,000	7-10 min	Rabbit	Reeling, weakness of forelegs, ataxia, flow of mucous fluid from mouth and nose, mydriasis, lacrymation, violent movement of body and extremities, cyanosis, death	Sakata et al. 1981
400,000	6 min	Swiss mouse (n = 108)	Cardiac arrhythmia only with simultaneous epinephrine administration	Aviado and Belej 1974

The available toxicity data have been used to establish safe concentrations for human exposures to HCFC-22 under various exposure scenarios. Table 9-2 presents the current limits been by various U.S. organizations. Table 9-3 presents spacecraft maximum allowable concentrations (SMACs) based on the data above. The rationale for the values in Table 9-3 immediately follow the table.

TABLE 9-2 Exposure Limits Set By Other Organizations

Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	1000 (TWA)	ACGIH 1998
OSHA's PEL	1000	ACGIH 1991b
OSHA's STEL	Not set	ACGIH 1991b
NIOSH's REL	1000 (TWA)	ACGIH 1991b
NIOSH's STEL	1250	ACGIH 1991b

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

TABLE 9-3 Spacecraft Maximum Allowable Concentrations[a]

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	1000	3500	CNS depression
24 h	1000	3500	CNS depression
7 d[b]	1000	3500	CNS depression
30 d	1000	3500	CNS depression
180 d	1000	3500	CNS depression

[a] These SMACs are ceiling values.
[b] Previous 7-d SMAC = 100 ppm (350 mg/m^3). The rationale for this value, which was set sometime before 1990, was not documented.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

To set SMAC values for HCFC-22, acceptable concentrations (ACs) were calculated for the induction of cardiac sensitization to arrhythmias and CNS

depression using the guidelines established by the NRC (1992). For each exposure time (1 h, 24 h, 7 d, 30 d, and 180 d), the lowest AC was selected as the SMAC value (Table 9-4). ACs were not set using data from Karpov's studies (1963a,b) for the reasons set forth below. Although decreases in weight gains were reported, they have never been considered an adverse effect, so no ACs were calculated for that end point. ACs were not calculated for developmental toxicity because NASA flight rules do not permit pregnant astronauts to fly.

Cardiac Sensitization to Arrhythmias

Speizer et al.'s (1975) data on human exposures could not be used to set ACs for cardiac effects because of the large uncertainty in the peak concentrations to which the medical students were exposed. It is quite likely that the effects reported by Speizer et al. were due to high peak exposures and that without such peaks, the measured 300 ppm average would actually be a NOAEL.

Thus, the ACs for sensitization to arrhythmias were based on data for dogs, the most sensitive species tested. The 25,000-ppm NOAEL for epinephrine-challenged dogs (Reinhardt et al. 1971) was adjusted for potential interspecies differences. An additional spaceflight factor of 5 was not applied to this NOAEL because the NRC SMAC Subcommittee concluded that it was unnecessarily conservative for cardiac sensitization data from epinephrine-challenged test animals (NRC 1996). For exposures to CFCs, cardiac sensitization to arrhythmias is generally dependent on blood concentrations but not on exposure duration after the blood concentration has reached a plateau (usually within 1 h). Therefore, no adjustment was made for duration of exposure.

Thus,

1-h, 24-h, 7-d, 30-d, and 180-d AC = 25,000 ppm ÷ 10 (species)
= 2500 ppm.

CNS Depression

Three reports of CNS effects were found: Weigland (1971) showed excitation, equilibrium disturbances, and narcosis in rats and guinea pigs; Sakata et al. (1981) showed dose-dependent severity of equilibrium disturbances, muscle weakness and violent movements in rabbits; and Nuckolls (1940) showed dose-dependent tremors, weakness, convulsions, and ataxia in guinea pigs. Of those

studies, the highest NOAEL (10,000 ppm) was reported by Nuckolls. No effects were reported by Weigland or Sakata et al. at lower doses. Thus, ACs for CNS effects are based on Nuckoll's 10,000 ppm NOAEL for a 2-h exposure. The 10,000 ppm NOAEL is divided by 10 for potential interspecies differences to set a 1-h AC. Because CNS effects are generally dependent only on blood concentration and independent of exposure time beyond about 1 h, the value for the 1-h AC is used for all exposure durations ≥ 1 h.

1-h, 24-h, 7-d, 30-d, and 180-d AC = 10,000 ppm ÷ 10 (species)
= 1000 ppm.

Pathology, Endurance, and Reflexes

A number of considerations lead to the conclusion that the data reported by Karpov (1963a,b) should not be used to set ACs for HCFC-22 exposures.

Karpov exposed rodents intermittently to HCFC-22 at 14,000 ppm for up to 10 mo and reported effects (tissue and organ pathology, decreased endurance, impaired reflexes, etc.) that were manifested only after several months of exposure. Karpov's descriptions of histopathology are often vague and non-quantitative, making it difficult to judge the true significance of the reported effects. Karpov uses nonspecific terms such as "dystrophic changes" and language such as "the number of eosinophiles also changed, but with a tendency toward an increase," which can only be interpreted to mean that the observed effect was not statistically significant. No description is given of what statistical methods, if any, were used, and although Karpov uses the term "significant" to describe some reported differences, the context in which it is used suggests that it is not meant in a rigorous statistical sense.

Despite the industrial importance of HCFC-22, it is unlikely that a long-term study such as Karpov's will be repeated, because the carcinogenicity studies by Tinston et al. (1981a,b), involving a lifetime intermittent exposure of rats and mice at concentrations up to 50,000 ppm, did not report any of the histopathology reported by Karpov in rats and mice. Other effects reported by Karpov, such as decreased endurance and impaired reflexes, were not measured by Tinston et al. (1981a,b).

Although the above arguments individually might not be sufficient to preclude the use of Karpov's data, taken together, they make it difficult to support the use of these data for setting ACs.

Carcinogenicity

HCFC-22 was reported by Tinston et al. (1981a) to cause an increase in the incidence of tumors in the salivary glands and Zymbal glands of male rats exposed for a lifetime to 50,000 ppm. Because a response was seen only in one sex of one out of two species tested and only at the highest dose and very late in the study, the investigators concluded that the observed response represents an increase in the spontaneous tumor incidence due to a nonspecific action or a weak promotional effect. That interpretation is supported by the lack of non-neoplastic histopathological changes in salivary glands and Zymbal glands in the Tinston et al.(1981a) study. Thus, HCFC-22 is judged not to present a significant risk of carcinogenicity, and no AC is set for this end point.

TABLE 9-4 Acceptable Concentrations

End Point, Exposure Data, Reference		Uncertainty Factors				Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d	
Cardiac effects	Dog	1	1	10	1	2500	2500	2500	2500	2500
NOAEL, 25,000 ppm for 5 min (epinephrine treated) (Reinhardt et al. 1971)										
CNS depression	Rat, guinea pig	1	1	10	1	1000	1000	1000	1000	1000
NOAEL, 10,000 ppm for 2 h (Nuckolls 1940)										
SMACs						**1000**	**1000**	**1000**	**1000**	**1000**

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B10 TRICHLOROFLUOROMETHANE (FREON 11)

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PHYSICAL AND CHEMICAL PROPERTIES

Trichlorofluoromethane is a colorless, nonflammable liquid or gas at room temperature and has a faint ether-like odor detectable at a threshold concentration of about 5 ppm (ACGIH 1991).

Formula:	$C1_3FC$
CAS no.:	75-69-4
Chemical name:	Trichlorofluoromethane
Synonyms:	Algofrene type 1, arcton 9, CFC-11, electro-CF 11, eskimon 11, F 11, FC-11, fluorocarbon 11, fluorotri-chloromethane, Freon 11, Freon 11A, Freon 11B, Freon HE, Freon MF, frigen 11, Genetron 11, halocarbon 11, isceon 131, isotron 11, ledon 11, monofluorotrichloromethane, NCI-C04637, trichloromonofluoromethane, ucon fluorocarbon 11, ucon refrigerant 11
Molecular weight:	137.36
Boiling point:	23.8°C
Melting point:	–111°C
Specific gravity:	1.494 g/mL (17.2°C)
Solubility:	Very slightly soluble in water (0.11 g/100 g at 20°C) Soluble in alcohol, ether, and other organic solvents
Conversion factors at 25°C, 1 atm:	1 ppm = 5.61 mg/m^3
	1 mg/m^3 = 0.175 ppm

OCCURRENCE AND USE

Trichlorofluoromethane (CFC-11) does not occur naturally. It is an ozonedepleting chlorofluorocarbon and is used principally as a plastic foam blowing agent, an aerosol propellant for pharmaceuticals for asthmatic patients and toiletries, a refrigerant, a heat-transfer medium, and a solvent-degreasing agent in the aerospace and electronics industries. Low concentrations of CFC-11 have been seen in the spacecraft atmosphere six times of 28 space-shuttle missions and five Spacelabs (once at $\leq$ 1.75 ppm, four times at $\leq$ 0.175 ppm, and once at $\leq$ 0.0175 ppm) (James et al. 1994).

UPTAKE, METABOLISM, AND EXCRETION

Uptake and excretion of CFC-11 have been studied in animals. During a 10-min inhalation exposure of dogs and rabbits to concentrations varying from 0 to 5%, CFC-11 rapidly diffused into the blood, cerebrospinal fluid, urine, and bile and reached steady-state concentrations in the blood within about 10 min. (Paulet et al. 1975a). After cessation of exposure, CFC-11 was eliminated, primarily (98%) through the breath, within 20-50 min. Small quantities of CFC-11 were eliminated in the urine and bile, the bile containing higher concentrations than the urine. Upon cessation of an inhalation exposure to CFC-11, its concentration in blood dropped sharply (Dollery et al. 1970). Although CFC-11 was rapidly cleared from the blood, it was retained for longer periods in some tissues. Niazi and Chiou (1975) showed that the kinetics of elimination of CFC-11 after intravenous infusion into dogs reflects three tissue compartments with half-lives of 3 min, 16 min, and 93 min for the initial, intermediate, and final phases of pulmonary clearance. Thus, multiple doses might result in accumulation of much higher concentrations of CFC-11 in some tissues than is evident by measuring blood concentrations.

Lack of appreciable metabolism has been demonstrated in humans. Radiolabeled CFC-11 inhaled for 7-17 min at 1000 ppm by a man and a woman was recovered quantitatively in the exhaled breath with only trace amounts of radioactivity found in exhaled carbon dioxide (0.13% and 0.10%) and recovered as nonvolatile materials in the urine (0.07% and 0.09%) (Mergner et al. 1975). It is likely that the trace amounts of metabolites were products of radiolabeled impurities. Cox et al. (1972) showed that, although CFC-11 is not significantly metabolized and does not produce free radicals, it exhibits characteristic binding spectra with hepatic microsomal preparations, giving a type I spectrum with a binding constant, K_s, value similar to carbon tetrachloride,

indicating that CFC-11 binds to cytochrome P-450. Although CFC-11 does not appear to be metabolized, Paulet and co-workers reported that CFC-11 had measurable effects on other metabolic processes—at 50,000 ppm, it produced corticosteronemia in the rat (Paulet and Rochcongar 1974)—and slight hyperglycemia with hyperlactacidemia and decreased oxygen consumption in rats, rabbits, and dogs (Paulet et al. 1975b).

TOXICITY SUMMARY

CFC-11 is considered one of the most cardiotoxic of the CFCs. Demonstrated toxic effects include sensitization to epinephrine, which results in induction of cardiac arrhythmia, and changes in respiration and narcosis at high doses. CFC-11 does not appear to be mutagenic or carcinogenic.

Acute and Short-Term Exposures

Cardiac Effects

In five groups of humans (9, 8, 10, 8, and 11 subjects in each group; a total of 46 subjects) exposed in a test chamber to CFC-11 at 1000 ppm for 1, 2, 8, or 10 h, including one group of 8 exposed 8 h/d for 18 d, no adverse effects were seen in EKGs or in a variety of other tests for toxic effects (Stewart et al. 1978).

In dogs inhaling CFC-11 at 3500 ppm, Reinhardt et al. (1971) reported ventricular fibrillation and cardiac arrest following injection of epinephrine. No cardiac sensitization was seen in dogs inhaling up to 1300 ppm. In a later report by Trochimowicz and Reinhardt (1975) from the same DuPont laboratory, cardiac sensitization to induction of arrhythmia by epinephrine was observed during experimental exposure of dogs to CFC-11 at 5000 ppm but not 1000 ppm. The average blood concentration associated with cardiac sensitization in these experiments was 25 μg/mL (arterial) or 20 μg/mL (venous). Arrhythmia was induced in dogs inhaling 800,000 ppm plus 20% oxygen when the dogs were frightened by a loud noise to induce release of endogenous epinephrine. Exercising on a treadmill, likewise known to induce release of endogenous epinephrine, did not induce arrhythmia in dogs inhaling CFC-11 at up to 10,000 ppm (Mullin et al. 1972).

In rats given CFC-11 with simultaneous injections of epinephrine, arrhythmia was seen at 25,000 ppm (Watanabe and Aviado 1975); pentobarbital anesthesia reduced the incidence of arrhythmia and increased the required

concentration of CFC-11 to 100,000 ppm (Doherty and Aviado 1975). Increased sensitivity was seen in rats with cardiac necrosis or pulmonary arterial thrombosis but not pulmonary emphysema (Watanabe and Aviado 1975).

Mice under pentobarbital anesthesia exhibited second-degree atrioventricular block upon inhalation of CFC-11 at 100,000 ppm (Aviado and Belej 1974). Injection of epinephrine reduced the required CFC-11 concentration to 50,000 ppm.

In anesthetized monkeys, CFC-11 at 50,000 ppm elicited cardiac arrhythmia. Epinephrine infusion lowered the required concentration to 25,000 ppm, and coronary arterial occlusion reduced it to 12,500 ppm (Belej et al. 1974). The combination of the two procedures further reduced the threshold concentration of CFC-11 to 5000 ppm

Respiratory Effects

The respiratory effects of exposure of humans to CFC-11 were reported by two laboratories. Stewart et al. (1978) reported finding no adverse effects in pulmonary-function tests, including computerized spirometry in two groups of humans (eight were exposed to CFC-11 at 1000 ppm for 6 h/d, 1 d/w for 4 w, and seven were exposed at 1000 ppm for a single 6-h exposure). Valic et al. (1977) studied the effects of much shorter exposures to higher concentrations. In 10 young male volunteers, a 15-s inhalation of 53,000 ppm led to highly significant but "not clinically alarming" reductions of up to about 10% in ventilatory capacity (maximum expiratory flow) lasting about 45 min (Valic et al. 1977). The magnitude of the effect did not change appreciably for a 45-s exposure. Exposure to 50:50 or 10:90 mixtures of Freon 11 and Freon 12 at individual concentrations of 3,000-18,000 ppm produced a greater effect (up to 14.1% and 11.0% reductions, respectively) than obtained with either Freon alone.

Aviado's laboratory studied the respiratory effects of CFC-11 in several animal species. In monkeys, 50,000 ppm caused a significant reduction in respiratory minute volume that was not preceded by stimulation of breathing (Belej et al. 1974). In dogs, rats, and mice, similar effects were seen at 25,000, 100,000, and 25,000 ppm, respectively. A concentration of 10,000 ppm was a no-observed-adverse-effect level (NOAEL) and 25,000 ppm was a lowest-observed-adverse-effect level (LOAEL) for decreased pulmonary resistance in anesthetized dogs exposed for 5 min (Belej and Aviado 1975). Bronchodilation was observed at 50,000 ppm in the anesthetized monkey (Aviado and Smith 1975) and at 25,000 ppm in the anesthetized dog; bronchoconstriction was seen

in rats and mice at concentrations of 25,000 and 10,000 ppm, respectively (Watanabe and Aviado 1975). Decreased pulmonary compliance was found in rats exposed to CFC-11 at 25,000 ppm (Watanabe and Aviado 1975) and mice exposed at 10,000 ppm (Brody et al. 1974) but not in monkeys (Aviado and Smith 1975) or dogs exposed at up to 50,000 ppm.

CNS Effects

In humans exposed to CFC-11 at 1000 ppm for up to 10 h, no adverse effects were seen in EEGs, neurological tests, visual-evoked responses, and 7 of 11 cognitive tests. The eight male subjects repetitively exposed at 1000 ppm for 8 h/d, 5 d/w for 18 d, however, showed statistically significant but not clinically significant performance decrements in a sound stimulus test (4%), light stimulus test (35%), 10-s estimation test (but not the 30-s estimation test), and the Flanagan arithmetic test (5%) (Stewart et al. 1978). No such decrements were seen in eight male subjects exposed for a single 8-h exposure. The authors interpret these results as showing no effect at the concentrations studied because of the absence of a consistent decrement in test performance or a dose-related response.

In guinea pigs exposed to CFC-11 at concentrations ranging from 8000 ppm to 106,000 ppm for durations of 5, 30, 60, or 120 min, the following signs were noted:

- A NOAEL for all signs at 8000 to 12,000 ppm for all durations up to 2 h.
- A NOAEL for all signs at 21,000 to 25,000 ppm for a 5-min exposure.
- Slight tremors progressing to increasingly severe CNS effects up to unconsciousness with increasing concentration or exposure duration beginning at 21,000 ppm for 30 min up to 106,000 ppm for 2 h.
- Unconsciousness with occasional weak convulsive movements and audible, irregular breathing at 100,000 to 106,000 ppm for 2 h, and apparent recovery within 2 d, but autopsy 8 d after 2-h exposures showed mottled area of resolved congestion or light hemorrhage in lungs (Nuckolls 1933).

Subchronic and Chronic Exposures

Other than carcinogenicity studies, no reports on the effects of long-term exposures to CFC-11 were found.

Carcinogenicity

No reports were found indicating that CFC-11 might be carcinogenic in either humans or animals. An analysis by Gold and Zeiger (1997) of the available literature on CFC-11 found no evidence of carcinogenicity in rats and mice. Epidemiological studies (e.g., of refrigeration workers) have been inconclusive because of the difficulty in finding a large enough study cohort with well-defined exposures to CFC-11 and without confounding exposures to other toxicants (Axelson 1985).

Maltoni et al. (1988) found that exposures to CFC-11 at 1000 or 5000 ppm for 4 h/d, 5 d/w for 104 and 78 w were not carcinogenic to Sprague-Dawley rats or Swiss mice, respectively. The TLV Committee of the American Conference of Governmental Industrial Hygienists (ACGIH 1991) and the World Health Organization (WHO Working Group 1990) reviewed the results of National Cancer Institute (NCI) studies in rats and mice fed CFC-11 at gavage doses of approximately 500 or 1000 mg/kg and 2000 or 3900 mg/kg for 78 w, respectively. The studies yielded no significant increase in tumor incidence (NCI 1978). The NCI considered the study in rats to be inadequate because of poor survival rates and the study in mice to be negative. There was no evidence of carcinogenicity in either male or female Swiss ICR/Ha mice given subcutaneous injections of CFC-11 in tricaprylin shortly after parturition and observed for the following year.

Genotoxicity

Negative results for genotoxicity have been obtained for CFC-11 in vitro using bacteria and mammalian cells with or without metabolic activation and in the dominant lethal test (WHO 1990). CFC-11 was shown to be nongenotoxic at doses up to 10,000 μg per plate in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 when tested in the Ames preincubation assay with and without rat liver and hamster liver homogenate (Zeiger et al. 1987; Gold and Zeiger 1997). CFC-11 was found to be negative for mutagenicity when tested in *S. typhimurium* G 46 strains TA1535 and 1538 at 3.6 mM for 60 min with active and inactive microsomes (Uehleke et al. 1977). It was negative for mutagenicity in the Ames *Salmonella* bacterial mutation assay when tested at a concentration of 1% for 72 h in strains TA1535 and TA100 with and without S9 (Longstaff et al. 1984); negative in *Escherichia coli* K12 and *S. typhimurium* strains TA1535 and TA1538 in the presence of microsomes at 3.6 mM for 1-2 h (Greim et al. 1977); and negative in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and *E. coli* WP2 uvra with and without

metabolic activation (Araki et al. 1994). CFC-11 gas was not mutagenic to cultured mammalian cells in the CHO/HGPRT assay (20-200 μL/3 mL culture medium, 5 h with and 18-19 h without metabolic activation) (Uehleke et al. 1977; Krahn et al. 1980) and did not transform mammalian cells in the Styles transformation assay in the presence of S9 (Longstaff 1988).

Reproductive and Developmental Toxicity

No studies were found on CFC-11's potential effects on reproduction or development.

Spaceflight Effects

Spaceflight, on rare occasions, has been accompanied by non-life-threatening cardiac dysrhythmias (but no life-threatening arrhythmias) at a higher frequency than observed in tests of the affected individuals on earth. Such a putative spaceflight-induced predisposition to cardiac dysrhythmias might enhance the arrhythmogenic effects of CFC-11 in a manner similar to the sensitization seen in animals upon injection of epinephrine.

Interaction with Other Chemicals

The interaction of CFC-11 with epinephrine in producing cardiac arrhythmia has been described in preceding sections. This effect appears to have a threshold concentration of CFC-11 (near 1000 ppm) below which arrhythmias are not produced, even with injection of epinephrine.

Table 10-1 presents a summary of the toxicity data on CFC-11.

TABLE 10-1 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
1000	1, 2, 8, 10 h	Human	NOAEL for EKG or EEG effects, clinical hematology and chemistry, neurological tests, visual evoked responses, ACTH stimulation tests and cognitive tests	Stewart et al. 1978
1000	8 h/d, 1 d/w up to 4 w	Human	NOAEL for pulmonary function effects	Stewart et al. 1978
1000	8 h/d; 18 d	Human	Minor decrements in cognitive tests	Stewart et al. 1978
53,000	15 s	Human	Decreased ventilatory capacity	Valic et al. 1977
10,000	NS	Mouse	Bronchoconstriction	Watanabe and Aviado 1975
10,000	NS	Mouse	Decreased pulmonary compliance	Brody et al. 1974
10,000	5 min	Dog	NOAEL for decreased pulmonary resistance	Belej and Aviado 1975
25,000	5 min	Dog	LOAEL for decreased pulmonary resistance	Belej and Aviado 1975
25,000	NS	Rat	Bronchoconstriction	Watanabe and Aviado 1975
25,000	NS	Dog	Bronchodilation	Aviado and Smith 1975
25,000	NS	Rat	Decreased pulmonary compliance	Watanabe and Aviado 1975
25,000	5 min	Dog, mouse	Decreased respiratory minute volume	Belej and Aviado 1975
21,000-25,000	30-60 min	Guinea pig	Occasional tremors; irregular breathing	Nuckolls 1933
21,000-25,000	120 min	Guinea pig	Occasional tremors; retching; irregular breathing	Nuckolls 1933
45,000-51,000	5 min	Guinea pig	Occasional retching	Nuckolls 1933
45,000-51,000	30 min	Guinea pig	Occasional tremors and retching	Nuckolls 1933
45,000-51,000	60 min	Guinea pig	Slight stupor; occasional tremors, irregular breathing	Nuckolls 1933
45,000-51,000	120 min	Guinea pig	Definite stupor; loss of coordination; difficulty standing; occasional tremors; irregular breathing	Nuckolls 1933

50,000	NS	Monkey, dog	NOAEL for decreased pulmonary compliance	Aviado and Smith 1975
50,000	NS	Monkey	Bronchodilation	Aviado and Smith 1975
50,000	30 min	Rat	NOAEL for CNS effects	Lester and Greenburg 1950
60,000	30 min	Rat	LOAEL for loss of postural reflex	Lester and Greenburg 1950
100,000	5 min	Rat	Decreased respiratory minute volume	Belej and Aviado 1975
100,000-106,000	1 min	Guinea pig	Sniffing	Nuckolls 1933
100,000-106,000	2 min	Guinea pig	Tremors and chewing movements	Nuckolls 1933
100,000-106,000	4 min	Guinea pig	Loss of coordination	Nuckolls 1933
100,000-106,000	5 min	Guinea pig	Difficulty standing	Nuckolls 1933
100,000-106,000	20 min	Guinea pig	Semi-consciousness	Nuckolls 1933
100,000-106,000	30 min	Guinea pig	Severe tremors; inability to stand	Nuckolls 1933
100,000-106,000	60 min	Guinea pig	Unconsciousness; severe tremors; convulsive movements; audible, iregular breathing	Nuckolls 1933
100,000-106,000	120 min	Guinea pig	Unconsciousness; occasional weak convulsive movements; audible, irregular breathing; congestion or light hemorrhage in lungs	Nuckolls 1933
100,000	20 min	Rat	TC_{Low}	Lester and Greenburg 1950
100,000	60 min	Cat	TC_{Low}	Scholz 1962
100,000	90 min	Rat	TC_{Low}	Scholz 1962
150,000	8 min	Rat	TC_{Low}	Lester and Greenburg 1950
150,000	A few min	Mouse	Lethality	Caujolle 1964
200,000	4 min	Rat	TC_{Low}	Lester and Greenburg 1950
500,000	1 min	Rat	TC_{Low}	Lester and Greenburg 1950

NS, not specified.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 10-2 presents exposure limits for CFC-11 set by other organizations and Table 10-3 presents the SMACs established by NASA.

To set SMAC values for CFC-11, acceptable concentrations (ACs) were calculated for the induction of each adverse effect (cardiac arrhythmia, respiratory effects, and CNS effects) using the guidelines established by the NRC (1992). For each exposure time (1 h, 24 h, 7 d, 30 d, and 180 d), the lowest AC was selected as the SMAC value (Table 10-4).

TABLE 10-2 Exposure Limits Set by Other Organizations

Organization	Exposure Limit, ppm	Reference
ACGIH's STEL	1000 (ceiling)	ACGIH 1997
OSHA's PEL	Not set	ACGIH 1991
OSHA's STEL	1000 (ceiling)	ACGIH 1991
NIOSH's REL	Not set	ACGIH 1991
NIOSH's STEL	1000 (ceiling)	ACGIH 1991
NRC's 1-h EEGL	1500	NRC 1984
NRC's 24-h EEGL	500	NRC 1984
NRCs' 90-d CEGL	100	NRC 1984

STEL, short-term exposure limit; PEL, permissible exposure limit; REL, recommended exposure limit; EEGL, emergency exposure guidance level; CEGL, continuous exposure guidance level.

TABLE 10-3 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	140	790	Cardiac arrhythmia
24 h	140	790	Cardiac arrhythmia
7 d[a]	140	790	Cardiac arrhythmia
30 d	140	790	Cardiac arrhythmia
180 d	140	790	Cardiac arrhythmia

[a] Previous 7-d SMAC = 100 ppm (560 mg/m^3).

Cardiac Sensitization to Arrhythmia

ACs for cardiac effects are based on the Stewart et al. (1978) report of a NOAEL for 46 humans exposed to CFC-11 at 1000 ppm for ≤ 1 h. Five groups comprised 9, 8, 10, 8, and 11 subjects. Safety factors of 10/√46 = 1.47 for the low number of human subjects and 5 for potential spaceflight effects on the cardiovascular system were applied. Paulet et al. (1975a) showed that steady-state concentrations in body fluids are achieved quickly; therefore, the resulting AC value of 140 ppm is used for all exposure durations ≥ 1 h.

1-h, 24-h, 7-d, 30-d, and 180-d ACs = 1000 ppm ÷ 1.47 ÷ 5 = 140 ppm.

Respiratory Effects

ACs for respiratory effects are based on the Stewart et al. (1978) report of NOAELs for respiratory effects in 15 humans (two groups of eight and seven subjects each) exposed to CFC-11 at 1000 ppm for 6 h/d, 1 d/w for up to 4 w. An adjustment for the low number of human subjects of 10/√15 = 2.56 was applied for all exposure durations. Because no effects were seen in the Stewart experiments even at the highest dose, although a reduced ventilatory capacity was reported by Valic at much higher concentrations of CFC-11, the ACs calculated below are probably quite conservative.

1-h and 24-h ACs = 1000 ppm ÷ 2.56 = 390 ppm.

CNS Effects

A 1-h AC for CNS effects is based on the Stewart et al. (1978) report of a NOAEL for 46 humans exposed to CFC-11 at 1000 ppm for ≥ 1 h. An adjustment of 10/√46 = 1.47 was applied for the low number of human subjects.

1-h AC = 1000 ppm ÷ 1.47 = 680 ppm.

The ACs for 24-h, 7-d, 30-d, and 180-d exposures were based on the Stewart et al. (1978) report of a NOAEL of 1000 ppm for 27 humans for ≤ 8 h exposures. (The groups comprised 3, 8, 4, 4, and 8 subjects each). An adjustment

of 10/`27 = 1.92 for the low number of human subjects was applied. No adjustment was made for exposure duration because the concentration of CFC-11 in the brain should parallel the concentration in the blood, which reaches steady state quickly (within 2 h).

24-h, 7-d, 30-d, 180-d ACs = 1000 ppm ÷ 1.92 = 520 ppm.

TABLE 10-4 Acceptable Concentrations

End Point, Exposure Data, Reference		Uncertainty Factors				Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d	
Cardiac effects	Human	10/`*n*	1	1	5	140	140	140	140	140
1000 ppm for ≥ 1 h (Stewart et al. 1978)										
Respiratory effects	Human	10/`*n*	1	1	1	390	390	NS	NS	NS
1000 ppm 6/h/d, 1 d/w, for up to 4 w (Stewart et al. 1978)										
CNS effects	Human	10/`*n*	1	1	1	680	520	520	520	520
1000 ppm for ≥ 1 h (Stewart et al. 1978)										
SMACs						**140**	**140**	**140**	**140**	**140**

NS, not set.

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B11 DICHLORODIFLUOROMETHANE (FREON 12)

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PHYSICAL AND CHEMICAL PROPERTIES

Dichlorodifluoromethane is a colorless, nonflammable gas with almost no odor (ACGIH 1991a).

Formula:	Cl_2F_2C
CAS no.:	75-71-8
Chemical name:	Dichlorodifluoromethane
Synonyms:	FC-12, fluorocarbon 12, CFC-12, Freon 12, Genetron 12, Halon
Molecular weight:	120.92
Boiling point:	–29.8°C
Melting point:	–158°C
Specific gravity:	1.1834 g/mL (57°C)
Vapor pressure:	4332 torr at 20°C
Solubility:	Insoluble in water (0.028 g/100 g at 25°C) Soluble in alcohol and ether
Reactivity:	Most halocarbons react violently with highly reactive materials, such as alkali and alkaline earth metals, sodium, potassium, and barium, in their free metallic form. Finely ground magnesium and aluminum might react at higher temperatures.
Conversion factors at 25°C, 1 atm:	1 ppm = 4.94 mg/m^3
	1 mg/m^3 = 0.202 ppm

OCCURRENCE AND USE

Dichlorodifluoromethane (CFC - 12) does not occur naturally. It is an ozonedepleting chlorofluorocarbon and has been used principally as a plastic foam blowing agent, an aerosol propellant, and a refrigerant. Low concentrations (≤ 0.175 ppm) of CFC - 12 have been seen in the spacecraft atmosphere in 5 of 28 shuttle missions and 5 Spacelabs (James et al. 1994).

UPTAKE, METABOLISM, AND TOXICOKINETICS

During a 10-min inhalation exposure of dogs and rabbits at concentrations of 200,000 or 500,000 ppm, CFC - 12 rapidly diffused into the blood, cerebrospinal fluid (CSF), urine, and bile and reached steady-state concentrations in the blood within 2 min for the rabbit and 5 min for the dog (Paulet et al. 1975). After cessation of exposure, CFC -12 is eliminated, primarily (98%) through the breath, within 20-50 min. Small quantities of CFC -12 are eliminated in the urine and bile, with the bile containing higher concentrations than the urine.

After intravenous injection of CFC-12 into dogs, about 1.5 h was required to achieve pseudo-distribution equilibrium in the tissue compartments (Niazi and Chiou 1977). Niazi and Chiou proposed a three-compartment open model for the disposition of CFC -12 in dogs with average half-lives of 1.47, 7.95, and 58.5 min for the three disposition phases. Disposition followed dose-independent kinetics after multiple dosing (Niazi and Chiou 1977). Thus, for multiple or continuous exposures, concentrations of CFC -12 in some tissues might accumulate to higher concentrations than would be apparent in blood.

Radiolabeled CFC -11 inhaled for 7-17 min at 1000 ppm by a man and a woman was recovered quantitatively in the exhaled breath with only trace amounts of radioactivity found in exhaled carbon dioxide (0.08% in both subjects) and recovered as nonvolatile materials in the urine (0.02% and 0.03%) (Mergner et al. 1975). It is likely that the trace amounts of metabolites were products of radiolabeled impurities. In tests on rats, rabbits, and dogs exposed at either 200,000 ppm for 20 min or 50,000 ppm for 2 h/d for 15 d, CFC -12 does not appear to disturb the basal metabolic rate or metabolic pathways (Paulet et al. 1975).

TOXICITY SUMMARY

A considerable amount of research has been done on CFC -12, mostly on cardiopulmonary effects induced by short-term exposures. Demonstrated toxic

effects of exposure to CFC-12 include sensitization to and induction of cardiac arrhythmias, reduced respiratory capacity, and central-nervous-system (CNS) effects at high concentrations.

Acute and Short-Term Exposures

Cardiac Arrhythmia

In two human volunteers, no adverse effects in continuous EKG monitoring were seen during a 2.5-h exposure to CFC-12 at 10,000 ppm (Azar et al. 1972). At 110,000 ppm, however, a "significant degree of cardiac arrhythmia" was reported in a single volunteer within the first 10 min of exposure (Kehoe 1943). A second volunteer in that same series of experiments was exposed at 40,000 ppm for 14 min, after which the concentration was reduced to 20,000 ppm for the remainder of an 80-min exposure. No cardiac effects were reported for that individual (Kehoe 1943).

Ten healthy young volunteers breathing CFC-12 at 134,000 to 135,000 mg/m^3 (27,000 to 27,300 ppm) for 15, 45, or 60 s experienced variations in heart rate exceeding those noted before exposure. In a few cases, inversion of the T wave and, in one case, atrioventricular block were observed; no life-threatening cardiac arrhythmias were observed (Valic et al. 1977).

Stewart et al. (1978) examined the effects of CFC-12 inhalation on a group of 43 male and 32 female volunteers. Subgroups of 2-11 volunteers were exposed at 0, 250, 500, or 1000 ppm for durations of 1, 2, 6, 8, or 10 h or repetitively for 8 h/d, 5 d/w for 3.5 w. A no-observed-adverse-effect level (NOAEL) of 1000 ppm (the highest concentration tested) was based on EKG results and a large number of other toxicity tests in 38 volunteers exposed for $\leq$ 1 h. Repetitive exposures (8 h/d, 5 d/w for 3.5 w) of eight males at 1000 ppm were similarly without adverse effects (Stewart et al. 1978).

Cardiac sensitization to epinephrine, resulting in multiple ventricular beats or cardiac arrest, has been seen in dogs inhaling CFC-12 at 5000 ppm for 5 min but not at 2500 ppm for 6 h/d for 5 d (Reinhardt et al. 1971; Trochimowicz and Reinhardt 1975). The average blood concentration associated with cardiac sensitization in these experiments was 25 μg/mL (arterial) or 20 μg/mL (venous). Arrhythmias were induced in dogs inhaling CFC-12 at 800,000 ppm plus 20% oxygen when the dogs were frightened by a loud noise to induce release of endogenous epinephrine. Exercising on a treadmill, likewise known to cause release of endogenous epinephrine, induced arrhythmias in dogs at CFC-12 concentrations two or three times higher than needed when using injections of epinephrine (Trochimowicz and Reinhardt 1975).

CFC-12 was also shown to be dysrhythmogenic in rats and monkeys but not in mice (Doherty and Aviado 1975). In unanesthetized rats, there was an acceleration of the heart rate but no abnormalities in the EKG pattern during inhalation of CFC-12 at 100,000, 200,000, and 400,000 ppm (Watanabe and Aviado 1975). In anesthetized rats, there was no alteration in heart rate and rare minor alterations in the EKG pattern. In rats with experimentally induced pulmonary emphysema, inhalation of CFC-12 at 400,000 ppm resulted in EKGs displaying ventricular extrasystoles (Watanabe and Aviado 1975).

Lessard and Paulet (1985) examined the mechanism of action of CFC-12 on cardiac fibers isolated from sheep hearts. They concluded that the most likely mechanism consistent with their results and those reported by others is a simple mechanical constraint on intramembrane structures by simple dissolution of CFC-12 in the internal lipid layer of biological membranes.

Respiratory Effects

Ten healthy young volunteers breathing CFC-12 at 134,000 to 135,000 mg/m^3 (27,000 to 27,300 ppm) for 45 s experienced a 3.4% reduction in maximum expiratory flow at 50% of the control vital capacity (MEF 50%) and a 5.6% reduction at MEF 75% (chest three-quarters empty) (Valic et al. 1977). Similar reductions (2.4% and 6.7%) were obtained for 15-s exposures to similar concentrations of CFC-12 (Valic et al. 1977).

Stewart et al. (1978) reported that exposure of 38 volunteers to CFC-12 at 1000 ppm (the highest concentration tested) for $\leq$ 1 h resulted in a NOAEL for effects on pulmonary function measured by computerized spirometry, which included the maximum mid-expiratory flow rate. Seventeen repetitive exposures (8 h/d, 5 d/w for 3.5 w) of eight males at 1000 ppm were similarly without adverse effects (Stewart et al. 1978).

At much higher concentrations, CFC-12 exposures produced respiratory effects in animals. In dogs, inhalation of 100,000 ppm for 5 min caused a significant increase in pulmonary resistance and 200,000 ppm also reduced the respiratory minute volume (Belej et al. 1974). Decreased pulmonary compliance and tidal volume and a variable effect on pulmonary resistance were found in rats exposed to CFC-12 at 50,000 ppm (Watanabe and Aviado 1975).

CNS Effects

Stewart et al. (1978) reported that exposure of 38 volunteers to CFC-12 at 1000 ppm (the highest concentration tested) for ≥ 1 h resulted in a NOAEL for

CNS effects (Stewart et al. 1978). Seventeen repetitive exposures (8 h/d, 5 d/w for 3.5 w) of eight males at 1000 ppm were similarly without adverse effects.

In guinea pigs exposed to CFC-12 at concentrations ranging from 21,000 to 304,000 ppm for durations of 5, 30, 60, or 120 min, 51,000 ppm was found to be a NOAEL for up to 2 h; and 193,000 ppm was a lowest-observed-adverse-effect level (LOAEL), inducing slight retching movements during a 30-min exposure (Nuckolls 1933). Longer exposure times at 193,000 ppm and increased concentrations up to 304,000 ppm led to increasingly severe signs of CNS effects, including deeper breathing, tremors, weakness, lethargy, and inability to stand. All effects appeared to be reversible within 1 d of exposure (Nuckolls 1933).

Subchronic and Chronic Exposures

Carcinogenicity

Maltoni et al. (1988) found no exposure-related cancers in male and female Sprague-Dawley rats exposed to CFC-12 at 0 (150 rats), 1000 (90 rats), and 5000 (90 rats) ppm and in male and female Swiss mice exposed at 0 (90 mice), 1000 (60 mice), and 5000 (60 mice) ppm for 4 h/d, 5 d/w for 104 and 78 w. The highest tested concentration (5000 ppm) might not have been sufficient, however, to firmly establish CFC-12 as noncarcinogenic. No other carcinogenicity studies of CFC-12 were found.

Genotoxicity

CFC-12 was found to be negative for genotoxicity in the Ames *Salmonella* bacterial mutation assay, the BHK21 cell-transformation assay (Longstaff et al. 1984; Longstaff 1988), and the CHO/HGPRT mammalian cell-mutation assay (Krahn et al. 1980).

Reproductive and Developmental Toxicity

No studies on CFC-12's potential effects on reproduction or development were found.

Spaceflight Effects

Spaceflight, on rare occasions, has been accompanied by non-life-threatening cardiac dysrhythmias at a higher frequency than observed in tests of the affected individuals on earth. Such a putative spaceflight-induced predisposition to cardiac dysrhythmias might enhance the arrhythmogenic effects of CFC-12 in a manner similar to the sensitization seen in animals upon injection of epinephrine.

Synergistic Effects

Ten healthy young volunteers breathing a 30:70 mixture of CFC-12 and CFC-114 (1,1,2,2-tetrafluoro-1,2-dichloroethane) at a CFC-12 concentration of 7070-8280 ppm for 15, 45, or 60 s experienced a reduction of ventilatory capacity, which was much more pronounced (more than additive) than after exposure to the individual compounds (Valic et al. 1977).

The sensitizing effect of epinephrine in lowering the concentration of CFC-12 required to induce cardiac dysrhythmias has been described in the preceding sections. There appears to be a threshold concentration of CFC-12 (near 2500 ppm) below which dysrhythmias are not produced in rats, even with injection of epinephrine.

Table 11-1 presents a summary of the toxicity data on CFC-12.

TABLE 11-1 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
1000	1-10h	Human (n = 38)	NOAEL for cardiac, pulmonary, CNS, hematology and clinical chemistry effects	Stewart et al. 1978
1000	8-10 h	Human (n = 23)	NOAEL for cardiac, pulmonary, CNS, hematology and clinical chemistry effects	Stewart et al. 1978
1000	8 h/d, 5 d/w, 3.5 w	Human (n = 8)	NOAEL for cardiac, pulmonary, CNS, hematology and clinical chemistry effects	Stewart et al. 1978
10,000	2.5 h	Human (n = 2)	NOAEL for cardiac dysrhythmia	Azar et al. 1972
27,000-27,300	15, 45, 60 s	Human (n = 10)	NOAEL for cardiac dysrhythmia; LOAEL for reduced ventilatory capacity and tachycardia	Valic et al. 1977
40,000 (14 min) + 20,000 (66 min)	14 min + 66 min	Human (n = 1)	NOAEL for cardiac dysrhythmia	Kehoe 1943
70,000	35 min	Human (n = 1)	NOAEL for cardiac dysrhythmia	Kehoe 1943
110,000	10 min	Human (n = 1)	LOAEL for cardiac dysrhythmia	Kehoe 1943
2500	6 h/d, 5 d	Dog	NOAEL for cardiac sensitization to dysrhythmia	Trochimowicz and Reinhardt 1975; Reinhardt et al. 1971
5000	5 min	Dog	LOAEL for cardiac sensitization to dysrhythmia	Trochimowicz and Reinhardt 1975; Reinhardt et al. 1971
5000	4 h/d, 5 d/w, 104 w	Rat, mouse	NOAEL for carcinogenicity	Maltoni et al. 1988

Concentration, ppm	Exposure Duration	Species	Effects	Reference
100,000	5 min	Dog	Increased pulmonary resistance	Belej et al. 1974
200,000	5 min	Dog	Increased pulmonary resistance; reduced minute volume	Belej et al. 1974
51,000	120 min	Guinea pig	NOAEL for CNS effects	Nuckolls 1933
193,000	30 min	Guinea pig	LOAEL for slight retching movements	Nuckolls 1933
193,000	60, 120 min	Guinea pig	Slight retching movements; slight tremors	Nuckolls 1933
285,000-304,000	30 min	Guinea pig	Weakness, lethargy, inability to walk	Nuckolls 1933
285,000-304,000	45, 120 min	Guinea pig	Inability to stand; occasional trembling	Nuckolls 1933
800,000	30 s	Dog	Cardiac dysrhythmia when frightened by a loud noise	Trochimowicz and Reinhardt 1975

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 11-2 presents exposure limits for CFC-12 set by other organizations and Table 11-3 presents the SMACs established by NASA.

To set SMAC values for CFC-12, acceptable concentrations (ACs) were calculated for the induction of each adverse effect (cardiac arrhythmia and CNS effects) using the guidelines established by the NRC (1992). For each exposure time (1 h, 24 h, 7 d, 30 d, and 180 d), the lowest AC was selected as the SMAC value (Table 11-4).

TABLE 11-2 Exposure Limits Set by Other Organizations

Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	1000 (TWA)	ACGIH 1998
OSHA's PEL	1000 (ceiling)	ACGIH 1991b
OSHA's STEL	Not set	ACGIH 1991b
NIOSH's REL	1000 (ceiling)	ACGIH 1991b
NIOSH's STEL	Not set	ACGIH 1991b

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

TABLE 11-3 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	540	2600	Tachycardia
24 h	95	470	Cardiac sensitization to arrhythmia
7 d[a]	95	470	Cardiac sensitization to arrhythmia
30 d	95	470	Cardiac sensitization to arrhythmia
180 d	95	470	Cardiac sensitization to arrhythmia

[a] Previous 7-d SMAC = 100 ppm (490 mg/m^3).

Cardiac Sensitization to Arrhythmias

ACs for cardiac effects are based on the reports of Azar et al. (1972), Valic* et al. (1977), and Stewart et al. (1978). The results of Valic* et al. show a LOAEL for heart-rate effects of 27,000 ppm for a 15-s exposure; symptoms did not increase in severity within 60 s of exposure. The results of Azar et al. suggest a NOAEL near 10,000 ppm. Using the data of Valic et al. as a starting point and dividing the LOAEL by 10 to estimate a NOAEL yields 2700 ppm. To set a 1-h AC, that value is then divided by 5 for potential spaceflight effects on cardiac arrhythmias.

1-h AC = 27,000 ppm ÷ 10 ÷ 5 = 540 ppm.

ACs for exposures that are > 1 h are based on the NOAEL for cardiac effects in 23 humans after exposure to CFC-12 for 8-10 h at 1000 ppm (Stewart et al. 1978). An adjustment of 10/`23 = 2.08 was applied for the low number of human subjects, and a spaceflight factor of 5 was applied.

≥ 24-h AC = 1000 ppm ÷ 2.08 ÷ 5 = 95.9 ppm rounded to 95 ppm.

Respiratory Effects

ACs for respiratory effects are based on the NOAEL for reductions in ventilatory capacity in 23 humans after exposure to CFC-12 for 8-10 h at 1000 ppm (Stewart et al. 1978). An adjustment of 10/`23 = 2.08 was applied for the low number of human subjects.

≥ 24-h AC = 1000 ppm ÷ 2.08 = 480 ppm.

The independence of respiratory symptoms from exposure duration is supported by the fact that the NOEAL of Stewart et al. (1978) occurred at a concentration times exposure duration (C × t) of 136,000 ppm•h (1000 ppm × 136 h), whereas the reduced ventilatory capacity reported by Valic et al. (1977) occurred at a C × t of 112.5 ppm•h (27,000 ppm × 0.0042 h).

TABLE 11-4 Acceptable Concentrations

End Point, Exposure Data, Reference	Species	Uncertainty Factors				Acceptable Concentrations, ppm				
NOAEL	Small *n*[b]	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d		
Tachycardia	Human	10	1	1	5	540	NS[a]	NS	NS	NS
LOAEL, 27,000 ppm for 15 s (Valic* et al. 1977)										
Cardiac sensitization to arrhythmia	Human	1	2.08	1	5	NS	95	95	95	95
NOAEL, 10,000 ppm ≥ 8 h (Stewart et al. 1978)										
Respiratory effects	Human	1	2.08	1	1	NS	480	480	480	480
NOAEL, 1000 ppm ≥ 8 h (Stewart et al. 1978)										
SMACs						**540**	**95**	**95**	**95**	**95**

[a] NS, not set.
[b] To correct for the small number of human test subjects, the factor is 10/ˆn.

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B12 4-METHYL-2-PENTANONE

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PHYSICAL AND CHEMICAL PROPERTIES

4-Methyl-2-pentanone or methyl isobutyl Ketone (MIBK) is a clear liquid with a sweet odor (ACGIH 1991). According to a review by Amoore and Hautala (1983), the odor threshold of MIBK in air is 0.68 ppm. In a study by Dick et al. (1992), 17 human volunteers were exposed to MIBK at a concentration of 88 ppm for 4 h, and they reported a statistically significantly higher incidence of strong odor than the 8 volunteers in the control group. The odor, however, was not unpleasant (Dick et al. 1992). Other properties of MIBK are listed as follows (ACGIH 1991).

Formula:	$CH_3COCH_2CH_2(CH_3)_2$
CAS no.:	108-10-1
Synonym:	Methyl isobutyl ketone; hexone
Molecular weight:	100.2
Boiling point:	115.8°C
Melting point:	–84.7°C
Vapor pressure:	15 torr at 25°C
Conversion factors at 25°C, 1 atm:	1 ppm = 4.09 mg/m^3
	1 mg/m^3 = 0.24 ppm

OCCURRENCE AND USE

As a solvent, MIBK is used in paints, varnishes, lacquers, aircraft dopes, rubber cements, and adhesives (ACGIH 1991). It is not used per se in Spacecraft, but

it has been found in air samples taken inside the spacecraft during some space-shuttle missions probably as a result of off-gassing (James et al. 1994). For instance, MIBK was detected at a high of 0.002 to 0.006 mg/m^3 (0.0005 to 0.015 ppm) in two missions and 0.01 to 0.06 mg/m^3 (0.0024 to 0.015 ppm) in another four missions (Huntoon 1990, 1991, 1992a,b,c, 1994). During a mission in 1992, MIBK was measured at a high of 0.41 mg/m^3 (0.10 ppm) (Huntoon 1992d).

TOXICOKINETICS AND METABOLISM

Absorption

MIBK vapor is absorbed relatively well in humans. Hjelm et al. (1990) measured the pulmonary retention of inhaled MIBK in eight men exposed to MIBK at 10, 100, or 200 mg/m^3 (2.4, 24, or 49 ppm) for 2 h by comparing the exhaled concentration with the inhaled concentration. Regardless of the exposure concentration, about 60% of the inhaled MIBK was retained by the body. The respiratory retention rate was fairly constant during the 2-h exposure. The blood concentration of MIBK rose quite rapidly, and no plateau was reached during the 2-h exposure.

Another study, however, shows that MIBK blood concentration can reach a plateau in 2 h. Dick et al. (1992) conducted a neurobehavioral study in which MIBK concentrations in the blood and the breath were measured in human volunteers exposed to MIBK at 88 ppm for 4 h. The mean concentrations in 13 men and 12 women combined are presented in Table 12-1. MIBK reached plateau concentrations in the blood and breath as early as 2 h into the exposure.

MIBK absorption is not as well studied in rodents as in humans. Duguay and Plaa (1993) measured the plasma concentration of MIBK given by inhalation

TABLE 12-1 Blood and Breath Concentrations in Volunteers Exposed to MIBK at 88 ppm (Dick et al. 1992)

	2-h Exposure	4-h Exposure	90-min Post-Exposure	20-h Post-Exposure
MIBK in blood (μg/mL)	10.6	10.5	0.2	ND[a]
MIBK in breath (ppm)	0.6	0.6	0.1	ND

[a] ND, below detection limit.

in a study on MIBK's potentiation of the cholestatic effects of taurocholate or manganese bilirubin in rats. MIBK reached about 5, 8, or 14 μg/mL in the plasma 1 h after a 4-h exposure of rats at 200, 400, or 600 ppm.

Distribution

The distribution of MIBK in blood was studied by Lam et al. (1990). In rats exposed to MIBK at 512 ppm for 2 h, MIBK reached a concentration of 25.3 μg/mL in blood with 51.2% distributed to red blood cells (RBCs) and the balance in plasma immediately after the exposure. Apparently, MIBK distributed similarly in human blood because Lam et al. (1990) found that 49.4% of MIBK added to human blood in vitro at 0.8 mg/mL resided with RBCs. For human RBCs, 68% of MIBK was associated with hemoglobin. In human plasma, 80% of MIBK was associated with plasma proteins. Therefore, the majority of MIBK in human blood was associated with proteins.

Metabolism

Based on studies in rodents, MIBK is metabolized by either oxidation at the omega-1 carbon to form a hydroxylated ketone or reduction of the carbonyl group to form an alcohol. DiVincenzo et al. (1976) identified 4-hydroxy-4-methyl-2-pentanone and 4-methyl-2-pentanol as the MIBK metabolites in the serum of guinea pigs administered MIBK at 450 mg/kg intraperitoneally. MIBK was cleared from the serum with a half-life of 66 min in guinea pigs.

The two metabolites of MIBK were not always found in MIBK-exposed rats. For instance, MIBK has been shown by Hirota (1991) to be metabolized into 4-methyl-2-pentanol in rats given MIBK intraperitoneally at 100-300 mg/kg. In contrast, Duguay and Plaa (1993) could not detect 4-methyl-2-pentanol in the plasma of rats 1 h after a 4-h inhalation exposure to MIBK at 200 ppm, but 4-hydroxy-4-methyl-2-pentanone was found at 5 μg/mL. However, both 4-hydroxy-4-methyl-2-pentanone (at about 6-7 μg/mL) and 4-methyl-2-pentanol (at about 4-5 μg/mL) were detected in the plasma of rats 1 h after a 4-h exposure to MIBK at 400 or 600 ppm (Duguay and Plaa 1993).

Excretion

In the eight men studied by Hjelm et al. (1990), about 0.04% of the MIBK dose was excreted in the urine as MIBK within 3 h after a 2-h inhalation

exposure to MIBK at 10, 100, or 200 mg/m^3 (2.4, 24, or 49 ppm). The urinary concentrations of MIBK's metabolites, 4-methyl-2-pentanol and 4-hydroxy-4-methyl-2-pentanone, were below the detection limit of 5 nmol/L at 0.5 or 3 h post-exposure. The total body clearance of MIBK was 1.6 L of blood per hour per kilogram of body weight in these men (Hjelm et al. 1990). However, in another study, Hjelm et al. (1991) found that the total body clearance was 12 L of blood per hour per kilogram of body weight in guinea pigs infused with MIBK intravenously. The reason for the large difference in MIBK's total body clearance between men and guinea pigs is unknown.

Hirota (1991) studied the elimination of MIBK in rats exposed intraperitoneally at 100-300 mg/kg. The major route of MIBK elimination was exhalation via the lungs, which accounted for 41% of the dose. The concentration of MIBK in the exhaled air declined with a half-life of 0.6 h after reaching a maximum at 0.5 h after the injection. Two minor routes of MIBK elimination were urinary excretion of MIBK and 4-methyl-2-pentanol. MIBK in the urine attained a maximum concentration within 3 h of the injection and then it declined with a half-life of 1.8 h. The concentration of 4-methyl-2-pentanol reached its peak within 3-6 h of the injection and then decreased with a half-life of 3.2 h.

TOXICITY SUMMARY

Most of what is known about MIBK's toxicity is from in vivo experimentation, but there is one report of an in vitro study. Huang et al. (1993) showed that about 45 µM of MIBK inhibited Na-K ATPase activity and the binding of dihydroalprenolol by 50% in mouse synaptosomes. Dihydroalprenolol binds to beta-adrenergic receptors, the binding of which is influenced by fluidity changes in membranes. The in vitro data suggest that MIBK can affect synaptosome membranes. However, whether the membrane effects are manifested as adverse effects in vivo is unknown.

Acute and Short-Term Exposures

In vivo studies demonstrated that MIBK exposures can lead to mucosal irritation, central-nervous-system (CNS) depression, renal damage, changes in hepatic weight, and death. Because negative findings in toxicity studies are as important as positive findings in the setting of exposure limits, some of the negative findings will first be discussed, followed by descriptions of positive findings. MacEwen et al. (1971) continuously exposed 4 monkeys, 8 dogs, 50

rats, and 40 mice to MIBK at 100 or 200 ppm at an atmospheric pressure of 725 mmHg (equivalent to 95 or 190 ppm at 760 mmHg) for 2 w. They detected no toxic signs during exposure. After the exposure, there were no changes in hematology (the red- and white-blood-cell counts, hematocrit, hemoglobin concentration) and clinical chemistry (the plasma concentrations of sodium, potassium, calcium, total phosphorus, chloride, cholesterol, total bilirubin, albumin, total protein, uric acid, creatinine, glucose, and alkaline phosphatase, as well as blood urea nitrogen) in dogs and monkeys. The exposure caused no changes in blood gases in dogs or in EEGs in monkeys.

Mucosal Irritation

There were four studies of MIBK's irritation properties in human volunteers, but three of them were conducted without sham-exposed controls (Hjelm et al. 1990; Dick et al. 1992; Iregren et al. 1993). Dick et al. (1992) reported a properly controlled human study in which 17 volunteers exposed to MIBK at 88 ppm for 4 h did not find the exposure objectionable. There were no significant differences in the incidence of throat irritation, lacrimation, nausea, or headache between the two groups. Dick et al. (1992) concluded that their data support the contention that the short-term exposure limit of 75 ppm proposed by the Occupational Safety and Health Administration would prevent irritation.

Silverman et al. (1946) exposed 12 human subjects to various concentrations of MIBK for 15 min while diverting their thoughts from the exposure by showing them a movie; however, no sham exposure was done. MIBK exposures at concentrations higher than 200 ppm produced nose or throat irritation in the majority of the subjects. An exposure at 200 ppm resulted in eye irritation and an objectionable odor in the majority of the subjects. Most of the subjects estimated that an exposure at 100 ppm would be tolerable for 8 h.

Iregren et al. (1993) exposed six men and six women to MIBK at 10 or 200 mg/m^3 (2.4 or 49 ppm) for 2 h (with light exercise at 50 W in the first 1.5 h and resting in bed in the remaining 0.5 h). Unfortunately, no air-exposed controls were used. Irritation was determined by asking the subjects to give a rating in a questionnaire one time before exposure and six times during the exposure. The irritation ratings during the 49-ppm exposure were consistently higher than those in the 2.4-ppm exposure (higher by at least one arbitrary unit) (Iregren et al. 1993). However, the irritation ratings at 49 ppm were not statistically different from those at 2.4 ppm. That is because "the irritation level is fairly high already at exposure to 10 mg/m^3 of MIBK" (in the first four questionnaires

administered during the exposure at 10 mg/m^3 (2.4 ppm), the irritation ratings were higher than the pre-exposure ratings by 1.5 to 2.5 arbitrary units) (Iregren et al. 1993). The authors stated that that "finding may be interpreted as an indication of a high potential for MIBK to induce irritation already at low concentrations." However, they did not reveal the severity of the irritation represented by one arbitrary unit. In addition, the absence of a sham-exposed control group makes the interpretation of the data of Iregren et al. (1993) difficult.

Another human study with no control exposure was conducted by Hjelm et al. (1990), who reported that nose and throat irritation were the most common symptoms. Three of eight men exposed to MIBK at 100 or 200 mg/m^3 (24 or 49 ppm) for 2 h during light exercise experienced nose and throat irritation, and one of eight experienced the irritation at 10 mg/m^3 (2.4 ppm) (Hjelm et al. 1990). Based on the ordinal data, no clear concentration-response relationship was seen. The subjects rated the irritation from 0 to 5 and gave an average rating of about 0.2, 0.4, or 0.3 at 20-50 min into the 2-h exposure to MIBK at 2.4, 24, or 49 ppm, respectively. Hjelm et al. (1990) did not reveal the qualitative equivalents of the numerical scores (e.g., whether a score of 1 represented mild irritation), so the severity of the irritation reported during MIBK exposure in the responsive men is unknown. More important, no sham-exposed control group was used by Hjelm et al. (1990). The same test subjects were exposed to MIBK at three concentrations on different days after having been informed of the maximum exposure concentration. Even though they did not know of the sequence of the exposure, the fact that they were aware that each 2-h exposure involved MIBK could create a bias in their reporting symptoms.

In addition to humans, laboratory animals can exhibit irritation signs when exposed to MIBK. McOmie and Anderson (1946) reported that all mice exposed to MIBK at 43-100 g/m^3 (10,300-24,000 ppm) exhibited signs of mucosal irritation, such as nose pawing, eye closing, the ruffling of fur, and the humping of back. According to de Ceaurriz et al. (1981), MIBK is a sensory irritant because a 5-min exposure at 3200 ppm was irritating in mice, reducing the respiratory rate by 50%. In guinea pigs exposed to MIBK at 10,000-28,000 ppm by Specht (1938), the animals squinted and rubbed their eyes and noses violently, indicating severe mucosal irritation in 1 min. Specht saw salivation after 2 min. The respiratory rate fell to 35/min after 25 min of exposure and the guinea pigs later died (Specht 1938). Finally, Phillips et al. (1987) exposed rats and mice to MIBK at 0, 100, 500, or 2000 ppm, 6 h/d, 5 d/w for 2 w. They observed lacrimation in rats and mice exposed to 2000 ppm.

MIBK's acute exposures at lower concentrations are not known to produce overt irritation in laboratory animals. In the 2-w study performed by Phillips

et al. (1987), no lacrimation was detected in rats and mice exposed to MIBK at 500 or 100 ppm. MacEwen et al. (1971) continuously exposed 4 monkeys, 8 dogs, 50 rats, and 40 mice at either 200 or 100 ppm at 725 mmHg, which was equivalent to 190 or 95 ppm, respectively, at 760 mmHg for 2 w. They monitored for any toxic sign and mortality during the exposure but failed to detect any, indicating the absence of overt mucosal irritation.

CNS Effects and Death

Three studies of the effects of MIBK on the CNS in humans were found (Hjelm et al 1990; Dick et al. 1992; Iregren et al. 1993). The overall conclusion from these three studies is that MIBK has no detrimental CNS effects for acute exposures at concentrations up to 88 ppm. A properly controlled study was conducted by Dick et al. (1992), who reported that during a 4-h exposure of 10 men and 7 women to MIBK at 88 ppm, there were no significant changes in the choice reaction time, simple reaction time, ability to simultaneously perform an auditory tone discrimination task together with a compensatory visual tracking task, memory scanning, postural steadiness, and mood states after 45 min or 2.75 h of exposure. The exposure also did not change the average score in visual vigilance. However, the performance of female volunteers in the visual vigilance test was positively correlated with the MIBK concentrations in blood (Dick et al. 1992). That means women with higher MIBK concentrations in blood performed better than women with lower MIBK concentrations, so it was not an adverse effect.

Iregren et al. (1993) exposed six men and six women to MIBK at 10 or 200 mg/m^3 (2.4 or 49 ppm) for 2 h (with light exercise at 50 W in the first 1.5 h and resting in bed in the remaining 0.5 h), but no air-exposed controls were used. During the exposure at 49 ppm, significantly more complaints of fatigue and other unnamed CNS symptoms were recorded than during the exposure at 2.4 ppm. However, there were no differences in simple reaction time and the ability to add between the 49- and the 2.4-ppm groups.

In a study without a sham-exposed control group, Hjelm et al. (1990) reported that a 2-h exposure at 10, 100, or 200 mg/m^3 (2.4, 24, or 49 ppm) had no effects on mood, reaction time, and ability to do addition in eight men. However, an exposure at 24 or 49 ppm resulted in headache and vertigo in two of the eight subjects, and 2.4 ppm caused vertigo but no headache in one subject. Because the test subjects knew that they would be exposed to MIBK, these isolated cases of headache and vertigo could be a result of their subjective bias toward chemical exposures.

Geller et al. (1979a) reported MIBK's effect on the discrimination behavior of juvenile male baboons. Four baboons were continuously exposed to MIBK at 50 ppm for 1 w. Except for the fourth and fifth day, a panel with three levers was presented to each of the baboons for 2 h everyday. A visual stimulus was presented periodically and a banana pellet would be rewarded if the baboon pressed the correct lever by matching the stimulus. The exposure did not affect the accuracy of discrimination (i.e., the percentage of correct matches) (Geller et al. 1979a,b). However, the mean response time was increased in one of the four baboons on the first and second days and in all four baboons on the third, sixth, and seventh days of exposure at 50 ppm (Geller et al. 1979a). The authors theorized that this "effect could be an early manifestation of the incoordination and narcosis which are observed at much higher concentrations."

De Ceaurriz et al. (1983, 1984) performed a series of experiments using the "behavioral despair" swimming test with industrial solvents. The test consisted of exposing groups of 10 mice to air or one of four concentrations of a solvent for 4 h. Every 5 min, one of the mice was placed in 10-cm deep water for about 30 min. In such a situation, mice tend to exhibit two types of behavior: an escape-directed behavior of intense swimming in the first 2 min and a continuous immobile posture after the first 2 min (de Ceaurriz et al. 1983). De Ceaurriz et al. (1983, 1984) found that industrial solvent vapors reduced the duration of immobility in the first 3 min. At 662 ppm, the immobility duration was reduced by 25% (de Ceaurriz et al. 1983). It took 803 ppm to halve the immobility duration (de Ceaurriz et al. 1983). The authors believed that such a reduction was an extension of the initial escape behavior of mice in water, but they admitted that more studies were needed to elucidate the meaning of the reduction of immobility (de Ceaurriz et al. 1983; de Ceaurriz et al. 1984).

Garcia et al. (1978) studied the behavioral effects of MIBK exposure in rats. Hungry rats were trained to press a lever to obtain food. A paired *t* test with the data presented by Garcia et al. (1978) shows that a 3-h exposure of these rats at 25 ppm did not cause any significant change in the number of times the lever was pressed per minute at a $p<0.05$ level.

McOmie and Anderson (1949) exposed mice to MIBK vapors at various high concentrations and observed the mice for the loss of righting reflex (as a determination of anesthesia), and death. Some of their data, including the mortality ratios, are summarized in Table 12-2.

Phillips et al. (1987) performed a 2-w study with rats and mice exposed to MIBK at 0, 100, 500, or 2000 ppm, 6 h/d, 5 d/w. There were no deaths in the exposure groups, but they observed isolated incidences of lethargy in rats and mice in the 2000-ppm group.

TABLE 12-2 Effects of Acute MIBK Exposures at High Concentrations on Mice (McOmie and Anderson 1949)

MIBK Concentration		Exposure Duration, h	Ratio[a]	Mortality Ratio[b]
g/m^3	ppm			
100	24,000	0.25	0:10	0:10
86	20,600	1.00	22:22	21:22
82	19,500	1.25	10:10	5:10
82	19,500	0.50	30:33	18:33
63	15,000	6.00	0:6	0:6
43	10,300	5.00	0:8	0:8

[a] Number of mice without righting reflex and number of mice exposed.
[b] Pulmonary congestion, hemorrhages, and pneumonia were found in dead mice.

Renal Effects

In the study by MacEwen et al. (1971) in which monkeys, dogs, rats, and mice of unspecified sex were continuously exposed to MIBK at 95 or 190 ppm for 2 w, there were no changes in the organ-weight-to-body-weight ratio for heart, lung, and spleen in rats. However, the ratio was raised for kidneys in rats exposed to MIBK at 95 or 190 ppm. The only histological change in the exposed monkeys, dogs, rats, and mice was toxic nephrosis (i.e., hyaline-droplet nephrosis) in the proximal tubules of rats exposed to MIBK at 95 or 190 ppm.

Phillips et al. (1987) also conducted a 2-w exposure with MIBK. Rats and mice were exposed for 6 h/d, 5 d/w for 2 w at 0, 100, 500, or 2000 ppm. There were no exposure-related changes in body weight. The kidney-weight-to-body-weight ratio was increased in male rats in the 2000-ppm group, but it was not changed in other groups. Phillips et al. (1987) detected an increased incidence of hyaline droplets and epithelial regeneration in proximal tubular cells in only the male rats exposed to MIBK at 2000 ppm.

Hepatic Effect

In rats exposed continuously to MIBK at 95 or 190 ppm for 2 w, the liver-weight-to-body-weight ratio was increased in rats exposed to MIBK at 190 ppm (MacEwen et al. 1971). Similarly, in the 2-w study performed by Phillips et al.

(1987), the liver-weight-to-body-weight ratio was raised in both female and male rats exposed to MIBK at 2000 ppm, 6 h/d, 5 d/w for 2 w. It was also increased in male rats exposed to MIBK at 500 ppm (Phillips et al. 1987). No hepatic histopathological change was detected by either MacEwen et al. (1971) or Phillips et al. (1987) in the exposed rats.

Subchronic and Chronic Exposures

Long-term MIBK exposures have been shown to cause toxic effects similar to those in acute exposures. For instance, renal and hepatic effects are known to be produced in long-term MIBK exposures. However, hypercholesterolemia has also been reported in long-term MIBK exposures, but it has never been found in short-term studies.

Hypercholesterolemia

Phillips et al. (1987) exposed rats and mice to MIBK at 0, 50, 250, or 1000 ppm, 6 h/d, 5 d/w for 14 w. According to the authors, there were no exposure-related hematological effects. Regarding serum chemistry, serum cholesterol increased 35% and 23% in the male rats of the 1000-ppm and the 250-ppm groups, respectively. There were, however, no MIBK-related changes in the serum concentrations of sodium, potassium, total calcium, glucose, total protein, and albumin.

Hypercholesterolemia is not detected in another long-term MIBK study. MacEwen et al. (1971) performed a subchronic study with male laboratory animals (100 rats, 8 dogs, and 2 monkeys per group). The animals were exposed to air or MIBK at 410 mg/m^3 at an atmospheric pressure of 5 psi with 68% oxygen and 32% nitrogen for 90 d. The MIBK exposure concentration was equivalent to 100 ppm at an atmospheric pressure of 14.7 psi or 760 mmHg. The exposure did not significantly change the hematology (the red-and white-blood-cell counts, hematocrit, and hemoglobin concentration) and clinical chemistry (the plasma concentrations of sodium, potassium, calcium, total phosphorus, chloride, cholesterol, total bilirubin, albumin, total protein, uric acid, creatinine, glucose, and alkaline phosphatase; the serum acid phosphatase and serum glucuronidase activities; as well as blood urea nitrogen) in dogs and monkeys. In the 90-d exposure of male rats, there were no differences in body growth between the exposed and control rats (MacEwen et al. 1971).

Renal Effects

In the study of male laboratory animals conducted by MacEwen et al. (1971), the 90-d continuous exposure to MIBK at 100 ppm equivalent caused no changes in organ-weight-to-body-weight ratios for organs of male rats except that the liver- and kidney-weight-to-body-weight ratios were raised. The exposure also produced no histopathological changes in male rats except for hyaline-droplet degeneration of the proximal tubules.

Similarly, in the 14-w exposure of rats and mice to MIBK at 0, 50, 250, or 1000 ppm for 6 h/d, 5 d/w, Phillips et al. (1987) reported that the only histopathological change was the presence of hyaline droplets in the kidney of male rats exposed to MIBK at 1000 or 250 ppm. The urinary excretion of glucose was increased in male rats exposed to MIBK at 1000 or 250 ppm by 55% or 37%, respectively. For some unknown reason, glucose urinary excretion also was increased 26% in female mice exposed to MIBK at 1000 ppm. The total protein urinary excretion was increased only in male rats exposed to MIBK at 1000 ppm. However, there were no exposure-related changes in the serum concentrations of creatinine and urea nitrogen.

Hepatic Effects

Chen et al. (1991) studied the liver function of 180 workers exposed to a mixture of solvents in paint manufacturing or spraying. The mean time-weighted-average concentrations of the solvents were the following: MIBK at 10 ppm, xylene at 75 ppm, toluene at 72 ppm, acetone at 24 ppm, benzene at 4 ppm, methyl ethyl ketone at 9 ppm, ethyl acetate at 4 ppm, and butyl acetate at 7 ppm. Chen et al. (1991) separated the workers into three groups based on the sum of the ratios of the exposure concentration of each compound versus its Threshold Limit Value (TLV). The low-exposure group had a sum of 0 to 0.38. The short-term high-exposure group also had a sum of 0 to 0.38, but the group members spent 30-90 min per day in a poorly ventilated painting booth exposed to MIBK at 115 ppm, toluene at 391 ppm, xylene at 192 ppm, ethyl acetate at 165 ppm, benzene at 40 ppm, and butyl acetate at 81 ppm. The long-term high-exposure group had a sum of 0.25 to 9.83. Chen et al. (1991) reported that the serum activities of gamma-glutamyl transferase were higher in the short-term and long-term high-exposure groups than in the low-exposure group. However, the serum activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, as well as the serum concentration of bile acid did not change with the exposure concentrations. Because the

exposures involved a number of compounds, it is difficult to draw any conclusion regarding MIBK's effects in this study.

In the subchronic study by MacEwen et al. (1971), the 90-d exposure to MIBK at 100 ppm equivalent failed to affect the liver function of dogs. There were no significant differences in the clearance of bromsulphalein between the control and exposed dogs. In rats, the 90-d exposure increased the liver-weight-to-body-weight ratio, but no hepatic histopathological change was detected.

In the study by Phillips et al. (1987) in which rats and mice were exposed to MIBK at 0, 50, 250, or 1000 ppm, 6 h/d, 5 d/w for 14 w, there were no changes in the serum concentrations of total bilirubin, direct bilirubin, lactate dehydrogenase, sorbitol dehydrogenase, and alkaline phosphatase. The liver-weight-to-body-weight ratio was increased in male rats and male mice exposed to MIBK at 1000 ppm. The liver, however, appeared normal histologically. Overall, the data suggest an absence of any major liver abnormality in rats and mice as a result of the subchronic MIBK exposure.

Carcinogenicity

Capurro (1979) performed an epidemiological study with 117 residents who had lived within an area of 1.5 km^2 surrounding a chemical plant for more than 5 y in the mid-1960s. The plant began operations in 1961, causing complaints of odors. In 1971, improvements were made to reduce the pollution substantially. The chemicals identified in the air before 1971 included ketones (MIBK, methyl ethyl ketone, and acetone), alcohols (methanol, isopropanol, butanol, amyl alcohol, isoamyl alcohol, and phenol), aromatic hydrocarbons (benzene, toluene, and xylene), halogenated hydrocarbons (chlorobenzene, nitrochlorobenzene, methyl chloride, methylene chloride, chloroform, carbon tetrachloride, trichloroethylene, trichloroethane, tetrachloroethane, and perchloroethylene), nitrogenous compounds (nitrophenol, acetonitrile, and acrylonitrile), and miscellaneous compounds (formaldehyde, acetic acid, furan, tetrahydrofuran, ethyl ether, and ethyl acetate). The chemicals "most easily detected" in the blood of 24 of the residents were MIBK, benzene, chloroform, and trichloroethylene. One cancer death was expected from 1968 to 1974, but Capurro found seven cancer deaths. The result was statistically significant ($p<0.03$) according to the Fisher's exact probability test (Gad and Weil 1988). However, it is not certain whether the excess cancer deaths were due to MIBK because of co-exposures to other compounds, some of which are known to be carcinogenic in humans or laboratory animals.

Genotoxicity

According to the data base Toxline from the National Library of Medicine at the National Institutes of Health, Microbiological Associates reported to the U.S. Environmental Protection Agency (EPA) that MIBK was devoid of genotoxicity. MIBK was not mutagenic in the Ames test with or without added metabolic activation by rat-liver S9 fraction (Microbiological Associates 1984a). MIBK did not increase the rate of unscheduled DNA synthesis in rat hepatocyte primary cultures (Microbiological Associates 1984b). Finally, MIBK tested negative in the micronucleus cytogenetic assay in mice injected with up to 1 mL/kg intraperitoneally (Microbiological Associates 1984c).

O'Donoghue et al. (1988) reported that MIBK tested negative in the Ames assay, micronucleus assay, and unscheduled DNA synthesis assay. MIBK produced only a marginal response in the L5178Y/TK$^{+/-}$ mouse lymphoma assay, so the authors concluded that "MIBK is unlikely to be genotoxic in mammalian systems" (O'Donoghue et al. 1988).

Souza and Puig (1987) studied the frequency of sister chromatid exchanges in the peripheral lymphocytes of 24 workers who used a mixture of solvents in the Mexican auto and paint industry. They were exposed to MIBK at 2.4 ppm, methanol at 0.6 ppm, isopropanol at 3.3 ppm, toluene at 3.3 ppm, benzene at 6.0 ppm, and hexane at 3.3 ppm in the factory air. Souza and Puig (1987) found no significant increase in the frequency of sister chromatid exchange.

Reproductive Toxicity

No data on MIBK's effect on reproduction were found in the open literature based on a search in Toxline (National Library of Medicine) and Chemical Abstracts (American Chemical Society).

Developmental Toxicity

Tyl et al. (1987) exposed Fischer 344 rats and CD-1 mice to MIBK at 0, 300, 1000, or 3000 ppm on gestational days (GDs) 6 through 15 at 6 h/d. The fetuses were examined when the dams were sacrificed on GD 18 for mice and GD 21 for rats. At the highest exposure concentration used, 3000 ppm, maternal toxicity was detected in both rats and mice based on clinical signs. Pregnant rats exposed to MIBK at 3000 ppm had decreased food consumption, decreased body-weight gain, and increased liver-weight-to-body-weight ratio. Pregnant mice in the 3000-ppm group had a 12% mortality and increased liver-

weight-to-body-weight ratio. Fetotoxicity was seen in the mice exposed to MIBK at 3000 ppm, as evidenced by decreased fetal body weight per litter, reduced skeletal ossification, and increased fetal deaths. There was no fetotoxicity in other groups of exposed animals. No embryotoxicity or fetal malformations were detected in any exposure groups.

Interaction with Other Chemicals

Abou-Donia et al. (1985) demonstrated that MIBK inhalation potentiated the neurotoxicity of *n*-hexane. In chickens exposed to *n*-hexane at 1000 ppm together with MIBK at 10-1000 ppm for 29 d, Lapadula et al. (1991) showed that "MIBK selectively induced cytochrome P-450 isozymes leading to the metabolic activation" of *n*-hexane. Cytochrome P-450 content and benzphetamine *N*-demethylase activity increased with increasing MIBK exposure concentration.

Table 12-3 presents a summary of the inhalation toxicity data on MIBK.

TABLE 12-3 Toxicity Summary of Inhalation Studies

Concentration, ppm	Exposure Duration	Species	Effects	Reference
24 or 49	2 h	Human (light exercise)	Nose and throat irritation; no effects on mood, reaction time, and ability to add	Iregren et al. 1993
49	2 h	Human (light exercise)	Higher rating for irritation than 2.4 ppm; more complaints of fatigue than 2.4 ppm	Iregren et al. 1993
88	4 h	Human	Strong odor; no irritation, nausea, headache, or CNS effects	Dick et al. 1992
200	15 min	Human	Eye irritation and objectionable odor	Silverman et al. 1946
25	3 h	Rat	No effect on the number of times a lever was pressed to get food	Garcia et al. 1978
50	24 h/d, 7 d/w for 1 w	Baboon	No effect on accuracy of discrimination; increased response time on the 3rd, 6th, and 7th d	Geller et al. 1979a
95 or 190	24 h/d, 7 d/w for 2 w	Monkey, dog, rat, and mouse	No toxic signs; no change in hematology and clinical chemistry; raised relative kidney weight and liver weight; toxic nephrosis in rats; normal clearance of bromosulphthalein	MacEwen et al. 1971
100 equivalent	24 h/d for 90 d	Monkey (n=2), dog, and rat (all male)	No significant changes in hematology and clinical chemistry Increased ratios of liver weight/body weight and kidney weight/body weight; hyaline droplet degeneration of proximal tubules in rats	MacEwen et al. 1971
500	6 h/d, 5 d/w for 2 w	Rat, mouse	No lacrimation of changes in body weight	Phillips et al. 1987
662 or 803	4 h	Mouse	Reduction in duration of immobility during first 3 min of swimming	De Ceaurriz et al. 1983

Concentration, ppm	Exposure Duration	Species	Effects	Reference
1000	6 h/d, 5 d/w for 14 w	Rat, mouse	Male rats: Increased serum cholesterol; hyalin droplets in kidneys; increased urinary glucose and protein; normal serum concentrations of alkaline phosphatase, aspartate aminotransaminase, lactate dehydrogenase, and bilirubin; normal liver histology Female rats: decreased eosinophils	Phillips et al. 1987
1000-28000	5 h	Rat	Signs of mucosal irritation in 1 min; salivation in 2 min; death in 85 min to 5 h; loss of auditory and corneal reflexes	Specht 1938
2000	6 h/d, 5 d/w for 2 w	Rat, mouse	Lacrimation; no change in growth; increased relative kidney weight; hyalin droplets and epithelial regeneration in proximal tubular cells in male rats	Phillips et al. 1987
3200	5 min	Mouse	Mucosal irritation (the respiratory rate declined 50%)	De Ceaurriz et al. 1983
10,300-24,000	0.25 to 22.6 h	Mouse	Signs of mucosal irritation; some deaths at 19,500 ppm or higher	McOmie and Anderson 1949

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 12-4 presents exposure limits for MIBK set by other organizations and Table 12-5 presents the SMACs established by NASA.

The SMACs are derived with the assistance of guidelines from the Committee of Toxicology, National Research Council (NRC 1992). Briefly, the procedure involves an identification of pertinent toxic end points for MIBK. For each toxic end point, an acceptable concentration (AC) is derived for each exposure duration of interest (i.e., 1 h, 24 h, 7 d, 30 d, and 180 d). The lowest AC for an exposure duration of interest is selected as the SMAC for that duration (Table 12-6). The toxic end points chosen for AC derivation are mucosal irritation and CNS depression.

Hypercholesterolemia reported by Phillips et al. (1987) in rats exposed to MIBK at 250 or 1000 ppm, 6 h/d, 5 d/w for 14 w, is not included in AC derivation because the 23-35% increases in serum cholesterol are not considered

TABLE 12-4 Exposure Limits Set by Other Organizations

Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	50 (TWA)	ACGIH 1991a
ACGIH's STEL	75	ACGIH 1991a
OSHA's PEL	50 (TWA)	ACGIH 1991b
NIOSH's REL	50 (TWA)	ACGIH 1991b

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

TABLE 12-5 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	35	143	CNS depression
24 h	35	143	CNS depression
7 d[a]	35	143	Irritation, CNS depression
30 d	35	143	Irritation, CNS depression
180 d	35	143	Irritation, CNS depression

[a] Current 7-d SMAC is 20 ppm.

clinically important. It should also be noted that MacEwen et al. (1971) failed to find hypercholesterolemia in rats, dogs, and monkeys exposed continuously to MIBK at a concentration equivalent to 100 ppm for 90 d.

According to MacEwen et al. (1971) and Phillips et al. (1987), continuous or repetitive MIBK exposures have been shown to lead to an increased liver-weight-to-body-weight ratio in rats. The histology of the liver, however, was normal in these animals. The increased relative liver weight was probably an adaptive change rather than an adverse effect. Lapadula et al. (1991) showed that MIBK is an inducer of cytochrome P-450 isozymes in chicken. Phillips et al. (1987) theorized that the increased relative liver weight might be a response to an increased metabolic load on the liver. Therefore, no ACs are established for the hepatic effects of MIBK.

The investigations by MacEwen et al. and Phillips et al. showed that continuous or repetitive MIBK exposures could increase the incidence of hyalin droplet degeneration in proximal tubules in the kidney of male rats (MacEwen et al. 1971; Phillips et al. 1987). Similar lesions, which were thought to be due to α-2-μ-globulin found only in rats, have been demonstrated in male rats exposed to other hydrocarbons (NRC 1992). This type of lesions in male rats is not considered a good model for humans (Alden 1986; NRC 1992). As a result, it is not used as a toxic end point in AC derivation.

Mucosal Irritation

Silverman et al. (1946) reported that a 15-min exposure to MIBK at 200 ppm produced nose or throat irritation in the majority of 12 human volunteers. Dick et al. (1992) showed that a 4-h exposure to MIBK at 88 ppm was not irritating to 17 human subjects. The ACs for mucosal irritation are derived without any time adjustment, because mucosal irritation is not believed to be time dependent. Because less than 100 human subjects were used in the study of Dick et al. (1992), an adjustment for the small number of test subjects is done in setting the long-term ACs in order to prevent mucosal irritation. However, no such adjustment is needed for 1-h and 24-h ACs, because a small degree of irritation is acceptable in contingencies.

1-h and 24-h ACs for mucosal irritation

= 4-h NOAEL

= 88 ppm (rounded up to 90 ppm)

7-d, 30-d, and 180-d ACs for mucosal irritation

= 4-h NOAEL × 1/small "*n*" factor

= 88 ppm × (square root of "*n*")/10

= 88 ppm × (square root of 17)/10

= 35 ppm.

CNS Depression

The studies of McOmie and Anderson (1949), Specht (1938), and Phillips et al. (1987) found that MIBK exposures at >1000 ppm could result in the loss of reflexes or lethargy in rats or mice. Dick et al. (1992), however, showed that a 45-min or 2.75-h exposure to MIBK at 88 ppm produced no CNS impairment in 17 human subjects. Because 88 ppm is a NOAEL for 45 min or 2.75 h, it should also be a NOAEL for a 1-h exposure. The CNS depressive effects of organic solvents are believed to be dependent on the CNS concentration of the chemical, which is directly dependent on the blood concentration. Because Dick et al. (1992) showed that the blood concentration of MIBK reached equilibrium as early as 2 h into the 4-h exposure in humans, the NOAEL of 88 ppm can be used to derive the ACs for 24 h and beyond.

1-h, 24-h, 7-d, 30-d, and 180-d ACs for CNS depression

= 2.75-h NOAEL × 1/small "*n*" factor

= 88 ppm × (square root of "*n*")/10

= 88 ppm × (square root of 17)/10

= 35 ppm.

Establishment of SMAC Values

The ACs for CNS depression and mucosal irritation are tabulated below. The 1-h, 24-h, 7-d, 30-d, and 180-d SMACs are all set at 35 ppm. Because these toxic end points are not expected to be potentiated by physiological changes induced by microgravity, no adjustments are needed for these SMACs.

TABLE 12-6 Acceptable Concentrations

End Point, Exposure Data, Reference		Uncertainty Factors			Acceptable Concentrations, ppm				
Species	Time	Species	Small *n*	1 h	24 h	7 d	30 d	180 d	
Mucosal irritation	Human	—	—	10/(Sq. Rt. 17)	90	90	35	35	35
NOAEL, 88 ppm for 4 h (Dick et al. 1992)									
CNS Depresion	Human	—	—	10/(Sq. Rt. 17)	35	35	35	35	35
NOAEL, 88 ppm for 2.75 h (Dick et al. 1992)									
SMACs					**35**	**35**	**35**	**35**	**35**

—, not applicable.

RECOMMENDATIONS

The excess cancer deaths found by Capurro in residents around a chemical plant exposed to MIBK and other compounds suggest that the carcinogenicity of MIBK needs to be studied further. More epidemiological studies and a chronic animal bioassay should be conducted.

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B13 CHLOROFORM

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PHYSICAL AND CHEMICAL PROPERTIES

Chloroform is a clear, colorless, volatile and mobile, highly refractive, dense liquid with a characteristic pleasant, nonirritating odor and a slight, sweet taste (ATSDR 1997).

Formula:	$CHCl_3$
CAS no.:	67-66-3
Chemical name:	Trichloromethane
Synonyms:	Chloroform, trichloroform, formyl trichloride, methenyl chloride, methenyl trichloride, methane trichloride, methyl trichloride, NCI-C02686, Freon 20, R-20, TCM
Molecular weight:	119.38
Boiling point:	61.3°C
Melting point:	–63.2°C
Liquid density:	1.485 g/m^3
Vapor density:	4.36 (air = 1)
Vapor pressure:	159 torr at 20°C
Solubility:	1 mL dissolves in 200 mL at 25°C
Odor threshold:	85 ppm (vapor) Miscible with alcohol, benzene, ether, petroleum ether, carbon tetrachloride, carbon disulfide, oils.
Conversion factors at 20°C, 1 atm:	1 ppm = 4.96 mg/m^3
	1 mg/m^3 = 0.20 ppm

OCCURRENCE AND USE

Chloroform is used as a raw material in the chemical industry for the manufacture of such materials as fluorocarbons, resins, and plastics; as an extractant for fats, oils, greases, resins, lacquers, rubber, alkaloids, gums, waxes, guttapercha, penicillin, vitamins, flavors, floor polishes, and adhesives; as a pharmaceutical solvent and dry cleaning spot remover; and as an intermediate in the manufacture of dyes and pesticides (ATSDR 1997). In the past, chloroform was used as a general anesthetic, a fire extinguisher, and a flavoring agent in toothpastes and cough syrups (ACGIH 1991). Trace amounts of chloroform are present in drinking water and in wastewater from sewage-treatment plants as a by-product of chlorine treatment to kill bacteria. Trace amounts of chloroform are also found almost ubiquitously in the environment. Small amounts of chloroform are sometimes carried on board the space shuttle as part of mid-deck or module experiments. Chloroform has been detected in the shuttle atmosphere in 6 of 27 missions at concentrations of 0.002 to 0.03 mg/m^3 (Huntoon 1987, 1993) and, in more recent missions, in about 10% of air samples at concentrations of 0.01 to 0.1 mg/m^3 (James et al. 1994).

TOXICOKINETICS AND METABOLISM

Considerable data are available on the uptake, processing, and elimination of chloroform in several species. The weight of evidence indicates that chloroform is rapidly distributed throughout the body and that its toxic effects are more dependent on the dose rate than the total dose or the route of administration.

Absorption

Due to chloroform's relatively high vapor pressure and high blood/air partition coefficient (8 to 10.3 at 37°C), inhalation is a primary route of entry into the body (EPA 1985). Raabe (1988) measured the uptake of ambient concentrations of chloroform (labeled with ^{14}C) in air inhaled through the nose (45.6%) and the mouth (49.6%) by four human subjects. Chloroform is also rapidly absorbed through the gastrointestinal tract from foodstuffs and drinking water (EPA 1985). Absorption of orally administered chloroform might be affected by the vehicle in which it is dissolved. In mice, tissue concentrations of chloroform after gavage dosing were consistently greater for aqueous versus corn-oil vehicles (Dix et al. 1997). Absorption of chloroform through the skin is significant (329 μmoles/min/cm^2 of skin exposed to the liquid) (EPA 1985).

Distribution

In humans (Smith et al. 1973) and animals (Cohen 1971; Brown et al. 1974), chloroform absorbed either by inhalation or orally is distributed to all tissues with relative tissue concentrations of body fat> brain> liver> kidney> blood, as expected owing to the lipophilic nature of chloroform. In studies in mice, the relative distribution in the organs was dependent on route of administration; oral dosing resulted in the highest concentrations being in the liver, possibly due to a first-pass effect, the time between dosing and measurement, and the metabolism and covalent binding of metabolites to cellular macromolecules (Brown et al. 1974; Taylor et al. 1974).

Excretion

Chloroform was detected in the exhaled air of volunteers exposed to a normal environment, to heavy automobile traffic, or to 2 h in a dry-cleaning establishment (Gordon et al. 1988). High chloroform concentrations in the breath corresponded to high exposure concentrations. The calculated biological half-time for chloroform was 7.9 h.

Excretion of radioactivity in mice and rats was monitored for 48 h following exposure to ^{14}C-labeled chloroform at 10, 89, and 366 ppm (mice) or 93, 356, and 1041 ppm (rats) (Corley et al. 1990). In this study, 92% to 99% of the total radioactivity was recovered in mice, and 58% to 98% was recovered in rats; percentage recovery decreased with increasing exposure. Of the total radioactivity, the percentages recovered as exhaled ^{14}C-labeled carbon dioxide were 80% to 85% for mice and 48% to 85% for rats. The fractions recovered as ^{14}C-labeled chloroform were 0.4% to 8% for mice and 2% to 42% for rats. The fractions recovered as urinary and fecal metabolites were 8% to 11% and 0.6% to 1.4% for mice and 6.4% to 8.9% and 0.6% to 1.1% for rats, respectively. A 4-fold increase in exposure concentration was followed by a 50- and 20-fold increase in the amount of exhaled, unmetabolized chloroform in mice and rats, respectively.

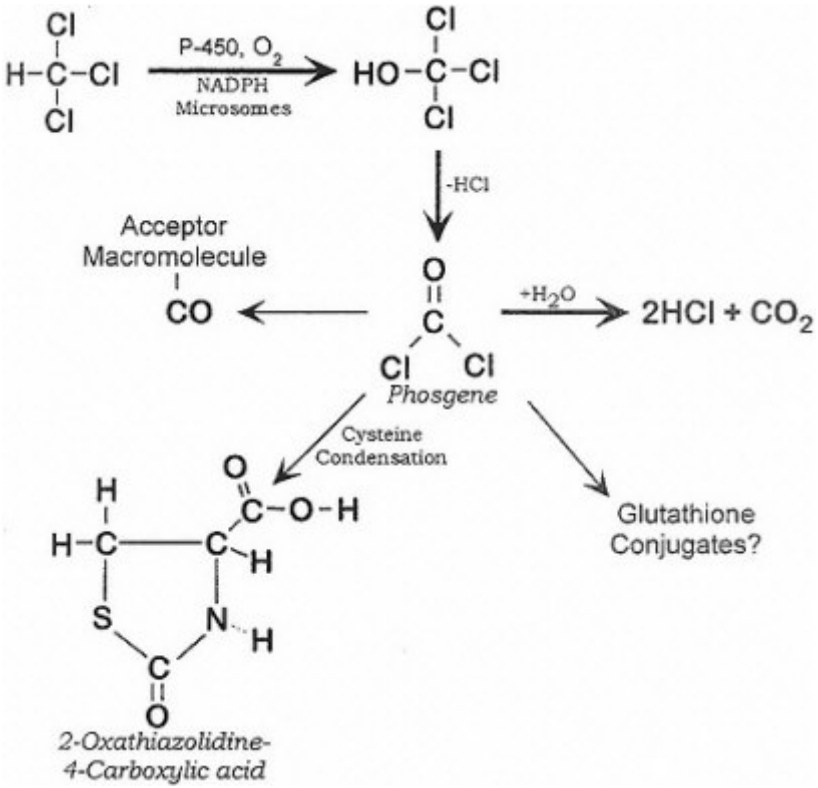
Metabolism

The metabolism of chloroform has been extensively studied and is fairly well understood. In humans, approximately 50% of an oral dose of 0.5 g chloroform was metabolized to carbon dioxide (Fry et al. 1972). Metabolism was dose

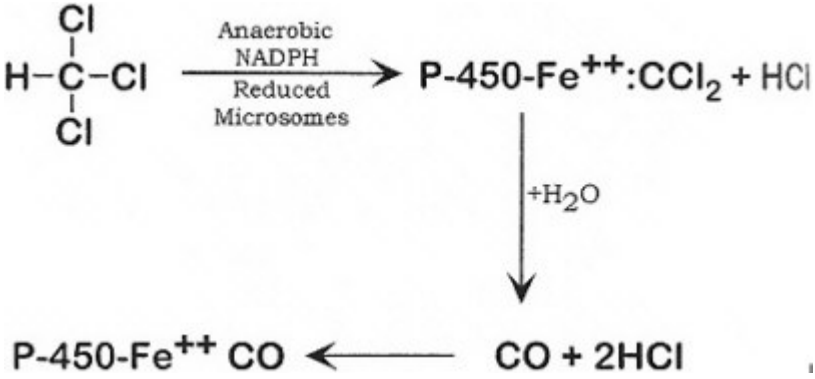
dependent, decreasing with higher exposure. A first-pass effect was observed after oral exposure (Chiou 1975). Approximately 38% of the dose was converted in the liver, and ≤ 17% was exhaled unchanged from the lungs. In a physiologically based pharmacokinetic modeling study of chloroform, Corley et al. (1990) defined in vivo metabolic rate constants (V_{max} C = 15.7 mg/kg/h, K_m = 0.448 mg) for humans by using experimental results obtained in rats and mice exposed to chloroform by inhalation and enzymatic studies in human tissues in vitro. Their results predicted that metabolic activation of chloroform to its toxic intermediate, phosgene, was slower in humans than in rodents (ATSDR 1997).

Chloroform can be metabolized aerobically and anaerobically as shown below:

Major Pathway (Aerobic). Source: Adapted from ATSDR (1997)



Minor Pathway (Anaerobic). Source: Adapted from ATSDR (1997)



The production of CO_2 by the aerobic pathway accounts for up to 85% of administered chloroform in mice, 65% in rats, and lesser amounts (28%) in squirrel monkeys and humans (50%) (Brown et al. 1974; Taylor et al. 1974). In mice, Brown et al. found greater amounts of radiolabeled chloroform in the kidneys of males than of females. Similarly, Culliford and Hewitt (1957) found that chloroform accumulated and metabolized to a greater extent in the renal cortex of males than of females; the results might have been influenced by testosterone concentrations. This effect was not observed in any other species. These species and sex differences in metabolism, distribution, and binding point out the dangers and difficulties in extrapolating studies in lower animals to humans.

Metabolism studies by Pohl (1977) and Stevens and Anders (1981) indicated that chloroform was exhaled from the lungs or was converted to phosgene in the liver and kidneys by cytochrome P-450 (Branchflower et al. 1984; Smith and Hook 1984). Phosgene might react with cellular elements, including lipids and proteins of the endoplasmic reticulum proximate to the cytochrome P-450. In phenobarbital-pretreated Sprague-Dawley rats, chloroform exposure yielded a covalent adduct to a single phospholipid, identified as phosphatidylethanolamine, in liver mitochondria (Guastedisegni et al. 1998). It was further demonstrated that chloroform can induce lipid peroxidation and inactivation of cytochrome P-450 in rat-liver microsomes under aerobic conditions (DeGroot and Noll 1989). This mechanism might also contribute to chloroform-induced hepatotoxicity in rats, although phosgene and other active metabolites are primarily responsible. The conversion of chloroform to reactive metabolites

occurs in nuclear preparations, as well as in microsomes (Gomez and Castro 1980). Covalent binding of chloroform to lipids can occur under anaerobic and aerobic conditions; binding to protein occurs only under aerobic conditions (Testai et al. 1987).

Covalent binding of chloroform metabolites to microsomal protein in vitro was intensified by microsomal enzyme inducers and prevented by glutathione (Brown et al. 1974). It was proposed that the reaction of chloroform metabolites with glutathione might act as a detoxifying mechanism. Phosgene might combine with two molecules of reduced glutathione (GSH) to form diglutathionyl dithiocarbonate, which is further metabolized in the kidneys (Sipes et al. 1977; Wolf et al. 1977). Chloroform doses that caused liver glutathione depletion produced liver necrosis (Docks and Krishna 1976). Furthermore, chloroform has been found to be more hepatotoxic in fasted animals, possibly due to decreased glutathione content in the liver (Brown et al. 1974; Docks and Krishna 1976; Wang et al. 1995). That might explain the clinical finding of severe acute hepatotoxicity in women exposed to chloroform via anesthesia during prolonged parturition.

Evidence that chloroform is metabolized at its carbon-hydrogen bond is provided by experiments using the deuterated derivative of chloroform (McCarty et al. 1979; Pohl et al. 1980; Branchflower et al. 1984). Deuterated chloroform was one-half to one-third as cytotoxic as chloroform, and its conversion to phosgene was much slower. The results confirmed that the toxicity of chloroform is primarily due to its metabolites (ATSDR 1997).

A recent in vitro study of mice hepatic microsomes indicated that a reductive pathway might play an important role in chloroform hepatotoxicity (Testai et al. 1990). It was demonstrated that radical chloroform metabolites bind to macromolecules (proteins, lipids) and the process can be inhibited by reduced glutathione (ATSDR 1997).

The final product of the aerobic metabolic pathway of chloroform is carbon dioxide (Fry et al. 1972; Brown et al. 1974), which is mostly eliminated through the lung, but some is incorporated into endogenous metabolites and excreted as bicarbonate, urea, methionine, and other amino acids (Brown et al. 1974). Inorganic chloride ion is an end product of chloroform metabolism found in the urine (Van Dyke et al. 1964). Carbon monoxide was a minor product of the anaerobic metabolism of chloroform in vitro (Ahmed et al. 1977) and in vivo in rats (Anders et al. 1978; ATSDR 1997).

Interspecies differences in the rate of chloroform conversion were observed in mice, rats, and squirrel monkeys. The conversion of chloroform to carbon dioxide was highest in mice (85%) and lowest in squirrel monkeys (28%) (Brown et al. 1974). Similarly, because of the lower relative rates of chloroform

metabolism, ventilation, and cardiac output (per kilogram of body weight) in the larger species, physiologically based pharmacokinetic (PBPK) calculations indicated that exposure to equivalent concentrations of chloroform vapor would lead to a lower delivered dose of active metabolites in humans compared with rats, which would have a lower delivered dose than mice (Corley et al. 1990; ATSDR 1997).

TOXICITY SUMMARY

Chloroform has pronounced effects on the central nervous system (CNS), most of which are reversible upon cessation of exposure. Short-term exposure to high concentrations causes liver necrosis, kidney degeneration, and cardiac arrhythmias, and possibly nasal lesions and immune-system depression. Exposures to lower concentrations, which do not cause liver or kidney pathology, can still cause cytotoxicity, as evidenced by increases in the labeling indices of these tissues. Long-term exposure to relatively high concentrations might lead to liver or kidney cancer.

Acute and Short-Term Exposures

Cardiac Effects

Chloroform anesthesia is associated with cardiac toxicity. In a 1965 epidemiological study by Whitaker and Jones (1965) of a cohort of 1502 patients (exposures at concentrations of 10,000 to 22,500 ppm), dose-related bradycardia developed in 8% of the cases, and cardiac arrhythmia developed in 1.3% of the cases. Hypotension was observed in 27% of the patients and was related to the duration of the anesthesia and to pretreatment with thiopentone. In 1973, Smith et al. reported that chloroform anesthesia (exposures at 8000 to 10,000 ppm) caused arrhythmia (nodal rhythm, first degree atrioventricular block, or complete heart block) in 50% of the cases from the cohort of 58 patients, and hypotension in 12% (Smith et al. 1973).

An EKG study of 66 patients anesthetized for at least 2 h at the State of Wisconsin General Hospital demonstrated that the effect of chloroform on the heart is not to induce ventricular fibrillation but rather depression of the myocardium to the point of asystole (Orth et al. 1951).

The EC50 (the concentration that induces a given affect in 50% of the exposed animals of a species in a given time) for sensitization to cardiac arrhythmia

in dogs exposed for 5 min to chloroform vapors was 16,000 ppm (Clark and Tinston 1982). This effect was rapidly reversed on cessation of exposure (Clark and Tinston 1982).

CNS

Evidence for chloroform's effects on the CNS come from occupational exposures and from the use in the past of chloroform as an inhalation anesthetic. The recommended method to induce anesthesia during surgery or childbirth involved increasing concentrations of chloroform gradually to 25,000 or 30,000 ppm during the first 2 or 3 min, with maintenance at much lower concentrations (ATSDR 1997). Concentrations < 1500 ppm are insufficient to induce anesthesia; 1500 to 2000 ppm causes light anesthesia (Goodman and Gilman 1980). Dizziness and vertigo occur after exposure to 920 ppm for 3 min; headache and slight intoxication occur at higher concentrations (Lehmann and Hasegawa 1910).

The EC_{50} for CNS depression (ataxia and loss of righting reflex) in rats exposed for 10 min to chloroform vapors was 16,000 ppm (Clark and Tinston 1982). That effect was rapidly reversed on cessation of exposure (Clark and Tinston 1982).

Hepatotoxicity

Several early studies reported acute hepatic necrosis in women exposed to chloroform via anesthesia (Royston 1924; Townsend 1939; Lunt 1953). The effects observed in women included jaundice, liver enlargement and tenderness, delirium, coma, and death. Centrilobular necrosis was found at autopsy in those who died. In 1973, Lieberman reported that there had not been any chloroform jaundice in 30,000 chloroform anesthesias at one hospital since 1942. Lieberman (1973) noted that other studies have documented hepatic necrosis due to all anesthetics, including cyclopropane, ether, ethylene, and nitrous oxide and reported a personal communication from a chief of pathology at a hospital that there had been no cases of chloroform hepatitis over a 15-y period from 1945 through 1960 during which 70,000 "open-drop" chloroform anesthesias were administered for obstetrics. In the open-drop technique, chloroform was administered on a handkerchief. Until precision vaporizers became available in the late 1950s, anesthetists had no means to measure or control the concentration of chloroform in inspired air, and cases of hepatotoxicity were attributed

to overdosing by inexperienced anesthetists. Whitaker and Jones (1965) studied over 1500 patients receiving chloroform anesthesia administered by a precision vaporizer at nominal inspired concentrations of no greater than 22,500 ppm for durations of less than 30 min (1164 patients) to over 120 min (24 patients). The only case of hepatotoxicity found was transient jaundice in one patient 36 h after exposure to chloroform for 6 min, but the patient was believed to have been incubating infectious hepatitis before anesthesia.

Increased sulfobromophthalein retention was observed in some patients exposed to chloroform via anesthesia (exposures at 8000 to 10,000 ppm), indicating impaired liver function (Smith et al. 1973).

Brown et al. (1974) found that exposure of rats for 2 h to chloroform at 5000 or 10,000 ppm produced hepatic necrosis and destruction of microsomal enzymes. Treatment of rats with phenobarbital to induce microsomal enzyme activity before exposure to chloroform markedly increased the hepatotoxic response to anesthesia and produced a 70% to 80% decrease in hepatic glutathione concentrations. In rats in which microsomal enzyme activity was not induced with phenobarbital, chloroform exposure did not result in depletion of glutathione or in hepatic necrosis 24 h after exposure (Brown et al. 1974). Experimental depletion of hepatic glutathione by pretreatment with diethyl maleate also resulted in centrilobular necrosis after exposure to chloroform (Brown et al. 1974). These results suggest that much or all of the hepatotoxicity of chloroform is due to the production of reactive chloroform metabolites (e.g., phosgene).

Studies at the Chemical Industry Institute of Toxicology (CIIT) found large species-specific differences in the dose-related hepatotoxicity of chloroform inhaled 6 h/d for 7 d by $B6C3F_1$ mice and Fischer 344 (F344) rats, with lowest-observed-adverse-effect levels (LOAELs) of 10 ppm and 300 ppm and no-observed-adverse-effect levels (NOAELs) of 3 ppm and 100 ppm, respectively (Larson et al. 1994b). They later tested for hepatotoxicity in male and female BDF_1 mice exposed to chloroform for 6 h/d for 4 d at 0, 0.3, 5, 30, or 90 ppm or males exposed for 6 h/d, 5 d/w for 2 w at 0, 30, or 90 ppm (Templin et al. 1996a). Mild centrilobular hepatocyte vacuolization was seen in one of five male mice exposed at 30 ppm for 2 w, and centrilobular vacuolization and focal areas of hepatocyte necrosis were seen in three of four male mice and three of five female mice exposed at 90 ppm for 4 d. Significant increases in hepatocyte labeling index (LI) were seen in male mice exposed to chloroform at 30 and 90 ppm and in female mice exposed at 90 ppm (Templin et al. 1996a). Unpublished results from CIIT indicated that similar dose responses were obtained for increases in hepatocyte LIs in $B6C3F_1$ mice exposed for periods of 7 d to 180 d (Butterworth 1997).

The observed species-specific differences in sensitivity to the hepatotoxic

effects of chloroform are not due to differences in the sensitivity of hepatocyte cells to the cytotoxic effects of chloroform or to differences in their ability to metabolize chloroform. When freshly isolated hepatocytes from $B6C3F_1$ mice and F344 rats were exposed to solutions of chloroform for up to 3 h, concentration-dependent cytotoxicity (lactate dehydrogenase release) was seen in culture at concentrations higher than 1 mM (Ammann et al. 1998). Cotreatment with the cytochrome P-450 inhibitor 1-phenylimidazole prevented both cytolethality and glutathione depletion, indicating that metabolism is necessary for chloroform-induced cytotoxicity. These results correlate well with simulations of a physiologically based dosimetry model for chloroform. The simulations indicated that after hepatotoxic oral bolus doses of chloroform at 477 mg/kg of body weight, the livers of mice and rats were exposed to chloroform at concentrations up to 5 mM for 3 h (Ammann et al. 1998). Hepatocytes from the two species exhibited similar sensitivity toward chloroform toxicity, indicating that toxicity is not sufficient to explain different susceptibility to heptocarcinogenicity.

Lethality

Acute exposures to relatively high concentrations of chloroform can cause immediate death due to cardiovascular toxicity or delayed death (1 to 4 d after exposure) due to hepatotoxicity or nephrotoxicity.

In humans, obstetric use of chloroform anesthesia earlier in this century occasionally caused fatal toxicity (Royston 1924). Obstetric deaths occurred either during anesthesia, due to cardiac arrhythmias, or a few days after anesthesia, due to hepatotoxicity. A 1973 report by an anesthesiologist stated that there had been no deaths at one hospital in 30 y from over 30,000 chloroform anesthesias for obstetrics since 1942 (Lieberman 1973) and attributed previous reports of chloroform-related deaths to use by inexperienced anesthetists of the crude open-drop technique of administering chloroform on a handkerchief, which made it difficult to control exposure concentrations.

The LC_{50} (lethal concentration for 50% of the exposed animals) for rats exposed for 15 min to chloroform vapor was 76,000 ppm (Clark and Tinston 1982). For a 6-h exposure to chloroform vapors, the LC_{50} was 1849 ppm in rats and 1260 ppm in mice (Bonnet et al. 1980).

Nausea

A frequent side effect of chloroform anesthesia (8000 to 22,500 ppm) was

nausea (Royston 1924; Townsend 1939; Whitaker and Jones 1965; Smith et al. 1973). Nausea was also reported by women employed in a lozenge factory while working in an atmosphere with chloroform concentrations ranging from 23 to 1163 ppm (average = 128 ppm) for a period of 3 to 10 y (Challen et al. 1958). Thirteen workers exposed to chloroform at >400 ppm for 1 to 5 mo and 18 workers exposed at 14.4 to 50.4 ppm for 1 to 4 mo developed nausea in association with toxic hepatitis (Phoon et al. 1983). The data available are insufficient to establish a NOAEL or LOAEL or a dose-response relationship for nausea.

Nephrotoxicity

Chloroform induces kidney toxicity, which, depending on the species and strain, can be more or less severe than the liver toxicity induced by the same dose. Reports of chloroform-induced kidney toxicity in humans are few and sketchy but always associated with severe liver toxicity. In case reports of women who died after exposure to chloroform anesthesia during childbirth, autopsy revealed fatty degeneration of the kidneys, indicating chloroform-induced damage (Royston 1924). Those deaths are most likely attributable to hepatotoxicity in fasted individuals rather than nephrotoxicity, because the same women were reported to have jaundice, liver enlargement and tenderness, and, at autopsy, centrilobular necrosis.

In laboratory animals, susceptibility to chloroform-induced nephrotoxicity varies greatly with species, strain, and sex. In BDF_1 mice, Templin et al. (1996a) found degenerative lesions and an increase of 7- to 10-fold in the percentage of cells in the S phase in the kidneys of males, but not of females, inhaling chloroform at 30 or 90 ppm, 6 h/d, 5 d/w for 2 w. In males exposed for 2 w, 40% of the 30-ppm group and 80% of the 90-ppm group died with severe kidney damage, indicating that 30 and 90 ppm exceeded the maximum tolerated dose. The NOAEL for male BDF_1 mice inhaling chloroform for 4 d, 6 h/d was 5 ppm. In contrast, $B6C3F_1$ mice were more resistant to chloroform nephrotoxicity and had a NOAEL of 100 ppm for a 6-h/d, 7-d exposure (Larson et al. 1994b). Similarly exposed F344 rats had a LOAEL of 30 ppm and a NOAEL of 10 ppm for exposure-induced kidney-cell proliferation (Larson et al. 1994b).

In certain strains of mice, renal tubular necrosis was reported in 100% of males after exposure to chloroform at ≤ 240 ppm for 2 h (Derringer et al. 1953; Culliford and Hewitt 1957). Mice surviving the exposure were found to have tubular calcifications when examined 12 mo after exposure. The kidneys of female mice of the susceptible strains and of both male and female mice of other strains were completely unaffected. Females of the susceptible strains

became susceptible when treated with testosterone. Immature males and castrated males were resistant to chloroform nephrotoxicity (Culliford and Hewitt 1957). Although this phenomenon is scientifically interesting, it is not a good model for the susceptibility of humans to chloroform nephrotoxicity. Clinical experience with thousands of patients, both males and females, who have undergone chloroform anesthesia (exposure at 8000 to 22,000 ppm) suggests a low incidence of nephrotoxicity in humans (Whitaker and Jones 1965; Lieberman 1973; Smith et al. 1973).

Resistance to Respiratory Infection

Mice (five groups of approximately 30 mice per group per dose for each exposure duration) exposed to Threshold-Limit-Value (TLV) concentrations (10.6 ppm) of chloroform for 3 h/d for 5 d and simultaneously challenged with an aerosol of *Streptococcus zooepidemicus* had significantly increased mortality (43.7% compared with 30.4% for filtered-air *Streptococcus* controls) over a 14-d observation period (Aranyi et al. 1986). Alveolar macrophage function (percentage of bacteria killed in 3 h), however, was not depressed under those conditions. Mice similarly tested for only 1 d (3 h) had no significant increase in mortality (6.7% vs. 5.7% for controls) (Aranyi et al. 1986).

Subchronic and Chronic Exposures

Long-term inhalation of chloroform vapors has been shown to be toxic to the liver and kidneys of several species, including humans. Other reported adverse effects include species-specific nasal toxicity, CNS effects, and lethality due to pneumonia.

Carcinogenicity

There are no compelling reports of chloroform-induced cancers in humans, despite extensive human exposure due to its use in the past in industry, as an anesthetic, and as an ingredient in medicinals. Chloroform is the major byproduct of chlorination of drinking water, and several studies have implicated chlorination by-products in the etiology of specific cancers. A 1992 epidemiological report suggests that consumption of chlorination by-products in drinking water is associated with an increased risk of rectal and urinary bladder cancers (Morris et al. 1992). A 1997 epidemiological study found a clear dose-response

relationship between increasing chloroform concentrations in finished drinking water and an increased risk of colon cancer (Doyle et al. 1997). Increasing chloroform concentrations in treated water, however, are likely to be accompanied by parallel increases in the concentrations of other chlorination by-products. Those epidemiological studies cannot specify which of the chlorination by-products is responsible for the increased cancer incidence. Some of the other by-products of water chlorination (e.g., bromodichloromethane) are genotoxic, whereas chloroform is not.

In animal studies, high doses and dose rates of chloroform have been shown to induce cancer in the liver and kidneys of mice and rats. Most studies involved gavage dosing, although several recent studies have examined the effects of chloroform inhalation. The most commonly observed cancers involved the liver and kidney in mice and rats. Striking differences were observed in the organ specificity between species and between different strains of the same species.

Long-term inhalation of high doses of chloroform vapors has been shown to induce kidney cancer in male BDF_1 mice (Yamamoto et al. 1994). In contrast, male and female F344 rats inhaling chloroform at 10, 30, or 90 ppm, 5 d/w for 2 y developed no tumors (Yamamoto et al. 1994). Studies at CIIT to elucidate the mechanisms for the lack of carcinogenicity in F344 rats found that under the conditions used in the Japanese bioassay, F344 rats showed only a marginal increase of cell proliferation in the kidneys of males and no exposure-induced histopathology or cell proliferation in the kidneys of females except at a highly toxic dose of 300 ppm, 7 d/w (Templin et al. 1996c). In BDF_1 mice, however, cancer induction appeared to correlate with cytotoxicity. Chloroform was found to be cytotoxic to both the liver and the kidney of BDF_1 and $B6C3F_1$ mice, yet BDF_1 mice develop kidney tumors but not liver tumors, and $B6C3F_1$ mice develop liver tumors but not kidney tumors. From that, Templin et al. (1996a) concluded that induced toxicity and regenerative-cell proliferation are necessary but not sufficient to induce cancer in a given organ. In another study, Larson et al. (1996) found a NOAEL of 10 ppm for increases in the LI of liver cells in female and male $B6C3F_1$ mice exposed to chloroform at 0, 0.3, 2, 10, 30, and 90 ppm, 6 h/d, 7d/w for up to 13 w and proposed that 10 ppm should also be a NOAEL for liver cancer in female $B6C3F_1$ mice (Larson et al. 1996). In other words, chloroform carcinogenicity should have a threshold if tumorigenesis is dependent on regenerative-cell proliferation (Golden et al. 1997). That proposal was challenged by Melnick et al. (1998), who argued that tumors can be produced at low doses of other trihalomethanes that do not produce increases in LIs (i.e., tumorigenesis is not dependent on regenerative-cell proliferation at low doses), and they asserted that the same would be true of chloroform also (Melnick et al. 1998). The trihalomethanes that they used to support that

contention, however, are DNA reactive, whereas chloroform is not, thus weakening their argument.

Numerous animal studies have shown that chloroform can be carcinogenic when given orally. The ability to induce cancer varied with the species, strain, and sex of the exposed animals, with the rate (bolus vs. intermittent) at which the chloroform was delivered, and with the dosing vehicle.

Female Wistar rats exposed for a lifetime to chloroform in drinking water at an average concentration of 200 mg/kg/d had an increased incidence of hepatic neoplastic nodules, and lymphosarcoma was increased in males (Tumasonis et al. 1987). Male Osborne-Mendel rats exposed to chloroform in drinking water for a lifetime at 160 mg/kg/d had an increased incidence of kidney tubular-cell adenoma and carcinoma but those exposed at 81 mg/kg/d did not (Jorgenson et al. 1985). A time-weighted-average chloroform dose of 263 mg/kg/d in drinking water for 1-4 w did not increase the incidence of hepatocellular carcinomas and adenomas in female $B6C3F_1$ mice (Jorgenson et al. 1985).

Male Osborne-Mendel rats exposed to chloroform at 90 mg/kg/d by gavage for 78 w developed kidney tubular-cell adenomas and carcinomas (NCI 1976). Sprague-Dawley rats, however, when exposed by gavage to chloroform in toothpaste at 60 and 165 mg/kg/d for 80 and 52 w, respectively, did not have an increased incidence of tumors (Palmer et al. 1979).

Dogs exposed to chloroform in toothpaste capsules at 30 mg/kg/d for 7.5 y had no increase in tumors (Heywood et al. 1979).

Mice exposed by gavage to chloroform in oil at 595 mg/kg/d for 30 d had an increased incidence of hepatomas, and those receiving 297 mg/kg/d did not (Eschenbrenner and Miller 1945). Female A/J mice exposed by gavage to chloroform in oil at 1800 mg/kg/d for 8 w had no increase in lung tumors (Stoner et al. 1986). Male $B6C3F_1$ mice exposed to chloroform in drinking water at 257 mg/kg/d for 52 w had no increase in lung or liver tumors (Klaunig et al. 1986).

ICI mice chronically exposed to chloroform by gavage at 60 mg/kg/d had an increased incidence of kidney tumors, but those exposed at 17 mg/kg/d did not (Roe et al. 1979). The overall incidence of all tumors, however, was lower in mice receiving the highest dose of chloroform than in controls. No significant differences were seen in the incidence or severity of nephrotoxicity in mice with kidney tumors and those without tumors. Under the same conditions, C57B1, CBA, and CF/1 mice had no change in the frequency of tumors (Roe et al. 1979). Thus, the significance of the increased incidence of kidney tumors in ICI mice is questionable.

$B6C3F_1$ mice exposed by gavage to chloroform in oil at ≥ 138 mg/kg/d for 78 w developed hepatocellular carcinomas (NCI 1976). $B6C3F_1$ mice exposed to

chloroform in drinking water at 263 mg/kg/d for 2 y had no increase in tumor incidence (Jorgenson et al. 1985), nor did they have increased liver-cell proliferation after 4 d or 3 w of exposure at up to 1800 ppm (Larson et al. 1994a); those given chloroform in corn oil at 238 or 477 mg/kg had both centrilobular necrosis and markedly elevated regenerative-cell proliferation (Larson et al. 1994a). Those studies support the mechanistic-based idea that chloroform's carcinogenicity is dependent on its capacity to induce necrosis and regenerative-cell proliferation. For the liver, Larson et al. (1994a) proposed that "the most straightforward risk assessment for chloroform for this tissue would assign no increased cancer risk for dosing regimens that do not induce cytolethality and cell proliferation." The NOAEL for histopathological changes from exposure to chloroform given in corn oil was 10 mg/kg/d, and for induced cell proliferation, the NOAEL was 34 mg/kg/d (Larson et al. 1994a).

CNS Effects

Challen et al. (1958) reported on two groups of workers in a British lozenge factory occupationally exposed to chloroform vapors. One group of eight workers (four half-time and four full-time) was exposed for ~2 h/d for 3 to 10 y to chloroform vapors at 77 to 237 ppm and experienced depression, irritability, a feeling of being dazed, "slow witted," "slowness in grasping things," lack of concentration, lassitude at the end of the day, and a desire to sleep. Additional symptoms included flatulence, nausea, dry mouth, thirst, and frequent and scalding urination. All these symptoms could be present at work but were usually worse in the evening at home (all exposed employees stated that at times they were unable to concentrate on household duties), and symptoms often persisted to some extent during the weekend, suggesting that chloroform was gradually being released from accumulated stores in the body. The second group of nine workers was exposed for 2 h/d for 10-24 mo to chloroform vapors at 22 to 71 ppm and complained of lassitude, flatulence, and dryness of the mouth and throat. It was not stated how soon symptoms developed after a new employee began working in the chloroform-contaminated atmosphere. Unexposed control workers did not have any of these symptoms.

Li et al. (1993) reported a variety of neurobehavioral effects in workers occupationally exposed for 1 to 15 y to chloroform vapors. These effects included dizziness, fatigue, somnolence, insomnia, hypomnesia, anorexia, palpitation, increased scores for depression, anger, and fatigue, and adverse effects on neurobehavioral functions, including increased simple visual reaction time, digit-symbol substitution, digit span, Bentsen retention, and pursuit aiming. The concentration of chloroform in the breathing zones of the workers

ranged from 4.3 to 148 mg/m^3 (0.87 to 30 ppm). The period of time over which the concentration measurements were taken was not stated, nor was any attempt made to temporally correlate effects in individuals with the exposure concentration at that time. The authors correlated the observed effects to the geometric means of the measured exposure concentrations (2.7 ppm and 5.9 ppm for two subgroups and 4.1 for the combined exposed groups). Because of the wide (>34-fold) variation in measured exposure concentrations, it is likely that the observed effects were seen only in individuals during exposure (for unknown durations) to concentrations at the high end of the range. Thus, it is inappropriate to ascribe the observed effects to the geometric mean of the measured concentrations.

Hepatotoxicity and Pneumonitis

There have been several reports of hepatotoxicity due to occupational exposures to chloroform vapors. A report of slight liver damage in factory workers exposed to chloroform at an average of 4.3 ppm for 1 to 15 y was based on a finding of increased serum concentrations of prealbumin and transferrin compared with 23 control workers (Li et al. 1993). Little weight is given to these results, however, because changes in serum concentrations of prealbumin and transferrin can be caused by many factors besides liver toxicity. In Singapore, 13 factory workers exposed to greater than chloroform at 400 ppm for 1 to 5 mo and 18 workers at another factory exposed at 14.4 to 50.4 ppm for 1 to 4 mo developed toxic hepatitis with symptoms, which included jaundice, nausea, and vomiting, without fever (Phoon et al. 1983). Those also tested negative for hepatitis B surface antigen. The total number of workers exposed to chloroform was not stated, but the department employing the 13 jaundiced workers had a total of 102 workers, and the factory employing the 18 jaundiced workers had a total of 360 employees in two departments, only one of which utilized chloroform. The measured exposure concentrations in these factories could therefore be considered LOAELs. In contrast, in a 1958 British study, relatively insensitive clinical tests for liver injury (thymol turbidity, serum bilirubin, and urine urobilinogen) gave negative or inconclusive results in two groups of workers at a lozenge factory exposed to chloroform at average concentrations of 71 to 237 ppm for 4 to 8 h/d for 3 to 10 y or 10 to 24 mo (Challen et al. 1958). Still another report of occupational chloroform-related hepatotoxicity was confounded by co-exposure to other solvents (Bomski et al. 1967).

Torkelson et al. (1976) exposed rats, guinea pigs, and rabbits to chloroform for 7 h/d, 5 d/w for 6 mo at 85, 50, or 25 ppm and dogs similarly at 25 ppm.

Additional groups of male rats were exposed for 4,2, or 1 h/d at 25 ppm. At 85 ppm, there were no adverse effects in male guinea pigs but marked microscopic pneumonitis in female guinea pigs. In rabbits exposed at 85 ppm, males had marked microscopic pneumonitis and foamy vacuolization and necrosis of the liver (Torkelson et al. 1976). Male rats exposed at 85 ppm had marked interstitial pneumonitis. Both male and female rats exhibited marked central lobular granular degeneration of the livers. At 50 ppm, no adverse effects were found in guinea pigs or rabbits; in rats, the effects were similar to but milder than those at 85 ppm. Female rats were affected less than males. At 25 ppm, male rats exposed for 7 h/d exhibited lobular granular degeneration with focal areas of necrosis throughout the liver. These effects were reversible within 6 W. No adverse effects were seen in male rats exposed to chloroform for 4, 2, or 1 h/d at 25 ppm. At 25 ppm, male guinea pigs showed microscopic granular degeneration and foamy vacuolization in the liver, and female guinea pigs showed foamy vacuolization centrally in the liver. Female rabbits showed slight microscopic changes in the lungs, liver, and kidneys.

In a recent study at CIIT, female B6C3F_1 mice exposed to chloroform at 100 or 300 ppm, 6 h/d, for 7 d exhibited centrilobular hepatocyte necrosis and severe vacuolar degeneration of midzonal and periportal hepatocytes, and exposure to 10 or 30 ppm resulted in mild-to-moderate vacuolar changes in centrilobular hepatocytes (Larson et al. 1994b). Slight, dose-related increases in the hepatocyte nuclear labeling indices (LIs) were observed for exposure concentrations of 10 and 30 ppm, and the LIs were increased more than 30-fold in the 100and 300-ppm groups (Larson et al. 1994b). The CIIT study also reported that mild centrilobular degeneration was observed only in the livers of rats exposed at 300 ppm (Larson et al. 1994b).

Lethality

Torkelson et al. (1976) exposed rats, guinea pigs, and rabbits to chloroform at 85 or 50 ppm, 7 h/d, for 138 to 144 d or those species plus dogs at 25 ppm, 7, 4, 2, or 1 h/d for 126 to 132 exposures in 180 to 186 d. At 85 ppm, they found excess mortality attributed to pneumonia only in male rats. At 50 ppm, there was no excess mortality in any of the tested species.

Nasal Toxicity

No reports of nasal toxicity due to chloroform inhalation were found in the literature before 1994. A 1994 CIIT report stated that in the nasal passages of

male F344 rats, chloroform concentrations of 10 ppm and above (6 h/d, 7 d) induced histopathological changes that exhibited clear concentration-related severity (Larson et al. 1994b). These lesions consisted of respiratory epithelial goblet-cell hyperplasia and degeneration of Bowman's glands in olfactory mucosa, with an associated osseous hyperplasia of the endo-and ecto-turbinates in the periphery of the ethmoid region (Méry et al. 1994; Larson et al. 1994b). Similarly exposed female $B6C3F_1$ mice did not exhibit such nasal lesions (Larson et al. 1994b). The only change noted in the nasal passages of exposed mice was increased cell proliferation without the osseous hyperplasia (Méry et al. 1994). In further studies at CIIT, edema and periosteal hypercellularity were observed in the nasal passages of Osborne-Mendel and F344 rats at single gavage doses of chloroform in corn oil at ≥ 90 mg/kg when necropsied 48 h after dosing (Templin et al. 1996b). No other studies were found that reported any nasal toxicity due to chloroform.

Nausea and Vomiting

Nausea and vomiting were reported in workers exposed to chloroform at 14 to 400 ppm for 1 to 6 mo (Phoon et al. 1983). Nausea as well as dry mouth and fullness of the stomach were reported by eight workers exposed to chloroform at 77 to 237 ppm for 3 to 10 y (Challen et al. 1958). Nausea and vomiting were commonly reported during or after chloroform anesthesia (Whitaker and Jones 1965).

Nephrotoxicity

The effects of chronic inhalation of chloroform vapors on the kidneys depends heavily on the sex, strain, and species being exposed as well as the exposure schedule. In a series of studies at CIIT, male F344 rats and male $B6C3F_1$ mice exposed to chloroform vapors for 7 d/w for 13 w had LOAELs for kidney toxicity of 30 ppm, and female F344 rats and female $B6C3F_1$ mice had NOAELs of 90 ppm (Larson et al. 1996; Templin et al. 1996c). When the exposure schedule was 5 d/w for 13 w, the LOAEL increased for male F344 rats from 30 to 90 ppm but decreased for male $B6C3F_1$ mice from 30 to 10 ppm (Larson et al. 1996; Templin et al. 1996c). Male BDF_1 mice exposed for 6 h/d for 4 d to chloroform at 0, 0.3, 5, 30, or 90 ppm had a LOAEL of 30 ppm and a NOAEL of 5 ppm for necrosis of the proximal convoluted tubules, tubule dilation, accumulation of hyaline casts, and focal mineralization of the kidneys (Templin et al. 1996a). Female BDF_1 mice showed no kidney toxicity at any

tested dose up to 90 ppm. A NOAEL of 5 ppm for nephrotoxicity, cell proliferation, and cancer was demonstrated in BDF_1 mice exposed for 2 y at concentrations of 5, 30, or 90 ppm for 6 h/d, 5 d/w (Templin et al. 1998).

Larson et al. (1994b) reported that about 25% to 50% of the proximal tubules were lined by regenerating epithelium in the kidneys of male F344 rats (females rats were not tested) and female $B6C3F_1$ mice (male mice were not tested) inhaling chloroform at 300 ppm for 6 h/d for 7 d but not in those inhaling 100, 30. or 10 ppm (Larson et al. 1994b).

Torkelson et al. (1976) exposed rats, guinea pigs, and rabbits to chloroform for 7 h/d, 5 d/w for 6 mo at 85, 50, or 25 ppm and dogs similarly at 25 ppm. Additional groups of male rats were exposed for 4, 2, or 1 h/d at 25 ppm. In rabbits exposed at 85 ppm, females had cloudy swelling in the kidneys. Both male and female rats exhibited cloudy swelling of the kidneys at 85 ppm. At 50 ppm, no adverse effects were found in guinea pigs or rabbits; in rats, the effects were similar to but milder than those at 85 ppm. Female rats were affected less than males. At 25 ppm, male rats exposed for 7 h/d exhibited cloudy swelling of the renal tubular epithelium. Those effects were reversible within 6 w. No adverse effects were seen in male rats exposed for 4, 2, or 1 h/d. In female rats exposed to chloroform for 7 h/d at 25 ppm, the relative weights, but not the absolute weights, of kidney and spleen were significantly increased. All other measurements were normal. At 25 ppm, male guinea pigs showed interstitial and tubular nephritis in the kidneys, and female guinea pigs showed significantly higher absolute and relative kidney weights, contrary to what had been observed at higher concentrations. Rabbits showed only an increase of interstitial and tubular nephritis in males and slight microscopic changes in the lungs, liver, and kidneys in females. Male dogs exposed at 25 ppm showed no changes, but female dogs exhibited microscopic pathological changes in the kidneys.

Genotoxicity

No data were found on the genotoxicity in humans of inhaled chloroform vapor.

A number of laboratories have tested chloroform for mutagenicity in *Salmonella* and *E. Coli* using a wide range of concentrations with and without metabolic activation. Rosenthal (1987) critically reviewed these studies and, despite noting some deficiencies in experimental procedures, concluded that chloroform is not mutagenic in bacteria—a conclusion that the current literature still supports (Roldan-Arjona and Pueyo 1993; Pegram et al. 1997).

Tests of chloroform's mutagenicity in various eukaryotes have given mixed

results. Callen et al. (1980) obtained only marginal effects in yeast for mitotic gene conversion and crossing over, as well as gene reversion, at chloroform concentrations of 21, 41, and 54 mM for 1 h. Crebelli et al. (1988 1992) reported the induction of aneuploidy by a threshold concentration of 0.16% vol/vol in the fungus *Aspergillus nidulans*, but that result is questionable because aneuploidy was not found at 0.20%.

Sturrock (1977) found that chloroform did not cause mutations at the HGPRT locus in Chinese hamster lung fibroblasts exposed to a 1-2.5% solution for 24 h, but no metabolic activation was used. Both negative results (White et al. 1979; Kirkland et al. 1981) and positive results (Morimoto and Koizumi 1983) have been reported for induction of sister chromatid exchanges in human lymphocytes in vitro and mouse bone marrow in vivo, but some aspects of the procedures used preclude reaching definitive conclusions.

In a tightly controlled study of mutation at the thymidine kinase gene in the L5178Y TK+/- mouse lymphoma cell, Mitchell et al. (1988) reported mixed results in experiments with and without metabolic activation. The mutant colonies might have resulted from chromosome loss (aneuploidy).

No increase in lacI mutant frequency was seen in hepatocytes isolated from B6C3F_1 lacI transgenic mice exposed by inhalation to chloroform at 0, 10, 30, or 90 ppm for 6 h/d, 7 d/w for up to 180 d (Butterworth et al. 1998).

No data have been reported that used tests recently designed for unequivocal detection of aneuploidy caused by chloroform, but, like other anesthetics, chloroform can disrupt the microtubules in the spindle of dividing cells. It is known that depolymerization of tubulin is involved in that action (see Liang et al. 1983), and low doses of depolymerizing agents can cause one or several chromosomes to come off the spindle, leading to aneuploid daughter cells. Chromosomes not attached to the spindle might form micronuclei, and that is probably the reason for the reports that chloroform causes small, statistically insignificant increases in micronuclei frequency, interpreted to be chromosomal aberrations (see Agustin and Lim-Sylianco 1978; Gocke et al. 1981). A 3.32-fold increase in the frequency of micronucleated kidney cells was reported by Robbiano et al. (1998) in male Sprague-Dawley rats given a single oral dose of 4 mmol/kg (476 mg/kg) of chloroform in corn oil.

Land et al. (1981) found statistically significant increases in the percentages of abnormal sperm heads in mice exposed to reagent grade chloroform for 4 h/d for 5 d at 400 or 800 ppm. That might be caused by one or more mutations, because abnormal sperm head shape in the mouse has been shown to be determined by genes. However, because cytoplasmic microtubules are still present in the early stages of sperm development (spermatids), abnormal sperm heads might alternatively be caused by depolymerized tubulin.

In summary, there are no convincing data that chloroform causes gene

mutation or chromosomal aberrations. It is more probable that it or its metabolic products act on proteins and not on DNA (Mersch-Sundermann et al. 1994) and are, therefore, likely to be aneugenic, but there are not yet definitive studies on that point. If it is an aneugen, it would have a concentration threshold for effect and would show a plateau at higher concentrations.

Reproductive Toxicity

No studies were found regarding reproductive effects in humans after inhalation exposure to chloroform vapors. An NTP study (Gulati et al. 1988) found that fertility was not affected in either of two generations of mice exposed by gavage to chloroform in corn oil at up to 41 mg/kg/d for 105 d, but at these doses, sperm morphology was not affected. In mice exposed by inhalation to chloroform at 400 or 800 ppm, 4 h/d for 5 d, Land et al. (1981) found statistically significant increases in the percentages of abnormal spermatozoa. In contrast, mice receiving five daily injections of chloroform intraperitoneally at 0.025, 0.05, 0.075, 0.1, and 0.25 mg/kg/d showed only nonreproducible, sporadic, small increases in abnormal sperm (Topham 1980).

Developmental and Fetal Toxicity

Embryotoxicity and fetotoxicity were found in pregnant Sprague-Dawley rats exposed to chloroform for 7 h/d at 100 or 300 ppm, but only minor embryo- and fetotoxicities were seen for exposure at 30 ppm on d 6 through 15 of gestation (Schwetz et al. 1974; Baeder and Hofmann 1988). A decreased ability to maintain pregnancy was observed in CF-1 mice exposed 7 h/d at 100 ppm on d 1 through 7 or 6 through 15, but no significant teratogenicity (Murray et al. 1979). When the exposure schedule was on d 8 through 15, however, no decrease was seen in the ability to maintain pregnancy, but a significant increase in the incidence of cleft palate was observed in the offspring.

No studies were found regarding developmental effects in humans after inhalation exposure to chloroform vapors. In rats exposed during gestation, chloroform-induced fetotoxicity and teratogenicity (decreased fetal crown-rump length and delayed ossification) were observed by Schwetz et al. (1974), but only at concentrations that produced maternal anorexia, with a LOAEL of 30 ppm. Murray et al. (1979) found increased incidences of cleft palate, decreased ossification, and decreased fetal crown-rump length in rats and an increased incidence of cleft palate in the offspring of mice exposed on d 8 through 15 of gestation to chloroform at 100 ppm.

Interaction with Other Chemicals

Chloroform-induced toxicity can be potentiated by several treatments. Some examples include ethanol, polybrominated biphenyls (PBBs), ketones, and steroids (EPA 1985). Chemicals, such as diethyl maleate, that deplete hepatic glutathione can greatly increase the hepatotoxicity of chloroform (Brown et al. 1974). Fasting, which also reduces glutathione, has a similar enhancing effect on chloroform hepatotoxicity (Brown et al. 1974).

Factors that appear to protect against toxicity include disulfiram and high carbohydrate diets (EPA 1985).

Table 13-1 presents a summary of the toxicity data on chloroform.

TABLE 13-1 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
			Effects in Humans	
2.7	1-15 y, occupational	Human, n = 14	Dizziness, fatigue, somnolence, insomnia, hypomnesia, anorexia, palpitation, increased serum concentrations of transferrin, increased scores for depression, anger, and fatigue	Li et al. 1993
5.9	1-15 y, occupational	Human, n = 46	Dizziness, fatigue, somnolence, insomnia, hypomnesia, anorexia, palpitation; increased serum concentrations of pre-albumin and transferrin; increased scores for depression, anger, and fatigue; adverse effects on neurobehavior (increased simple visual reaction time, digit-symbol substitution, digit span, Bentsen retention, and pursuit aiming)	Li et al. 1993
10-200	1-4 y, occupational	Human, n = 68	Hepatomegaly in 16 of 68	Bomski et al. 1967
14-400	1-6 mo, occupational	Human, n = 31	Toxic hepatitis, jaundice, nausea, vomiting, no fever	Phoon et al. 1983
22-71	10-24 mo, occupational	Human, n = 10	Lassitude; no evidence of liver toxicity	Challen et al. 1958
77-237	3-10 y, occupational	Human, n = 10	No evidence of liver toxicity; lassitude, digestive disturbances, frequent burning urination, depression, lack of concentration, and irritability	Challen et al. 1958
920	3 min	Human	Dizziness and vertigo	Lehmann and Hasagawa 1910
1000	<1 h	Human	Dizziness, nausea, and after-effects of fatigue and headache	Hathaway et al. 1991
<1500	NS	Human	NOEL for anesthesia	Goodman and Gilman 1980
1500-2000	NS	Human	Light anesthesia	Goodman and Gilman 1980

4100	<1 h	Human	Serious disorientation	Hathaway et al. 1991
8000-10,000	Anesthesia	Human	Increased sulfobromophthalein retention (impaired liver function); EC_{50} for anesthesia; cardiac arrhythmia in 50% of 58 patients	Smith et al. 1973
10,000-22,500	Anesthesia	Human	Bradycardia (<60 beats/min) in 8% of 1502 patients; cardiac arrhythmia in 1.3% of 1,502 patients; hypotension in 27% of 1502 patients (related to pretreatment with thiopentone)	Whitaker and Jones 1965
14,000-16,000	<1 h	Human	Rapid loss of consciousness	Hathaway et al. 1991
20,000-40,000	<1 h	Human	Induction of anesthesia; continued exposure to 20,000 ppm results in respiratory failure, cardiac arrhythmia, and death	Hathaway et al. 1991
			Effects in Animals	
2	6 h/d, 7 d/w, 13 w	Rat, F-344	LOAEL for atrophy of nasal ethmoid turbinates	Larson et al. 1996
3	6 h/d, 7 d	Rat	NOAEL for histopathological changes in nasal passages	Larson et al. 1994b
3	6 h/d, 7 d	Mice, $B6C3F_1$, female	NOAEL for vacuolar changes in centrilobular hepatocytes, hepatocyte DNA synthesis/repair, nasal lesions	Larson et al. 1994b
5	6 h/d, 4 d	Mice, BDF_1, male and female	NOAEL for hepatocyte vacuolization or necrosis	Templin et al. 1996a
5	6 h/d, 5 d + 4 d	Mice, BDF_1, male	NOAEL for necrosis of the proximal convoluted tubules, tubule dilation, accumulation of hyaline casts, and focal mineralization of the kidneys	Templin et al. 1996a
10	6 h/d, 7 d	Rat, F-344, male	LOAEL for histopathological changes in nasal passages	Méry et al. 1994
10	6 h/d, 7 d/w, 13 w	Rat, F-344, male	LOAEL for histopathological changes in nasal passages	Larson et al. 1994b
10	6 h/d, 7 d	Rat, F-344, male	NOAEL for increased LI in kidney	Larson et al. 1994b

Concentration, ppm	Exposure Duration	Species	Effects	Reference
10	6 h/d, 7 d/w, 13 w	Rat, F-344, male	NOAEL for increased LI in kidney	Larson et al. 1996
10.6	3 h	Mice	NOAEL for increased mortality with bacterial challenge	Aranyi et al. 1986
10.6	3 h/d, 5 d	Mice	Increased mortality with bacterial challenge	Aranyi et al. 1986
25	7 h/d, 5 d/w, 6 mo	Rat, male	Lobular granular degeneration with focal areas of necrosis of liver; cloudy swelling of renal tubular epithelium	Torkelson et al. 1976
25	7 h/d, 5 d/w, 6 mo	Rat, female	Increased relative, but not absolute weights of kidney and spleen	Torkelson et al. 1976
25	4 h/d, 5 d/w, 6 mo	Rat, male	NOAEL for liver, kidney, lung, organ weight, and blood effects	Torkelson et al. 1976
25	7 h/d, 5 d/w, 6 mo	Guinea pig, male	Granular degeneration and vacuolization in the liver; interstitial and tubular nephritis	Torkelson et al. 1976
25	7 h/d, 5 d/w, 6 mo	Guinea pig, female	Foamy vacuolization in the liver; increased absolute and relative kidney weights	Torkelson et al. 1976
25	7 h/d, 5 d/w, 6 mo	Rabbit, male	Increased interstitial and tubular nephritis	Torkelson et al. 1976
25	7 h/d, 5 d/w, 6 mo	Rabbit, female	Slight microscopic changes in the lungs, liver, and kidneys	Torkelson et al. 1976
25	7 h/d, 5 d/w, 6 mo	Dog, male	NOAEL for liver, kidney, lung, organ weight, and blood effects	Torkelson et al. 1976
25	7 h/d, 5 d/w, 6 mo.	Dog, female	Microscopic pathological changes in the kidneys	Torkelson et al. 1976

Concentration, ppm	Exposure Duration	Species	Effects	Reference
30	6 h/d, 2 w	Mice, BDF_1, male and female	LOAEL for hepatocyte vacuolization	Templin et al. 1996a
30	6 h/d, 7 d/w, 90 d	Rat, F-344	NOAEL for kidney cytotoxicity and regenerative-cell proliferation	Templin et al. 1996c
50	7 h/d, 5 d/w, 6 mo	Guinea pig, rabbit	NOAEL for liver, kidney, lung, organ weight, and blood effects	Torkelson et al. 1976
50	7 h/d, 5 d/w, 6 mo	Rat	Cloudy swelling of kidney, granular degeneration of liver, LOAEL for weight loss	Torkelson et al. 1976
75	4 h	Rat, male	NOAEL for increased serum concentrations of sorbitol dehydrogenase	Lundberg et al. 1986
85	7 h/d, 5 d/w, 6 mo	Rat, male	Cloudy swelling of kidney, granular degeneration of liver, LOAEL for mortality due to interstitial pneumonitis	Torkelson et al. 1976
85	7 h/d, 5 d/w, 6 mo	Rat, female	Cloudy swelling of kidney, granular degeneration of liver, NOAEL for interstitial pneumonitis	Torkelson et al. 1976
85	4 h/d, 5 d/w, 6 mo	Rat, male	NOAEL for liver, kidney, lung, organ weight, blood effects	Torkelson et al. 1976
90	6 h/d, 4 d	Mice, BDF_1, male and female	LOAEL for hepatocyte vacuolization and necrosis and for increased hepatocyte LI in females	Templin et al. 1996a
90	6 h/d, 5 d/w, 2 y	Rat, F344, male and female	NOAEL for kidney cancer	Yamamoto et al. 1994
90	6 h/d, 5 d/w, 90 d	Rat, F-344, male and female	LOAEL for kidney cytotoxicity and regenerative-cell proliferation in males	Templin et al. 1996c

Concentration, ppm	Exposure Duration	Species	Effects	Reference
100	6 h/d, 7 d	Mouse, B6C3F$_1$, female	NOAEL for kidney cytotoxicity	Larson et al. 1994b
100	6 h/d, 7 d	Rat, F-344, male	LOAEL for liver cytotoxicity	Larson et al. 1994b
100	6 h/d, 7 d/w, 13 w	Rat, F-344, male	NOAEL for hepatic lesions and increased LI	Larson et al. 1996
100	7 h/d, 7-10 d	Mouse	Decreased ability to maintain pregnancy, increased incidence of cleft palate, decreased ossification, decreased incidence of resorptions, and reduced fetal body measurements	Murray et al. 1979
100	7 h/d, 7-10 d	Rat	Retarded fetal development; low incidence of acaudate fetuses	Murray et al. 1979
149	4 h	Rat	LOAEL for increased serum concentrations of sorbitol dehydrogenase	Lundberg et al. 1986
300	6 h/d, 7 d	Mouse, B6C3F$_1$, female	LOAEL for kidney cytotoxicity	Larson et al. 1994b
300	6 h/d, 7 d/w, 13 w	Rat, F344	LOAEL for hepatic lesions and increased LI	Larson et al. 1996
300	7 h/d, 10 d	Rat	Decreased incidence of pregnancy; increased incidence of resorptions	Schwetz et al. 1974
400	4 h/d, 5 d	Mouse	Increased percentage of abnormal spermatozoa	Land et al. 1981
1260	6 h	Mouse	LC_{50}	Bonnet et al. 1980
1849	6 h	Rat	LC_{50}	Bonnet et al. 1980

Concentration, ppm	Exposure Duration	Species	Effects	Reference
1849	6 h	Rat	LC_{50}	Bonnet et al. 1980
5000	2 h	Rat	Hepatic necrosis; destruction of microsomal enzymes	Brown et al. 1974
9540	4 h	Rat	LC_{50}	Lundberg et al. 1986
16,000	5 min	Dog	Sensitization to cardiac arrhythmia	Clark and Tinston 1982
16,000	10 min	Rat	EC_{50} for ataxia and loss of righting reflex	Clark and Tinston 1982
76,000	15 min	Rat	LC_{50}	Clark and Tinston 1982

NS, not specified.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 13-2 presents exposure limits for chloroform set by other organization and Table 13-3 presents the SMACs established by NASA.

The SMAC values listed above were set based on the lowest acceptable

TABLE 13-2 Exposure Limits Set by Other Organizations

Organization	Exposure Limit, ppm	Reference
ACGIH's TLV	10 (TWA)	ACGIH 1997
ACGIH's STEL	Not set	ACGIH 1997
OSHA's PEL	2 (TWA)	ACGIH 1991
NIOSH's REL	2 (60-min ceiling)	ACGIH 1991
NIOSH's IDLH	1000	NIOSH 1990
NRC's 1-h EEGL	100	NRC 1984
NRC's 24-h EEGL	30	NRC 1984
NRC's 90-d CEGL	1	NRC 1984

TLV, Theshold Limit Value; TWA, time-weighted average; STEL, short-term exposure limit; PEL, permissible exposure limit; REL, recommended exposure limit; IDLH, immediately dangerous to life and health; EEGL, emergency exposure guidance level; CEGL, continuous exposure guidance level.

TABLE 13-3 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	2	10	CNS depression
24 h	2	10	CNS depression
7 d[a]	2	10	CNS depression, hepatotoxicity, nephrotoxicity
30 d	1	5	CNS depression, hepatotoxicity
180 d	1	5	CNS depression, hepatotoxicity

[a] Previous 7-d SMAC = 1 ppm.

concentration (AC) among those calculated for the various adverse effects at each exposure duration, following the guidelines of the National Research Council (1992). The evidence and logic used to determine the ACs for each adverse effect and exposure duration are documented below. ACs were set for CNS effects, hepatotoxicity, nephrotoxicity, cardiac arrhythmia, and carcinogenicity (Table 13-4). No ACs were set for the following end points:

- *Nausea*. Insufficient data are available to establish a dose-response relationship. In addition, it was seen in humans only in conjunction with CNS effects or hepatotoxicity.
- *Genotoxicity*. There is no convincing evidence of chloroform's genotoxicity.
- *Resistance to respiratory infection*. It is not clear to this author how to derive an AC for human exposures to chloroform under conditions of low level (ambient) bacterial challenge from the results reported by Aranyi et al. (1986). Attempts to use their data directly without adjustment for the massive bacterial challenge results in a 1-h AC of about 1 ppm (10.6 ppm NOAEL ÷ 10 (species)). Experience with human exposures to much higher concentrations for much longer exposures, including multi-year occupational exposures, has not produced any indication of increased susceptibility to respiratory infections, even when nausea and liver toxicity have been evident. No reports were found, however, that specifically looked for increases in respiratory infections.
- *Nasal lesions*. Such lesions appear to be specific to rats and mice, and no nasal toxicity has ever been seen in humans or other animal species.
- *Developmental toxicity*. Pregnant astronauts are not permitted to fly.

Carcinogenicity

The weight of evidence indicates that chloroform exposure results in tumors only under conditions resulting in exposure-induced cytotoxicity and cell regeneration (Golden et al. 1997), and even then, those effects appear to be necessary but not sufficient for tumorigenesis. The evidence from many studies supports the conclusion that chloroform exposures that do not produce cytotoxicity and cell regeneration will not result in tumorigenesis—a NOAEL for chloroform-induced cytotoxicity in a chloroform-sensitive species, strain, sex, and organ will be a NOAEL for carcinogenicity.

In humans, the organ most sensitive to chloroform toxicity appears to be the liver rather than the kidney. Despite numerous reports of chloroform-induced liver damage and even deaths in humans, there are no known reports of chloro-

form-induced liver cancer in humans, even those with substantial, multi-year occupational exposures. From that, one may surmise that it is likely that liver cytotoxicity and hepatocyte regeneration in humans is not sufficient to produce liver tumors. To err on the side of conservatism, however, the AC values will be derived assuming that humans might be sensitive to chloroform-induced hepatocarcinogenisis and basing them on NOAEL values for liver cytotoxicity in rodents inhaling chloroform vapors. A NOAEL for liver cancer can be deduced from the NOAEL of 10 ppm observed for liver cytotoxicity in female and male $B6C3F_1$ mice exposed to chloroform at 0, 0.3, 2, 10, 30, and 90 ppm for 6 h/d, 7 d/w for up to 13 w (Larson et al. 1996). Because LI has not been found to increase with increasing exposure duration (Butterworth 1997), no adjustment is made for exposure duration. A species extrapolation factor of 1, rather than 10, is applied to the 10-ppm mouse NOAEL, because, at a given atmospheric concentration of chloroform vapor, the effective dose in the liver is lower in humans than in mice (Reitz et al. 1990) and because humans do not appear to be more sensitive than rodents to chloroform-induced carcinogenicity. No ACs are set for 1 h or 24 h exposures to carcinogens. Thus,

7-d, 30-d, and 180-d ACs = 10ppm ÷ 1 = 10ppm.

Cardiac Arrhythmia

An AC can be set based on the EC_{50} of 8000 ppm reported by Smith et al. (1973) for arrhythmia in patients receiving chloroform anesthesia. The EC_{50} is divided by 10 to estimate a NOAEL and divided again by an additional factor of 5 for possible spaceflight-enhanced sensitivity to noncritical arrhythmias. Thus,

AC = 8000 ppm (EC_{50}) ÷ 10 (to NOAEL) ÷ 5 (spaceflight) = 160 ppm.

Because this effect is dependent only on the concentration of chloroform, is inducible within the first few minutes of exposure and is rapidly reversible on cessation of exposure, a concentration that would not induce arrhythmia for a 1-h exposure would also not induce arrhythmia for exposures of longer duration. Thus, the AC would apply for all exposure durations.

CNS Depression

ACs for CNS depression can be based on the estimated LOAEL of 22 ppm in workers reported by Challen et al. (1958). A 10-fold factor is applied to

estimate a NOAEL. Because CNS effects of solvents are generally dependent on blood concentration and independent of exposure duration, the calculated AC is applicable for all exposure durations ≤ 1 h. Although mild effects, such as irritation, which would not compromise a crew member's ability to safely perform his duties, might be tolerable for exposures of 24 h or less, mild CNS depression could affect a crew member's judgment and reaction time and thus would compromise safety and would be unacceptable, even for short exposures. Thus, for all exposure durations,

AC = 22 ppm ÷ 10 = 2 ppm.

Hepatotoxicity

No data are available to calculate an AC for 1 h or 24 h.

ACs for 7 d and 30 d can be calculated from the data of Phoon et al. (1983) for jaundice in 18 workers exposed to chloroform at an average of 32 ppm for 1 to 4 mo for an unstated number of hours per week; 8 of the cases occurred within the first month of exposure. Typical work schedules for such industries in Singapore during that time involved at least 8 h/d for 6 d/w. Using 32 ppm as a LOAEL for exposures of 206 h/mo (8 h/d × 6 d/w × 4.3 w/mo), a 7-d NOAEL is estimated by applying a safety factor of 10 to the 1-mo LOAEL. Thus,

7-d AC = 32 ppm ÷ 10 (to NOAEL) = 3 ppm.

A 30-d AC is calculated similarly, adjusting for the duration of exposure.

$$\text{30-d AC} = 32\ \text{ppm} \div 10\ (\text{to NOAEL}) \times \frac{206\ \text{h} \times 1\ \text{mo} \div 24\ \text{h/d}}{30\ \text{d}} = 1\ \text{ppm}.$$

In a 1958 study, 17 workers in a lozenge factory exposed occupationally to chloroform at 23 to 71 ppm for 10 to 24 mo showed minimal evidence of liver toxicity. The tests used, however, were rather insensitive, so minor liver damage cannot be excluded. Because the exposures produced CNS and gastrointestinal-tract symptoms, which often precede symptoms of hepatotoxicity, 22 ppm will be treated as a LOAEL for subclinical hepatotoxicity for 4-h/d exposures up to 24 mo. The 180-d AC is set at the human LOAEL of 22 ppm, divided by 10 to estimate a NOAEL, and adjusted for exposure duration.

Thus,

$$\text{180-d AC} = 22 \text{ ppm} \div 10 \text{ (to NOAEL)} \times \frac{4 \text{ h/d} \times 5 \text{ d/w} \times 104 \text{ w}}{180 \text{ d} \times 24 \text{ h/d}}$$

$$= 1 \text{ ppm.}$$

Nephrotoxicity

ACs for nephrotoxicity are set based on data from animal experiments because few or no data exist on the nephrotoxic effects of inhalation exposures to chloroform in humans other than autopsy reports on women who died as a result of chloroform anesthesia during childbirth. Those reports found fatty degeneration of kidneys believed to be due to chloroform exposure in some of the patients (Royston 1924), but the exposure concentrations that produced such toxicity were not reported.

In males of the most sensitive species of mice, Templin et al. (1996a) found an experimental NOAEL of 5 ppm for kidney necrosis after exposures of 6-h/d for 4 d or for 2 w (5 d + 4 d). Their NOAEL is used to derive an AC for nephrotoxicity. A species extrapolation factor of 1 is used because PBPK modeling of the rate at which inhaled chloroform reaches the liver shows that, at any given atmospheric concentration, humans will achieve lower hepatic (and, by extension, kidney) chloroform concentrations than will mice. In addition, the data available suggest that the susceptibility of human tissues to injury by the active metabolites of chloroform does not exceed that of mouse tissues (Reitz et al. 1990). The total exposure time in the Templin et al. (1996a) experiment was 54 h, (6 h/d × 9 d). Thus, for exposure durations shorter than 54 h, (i.e., 1 h and 24 h), the ACs are set equal to the 5-ppm NOAEL, and for the 7-d AC, the NOAEL is adjusted for exposure duration using Haber's rule. Thus,

$$\text{1-h and 24-h ACs} = 5 \text{ ppm.}$$

$$\text{7-d AC} = 5 \text{ ppm} \times \frac{54 \text{ h}}{24 \text{ h/d} \times 7 \text{ d}} = 1.6 \text{ ppm.}$$

No 30-d or 180-d AC was set using these data because they would require a time extrapolation of greater than 10-fold.

Reproductive and Developmental Toxicity

ACs are not calculated for chloroform's ability to cause changes in sperm morphology, because Land et al. (1981) stated that the clinical significance of their findings (up to 3.5% abnormal sperm in mice exposed 4 h/d for 5 d at 800 ppm) cannot be evaluated because they did not study mating outcomes. ACs are also not calculated for decreased ability to maintain pregnancy, decreased conception rates in females, or teratogenic effects because NASA policy does not permit pregnant astronauts to fly.

Spaceflight Effects

Spaceflight is believed to increase the susceptibility of crew members to noncritical cardiac arrhythmias and could amplify the arrhythmogenic effects of chloroform.

TABLE 13-4 Acceptable Concentrations

End Point, Exposure Data, Reference		Uncertainty Factors			Acceptable Concentrations, ppm					
Species	NOAEL	Species	Time	Spaceflight	1 h	24 h	7 d	30 d	180 d	
Carcinogenicity (liver cytotoxicity)	Mouse	1	1	1	1	NS	NS	10	10	10
NOAEL, 10 ppm (Larson et al. 1996)										
Cardiac arrhythmia	Human	10	1	1	5	160	160	160	160	160
8000 ppm = EC_{50} (Smith et al. 1973)										
CNS depression	Human	10	1	1	1	2	2	2	2	2
LOAEL, 22 ppm (Challen et al. 1958)										
Hepatotoxicity	Human	10	1	HR	1	NS	NS	3	1	NS
LOAEL 32 ppm, 8 h/d, 6 d/w, 1 mo (Phoon et al. 1983)										
LOAEL, 22 ppm, 4 h/d, 5 d/w for 24 mo (Challen et al. 1958)	Human	10	1	HR	1	NS	NS	NS	NS	1
Nephrotoxicity	Mouse, BDF_1, male	1	1	1	1	5	5	1.6	NS	NS
NOAEL increases in LI, 5 ppm, 6 h/d, 4 d (Templin et al. 1996a)										
SMACs						**2**	**2**	**2**	**2**	**1**

—, not applicable; NS, not set; HR, Haber's rule.

RECOMMENDATIONS

Because chloroform's toxicity to the liver, kidney, and possibly the respiratory tract is due to its metabolites produced by cytochrome P-450, research is needed to quantitate the organ-specific levels of metabolism in humans and to elucidate factors, such as glutathione concentrations, which could modulate the threshold concentration of chloroform required for toxicity. Once those are determined, a PBPK model incorporating these values would be useful.

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B14 FURAN

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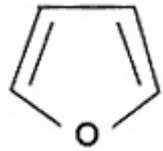
PHYSICAL AND CHEMICAL PROPERTIES

Furan is a volatile, clear, colorless liquid that turns brown upon standing. It is soluble in alcohol and ether and insoluble in water (Windholz 1976; Sax and Lewis 1989). It forms resins on evaporation or when in contact with mineral acids but is stable to alkalies (Sax and Lewis 1989).

Formula:	C_4H_4O
CAS no.:	110-00-9
Synonyms:	1,4-Epoxy-1,3-butadiene, furfuran, oxole, tetrole, divinylene oxide, oxacyclopentadiene, NCI-C56202
Molecular weight:	68.07
Boiling point:	31.36°C
Melting point:	–85.65°C
Saturated vapor concentration:	650,000 ppm at 20°C
Lower explosive limit:	2.3%
Upper explosive limit:	14.3%
Density:	0.9371; vapor density, 2.35
Conversion factors:	1 ppm = 2.78 mg/m^3 1 mg/m^3 = 0.359 ppm



OCCURRENCE AND USE

Furan occurs in oils obtained by the distillation of rosin-containing pine wood (Windholz 1976). Commercial production of furan is performed by the

decarbonylation of furfural or by the oxidation of butadiene (NTP 1993). Furan is widely used industrially, primarily as a solvent for resins, in the formation of lacquers, a binder in foundry applications, and a chemical intermediate for organic syntheses. It is also found in the vapor phase of cigarette smoke at a concentration of about 8.4 μg/40 mL puff (Egle and Gochberg 1979).

Furan has been identified as a major component of certain processed foods and drinks. It has been found in the volatile fraction from roasted ground coffee, in the vapor from baked white bread, and in head-space samples from canned meat (Merritt et al. 1963; Mulders et al. 1972; Persson and von Sydow 1973).

Derivatives of furan, such as tetrahydrofuran and 2-methylfuran, have been detected occasionally in spacecraft atmospheres (James 1991). Furan has not been detected in the atmosphere of American spacecraft but has been detected occasionally at low concentrations (0.12 mg/m^3) in the Russian space-station Mir core module.

TOXICOKINETICS AND METABOLISM

Absorption

Anesthetized dogs exposed for 1 to 2 min to furan vapors at concentrations of 400 to 600 mg/m^3 were found to have total respiratory-tract retentions between 90% and 95% and lower tract retentions of 87% to 93% (Egle and Gochberg 1979). The fraction of inhaled furan retained was not affected by tidal volume changes but was directly related to concentrations inhaled (Egle and Gochberg 1979).

Administration of furan by oral gavage leads to absorption from the gastrointestinal tract into the portal vein and to the liver. In rats, it has been shown that the rate of furan metabolism in hepatocytes greatly exceeds the ability of furan to be delivered by the blood flow to the liver (Kedderis and Held 1996); hence, little unmetabolized furan should reach the general circulation after passing through the liver. It appears, however, that some furan might bypass the liver. In CIIT studies of mice receiving a single gavage dose of furan at 50 mg/kg, histopathology showed that the subcapsular parenchyma of the visceral surface of the left and caudate hepatic lobes had necrosis with some inflammatory cell infiltrate primarily at 12 hafter exposure, suggesting that some of the furan diffused through the stomach wall and into the liver subsequent to gavage (Wilson et al. 1992). Some unmetabolized furan reaches the lungs, either by diffusion or through the general circulation, because unmetabolized furan is found in the expired air of rats given furan by oral gavage (see below).

Distribution

The tissue distribution of radioactivity 24 h after oral gavage of [2, 5-^{14}C-furan] to male Fischer 344 (F344) rats at 8 mg/kg has been reported (Burka et al. 1991). The nmol eq/g of tissue were as follows: liver, 307; kidney, 60; large intestine, 25; small intestine, 13; stomach, 6; blood, 6; and lung, 4. The labeling distributed in the tissues represented approximately 15% of the total dose administered. The label in liver included no unmetabolized furan, and none of the label was bound to DNA; most of the label was associated with protein and could not be extracted from it. Over the next 7 d, the radioactivity in liver, kidney, and blood declined to near the detection limits. Repeated administration of the same dose of furan for 8 d resulted in the accumulation of labeling to 1100 nmol eq/g of liver tissue, 320 nmol eq/g of kidney, and 37 nmol eq/g of blood.

Elimination

During the first 24 h after an 8-mg/kg oral gavage in male rats, approximately 80 % of the radioactivity derived from furan was eliminated in the expired air, urine, and feces (Burka et al. 1991). The radioactivity in expired air consisted of unchanged furan (14 %) and carbon dioxide (26%). The urine contained 20% of the radioactivity administered, and HPLC analysis suggested at least 10 metabolites were present. Approximately 22% of the single dose was eliminated in the feces. The fraction eliminated in the feces was unchanged during 8 d of administration, but the fraction found in the urine increased to 33% of the total dose after the eighth dose.

Metabolism

There is convincing evidence that, in F344 rats, furan is bioactivated in vitro and in vivo to cis-2-butene-1,4-dial by cytochrome P-450 2E1-catalyzed oxidation (Burka et al. 1991; Carfagna et al. 1993; Chen et al. 1996). Cytochrome P-450 2E1 is present not only in hepatocytes but also in nasal, lung, and other tissues. Thus, the route of exposure might influence the site of toxic lesions.

Kedderis and colleagues at the Chemical Industry Institute of Toxicology (CIIT) used computational models to study interspecies differences in the rate of metabolism of furan by estimating the effect of body size and blood-flow rates on the delivery of furan to the liver (and hence, the rate of production of

active metabolites by hepatocytes of the liver). Using a physiologically based pharmacokinetic (PBPK) model, Kedderis and Held (1996) showed that, although furan is rapidly metabolized by isolated hepatocytes from humans and rodents, the rate of metabolism of inhaled furan in humans is 10-fold lower than that in mice and 3-fold lower than that in rats. That result was based on a simulated exposure to furan at a concentration of 10 ppm for 4 h. The lower rate in humans is due to the comparative kinetics of hepatic blood flows in rodents and humans using a model in which inhaled furan vapor is metered into the bloodstream via the breathing rate and distributed throughout the organism at rates that are a function of body size (Kedderis and Held 1996).

In rats, furan is bioactivated by a cytochrome-P-450-dependent pathway to one or more metabolites that uncouple oxidative phosphorylation, deplete cellular ATP, and induce DNA double-strand breaks before cell death (Mugford and Kedderis 1997). Errors in repair of the DNA breaks have been hypothesized to result in mutations and contribute to the neoplastic effects of furan (Mugford and Kedderis 1997).

TOXICITY SUMMARY

The toxicity of furan in animals has been studied primarily by using the oral exposure route. A few studies examined the toxic effects of intraperitoneal (ip) injection of furan, but only two reports investigated the inhalation toxicity of furan vapors in animals. No studies were found on the effects of furan in humans by any route of exposure.

Acute and Short-Term Exposures

General Toxicity

Since the mid-1920s, various investigators have shown that furan is quite toxic to many organ systems by various routes of exposure. Furan can be absorbed through the skin, and its vapors are narcotic (anesthetic and convulsant) (Koch and Cahan 1925; Sax and Lewis 1989). Because its narcotic properties were recognized first, early investigators had hoped to use furan as an anesthetic but found that, although it has some anesthetic effects, it is extremely toxic (Koch and Cahan 1925).

Furan injected ip has been found to cause acute pulmonary edema (Egle and Gochberg 1979) and liver and kidney damage in mice (Wiley et al. 1984). Early studies (Koch and Cahan 1925) reported that furan has a corrosive effect

on mucous membranes of the gastrointestinal tract when given orally in capsules (0.25 mL/kg) to dogs and rabbits. Oral exposure induced a copious flow of bloody saliva, watery fluid from the nose, marked hyperemia of lungs and dilation of blood vessels, and gastrointestinal-tract hemorrhages (Koch and Cahan 1925). Other symptoms of oral exposure include cherry-red blood, soft swollen kidneys, and liver resembling the first stages of chloroform poisoning (Koch and Cahan 1925).

Lethality

Since the mid-1920s, studies have reported on the acute lethality of furan in animals. With intravenous injection (1.5 mL) in dogs, furan caused symptoms and postmortem changes similar to acute cyanide poisoning (Koch and Cahan 1925). Convulsions were followed rapidly by death. Intraperitoneal injection of 0.2 mL furan into a rat stopped respiration within 30 s, although the heart continued to beat for 3 min (Koch and Cahan 1925). Effects noted by the investigator included hyperemic liver and intestines, cherry-red blood, and dilation of the blood.

Furan vapor is highly toxic to Sprague-Dawley rats and has a steep dose-response curve with a 1-h LC_0 of 2850 ppm and a 1-h LC_{50} of 3464 ppm (Terrill et al. 1989). The symptoms of intoxication by inhalation are an increase in respiratory rate, fall of blood pressure, convulsions, complete anesthesia, and death from asphyxia (Koch and Cahan 1925; Terrill et al. 1989). Although no concentrations were given, an early report indicated that rats and rabbits inhaling furan from a saturated cotton wad collapsed after a short struggle and died shortly thereafter (Koch and Cahan 1925). Similarly, dogs anesthetized with ether died suddenly when furan was substituted for ether after induction of light anesthesia (Koch and Cahan 1925). Another early paper reported that a lethal concentration (presumably by inhalation for an unspecified exposure time) for cats was 136 mg/m^3 (Johnston 1931).

Two LC_{50} studies have been published for furan. The 1-h LC_{50} for furan inhaled by mice was reported to be 0.12 μg/mL (43 ppm) (Egle and Gochberg 1979). The exposure methods used in the study, however, would be unacceptable today. The mice in the study were exposed in 5.2-L sealed glass desiccators into which furan vapor was injected from a 100-cc glass syringe. Calculations of the volume of air breathed in 1 h by four mice (4×40 mL/min $\times$ 60 min = 9.61) suggest that hypoxia might have contributed to the observed deaths of mice in this early study. The authors stated, "Behaviorally, in all mouse inhalation experiments, those mice that did not survive the hour became hyperactive for periods of 10 to 15 minutes, then their breathing appeared

labored, and they died soon after . . . . Examination of the lungs revealed inflammation and fluid accumulation." The relative contributions of furan exposure and oxygen deprivation to those effects cannot be determined from that study. Therefore, it was not used in determining an AC for prevention of lethality.

Ten years after Egle and Gochberg's report, Terrill et al. (1989) reported an 80-fold higher 1-h LC_{50} (3500 ppm) for Sprague-Dawley rats exposed to analytical concentrations of 1000, 2850, or 4050 ppm in a dynamic Hinnerstype chamber; lethality was observed only at the highest exposure concentration. Signs of toxicity during exposure included respiratory distress, increased secretory responses, and, at the highest concentration, death, which in many instances, was delayed until the end of the first week or the beginning of the second week after exposure. No exposure-related lesions were found in gross postmortem evaluations of 14-d survivors (Terrill et al. 1989).

Nephrotoxicity and Hepatotoxicity

Severe kidney and liver toxicity were seen in animals following high doses of furan. In experiments designed to examine the ability of two chemicals to inhibit the liver and kidney toxicity of furan, furan was injected ip into eight mice at a dose of 0.2 mL/kg (187 mg/kg). When the animals were sacrificed 24 h after injection, they were found to have severe coagulation necrosis of tubular cells of the kidney cortex, atrophy of the glomeruli of the kidneys, and massive coagulation necrosis of centrilobular parenchymal cells of the liver (Masuda et al. 1984). Similar results were found 24 h after ip injection of furan into mice at 5.1 mmol/kg (347 mg/kg) (Wiley et al. 1984).

In 16-d gavage studies conducted by the National Toxicology Program (NTP 1993), mottled and enlarged livers were observed at necropsy in male rats receiving furan at 20, 40, or 80 mg/kg and in females receiving 40, 80, or 160 mg/kg. In similarly exposed mice, no furan-related lesions were observed at necropsy.

Subchronic and Chronic Exposures

Nephrotoxicity and Hepatotoxicity in Gavaged Rodents

In 13-w gavage studies conducted by the NTP, rats exposed to furan in corn oil for 5 d/w at 0, 4, 8, 15, 30, or 60 mg/kg had dose related increases in the incidence and severity of toxic lesions of the liver, including bile-duct hyper

plasia, cholangiofibrosis, cytomegaly and degeneration of hepatocytes, and nodular hyperplasia of hepatocytes (NTP 1993). Kidney lesions were present in rats receiving 30 or 60 mg/kg. Toxic liver lesions (cytomegaly, degeneration, and necrosis of hepatocytes) were also present in all groups of mice exposed to furan at the same doses as the rats.

Dose-related increases in the incidence of numerous non-neoplastic lesions were noted in rats of both sexes exposed by gavage for 5 d/w with furan in corn oil at 0, 2, 4, or 8 mg/kg in the NTP 2-y bioassay. These lesions included multiocular cysts, fibrosis, metaplasia, hyperplasia, and cytomegaly of the biliary tract and chronic inflammation, proliferation and hepatocellular cytomegaly, cytoplasmic vacuolization, degeneration, nodular hyperplasia, and necrosis in the liver (NTP 1993). In mice, non-neoplastic hepatocellular lesions included hepatocyte cytomegaly, degeneration, necrosis, multifocal hyperplasia, and cytoplasmic vacuolization and biliary-tract dilatation, fibrosis, hyperplasia, and inflammation (NTP 1993).

More recent studies at the National Institute of Environmental Health Sciences (NIEHS) and CIIT have examined the effects of lower doses of furan. A lowest-observed-adverse-effect level (LOAEL) of 4 mg/kg and a no-observed-adverse-effect level (NOAEL) of 2 mg/kg for histological changes and hepatocyte proliferation were found in male mice exposed by gavage for 5 d/w for 3, 6, and 13 w with furan at 0, 0.5, 2, 4, 8 or 15 mg/kg (R. Maronpot, National Toxicology Program, NIEHS, personal commun., 1994).

Carcinogenicity

No studies have been reported on the carcinogenicity of inhaled furan vapors; however, furan has been shown to be a potent, multi-target carcinogen by the oral route. Furan has been tested by gavage in a 2-y bioassay by the NTP (1993). Groups of 70 rats of each sex were administered furan in corn oil by gavage at 0, 2, 4, or 8 mg/kg for 5 d/w for 2y. Groups of 50 mice of each sex received doses of furan at 0, 8, or 15 mg/kg, for 5 d/w for 2 y. Exposure was found to induce a variety of neoplastic lesions in both rats and mice (Table 14-1).

Rats were found to be the more sensitive species for induction of neoplasias, the most prevalent neoplasm being cholangiocarcinomas. Interim sacrifices revealed high (but less than 100%) incidences of cholangiocarcinomas in rats given 2 mg/kg for 9 or 15 mo (Table 14-2 and Figure 14-1).

Exposure of male rats to furan at 30 mg/kg for 13 w and observation for the remainder of 2 y yielded an overall incidence of cholangiocarcinoma at 100% (NTP 1993). As shown in Table 14-3, furan also induced hepatocellular

adenomas and hepatocellular carcinomas in male and female mice and rats and mononuclear-cell leukemias in male and female rats. The NTP (1993) concluded that there was clear evidence of carcinogenic activity of furan in male and female F344/N rats and male and female $B6C3F_1$ mice.

TABLE 14-1 Neoplastic Lesions in 2-y Study of 50 Rats and 50 Mice

			Doses, mg/kg				
Species	Sex	Neoplasm	0	2	4	8	15
Rat	Male	Monocytic leukemia	8	11	17	25	—
		Cholangiocarcinoma	0	43	48	49	—
		Hepatocellular adenoma/ carcinoma	1	5	22	35	—
Rat	Female	Monocytic leukeumia	8	9	17	21	—
		Cholangiocarcinoma	0	49	50	48	—
		Hepatocellular adenoma/ carcinoma	0	2	4	7	—
Mice	Male	Hepatocellular adenoma/ carcinoma	26	—	—	44	50
	Female	Hepatocellular adenoma/ carcinoma	7	—	—	34	50

TABLE 14-2 Incidences of Cholangiocarcinomas in Rats in the NTP Bioassay

	9 months		15 months		24 months	
Dose, mg/kg	Male	Female	Male	Female	Male	Female
0	0/10	0/10	0/10	0/10	0/50	0/50
2	5/10	4/10	7/10	9/10	43/50	49/50
4	7/10	9/10	9/10	9/10	48/50	50/50
8	10/10	10/10	6/10	7/10	49/50	48/50

Genotoxicity

Although furan is not mutagenic in the *Salmonella*-microsome preincubation test, it is a mutagen in the L5178Y $TK^{+/-}$ mouse lymphoma-cell forward-

mutation assay without the need for metabolic activation by rat liver S9 mix (McGregor et al. 1988). It is also genotoxic in the phage T7 inactivation test (Ronto et al. 1989). Furan does not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* (NTP 1993). Furan does induce chromosomal aberrations and sister chromatid exchanges in cultured Chinese hamster ovary cells in vitro, with and without metabolic activation, and induces forward mutations in cultured mouse L5178Y lymphoma cells (NTP 1993). It induces chromosomal aberrations, but not sister chromatid exchanges, in bone-marrow cells of mice injected ip (NTP 1993). Furan produces chromosomal aberrations in cultured Chinese hamster ovary cells after a 3-h exposure only in the presence of complete S9 mixture; removal of NADP from the S9 abolished furan's clastogenicity (Stich et al. 1981).

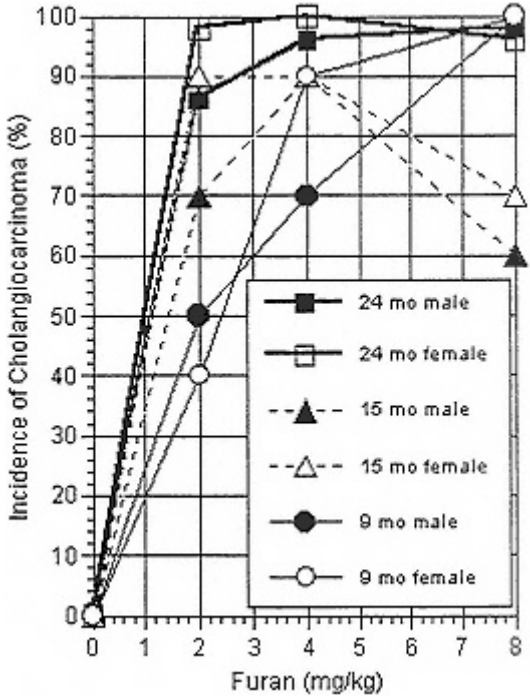


FIGURE 14-1 Results of NTP bioassay of furan in F344 rats.

Comparison of activated oncogenes from furan-induced B6C3F$_1$ mouse-liver tumors with oncogenes from spontaneously developing mouse-liver tumors suggests that furan causes an increased incidence in mouse-liver tumors at least in part by induction of novel weakly activating point mutations in H-*ras* genes

(Reynolds et al. 1987). Slower than normal electrophoretic mobilities were observed for some of the mutated c-H-ras proteins (e.g., p21) from furaninduced liver-tumor DNA transfectants, whereas faster than normal mobilities were observed for mutated c-H-ras proteins from spontaneously developed liver-tumor DNA transfectants (Reynolds et al. 1987). It should be noted that the $B6C3F_1$ strain of mice used in the Reynolds et al. study have a background incidence of hepatocellular carcinomas of 50%. In that case and on the basis of the data alone, furan appears to induce mouse-liver tumors by a direct genotoxic mechanism involving activation of oncogenes.

Reproductive Toxicity

No data were found on the reproductive toxicity of furan.

Developmental Toxicity

No data were found on the developmental toxicity of furan.

Interactions with Other Chemicals

No data were found on the interaction of furan with other chemicals.

TABLE 14-3 Toxicity Summary

Dose or Concentration	Exposure Duration	Species	Effects	Reference
			Inhalation	
43 ppm	1 h	Mouse	LC_{50}	Egle and Gochberg 1979
3,464 ppm	1 h	Rat	LC_{50}	Terrill et al. 1989
			Oral (Capsules)	
0.25 cc/kg	Once	Dog, rabbit	Copious flow of bloody saliva; bright cherry-red blood; hyperemic liver, kidneys, lungs; lethal to 4 of 5 dogs and 2 of 2 rabbits	Koch and Cahan 1925
			Oral (Gavage)	
2 mg/kg	5 d/w, 13 W	Mouse, male	NOAEL for histological changes and hepatocyte proliferation	R. Maronpot, personal commun., 1994
4 mg/kg	5 d/w, 13 W	Mouse, male	LOAEL for histological changes and hepatocyte proliferation	R. Maronpot, personal commun., 1994
20, 40, 80 mg/kg	16 d	Rat, male	Mottled and enlarged livers	NTP 1993
40, 80, 160 mg/kg	16 d	Rat, female	Mottled and enlarged livers	NTP 1993
20, 40, 80 mg/kg	16 d	Mouse	NOAEL for lesions at necropsy	NTP 1993
2, 4, 8 mg/kg	24 mo	Rat	Various neoplasms (see Table 14-1)	NTP 1993
4 mg/kg	13 w	Rat	Cholangiofibrosis, biliary-tract hyperplasia, and Kupffer cell pigmentation in 4 of 10 males; biliary-tract hyperplasia in 7 of 10 females	NTP 1993

Dose or Concentration	Exposure Duration	Species	Effects	Reference
8 mg/kg	13 w	Rat	Cholangiofibrosis in 7 of 10 females, 7 of 10 males; biliarytract hyperplasia in 9 of 10 males, 10 of 10 females; hepatocyte degeneration in 7 of 10 males; Kupffer cell pigmentation in 6 of 10 males and 8 of 10 females	NTP 1993
8 mg/kg	24 mo	Rat	Urinary bladder pappilomas in 3 of 50 females	NTP 1993
8, 15 mg/kg	24 mo	Mouse	Hepatocellular adenomas or carcinomas (see Table 14-1)	NTP 1993
15, 30, 60 mg/kg	13 w	Rat	Cholangiofibrosis and biliary tract hyperplasia; hepatocyte cytomegaly, degeneration, necrosis, and Kupffer cell pigmentation	NTP 1993
60 mg/kg	13 w	Rat	Renal tubule dilation and necrosis; thymus atrophy; testicular atrophy; ovarian atrophy	NTP 1993
			Injection	
187 mg/kg	Single ip injection	Mouse	Severe kidney necrosis; massive liver necrosis	Masuda et al. 1984
347 mg/kg	Single ip injection	Mouse	Liver necrosis and kidney toxicity	Wiley et al. 1984
937 mg/kg	Single ip injection	Rat	Respiration stopped in 0.5 min; heart stopped in 3 min; hyperemic liver and intestines, cherry red blood, dilated blood vessels	Koch and Cahan 1925

No exposure limits have been set by other organizations.

TABLE 14-4 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	4	1.4	Hepatotoxicity
24 h	0.4	1	Hepatotoxicity
7 d[a]	0.025	0.07	Carcinogenicity
30 d	0.025	0.07	Carcinogenicity
180 d	0.025	0.07	Carcinogenicity

[a] Previous 7-d SMAC = 0.11 mg/m^3.

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

To the extent possible, the guidelines from the National Research Council (NRC 1992) were used to develop the rationale for furan SMACs (Tables 14-4 and 14-5). Setting long-term exposure standards for furan inhalation is a challenge because of the serious deficiencies in the data base and the implications that it could be highly carcinogenic based on liver tumors induced in rodents given furan by oral gavage. The data base is deficient because there are no inhalation exposures except for two acute exposures that report very different LC_{50} values. Although some workers seem to have potential exposure to the vapor, there are no epidemiological studies. The data base is strongest in the areas of metabolism and molecular mechanisms involved in cancer induction by furan. The weight of evidence, which is summarized below, suggests that furan will not induce cancer if its toxic metabolites are kept below concentrations that cause cytotoxicity. Furan has been reported in several types of foods at relatively high concentrations; however, the quantification of furan in the food was not sufficiently complete to assist the risk assessment.

Ordinary Human Exposures

By way of background to the risk assessment, it is essential to note that humans are exposed to significant quantities of furan when they consume certain types of food or drink. Merritt et al. (1963) used gas chromatogra

phy-mass spectrometry (GC-MS) to show that the volatile compounds released from dry roasted ground coffee in room-temperature vacuum distillation consisted of 2.3% furan and 4.0% 2-methyl furan (mole percentages). Mulders et al. (1972) used GC-MS analysis of vapor from freshly baked white bread to show that furan was a major component of the 15 compounds identified. Persson and von Sydow (1973), using GC-MS analyses, found furan from 0.75 to 3.9 ppm in the headspace of canned meat. The variations were caused by changes in the formulations used in the canning process and the time held at the process temperature of 121°C. Furan was the second or third most-concentrated compound in the headspace sample of the canned beef. All three studies were designed to characterize the chemical basis for the odors from the foods, so the results cannot be used to quantify human exposures to furan; however, furan is clearly a component of processed foods consumed by many people.

Acute Lethality and Hepatotoxicity

The 1-h LC_{50} value of 3500 ppm (9700 mg/m^3) of Terrill et al. (1989) was used to derive an acceptable concentration (AC) for hepatotoxicity. That was done to avoid setting an AC based on lethality. Data from oral exposures indicate that hepatotoxicity is the most likely effect at lower exposures. To extrapolate from the LC_{50} to a nonhepatotoxic concentration, the dose of furan retained by rats during the 1-h exposure was estimated and compared with the oral NOAEL as follows:

$$\text{Dose} = R \times LC_{50} \times V_h = 0.9 \times 9700 \text{ mg/m}^3 \times 0.01 \text{ m}^3\text{/h} = 90 \text{ mg}.$$

The V_h was calculated from the minute volume of 0.16 L/min (Crosfill and Widdicombe 1961) for 250-g rats, and the respiratory retention, indicated by "R," was estimated from studies on dogs (Egle and Gochberg 1979).

The single oral doses of furan that are considered "severely toxic" to the livers of male rats are those above 100 mg/kg (Wilson et al. 1992). The stated age of the rats dosed with furan was 10 w to 1 y, so the weight range was approximately 350 to 450 g; hence, the 100 mg/kg dose averaged about 40 mg per rat. That seems consistent with the calculation above showing that the LC_{50} dose was about 90 mg per rat. Studies with the same strain of rat show that 8 mg/kg (about 3 mg/400 g of rat) given orally is a high NOAEL based on increased liver enzymes in serum (Wilson et al. 1992). Based on the comparison of the LC_{50} and the oral NOAEL, the factor needed to extrapolate from the

LC_{50} to an inhalation NOAEL for hepatotoxicity is estimated to be 90 mg ÷ 3 mg = 30. The NRC Committee on Toxicology discussed factors of 20 to 50 for extrapolation of an LC_{50} to a NOAEL for sublethal effects (Paulson 1998), and the value of 30 for furan is within that expected range. The 1-h AC to avoid hepatotoxicity was estimated as follows:

1-h AC = 9700 mg/m^3 × 1/30 × 1/3 × 1/10 = 11 mg/m^3 = 4 ppm.

In addition to the factor of 30 for extrapolation of the LC_{50} to a NOAEL, factors of 3 and 10 were used. The factor of 3 was applied for species extrapolation from rats to humans. The species factor was less than the usual factor of 10 because pharmacokinetic data indicate that, on a milligram-per-kilogram body-weight basis, humans have a lower rate of metabolism of inhaled furan vapors than do rats when exposed to 10 ppm (Kedderis and Held 1996). The species extrapolation factor was not reduced to 1 because it was uncertain whether human liver would be more susceptible than rat liver to furan toxicity. A factor of 10 was applied because of the inadequate data on the sublethal effects of inhaled furan vapors, the lack of data on effects in humans by any route of exposure, and the need to be more consistent with the very low AC values calculated for exposure durations of 7 d, 30 d, and 180 d (see below). The NRC does not normally recommend the use of a factor for lack of data; however, the nature of the data base for the toxicity of furan suggests the need for such a factor in this case.

AC for a 24-h Exposure

There is a 160-fold difference between the 1-h AC to protect against hepatotoxicity and the 7-d AC to protect against liver cytotoxicity and cholangiocarcinomas (see below). The 24-h AC should be suitably placed between the 1-h value and the 7-d value. A 24-h AC of 1 mg/m ^{3} (0.4 ppm) seems reasonable in this circumstance. That value is 11 times less than the 1-h AC and 14 times greater than the 7-d AC of 0.07 mg/m^3 (0.025 ppm). This AC is set to protect against hepatotoxicity.

Carcinogenicity

Inspection of Table 14-1 shows that furan is a potent carcinogen when given by gavage to either rats or mice. The most prevalent neoplasms are the

cholangiocarcinomas in rats; however, the incidence of tumors is so high that rational application of the linearized multistage model is impossible. In assessing the risk of furan-induced cancers, we began by making the assumption that an exposure concentration that adequately controls the risk of cholangiocarcinomas will also control leukemias and hepatocellular adenomas and carcinomas. Several recent investigations have elucidated the mechanisms of furan carcinogenesis, and those findings can be used to estimate safe concentrations for bolus oral ingestion.

The weight of evidence is that furan, or an active metabolite of furan, affects DNA indirectly through a mechanism involving cytotoxicity and does not react directly with the DNA in target cells. The evidence is as follows:

- Furan was negative in in vitro genotoxicity assays using four strains of *Salmonella typhimurium* with and without S9 fraction (Mortelmans et al. 1986).
- Radiolabeled furan given once orally to male F344 rats at 8 mg/kg did not produce metabolites that covalently bound to DNA. That result was shown after the DNA fraction was digested to remove DNA associated proteins (Burka et al. 1991). It does not preclude mechanisms involving noncovalent reactions with DNA.
- Furan did not cause unscheduled DNA synthesis in hepatocytes isolated from male F344 rats or male B6C3F$_1$ mice given a single gavage dose (5-100 mg/kg in rats and 10-200 mg/kg in mice) (Wilson et al. 1992). This type of evaluation has not been reported for bile-duct cells, which seem to be the most sensitive target for furan.
- Cytotoxicity to the liver, as indicated by the release of liver-associated enzymes into plasma, was induced by a single dose of furan at one of the higher concentrations that induced cancer in the 2-y bioassay (Wilson et al. 1992). Note that the radiolabel from furan accumulates in male rats from about 300 nmol eq/g of liver after a single administration of 8 mg/kg to 1100 nmol eq/g of liver after eight administrations (Burka et al. 1991). Taken together, the data suggest that all doses used in the 2-y bioassay were cytotoxic to the liver. That possibility seems to have been confirmed by Fransson-Steen et al. (1997) in mice given 3 w (15 doses) of exposure at or below the NTP bioassay doses of 8 and 15 mg/kg/d, 5 d/w. Female mice showed increased liver-associated plasma enzymes if given 8 or 15 mg/kg/d, 5 d/w, but the increase was not statistically significant when they were given 4 mg/kg/d, 5 d/w for 3 w.
- Attempts have been made to understand the sequence of events that could lead to indirect effects on DNA. Furan-treated hepatocytes in vitro and mitochondria isolated from furan-exposed rats show 95% loss of ATP due to uncoupling of oxidative phosphorylation (Mugford et al. 1997). That depletes the energy needed to maintain calcium "pumps" in cellular membranes, calcium

increases within the cell, and cytotoxic enzymes are activated, including endonucleases. Preliminary results show that the endonuclease inhibitor aurintricarboxylic acid reduced the formation of DNA double-strand breaks because of endonuclease activity (Mugford and Kedderis 1997). Although this study does not prove the absence of a direct mechanism of DNA interaction, the weight of evidence favors an indirect mechanism.

Two pieces of evidence, considered at face value, suggest that furan or its proximate metabolite can react directly with DNA. They are considered below:

- Liver tumor cells from furan-exposed and unexposed rodents have differing patterns of mutations leading to oncogene activation, suggesting that furan (or a metabolite) can directly activate proto-oncogenes (Reynolds et al. 1987; Butterworth et al. 1994). An alternative explanation is that error-prone repair of the double-strand DNA lesions resulting from endonuclease induction leads to the mutations (Mugford and Kedderis 1997). Another interpretation is that increased rates of cell proliferation (secondary to necrosis and apoptosis) amplify background mutation rates, leading to the observation of rare mutations (Ames and Gold 1990).
- Furan is positive in many in vivo chromosomal-aberration tests and in mutagenicity tests in some eukaryotic cells, suggesting that it or its metabolite can react directly with DNA (summarized in the Genotoxicity section). Evidence cited above for the cytotoxicity mechanism involving double-strand DNA breaks can explain these observations without the need for a direct interaction with DNA.

If one accepts the postulate that furan is an indirect carcinogen, as the weight of evidence above suggests, then a failure to complete that indirect process effectively eliminates the potential for furan to cause cancer. To complete the indirect process, the reactive metabolite(s) of furan must be in sufficient concentration in the target tissue to uncouple oxidative phosphorylation and substantially reduce ATP production. That will occur only if the concentration of metabolite(s) at the target site is above some threshold, and this threshold will be reached only if the amount of furan administered is above the threshold to deliver that amount of metabolite(s) to the site. To complete the risk assessment, that threshold must be estimated.

Estimation of a NOAEL for Cancer Risk in Gavaged Rats

There are no long-term exposures that demonstrate a NOAEL for injury to bile-duct cells by any route of administration. The 9-mo oral gavage study

demonstrated a LOAEL (5/10 incidence) for cholangiocarcinomas of 2 mg/kg/d. The 13-w study showed that 4 mg/kg/d, 5 d/w was a LOAEL (4/10) for biliary hyperplasia. To estimate a NOAEL, one can assume that this lesion is a precursor to the cholangiocarcinomas found in rats given furan by oral gavage. In male rats, the data were as follows (NTP 1993):

Dose	Biliary hyperplasia 13 w exposure	Cholangiocarcinomas 9 mo exposure
Control	0	0/10
2 mg/kg	—	5/10
4 mg/kg	4	7/10
8 mg/kg	9	10/10
15 mg/kg	10	—

The 1% benchmark dose rate (BD_{01}), using a probit model and the 13-w data, was found to be 1.63 mg/kg/d (central estimate), with a 95% lower confidence limit of 0.09 mg/kg/d. The 95% lower confidence on the BD_{01}, 0.09 mg/kg/d, was taken to be a NOAEL in rats by oral gavage.

Alternatively, the NRC SMACs subcommittee noted that 2 mg/kg/d, 5 d/w for 9 mo seemed to be a LOAEL for a severe effect (cancer). Its recommendation was to reduce that value by a safety factor of 30 rather than the usual 10 because of the seriousness of the effect, the high incidence (50%) even in the lowest-dose group, and the lack of lifetime observation for tumors after 9 mo of exposure. Thus, using that approach, the NOAEL was estimated to be 0.07 mg/kg/d. The benchmark approach and the safety-factor approach gave comparable estimates for the NOAEL, so the estimates were averaged to 0.08 mg/kg/d. Because exposures were for 5 d/w, the weekly dose was 0.4 mg/kg/w.

Extrapolation from Rat NOAEL to Human Inhalation ACs

Assuming that a human weighs 70 kg, that 90% of inhaled furan is absorbed, and that a human inhales 20 m^3/d (140 m^3/W), the equivalent human inhalation NOAEL was found as follows:

$NOAEL_{inhaled}$ = (0.4 mg/kg/w × 1/3 × 70 kg)/(140 m^3/w × 0.9)

7-d, 30-d, and 180-d AC = 0.07 mg/m^3 (human) = 0.025 ppm

The species extrapolation factor was reduced from the usual 10 to 3 because the dose of furan of 10 ppm absorbed by inhalation was estimated on a milligram-per-killogram basis, to be 3-fold *less* in humans than in rats (Kedderis and Held 1996); a species extrapolation factor of 3 was retained because the relative susceptibility of human liver tissue and rat liver tissue is unknown. Furthermore, when protecting against an end point, such as cytotoxicity, leading to cancer, equating a bolus oral dose, which is rapidly absorbed, to an inhalation dose spread over 24 h provides a wide margin of safety. The "threshold" NOAEL estimated above is applicable to 7-d, 30-d, 180-d ACs. ACs for carcinogenicity are not set for 1-h or 24-h exposures.

Is the Respiratory System a Target by Inhalation?

One must consider whether the respiratory system could be a target for inhaled furan. Some of the descriptions of the acute inhalation exposures suggest that possibility, certain furan analogues (e.g., 3-methylfuran) specifically target the respiratory system (Boyd et al. 1978), and the respiratory system has the enzymes necessary to oxidize furan to cis-2-butene-1, 4-dial. There were no furan-induced respiratory-system neoplasms in male rats in the 2-y bioassay (NTP 1993), and approximately one-seventh of a single 8-mg/kg oral dose, which was the highest dose used in rats in the bioassay, is exhaled as unchanged furan (Burka et al. 1991). Hence, the respiratory system of the rats received substantial exposure to furan during the oral gavage study, but there were no furan-associated neoplasms or other lesions in the respiratory system. That is sufficient evidence that the respiratory system would not be a target of inhaled furan, particularly at the 0.07 mg/m^3 (0.025 ppm) AC proposed above.

Spaceflight Effects

Lift-off, microgravity, and re-entry are not anticipated to have any effect on the carcinogenicity, toxicity, or lethality of furan.

RECOMMENDATIONS

Substantial research efforts are under way in several laboratories to understand the mechanisms associated with certain carcinogens that do not directly bind DNA. Some of that work is focused on the mechanisms of furan carcinogenesis

in rodents. It is expected that these efforts will answer many of the questions that must be answered to improve the risk analysis. Both acute and chronic inhalation studies are needed to improve the descriptive toxicity database. These studies should include assessment of sublethal end points, tissue-specific metabolic activation, and DNA lesions in target tissues. Humantissue studies in vitro would provide a valuable adjunct to improve species extrapolations.

TABLE 14-5 Acceptable Concentrations

End Point, Exposure Data, Reference		Uncertainty Factors				Acceptable Concentrations, ppm				
Species	NOAEL	Time	Species	Spaceflight	1 h	24 h	7 d	30 d	180 d	
Hepatotoxicity	Rat	30	(see text)	3	1	4[a]	0.4[b]	NS[c]	NS	NS
LC_{50} = 9700 mg/m^3(Terrill et al. 1989)										
Biliary hyperplasia	Rat	BD_{01}	1	3	1	NS	NS	0.025[d]	0.025	0.025
Dose response, 13 w (NTP 1993)										
Cholangiocarcinomas	Rat	30	1	3	1	NS	NS	0.025[d]	0.025	0.025
LOAEL, 2 mg/kg, 9 mo (NTP 1993)										
SMACs						**4**	**0.4**	**0.025**	**0.025**	**0.025**

[a] An additional uncertainty factor of 10 was applied due to weakness in the inhalation data base.
[b] Set between 1-h and 7-d values (see text).
[c] NS, not set.
[d] NOAELs from benchmark and safety-factor approaches were averaged to give 0.08 mg/kg/d or 0.4 mg/kg/w (for rats exposed by gavage, see text).

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B15 HYDROGEN CYANIDE

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PHYSICAL AND CHEMICAL PROPERTIES

Hydrogen cyanide (HCN) is a colorless liquid with a boiling point close to room temperature. Thus, at ambient temperature, HCN exists essentially as a gas. HCN has a faint odor of bitter almonds (Sax 1984; ACGIH 1991).

Synonyms:	Hydrocyanic acid, prussic acid, formonitrile
Formula:	HCN
CAS no:	74-90-8
Molecular weight:	27.0
Boiling point:	25.7°C
Melting point:	–13.2°C
Vapor pressure:	400 torr at 9.8°C
Solubility:	Miscible with water
Odor threshold:	2 to 5 ppm (Hartung 1982)
Conversion factors at 25°C, 1 atm:	1 ppm = 1.10 mg/m^3 1 mg/m^3 = 0.91 ppm

OCCURRENCE AND USE

HCN is used mainly in the production of acrylonitrile, methyl methacrylate, sodium cyanide, and cyanuric chloride (Hartung 1982). HCN can be generated when acid is added to cyanide salts (Gosselin et al. 1984) or, to a lesser extent, when alkaline cyanide, especially calcium cyanide, is exposed to water or moisture (NIOSH 1976). HCN is volatile; its volatilization increases as pH

decreases or as temperature increases. Potassium cyanide (KCN) and sodium cyanide (NaCN) are basic; the pH of a 0.1N KCN solution is 11. When these alkaline salts are neutralized, the majority of cyanide ions (CN^{-}) are converted to HCN. At pH 8,93% of cyanide exits as HCN; at pH 7,99% is HCN (Towill et al. 1978, as cited in ATSDR 1997). Alkaline cyanide salts are used extensively in industrial processes, including the manufacture of dyes, pigments, or nylon; cleaning; and electroplating (NTP 1993). Neither HCN nor cyanide salts have ever been used in any payload experiment aboard the space shuttle.

Pyrolysis of polyurethane foam or polyacrylonitrile can produce HCN (Wooley et al. 1979). Polyurethane foam that was pyrolyzed at 700°C released HCN at 0.2% of the polymer weight; the amount of HCN increased 10-fold at 900°C. When polyacrylonitrile was pyrolyzed at 400°C, 700°C, or 900°C, HCN yields were 1%, 6%, or 36% of the polymer weight, respectively. Burning wool, silk, paper, or nylon also can generate HCN (Terrill et al. 1978). HCN was detected in thermodegradation of electrical cables containing polyimide and polyfluorocarbon (Bourdin 1991); these cables were similar to those used aboard U.S. spacecraft. However, HCN has not been generated in the few thermodegradation events that occurred aboard spacecraft; the pyrolysis did not involve urethane foam or other polymers containing nitrogen atoms.

Lowry et al. (1985) detected HCN in 12% of the fires examined in Dallas. HCN concentrations reached 15 ppm in 10% of those fires; the maximum HCN concentration detected was 40 ppm. Increased serum concentrations of thiocyanate (a metabolite of HCN) was found in 12% of the fire fighters, after accounting for the contributions from cigarette smoking.

TOXICOKINETICS, METABOLISM, AND MECHANISM OF TOXICITY

Toxicokinetics

HCN is a very weak acid with a dissociation constant of 4.93×10^{-10} and pKa of 9.31 (CRC 1985). At pH 2, the ratio of CN^{-} to HCN is 4.89×10^{-8}; that value indicates that essentially all of any ingested KCN or NaCN is converted to HCN in the stomach. The nonionized form is rapidly absorbed, and thus cyanide salts are rapidly fatal by the oral route (ATSDR 1997). At pH 7.4, the ratio of CN^{-} to HCN is 0.012, which indicates that about 1% of the HCN absorbed from the stomach or lung is ionized in the blood or intracellular fluid, and about 99 mole-percent of KCN or NaCN given parenterally exists as HCN in the body fluid. Thus, data on KCN or NaCN given enterally or parenterally are useful for

toxicological assessment of HCN exposures. However, given the rapid conversion of cyanide ions to HCN and the rapid absorption of HCN in the stomach, a bolus dose will generate a sudden high blood concentration, whereas continuous uptake from inhalation exposures will produce a different blood profile. Moreover, cyanide given orally (such as in drinking water) is subject to the first-pass effect through the liver uptake and detoxification. The first-pass processes can greatly affect the distribution of HCN to target organs. For example, Gettler and Baine (1938) reported that the proportionate tissue concentrations of HCN were lung > brain> heart>liver in two dogs and one human who died after exposure to HCN vapor at unknown concentrations. HCN concentration in the brain was half that in the lung in both dogs and the human. Liver HCN concentrations in both dogs were half those in the brain; for the human, the liver HCN was two-thirds that in the brain. In contrast to those results from inhalation exposures, six rats given KCN or NaCN at 4 mg CN^-/kg orally and an unspecified number of rabbits gavaged with 11.9-20.3 mg CN^-/kg had five times the HCN in the liver as in the brain (Ahmed and Farooqui 1982; Ballantyne 1983). These data demonstrate that the first-pass effect must be considered in assessing the effects of cyanide in the brain, the primary target organ. However, the first-pass effect will have little influence on the toxicity outcome resulting from the metabolic products of cyanide (such as thiocyanate) on extrahepatic organs.

Wolfsie and Shaffer (1959) predicted that HCN can readily diffuse across cellular membranes and be absorbed in the lung. Landahl and Herrmann (1950) compared HCN concentrations in inhaled versus exhaled gases from two volunteers who inhaled (by mouth) 450 mL of HCN at 0.46-4.6 ppm in 1.5 s and held their breath for 2 s. Under those conditions, the lung retained 58.5% of the inhaled HCN. When the holding time was doubled, the absorption increased to 73%. Nasal inhalation and mouth exhalation yielded nasal absorption estimated at 10-20%. Thus, about 75% of HCN inhaled through the nose in normal breathing would be retained in the body. In monkeys exposed to HCN via face mask, HCN uptake was rapid, and the blood cyanide concentration reached steady state in only 10-20 min (Purser et al. 1984).

Trace amounts of cyanide are present normally in healthy subjects; it probably comes from the breakdown of cyanogenic food, bacterial actions in the gastrointestinal tract, and cigarette smoking (Ansell and Lewis 1970). Yamanaka et al. (1991) reported that mainstream cigarette smoke contains HCN at 40-70 ppm, and side-stream cigarette smoke contains less than 5 ppm. Urinary cyanide concentrations average about 0.07 µg/mL in nonsmokers and 0.17 µg/mL in smokers (Ansell and Lewis 1970).

In adult humans, the half-lives of cyanide and thiocyanate in blood have been estimated at 20-60 min and 4-8 d, respectively (Ansell and Lewis 1970; Levine

and Radford 1978). The large difference between the two supports the notion that the metabolism of cyanide to thiocyanate is favored over the reverse reaction. By monitoring the blood cyanide concentration in rats for 2 to 60 min after gavage with KCN (1 mg/kg), Leuschner et al. (1991) calculated a half-life of 14 min. Cyanide concentrations in blood samples collected from dogs 5 to 245 min after a KCN intravenous (iv) injection (0.82 mg/kg) showed a biphasic elimination ($T1/2\alpha$ = 23 min; $T1/2\beta$ = 5.5 h). Although these findings suggest that blood cyanide concentrations should decline significantly in the first 60 min after the exposure ends, Purser et al. (1984) found that exposing monkeys to HCN gas at 100-147 ppm for 30 min via face mask produced no appreciable decline in blood cyanide concentrations 1 h after the exposure. These authors concluded that cyanide has a fairly long half-life in monkeys. At exposure concentrations that high and for that length of time, the amounts of thiosulfate and 3-mercaptopyruvate, the two endogenous compounds that normally react with cyanide in the body (see below), might have been depleted to the extent that blood cyanide concentrations showed no appreciable decline 1 h after the exposure. In any event, all the exposed monkeys were incapacitated within 15 min.

Metabolism and Disposition

Cyanide is metabolized through several pathways. Thiocyanate, the major metabolite, is formed from the reaction of cyanide either with thiosulfate, catalyzed by rhodanase (a mitochondrial enzyme found in many tissues, particularly liver), or with 3-mercaptopyruvate catalyzed by cyanide-sulfur transferase (Baumann et al. 1934; Himwich and Saunders 1948; Wood and Cooley 1956; Singh et al. 1989). A minor metabolic pathway is the reaction of HCN with cystine to form 2-aminothiazoline-4-carboxylic acid and 2-iminothizolidine-4-carboxylic acid (Wood and Cooley 1956). Other minor pathways include oxidation of HCN or thiocyanate to CO_2, reaction with hydroxocobalamine to form cyanocobalamin, and conversion of HCN to formic acid, which enters one-carbon metabolism in the body (Boxer and Richards 1952; Ansell and Lewis 1970; Baumeister et al. 1975).

After a cyanide exposure, some of the body burden of HCN is exhaled unchanged, producing the characteristic odor of bitter almonds on the breath (Ansell and Lewis 1970). Boxer and Richards (1952) showed that rats exhaled cyanide gas following a subcutaneous injection of KCN at 0.65 mg/kg. However, most of the body burden is excreted in the urine as thiocyanate, and a small amount is metabolized to CO_2 (Ansell and Lewis 1970). Wood and Cooley (1956) reported that 80% of cyanide injected intraperitoneally in rats

was recovered as thiocyanate and 15% was recovered as 2-aminothiazoline-4-carboxylic acid.

Thiocyanate formation from cyanide is quite rapid. Pettersen and Cohen (1985) showed that the thiocyanate concentration in plasma of mice doubled in 5 min and peaked at 30 min after a subcutaneous injection of KCN at 4 mg/kg (a sublethal dose). Some thiocyanate can be converted back to cyanide by thiocyanate oxidase in red blood cells (Goldstein and Reiders 1953).

When rats were given potassium cyanide in drinking water at a daily dose of 40, 80, or 150 mg/kg for 13 w, both blood cyanide and urinary thiocyanate concentrations increased proportionally with the doses (Leuschner et al. 1991). The authors concluded that exposing rats to the maximum tolerated dose did not saturate cyanide detoxification pathways. Blood cyanide concentrations remained fairly constant in each dosing group during the test.

Okoh (1983) showed that rats fed 2 mg KCN daily for 6 months showed no statistically significant changes in the fractions of the dose excreted in urine, feces, and expired air. These results suggest that neither the metabolism nor the pattern of excretion of cyanide is affected by long-term cyanide intake.

Mechanism of Toxicity

HCN, the nonionized form of cyanide, can permeate tissues much more readily than cyanide ions and is distributed widely throughout the body (Wolfsie and Shaffer 1959). The toxicity, especially the acute one, of cyanide is due mainly to its inhibition of cytochrome oxidase. HCN, a small molecule, can diffuse very rapidly into the mitochrondria, where it binds to cytochrome C oxidase and forms a stable but reversible coordination complex with the heme (F^{+++}) sites. The inhibition of cytochrome oxidase by cyanide in the tissue prevents oxygen utilization in situ (Albaum et al. 1946). Organs that are very sensitive to tissue hypoxia, such as the brain and heart, are the primary targets of cyanide toxicity. Blockage of oxygen utilization in tissues resulted in an accumulation of oxyhemoglobin. Venous blood becomes bright red or cherryred, a characteristic sign of cyanide intoxication (Gosselin et al. 1984).

Ballantyne and Bright (1979) showed that cytochrome C oxidase activities were reduced by 76% in the myocardium and 54% in the cerebral cortex of rabbits immediately after they were killed by an intramuscular injection of KCN at 8 mg of CN^- per kilogram. In another study, rats injected subcutaneously with NaCN at 3, 6, 9 or 12 mg/kg ($LD_{50} \cong 9$ mg/kg) showed inhibition of brain cytochrome C oxidase by about 2%, 22%, 35%, or 42%, respectively, 20 min after the injection (Tadic 1992). It took only 3.5 to 8 min to inhibit 50% of the cytochrome oxidase in the brains of rats injected intraperitoneally with a lethal

dose of NaCN (5 mg/kg) (Albaum et al. 1946). In mice administered KCN intraperitoneally at 10 mg/kg, 83% of the brain cytochrome oxidase was inhibited 3 min after the injection (Isom and Way 1976). Rapid inhibition of cytochrome oxidase in other organs also has been reported; hepatic cytochrome oxidase was inhibited maximally (about 80%) in mice 5 min after an intraperitoneal injection of KCN at 5 mg/kg (Isom et al. 1982).

In the study by Pettersen and Cohen (1985), 30 min after mice were given a subcutaneous injection of KCN at 4 mg/kg (i.e., 10 min following the peak inhibition), cytochrome oxidase activity in the heart recovered fully, and that in the brain recovered to 85% of the pre-injection level. From this trend of recovery, the brain cytochrome oxidase could be expected to have recovered fully 40 min after the injection (i.e., 20 min after the peak inhibition). The hepatic cytochrome oxidase activity in mice recovered fully by 10, 20 or 25 min after a KCN injection intraperitoneally at 1, 3, or 5 mg/kg, respectively. In rats exposed to KCN, the enzyme recovered fully 1 h after a 3 or 5 mg/kg injection; recovery time was doubled when the dose was increased to 8 mg/kg.

TOXICITY SUMMARY

Cyanide is an extremely potent and fast-acting poison regardless of the route of exposure. Typical symptoms of acute poisoning from a lethal dose include headache, vertigo, lack of motor coordination, nausea, vomiting, tachypnea, weak pulse, cardiac arrhythmia, convulsion, coma, and death. Pathological findings might include petechiae of the brain, meninges, and pericardium; cerebral and pulmonary edema; and tracheal congestion with hemorrhage (NTP 1993). These effects result mostly from direct inhibition of cellular respiration by binding cytochrome oxidase in the brain and heart, the two primary targets of cyanide poisoning. The toxic effects of this direct-acting poison are similar in humans and in animals (NTP 1993).

Acute or Short-Term Exposures

Neurological Effects

Humans

Being the most sensitive to tissue hypoxia, the brain is a primary target of cyanide toxicity. Signs and symptoms of acute cyanide poisoning reflect cellular hypoxia and anoxia and often are nonspecific. Exposures to high

concentrations of HCN vapor can produce flushing, headache, tachypnea, and dizziness within 30 s. Toxic effects progress to stridor, irregular breathing, coma, seizures, and death within 10 min (Ellenhorn and Barceloux 1988). The onset of toxic sequelae is less rapid in oral exposures than in other routes of exposure because of slower entry of cyanide into the circulation and passage through the portal system, where the liver detoxifies some cyanide through the first-pass effect. However, the rapid uptake of HCN from the stomach and the ease with which HCN passes through the blood-brain barrier make this toxin rapidly dangerous even by the oral route. Because of the dangerous nature of cyanide and the small margin of safety, very few controlled human studies have been conducted on the cyanides.

Flury and Zernik (1931) and Henderson and Haggard (1943) stated that exposing humans to HCN at 20-40 ppm for several hours produced only slight symptoms. The authors also noted that a 1-h exposure to HCN at 50-60 ppm could be tolerated without serious consequences but that an exposure at 100-240 ppm for 30 min or more is dangerous. However, it is not clear whether this information, which referred to work originally done by Lehman (1919) and cited by McNamara (1976), had been obtained from human experiments or from extrapolations from rabbit data.

Peden et al. (1986) reported that 12 men who were exposed to unknown concentrations of HCN in separate industrial accidents experienced dizziness (n = 8), dyspnea (n = 8), a shaky feeling (n = 6), headaches (n = 4), nausea (n = 4), and unconsciousness (n = 5). All the unconscious victims rapidly regained consciousness "probably less than 10 min" after having been removed from the accident sites. However, the headaches persisted for up to 8 h after hospital admission. Similar symptoms and toxic signs also were reported by Nagler et al. (1978) in three cases of HCN poisoning at unknown concentrations from the accidental addition of 0.5 kg of a cyanide salt to a sulfuric acid bath in the electroplating department of a factory in Belgium. Those victims experienced semiconsciousness, headaches, nausea, sinus bradycardia, and atrial fibrillation.

Grubbs (1917) generated HCN gas in a practically airtight room by dropping 0.5 NaCN per 1000 ft^3 space into acid, which generated HCN at about 240 ppm. Several human volunteers (the exact number was not specified) breathed the HCN atmosphere for 2 min without showing any symptoms, but a similar HCN atmosphere had "at other times caused dizziness." Also, no toxic effects were noted for human volunteers breathing HCN gas generated by dropping 0.75 oz of NaCN into acid per 1000 ft^3 of space (estimated HCN concentration of 360 ppm) for 1.5 min. Barcroft (1931) quoted a 1923 report by Katz and Longfellow: "Men employed in fumigation with HCN have been tested while at rest in 250 ppm of air for 2 min and in 350 ppm for 1.5 min, but felt no dizziness, although possibly on exertion they might have done so." According to

McNamara (1976), Katz and Longfellow were describing the data that Grubbs had gathered with human subjects.

Bonsail (1984) reported the case of a worker poisoned in a tank that contained residual hydrazodiisobutyronitrile, which decomposes to HCN upon exposure to water. This man was in the tank for about 6 min, during which time he was exposed to HCN at about 500 mg/m^3 (460 ppm) and probably also to hydrazodiisobutyronitrile vapor. He collapsed in the tank and was rescued; he was comatose, with marked conjunctivitis, vomiting, and paralysis upon arrival at a hospital. After being placed on a ventilator and treated with sodium thiosulfate and phenytoin, the victim regained full consciousness within 72 h. He was discharged from the hospital 2 w later with only minor loss of peripheral vision.

Barcroft (1931), of the U.S. War Department, exposed a man and a dog to HCN at a nominal concentration of 625 ppm (the actual concentration was between 500 and 625 ppm) in an airtight chamber. The man was more tolerant than the dog. The dog became unsteady at 50 s and unconscious at 1 min 15 s. It made "crying sounds" and went into tetanic convulsions at 1 min 30 s, at which time the man left the chamber. He put on a respirator and went back into the chamber at 1 min 30 s to retrieve the dog and remained outside the chamber thereafter. At 5 min after the start of the experiment, the man developed a "momentary feeling of nausea." At 10 min, he had difficulty in concentrating in close conversation.

Animals

Exposing monkeys to HCN at 100, 125 or 150 ppm produced semiconsciousness or unconsciousness in 17, 14, or 8 min, respectively (Purser et al. 1984). When the exposure was terminated, the animals recovered within 10 min to a fairly active state. The effects of HCN in monkeys and other laboratory animals were compared in an earlier study conducted by Dudley et al. (1942). The symptoms in monkeys exposed at 125 ppm for 12 min were described as "distinctly toxic." Clinical signs in cats, according to the authors, were "markedly toxic" after exposure to the same concentration for 7 min, but no symptoms were observed in rabbits. Exposing dogs at 35-60 ppm (for an unspecified duration) led to vomiting, convulsions, or death, but the dogs could tolerate HCN at 30 ppm. Guinea pigs could tolerate HCN at 200 ppm for 1.5 h without toxic signs. Dudley et al. (1942) concluded that sensitivity to HCN toxicity increases progressively in guinea pigs, rabbits, monkeys, cats, and dogs.

One of two rats exposed to HCN at 50 ppm showed violent agitation, paralysis, unconsciousness, and gasping after 3 min of exposure (Moss et al. 1951).

The other rat survived the exposure for an unspecified duration without paralysis. Rats and mice injected intraperitoneally with KCN at 3-5 mg/kg became unconscious in 1-1.5 min and recovered at about the same time as the hepatic cytochrome oxidase activity had returned to normal (Schubert and Brill 1968).

Histopathological Lesions in the Brain

HCN produces central-nervous-system (CNS) effects through blocking cytochrome C oxidase. Prolonged hypoxia or anoxia, which can occur when HCN concentrations are high enough or the exposure duration is long enough, can result in permanent brain damage.

Humans

Long-term cerebellar spasmodic symptoms were documented in one man after he recovered from a coma after acute HCN intoxication (Fiessinger et al., 1938). Another man was comatose for 7 h after ingesting about 1 g of KCN in an attempted suicide; he acquired parkinsonism after the incident (Uitti et al. 1985). This man had hallucinations and delusions over the first few days after regaining consciousness; in the weeks that followed, he had difficulty expressing himself, personality changes, and depression. Four months after the cyanide ingestion, he experienced marked and generalized rigidity, bradykinesia, and tremors of the tongue, eyelids, and arms. He died 2 years later from an overdose of imipramine and alcohol. Autopsy revealed a shrunken striatum of spongy consistency with widespread lacunar formation, focal atrophy of cerebellar folia, and resolved laminar necrosis in the occipital lobes.

Animals

When 11 rats and 11 monkeys were infused with NaCN solution (0.07-0.15 mg/min-kg for rats and 0.05-0.10 mg/min-kg for monkeys) for approximately 35-120 min, 4 rats and 4 monkeys developed brain damage (Brierley et al. 1976, 1977). The animals were killed within 4 d after the exposure. All eight animals showed white-matter brain damage, but only one rat and one monkey suffered gray-matter damage.

The time course of brain damage induced by acute HCN intoxication was investigated by Levine and colleagues (Levine and Stypulskowski 1959;). Rats exposed for 20-45 min to an unspecified HCN concentration (sufficient to

incapacitate the rats within 10 min) showed initial neuronal damage followed by incomplete myelination loss of the damaged neurons. Extensive brain-tissue injury was observed 1 to 2 d after the exposure. At 4 mo after the exposure, the lost neural cells in corpus callosum, striatum, and hippocampus had been replaced by glial cells and vessels (Levine and Stypulskowski 1959; Hirano et al. 1968). However, the remyelination process in the CNS was slow and incomplete (Hirano et al. 1968).

Cardiac Effects

Blockage of oxygen utilization resulting from binding of HCN on cytochrome C has a devastating effect on the heart. The initial effect is tachycardia, followed by bradycardia. Dysrhythmias and hypotension often precede peripheral vascular collapse (Ellenhorn and Barceloux 1988).

Humans

Electrocardiographic (EKG) changes in four men executed by HCN inhalation at unspecified concentrations were monitored and documented by Wexler et al. (1947). During the first 7 min of the inhalation, the heartbeat occasionally slowed to varying degrees with periods of either absence of P waves or irregular P waves. All four men also experienced A-V dissociation. After the first 7 min, the heartbeat slowed even further, followed by either heart block or ventricular fibrillation.

Three workers were poisoned by HCN vapor when one accidentally added 0.5 kg of cyanide salt into a sulfuric acid bath, generating 280 g HCN (Nagler et al. 1978). One worker, who was about 1 m away from the bath, immediately became semiconscious and developed atrial fibrillation. Another worker rushed to rescue the man and soon complained of headache, nausea, and throat irritation; when admitted to a hospital, the rescuer had crushing chest pain, profuse diaphoresis, vomiting, and tachycardia. Another worker who had been standing 3 m from the bath experienced throat irritation, nausea, vomiting, profuse diaphoresis, sinus bradycardia (36 beats per min), and crushing chest pain.

Animals

Purser et al. (1984) observed that cardiac changes after HCN exposures were accompanied by incapacitation and were always preceded by a hyperventilatory

episode. Hyperventilation was inevitable upon incapacitation. During a 30-min exposure of monkeys to HCN at 147 ppm, the heart rate slowed and the amplitude of T waves either increased or decreased. EKG data from the monkeys showed that HCN could produce EKG changes only in severe intoxication.

Effects on the Respiratory System

Humans

HCN poisoning in humans produces biphasic changes in respiration (i.e., initial rapid and deep respiration), followed by slow and irregular respiration (Parmenter 1926; Wood and Cooley 1956).

Animals

In monkeys and mice, HCN's respiratory effects are monophasic. Respiration rate was not affected in monkeys exposed to HCN for 30 min at 80 ppm or less. At 90 ppm, hyperventilation was noted 20 min into the exposure. At 180 ppm, however, hyperventilation was almost immediate. In monkeys exposed to HCN at 147 ppm for 30 min, hyperventilation (about 130% increase in minute volume most of the time) developed within 0.5 min and lasted until 13 min into the exposure (Purser et al. 1984). Purser and co-workers attributed the hyperventilatory response to HCN's stimulatory effect on respiration. In contrast, Matijak-Schaper and Alarie (1982) found that HCN in mice slowed respiration, which they hypothesized as being due to depression of the respiratory centers. In mice exposed for 30 min to HCN at 23 or 120 ppm, the respiratory rate was reduced by 20% or 80%, respectively.

Lethality

Humans

HCN is rapidly lethal if inhaled at sufficient concentrations. NcNamara (1976) estimated the average fatal concentration for humans to be 546 ppm for a 10-min exposure. According to Henderson and Haggard (1943), an exposure at 200 to 480 ppm for 30 min is fatal to humans. However, according to Dudley et al. (1942), HCN at 270 ppm can cause death immediately.

Animals

When Grubbs (1917) exposed four rats to an estimated HCN concentration of 240 ppm for 1 h, two rats died in 50 min and one died after the 1-h exposure. Moss et al. (1951) reported that one rat exposed to HCN at 25 ppm and one of two rats exposed at 50 ppm for unspecified periods survived the exposure; the other rat died after being exposed at 50 ppm for 8 min. Moss and co-workers further noted that exposure at 100 ppm for 5.5 min or at 200 ppm for 10 min was also lethal to rats.

The lethality of HCN also has been investigated in dogs exposed at 530 to 2200 mg/m^3 (480 to 2000 ppm) for up to 10 min (Jandorf and Bodansky 1946) (Table 15-1). Etteldorf (1939) reported that dogs exposed to HCN at 40 mg/m^3 (36 ppm), a concentration that induced convulsions, for 15 min or longer usually died. A 10-min exposure to HCN at 40 mg/m^3 killed one of three dogs; the two survivors required 24 to 72 h to recover. None of four dogs exposed to HCN at 40 mg/m ^{3} for 8 min died; however, one developed CNS sequelae manifested by weakness and "dementia" 48 h after the exposure. In dogs, convulsions are always preceded by prodromal signs, such as lacrimation, salivation, defecation, and urination. If the HCN exposure was stopped during the prodromal stage, dogs always recovered.

Dudley et al. (1942) reported that an exposure to HCN at 90 ppm for several hours also was fatal to dogs. Exposing rats to HCN at 110 ppm for 1.5 h produced death; 315 ppm was fatal to guinea pigs, rabbits, and cats. The relative sensitivities of guinea pigs, rats, and rabbits to the effects of HCN also was determined by exposing these animals to HCN in a static gas chamber (Thautman 1933). For guinea pigs exposed to HCN at a nominal concentration of 900 ppm for an average duration of approximately 6 min, 10 of 25 (40%) died. Exposing rats and rabbits to HCN at a nominal concentration of 450 ppm for about 3 min killed 10 of 32 (31%) rats and 2 of 17 (12%) rabbits. Thautman concluded that rats were more sensitive than guinea pigs and rabbits to the lethal effects of HCN.

Species differences in the lethality of HCN also have been reported by others. According to Vernot et al. (1977), mice were more sensitive than rats; the 1-h LC_{50} was 323 ppm for mice (95% confidence limits 276-377 ppm) and 484 ppm for rats (95% confidence limits 442-535 ppm). In another study, Barcroft (1931) noted that "the monkeys were only beginning to show signs of unsteadiness when the dogs died." According to Barcroft, sensitivity to HCN, from least to most, follows the order of monkey, goat, rabbit, rat, dog, pigeon, and canary. Barcroft's data on monkeys, rats, and dogs are shown in Table 15-2.

Table 15-1 Acute Lethality of HCN in Dogs[a]

Concentration, ppm	Exposure Duration, min	C × T, ppm-min	Death Rate
1220-1900	0.48-0.75	900-910	2/4
540-1910	0.53-2.00	1000-1270	9/10
480-2000	0.87-4.00	1730-1910	3/3

[a] Jandorf and Bodansky 1946.

TABLE 15-2 Acute Lethality of HCN in Laboratory Animals

Monkeys Exposure			Rats Exposure			Dogs Exposure		
ppm[a]	min	Death Rate	ppm[a]	min	Death Rate	ppm[a]	min	Death Rate
400	3	0/3	500	10	6/6	200	10	2/3
200	30	1/3	500	3	3/6	200	5	0/3
170	60	3/3	200	30	6/6	100	30	2/2
140	30	1/3	200	15	1/4	100	15	1/2
100	60	0/8	100	60	3/6	70	30	0/2
			100	45	5/6	60	60	0/4
			100	30	2/6			
			50	10	1/6			

[a] Nominal concentrations.

Subchronic and Chronic Exposures

Clinical Neurological Effects

Humans

The toxicity of HCN in 36 chronically exposed workers in three electroplating factories in Egypt was reported by E1 Ghawabi et al. (1975). These subjects, all nonsmokers, were exposed to HCN and probably also to cyanide salt mist in the course of their 5 to 15 y of work in the factories. During the 2-mo study, breathing-zone air samples and urine samples were collected three times a week from each worker. Average HCN concentrations in the three facilities were 6.4 ± 6.9, 8.1 ± 8.2, and 10.4 ± 10.9 ppm (mean ± standard deviation).

Atmospheric cyanide concentrations correlated well with urinary thiocyanate concentrations. Past and present medical histories showed that these workers had significantly higher incidences of headaches, weakness, changes in taste and smell, giddiness, vomiting, and dyspnea than 20 male control subjects who were of the same age range and socioeconomic status and who had "never been exposed to any chemical hazard." Because the articles to be electroplated were washed in petrol (gasoline) baths before being treated in the cyanide baths, workers could have been exposed to a variety of volatile organic compounds as well as to cyanide. The authors did not say whether workers in factory A, where HCN concentrations were the highest, had a higher incidence of complaints, nor did they provide any indication of whether any of the symptoms noted had been experienced during the 2-mo survey period. Lacking this information and other details, assessment of any correlation between symptoms and exposure concentrations is difficult. Nevertheless, the symptoms noted above are similar to those resulting from acute cyanide exposures; therefore, it cannot be ruled out that the clinical toxicity experienced by these workers might have resulted from periods of exposures to higher concentrations than those measured during the survey.

CNS symptoms similar to those documented by E1 Ghawabi et al. (1975) were reported by Radojicic (1973, as cited in NIOSH 1976). Radojicic's subjects were 43 cyanide-exposed workers and 20 nonexposed workers in a Yugoslavian electroplating plant. According to the NIOSH report, Radojicic's subjects were exposed to cyanide at concentrations of 6 to 11 mg/m^3 (5.5 to 10.0 ppm), and most of the exposed workers complained of headaches, fatigue, weakness, pain, tremors of the hands and feet, and nausea.

A fatality in a factory where NaCN solution was used to extract silver from photographic films led the U.S. Occupational Safety and Health Administration (OSHA) to suspend the factory's operations (Blanc et al. 1985). (OSHA cited the factory for failing to provide protective gloves and chemical vapor masks for its workers.) One day after the suspension, 24-h environmental monitoring showed an airborne HCN concentration of 15 ppm (24-h time-weighted average (TWA)), a result that suggests that workers had been exposed to cyanide at more than 15 ppm. The factory never reopened. A later survey was conducted with 36 former employees who had worked an average of 11 ± 10.4 mo (mean ± standard deviation) at that factory. Responses to questionnaires revealed that between 42% and 72% of the workers experienced, during their active employment, headache, nausea or vomiting, almond or bitter taste, eye irritation, loss of appetite, weight loss, epitaxis, easy fatigue, and rash (symptoms listed in decreasing order of occurrence). During the month before the interview, 11-36% of these former workers still experienced those symptoms, except that none were still losing weight. Positive correlations were noted between symptoms

and doses when the extent of cyanide exposure was classified on the basis of job category, the number of times powdered cyanide was handled monthly, the number of direct skin contacts with cyanide solution per month, and the ingestion of food or beverages in production areas. Unfortunately, no airborne HCN concentrations were measured in the factory during production, so exposure concentration-response information was not available.

NIOSH (1976) reviewed two studies and concluded that HCN produced no toxic effects of HCN in the workers being exposed at 0-17 ppm (5 ppm, mean) in the Grabois (1954) study or at 4-6 ppm in the Hardy et al. (1950) study. In fact, Grabois measured only airborne HCN concentrations in an apricot-kernel processing plant and did not assess health effects. Hardy et al. cited the results from field studies conducted by Massachusetts State chemists, who measured air samples and did not study health effects. The exact concentrations to which Hardy's subjects were exposed were not determined.

In a study of 23 cyanide-exposed male workers and 20 control workers in an electroplating and case-hardening factory in India, Chandra et al. (1980) noted that cyanide-exposed workers experienced "typical symptoms of poisoning." Neither the types nor the incidence of symptoms were specified. The average airborne cyanide concentration in the factory was reported to be 0.45 mg/m^3 (range 0.2 to 0.8 mg/m^3). That concentration, equivalent to 0.4 ppm, is an order of magnitude lower than those reported by E1 Ghawabi et al. (1975), Radojicic (1973), and Hardy et al. (1950). That measurement is probably not correct. As Hardy et al. noted, an electroplating factory equipped with adequate engineering devices could maintain HCN at not more than 4-6 ppm in workers' breathing zones. It is doubtful that an electroplating factory operating in India nearly 20 y ago would have had engineering devices that could maintain HCN at less than one-tenth that concentration. Moreover, the average thiocyanate concentration in 24-h urinary samples collected from the cyanide-exposed workers (who were nonsmokers) in the Chandra et al. (1980) study was 0.57 mg/100 mL. If the average daily output of urine was about 1360 mL (690-2690 mL/d, n = 33 subjects; Liappis 1973), then Chandra et al. subjects excreted about 7.8 mg of thiocyanate daily. That value corresponds to about 13 ppm (12 mg/m^3) of airborne cyanide in the workplace, as calculated from an equation derived by E1 Ghawabi et al. (1975). The estimated HCN concentration (13 ppm) is comparable with those reported by others (E1 Ghawabi et al. 1975; Radojicic 1973; Hardy et al. 1950). Thus, the symptoms observed in the Chandra et al. (1980) study were probably induced by exposures to cyanide at concentrations much more than 0.45 mg/m ^{3}.

Chandra et al. (1988) also reported a case-controlled study of 111 cyanide-exposed workers in the electroplating and heat-treatment plants of two Indian factories (40 in factory A and 71 in factory B); 30 control workers were from

factory B. From clinical, behavioral, and biochemical data, Chandra and co-workers proposed an exposure limit for CN of 0.35 mg/m^3 for workers in India. However, the rationale for this recommendation is questionable, because cyanide and thiocyanate concentrations in blood and urine (biochemical data) were treated as "indicators of cyanide toxicity." In addition, no clinical data were collected from the control group.

Animals

When cyanide at up to 300 ppm in drinking water was given to rats and mice for 13 w, clinical observations showed no evidence that cyanide produced CNS toxicity (NTP 1993; see below).

Neurological Damage

Humans

Cyanide in cigarette smoke is thought to cause tobacco amblyopia in certain smokers (Baumeister et al. 1975). This condition is characterized by central scotomata with ganglion-cell degeneration and vascular changes in the retina, as well as degeneration of the optic nerve. However, in addition to long-term cyanide exposure, deficiency of hydroxocobalamin, which is involved in one of cyanide's metabolic pathways, also is thought to contribute to this condition.

Animals

The effects of cyanide and thiocyanate on the CNS was assessed in rats that were fed a diet containing potassium cyanide at 500 ppm or potassium thiocyanate at 2240 ppm for 11.5 mo. Both compounds produced demyelination in the white matter of the spinal cord (Philbrick et al. 1979).

Crampton et al. (1979) reported that baboons exposed to KCN at 1 mg/kg subcutaneously for 5 d/w for 42 mo showed no neurological damage as assessed by neurological and histological examination. Lessell (1971) reported that subcutaneous exposure of rats to cyanide at increasing doses three times a week for 3 mo produced segmental demyelination of the optic nerve and necrosis in the corpus callosum. However, the doses required to produce these neurological lesions were so high (cumulative dose 200 to 300 mg/kg) that the exposure was lethal to 70-80% of the rats. However, in another study, giving cyanide

salts to rats and mice at up to 300 ppm in drinking water over a 13-w period produced no evidence of brain lesions on histopathological examination (NTP 1993).

General Toxicity and Pathology

The long-term effects of cyanide were evaluated by the National Toxicology Program (NTP 1993) in groups of 20 rats and 20 mice (10 of each sex) given NaCN in drinking water at concentrations of 0, 3, 10, 30, 100, or 300 ppm for 13 w. The average daily intake of NaCN is shown in Table 15-3. As noted above in the Toxicokinetics section, essentially all ingested cyanide is absorbed as HCN in the stomach. Thus, the intake of HCN can be calculated from the NaCN values (Table 15-3).

If the intake of NaCN from drinking water is assumed to be roughly continuous, then an equivalent continuous inhalation exposure concentration of HCN that would produce the same body burden can be estimated from the amount of water consumed. To accomplish that, we first used the estimates of daily HCN oral doses (in milligrams per kilogram) (Table 15-3) to calculate the daily HCN consumption for a 300-g rat or a 30-g mouse (Table 15-4). Then, the airborne HCN concentration for continuous exposure that yields the same daily HCN dose in the rat or mouse can be estimated. No data are available on the uptake efficiency of inhaled HCN in rodents; however, humans retain about 75% of

TABLE 15-3 Daily Consumption of NaCN in Drinking Water by Rodents in a 13-W Study and the Calculated HCN Intake (NTP 1993)

NaCN, ppm	Male Rats		Female Rats		Male Mice		Female Mice	
	NaCN[a]	HCN[a]	NaCN[a]	HCN[a]	NaCN[a]	HCN[a]	NaCN[a]	HCN[a]
0	0	[0.0]	0	[0]	0	[0]	0	[0]
3	0.3	[0.2]	0.3	[0.2]	0.5	[0.3]	0.6	[0.3]
10	0.9	[0.5]	1.0	[0.6]	1.8	[1.0]	2.1	[1.2]
30	2.7	[1.5]	3.2	[1.8]	5.1	[2.8]	6.2	[3.4]
100	8.5	[4.7]	9.2	[5.1]	15.2	[8.4]	19.1	[10.5]
300	23.6	[13.0]	23.5	[13.0]	45.9	[25.3]	54.3	[29.9]

[a] In milligrams per kilogram.
Note: Values in brackets are the daily equivalents of HCN dose (calculated from NaCN).

inhaled HCN in the body (Landahl and Herrmann 1950). If the same absorption efficiency is assumed for rodents and if the corresponding ventilation rates are 210 mL/min (0.30 m^3/d) for a 300-g rat and 20 mL/min (0.029 m^3/d) for a 30-g mouse (Lai 1991), then airborne concentrations for the rat or mouse can be estimated. The estimates are shown in Table 15-4.

TABLE 15-4 NaCN Concentrations in Drinking Water in a 13-W Rodent Study and the Calculated Equivalent Airborne HCN Concentrations that Could Produce the Same Body Burden

	Rats				Mice			
NaCN, ppm	HCN Oral Dose		Airborne HCN		HCN Oral Dose		Airborne HCN	
mg/kg/d[a]	mg/kg	mg/m^3	ppm	mg/kg/d[a]	mg/kg	mg/m^3	ppm	
0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0
3	0.2	0.06	0.26	0.24	0.3	0.009	0.4	0.3
10	0.5	0.15	0.66	0.6	1.1	0.033	1.5	1.4
30	1.6	0.48	2.1	1.9	3.1	0.09	4.1	3.8
100	4.9	1.47	6.5	5.9	9.5	0.029	13.3	12.1
300	13.0	3.90	17.2	15.6	27.6	0.83	38.2	34.7

[a] Averages of daily HCN doses for female and male rats or mice (values from Table 15-3)

No deaths were attributed to cyanide treatment in this 13-w study. No clinical signs or microscopic changes were noted in any organs, including brain or thyroid. Minimal changes were present in hematological, clinical chemistry, and urinalysis values; however, these changes were considered neither biologically significant nor related to the effects of cyanide. Weights of the left caudal epididymis in all groups of exposed males were significantly lower than those of the controls (see section on developmental toxicity).

In another study, no morphological or hematological changes were noted over a 2-y period in which rats were given food containing HCN (Howard and Hanzal 1955). The HCN was added to the feed by fumigation, after which the feed was stored in special jars. HCN concentration in the feed was either 190 ppm (range 80 to 300 ppm) or 76 ppm (52 to 100 ppm). Because a 250-g rat eats about 15 g of feed per day and inhales approximately 240 mL of air per minute (Sweet 1987; Mauderly 1986), the consumption of HCN at 190 or 76 ppm in the feed is equivalent to inhaling airborne HCN at 11 or 4.4 ppm, again assuming 100% absorption for the oral route and 75% for inhalation. Body weight was not affected during the first 91 w, but was lower at w 104.

Thyroid Effects

Humans

In the study of Egyptian electroplating workers (E1 Ghawabi et al. 1975), 20 of the 36 HCN-exposed workers (56%) had goiter of a mild or moderate degree. The enlarged thyroids were reportedly firm and slightly nodular in 4 workers but soft and smooth in the remaining 16 workers. These 20 individuals had "no clinical manifestations of hypo-or hyperthyroidism." No correlation was found between thyroid size or goiter incidence and duration of employment: 14 subjects were employed for less than 5 y, 14 for 5-10 y, and 8 for more than 10 y. The authors of this report attributed the goiter to thiocyanate formation in the body, quoting the finding of goiter in hypertension patients who had been treated with thiocyanate for 4 mo or longer (Potter 1944; Rawson et al. 1943). According to E1 Ghawabi and co-workers, the HCN-exposed workers accumulated iodine-131 in thyroid more rapidly than controls when the exposed workers returned to work after 2 d of nonexposure over the weekend.

Goiter also was reported by Hardy et al. (1950) in two workers exposed to HCN while they dipped red-hot metallic tools into a cyanide solution. The exposure concentrations of HCN were unknown. No palpable thyroid abnormalities were found among those who had worked for about a year in a silver recovery plant that used cyanide salt.

Animals

In the 13-w drinking-water study discussed above, the equivalent continuous inhalation exposure concentrations of HCN were estimated to be 0.24, 0.6, 1.9, 5.9, or 15.6 ppm for the rats and 0.3, 1.4, 3.8, 12.1, or 34.7 ppm for the mice. Histopathological examination of thyroid glands in both species revealed no evidence of toxicity. In rats, urinary thiocyanate concentrations increased as exposure concentrations increased (NTP 1993).

Longer exposures to higher concentrations of cyanide produced effects on the rat thyroid gland. Rats fed a diet containing potassium at 1500 ppm for 11.5 mo showed decreased rates of thyroxine secretion but no changes in plasma thyroxine concentration (Philbrick et al. 1979). However, exposure to potassium thiocyanate at 2240 ppm reduced thyroxine measurements and produced thyroid enlargement. The cyanide exposure also suppressed body-weight gain. Calculations of equivalent airborne HCN exposure concentrations reveal that daily ingestion of food containing KCN at 1500 ppm would give the same body burden of HCN as inhaling air containing HCN at 86.8 ppm.

Reproductive and Developmental Toxicity in Animals

In the aforementioned NTP (1993) study, sperm morphology and vaginal cytology were assessed in rats and mice that consumed cyanide at 30, 100, or 300 ppm in drinking water. As shown in Table 15-4, the equivalent inhalation exposure concentrations of HCN for rats were 1.9, 5.9, or 15.6 ppm; those for mice were 3.8, 12.1, or 34.7 ppm. Mild but statistically significant decreases in the weight of the left caudal epididymis were seen in the exposed male rats and in some of the male mice; marginal reduction of sperm motility was seen in the exposed rats but not in the mice (see Table 15-5). NTP (1993) concluded that these mild reproductive changes in rats are probably not biologically significant and might not decrease fertility. However, humans are considered more sensitive to such effects (NTP 1993).

Female rats in the 100- and 300-ppm groups had prolonged proestrus and diestrus. However, the lack of a dose-response relationship in these variables led the NTP to conclude that "these differences are spurious and the results of this study would need to be replicated before such changes could be unequivocally attributed to sodium cyanide exposure."

In vivo developmental toxicity evaluations of HCN have not been reported in the literature. In one study of chick embryo explants (Spratt 1950), sodium cyanide at more than 5 µM inhibited CNS development. Hamsters infused with sodium cyanide at 0.13 mmole/kg-h on d 6-9 of gestation showed fetal malformations, including limb and tail defects, hydropericardium, and encephaloceles, on d 11 of gestation (Doherty et al. 1982).

TABLE 15-5 Effects on Reproductive Tissues from Rodents Exposed to Cyanide in Drinking Water

	Dosage Groups							
	0 ppm		30 ppm		100 ppm		300 ppm	
	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice
Left epididymis (g)	0.448	0.049	0.437	0.047	0.425	0.047	0.417[a]	0.044[a]
Left caudal epididymis (g)	0.162	0.017	0.150[a]	0.016	0.148[a]	0.015	0.141[a]	0.014[a]
Left testis (g)	1.58	0.121	1.56	0.113	1.52	0.117	1.46[a]	0.118
Spermatid head count (10^7/testis)	17.86	2.24	16.94	2.26	16.58	2.03	15.42[a]	2.11
Sperm motility %	94.24	92.38	90.67	90.63	92.09[a]	91.43	90.66[a]	89.52

[a] Significantly different ($p \leq 0.05$) from the control group.
Note: Values are means (see original reference for SDs).

Carcinogenicity and Mutagenicity

Rats fed food containing HCN at 190 ppm (range 80 to 300 ppm) or 76 ppm (80 to 300 ppm) for 2 y showed no increase tumor incidence (Howard and Hanzal 1955). The equivalent inhalation concentrations of HCN were 11 or 4.3 ppm, respectively.

Kushi et al. (1983) reported that HCN is weakly mutagenic in *Salmonella typhimurium*.

Interactions with Other Chemicals

HCN might potentiate the lethal effect of carbon monoxide (Moss et al. 1951). A 30-min exposure to CO at 2000 ppm produced no ill effects in rats; however, CO exposure at 2000 ppm with HCN at 10 or 20 ppm was lethal to some rats. Moss attributed the synergistic effect to respiratory stimulation by HCN. Synergism also was demonstrated for CO and potassium cyanide: Moore et al. (1991) reported a higher mortality in rats exposed to both CO (2000 to 3750 ppm) and KCN (3 to 7 mg/kg, intraperitoneal injection) than could be explained by an additive effect. The combination also resulted in higher concentrations of carboxyhemoglobin than CO poisoning alone, and more severe lactic acidosis than KCN intoxication alone.

Table 15-6 presents a summary of the toxicity data on HCN.

TABLE 15-6 Toxicity Summary

Concentration, ppm	Exposure Duration	Species	Effects	Reference
Human Studies				
6.4 ± 6.9 8.1 ± 8.2 10.4 ± 10.9	Occupational exposure (5-15 y)	Human (n = 36)	Headaches, weakness, changes in taste and smell, giddiness, throat irritation, lacrimation, vomiting, dyspnea; thyroid enlargement, increased rate of iodine accumulation in thyroid after 2 d of nonexposure	El Ghawabi et al. 1975
240 (nominal)	2 min	Human	No symptoms	Grubbs 1917
360 (nominal)	1.5 min	Human (n = 1)	No symptoms	Grubbs 1917
625 (nominal)	1.5 min	Human	Nausea and difficulty in concentrating	Barcroft 1931
1000	Not stated	Human	Impossible or too toxic to breathe for many min	Barcroft 1931
Animal Studies				
0.5	4 w	Rabbit	No ultrastructural change in heart muscle	Hugod 1981
23	30 min	Mouse	Respiratory rate reduced by 20%	Matijak-Schaper and Alarie 1982
35-60	Not stated	Dog	Vomiting, convulsions, or death	Dudley 1942
36	8 min	Dog	All 4 dogs survived, but one developed CNS sequelae (weakness and "dementia")	Etteldorf 1939
36	10 min	Dog	1 of 3 died	Etteldorf 1939
46	Not stated	Dog	Convulsions	Etteldorf 1939

Concentration, ppm	Exposure Duration	Species	Effects	Reference
50	3 min	Rat (n = 2)	1 paralyzed at 2.5 min; the other not paralyzed	Moss et al. 1951
50 (nominal)	60 min	Rat	1 of 6 died	Barcroft 1931
60 (nominal)	1 h	Dog	All 4 survived	Barcroft 1931
70 (nominal)	30 min	Dog (n = 2)	Both survived	Barcroft 1931
80	30 min	Monkey	No significant hyperventilation and no incapacitation	Purser et al. 1984
90	30 min	Monkey	Hyperventilation began at 20 min	Purser et al. 1984
100 (nominal)	15 min	Dog	1 of 2 died	Barcroft 1931
100	17 min	Monkey	Incapacitation	Purser et al. 1984
100 (nominal)	30 min	Dog	Both died	Barcroft 1931
100 (nominal)	30 min	Rat	2 of 6 died	Barcroft 1931
100 (nominal)	45 min	Rat	5 of 6 died	Barcroft 1931
100 (nominal)	60 min	Monkey, rabbit, rat	All 8 monkeys and both rabbits survived; 3 of 6 rats died	Barcroft 1931
110	1.5 h	Rat	Death	Dudley et al. 1942
120	30 min	Mouse	Respiratory rate reduced by 80%	Matijak-Schaper and Alarie 1982
120	Not stated	Rabbit	No "markedly toxic symptoms"	Dudley et al. 1942
125	14 min	Monkey	Incapacitation	Purser et al. 1984
125	12 min	Monkey, cat	"Distinctly toxic" in monkeys at 12 min; "markedly toxic" in cats after 6-7 min	Dudley et al. 1942

140 (nominal)	30 min	Monkey	1 of 3 died	Barcroft 1931
140 (nominal)	60 min	Goat, rabbit	No deaths in 8 goats tested; 2 of 4 rabbits died	Barcroft 1931
147	30 min	Monkey	Hyperventilation developed within 0.5-13 min; heart rate reduced; heartbeat "became arrhythmic"; T-waves changed in amplitude; increased slow EEG activity; decreased fast EEG activity	Purser et al. 1984
150	8 min	Monkey	Incapacitation	Purser et al. 1984
170 (nominal)	60 min	Monkey	All 3 died	Barcroft 1931
180	30 min	Monkey	Hyperventilation developed immediately	Purser et al. 1984
200	5 min	Dog	All 3 survived	Barcroft 1931
200	10 min	Dog	2 of 3 died	Barcroft 1931
200 (nominal)	15 min	Rat, rabbit	1 of 4 rats and 3 of 7 rabbits died	Barcroft 1931
200 (nominal)	30 min	Monkey, goat, rat	1 of 3 monkeys, 3of 4 goats, and all 6 rats died	Barcroft 1931
200 (nominal)	60 min	Goat	4 of 8 died	Barcroft 1931
200	1.5 h	Guinea pig	No symptoms	Dudley et al. 1942
240 (nominal)	6-12 min	Rat	Unspecified symptoms; 3 of 4 died	Grubbs 1917
300 (nominal)	10 min	Rabbit	2 of 4 died	Barcroft 1931
300 (nominal)	15 min	Goat	1 of 4 died	Barcroft 1931
323	1 h	Mouse	LC_{50}	Vernot et al. 1977
400 (nominal)	3 min	Monkey	All 3 died	Barcroft 1931
400 (nominal)	10 min	Goat	3 of 4 died	Barcroft 1931

Concentration, ppm	Exposure Duration	Species	Effects	Reference
484	1 h	Rat	LC_{50}	Vernot et al. 1977
500 (nominal)	3 min	Rabbit, rat	3 of 4 rabbits died; 3 of 6 rats died	Barcroft 1931
500 (nominal)	10 min	Rabbit, rat	All 4 rabbits died; all 6 rats died	Barcroft 1931
625 (nominal)	1.5 min	Dog	Unsteady at 50 s; unconscious at 75 s; convulsions at 90 s	Barcroft 1931

RATIONALE FOR ACCEPTABLE CONCENTRATIONS

Table 15-7 presents exposure limits for HCN set by other organizations and Table 15-8 presents the SMACs established by NASA.

SMACs are derived in accordance with guidelines developed by the SMAC subcommittee of the Committee on Toxicology (NRC 1992). The SMACs are set by choosing the lowest values among the acceptable concentrations (ACs) (see Table 15-9). The major difficulty in setting exposure limits for HCN is the lack of good dose-response inhalation data from human and animal studies. Even with the data from a few epidemiological studies on HCN-exposed workers, the correlation between exposure concentrations and cyanide toxicity cannot be established with certainty. Most of the human studies were conducted to investigate the consequences of brief exposures to high concentrations. Most of the animal inhalation data also were obtained from brief exposures to high concentrations; these results on lethality or serious toxicity are of little value in setting exposure limits.

TABLE 15-7 Exposure Limits Set by Other Organizations

	Exposure Limit		
Organization	ppm	mg/m^3	Reference
ACGIH's TLV	10 (TWA) (ceiling)	11	ACGIH 1991
OSHA's PEL	4.7	5	NIOSH 1990
NIOSH's IDLH	50	55	NIOSH 1990

TLV, Theshold Limit Value; TWA, time-weighted average; PEL, permissible exposure limit; IDLH, immediately dangerous to life and health.

TABLE 15-8 Spacecraft Maximum Allowable Concentrations

Duration	Concentration, ppm	Concentration, mg/m^3	Target Toxicity
1 h	8	9	CNS effects
24 h	4	4.5	CNS effects
7 d	1	1.1	CNS effects
30 d	1	1.1	CNS effects
180 d	1	1.1	CNS effects, testicular toxicity, thyroid effects

The brain is known to be the organ most sensitive to HCN toxicity. However, a recent NTP rodent study showed that the reproductive system also is sensitive to HCN. Chronic cyanide exposures have resulted in goiters in humans. Data on these toxicity end points will be considered in setting the SMAC values.

ACs Set Based on CNS Effects in Humans

The most relevant data for setting exposure limits come from the reports of 36 workers in three electroplating factories where cyanide salts were used (El Ghawabi et al. 1975). Unfortunately, the subjects in those factories were exposed to other chemicals besides HCN. Symptoms reported from past and "present" medical histories and from interviews of these workers, who had worked for periods of a few years to more than 15 y in those facilities. HCN concentrations in the three factories during the 2-mo survey were measured at 6.4 ± 6.9, 8.1 ± 8.2, and 10.4 ± 10.9 ppm; the highest concentration was found in factory A, which had no ventilation. The most prevalent and least-severe symptoms were headache and weakness, which were reported by 80% of the workers (20-30% in controls). Vomiting and more-serious CNS effects were reported by 44% of the subjects during their tenures. The authors did not mention whether any symptoms were present during the survey period. The symptoms were reported by the workers who had worked for many years in these factories. The concentrations reported were obtained by analyzing air samples taken several times from each factory during the 3-mo survey. It is likely that the more serious symptoms, such as vomiting, were the result of brief exposures to high HCN concentrations at work. Therefore, it is reasonable to conclude that 8 ppm would likely produce no more than mild CNS effects (e.g., mild headache), which would be acceptable for 1-h exposures in a spacecraft. Therefore, 8 ppm is set as the 1-h AC for HCN. The concentration is reduced by half to 4 ppm to ensure that exposure would produce no more than slight CNS effects. This AC is further reduced to 1 ppm as the AC for 7-d, 30-d and 180-d exposures. HCN at 1 ppm is not expected to produce any CNS effects.

Testicular Effects in Rats

When rats and mice were fed drinking water containing NaCN at 300 ppm, which is equivalent to HCN at 15.6 ppm in the air (see Table 15-4), they had

mild but statistically significant decreases in the weights of the left epididymis and left cauda epididymis. Statistically significant changes in testicular weight, sperm counts and sperm motility were also observed in rats but not in mice (NTP 1993). NTP concluded that those changes are probably not biologically significant and are insufficient to decrease fertility in rats; however, NTP cautioned that "humans are considered to be relatively more sensitive than rats to such changes." Thus, 300 ppm in drinking water or the equivalent of 16 ppm continuous (24-h) inhalation exposure was treated as the no-observed-adverse-effect level (NOAEL) for rats. A species factor of 10 is used to account for the possibility that humans are more sensitive to the reproductive toxicity of HCN. Therefore, the AC for 7 d and 30 d is set at 1.6 ppm (NOAEL ÷ 10). The 180-d AC is calculated below:

180-d AC = 16 ppm ÷ 10 × (90 d/180 d) = 0.8 ppm

= 1 ppm (rounded up from 0.8 ppm).

Thyroid Effects

El Ghawabi et al. (1976) reported that mild goiter was detected in 44% of the 36 Egyptian workers working for up to 15 y in poorly ventilated electroplating factories where the HCN concentration varied greatly. The great variations in exposure length and concentrations make these data unsuitable for setting SMACs. A recent 13-w study on HCN toxicity to all major organs and tissues, including thyroid, has more direct relevance to the assessment of HCN exposures (≤ 180 d) in spacecraft. In the latter study, rats and mice were fed water containing as much cyanide as 300 ppm; histopathological examination revealed no thyroid lesions. As discussed above, the exposure concentration is roughly equivalent to a 24-h continuous airborne HCN concentration of 15.6 ppm for the rats or 34.7 ppm for the mice. Rats are known to be more sensitive than mice to the effects of chemicals on the thyroid; therefore, the equivalent exposure of 15.6 ppm to rats are used to establish ACs. A species factor of 10 is applied to obtain an AC of 1.6 ppm for a 30-d exposure. A factor of 2 is applied to obtain an AC of 0.8 ppm (which was rounded up to 1 ppm) for 180-d exposure, which is twice the duration of that in the NTP study. It is very unlikely that exposures to low concentrations of HCN for 7 d or less could alter thyroid function. Therefore, ACs for exposures of 7 d or less will not be set on the basis of thyroid toxicity.

RECOMMENDATIONS

The major difficulty in setting exposure limits for HCN is the lack of controlled human inhalation exposure data, or good dose-response inhalation data from animals. It is recommended that experiments be carried out to better elucidate the concentration response of HCN at exposure concentrations at 20 ppm and below. These results will be useful for reevaluating SMACs, TLVs, and the OSHA PEL. The current TLV and PEL for HCN are 10 and 4.7 ppm, respectively.

TABLE 15-9 Acceptable Concentrations

End Point, Exposure Data Reference		Uncertainty factors		Acceptable Concentrations, ppm				
Species	Species	Time	1 h	24 h	7 d	30 d	180 d	
Headache and weakness	Human	1	1	8	4	1	1	1
6.4 ± 6.9, 8.1 ± 8.2, and 10.4 ± 10.9 ppm for 5-15 y (El Ghawabi et al. 1975)								
Testicular toxicity	Rat	10	2[b]	—	—	1.6	1.6	1[c]
NOEAL, 15.6 ppm × 13 w[a] (NTP 1993)								
Thyroid Effect	Rat	10	2[b]	—	—	—	1.6	1[c]
NOEAL, 15.6 ppm × 13 w[a] (NTP 1993)								
SMACs				**8**	**4**	**1**	**1**	**1**

[a] See text for detail.
[b] For 180 d.
[c] Rounded up from 0.8 ppm.

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