Spacecraft Water Exposure Guidelines for Selected Contaminants: Volume 2

Committee on Spacecraft Exposure Guidelines, Committee on Toxicology, National Research Council

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SPACECRAFT WATER EXPOSURE GUIDELINES FOR SELECTED CONTAMINANTS

VOLUME 2

Committee on Spacecraft Exposure Guidelines

Committee on Toxicology

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

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Preface

The National Aeronautics and Space Administration (NASA) maintains an active interest in the environmental conditions associated with living and working in spacecraft and identifying hazards that might adversely affect the health and well-being of crew members. Despite major engineering advances in controlling the spacecraft environment, some water and air contamination is inevitable. Several hundred chemical species are likely to be found in the closed environment of the spacecraft, and as the frequency, complexity, and duration of human space flight increase, identifying and understanding significant health hazards will become more complicated and more critical for the success of the missions.

NASA asked the National Research Council (NRC) Committee on Toxicology to develop guidelines, similar to those developed by the NRC in 1992 for airborne substances, for examining the likelihood of adverse effects from water contaminants on the health and performance of spacecraft crews. In 2000, the NRC report *Methods for Developing Spacecraft Water Exposure Guidelines* was published, and NASA now uses those methods for developing spacecraft water exposure guidelines (SWEGs) for individual water contaminants. NASA is responsible for selecting the water contaminants for which SWEGs will be established. To ensure that the SWEGs are developed in accordance with the NRC guidelines, NASA requested that the NRC committee independently review the draft SWEG documents. In its evaluations, the committee reviews the documents as many times as necessary until it is satisfied that the SWEGs are scientifically justified. This report is the second volume in the series, *Spacecraft Water Exposure Guidelines for Selected Contaminants*. *Spacecraft Water Exposure Guidelines Volume 1*, published in 2004, used the NASA guidelines to establish exposure concentrations for phenol, *N*-phenyl-beta-naphthylamine, dichloromethane, chloroform, di(2-ethylhexyl) phthalate, di-*n*-butyl phthalate, silver, and 2-mercaptobenzothiazole. This second volume presents SWEGs for acetone,

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alkylamines, ammonia, barium, cadmium, caprolactam, formate, formaldehyde, manganese, total organic carbon, and zinc.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report: James V. Bruckner, University of Georgia; Barbara Callahan, University Research Engineers and Associates; Samuel Kacew, University of Ottawa; John O'Donoghue, University of Rochester, School of Medicine and Denistry; and Robert Young, Oak Ridge National Laboratory.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Ernest McConnell, ToxPath, Inc. Appointed by the NRC, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Special thanks are extended to John James and Torin McCoy (NASA); Hector Garcia and Raghupathy Ramanathan (Wyle Laboratories); and Jean Hampton (Texas Southern University) for preparing and revising the SWEG documents. We would also like to thank previous members of the committee who contributed to the development of this document including Joseph Brady, The Johns Hopkins University; Gary Carlson, Purdue University; Donald Gardner, Inhalation Toxicology Associates; Elaine Faustman, University of Washington; Charles E. Feigley, University of South Carolina; Mary Esther Gaulden, The University of Texas Southwestern Medical Center at Dallas; William Halperin, New Jersey Medical School; Ralph Kodell, Food and Drug Administration; Robert Snyder, Rutgers, The State University of New Jersey; Bernard Wagner, Independent Consultant; and Bernard Weiss, University of Rochester School of Medicine and Dentistry.

We are grateful for the assistance of the NRC staff in supporting this project and preparing the report. Staff members who contributed to this effort are James J. Reisa, director of the Board on Environmental Studies and Toxicology and Alexandra Stupple, senior editorial assistant. We especially wish to recognize the contributions of senior program officers, Eileen Abt and Susan N.J.

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Martel, project director, Jennifer Saunders, and senior project assistant, Tamara Dawson.

Finally, we would like to thank all the members of the committee for their dedicated efforts throughout the development of this report.

Garold S. Yost, Ph.D.
Chair, Committee on Spacecraft Water Exposure Guidelines

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SPACECRAFT WATER EXPOSURE GUIDELINES
FOR SELECTED CONTAMINANTS

VOLUME 2

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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 three to six astronauts for up to 180 days (d). Because the space station will be a closed and complex environment, some contamination of its internal atmosphere and water system is unavoidable. Several hundred chemical contaminants are likely to be found in the closed-loop atmosphere and recycled water of the space station.

To protect space crews from contaminants in potable and hygiene water, the National Aeronautics and Space Administration (NASA) requested that the National Research Council (NRC) provide guidance on how to develop water exposure guidelines and subsequently review NASA's development of the exposure guidelines for specific chemicals. The exposure guidelines are to be similar to those established by the NRC for airborne contaminants (NRC 1992; 1994; 1996a,b; 2000a). The NRC was asked to consider only chemical contaminants, and not microbial agents. The NRC convened the Committee on Spacecraft Water Exposure Guidelines to address this task. The Committee published its first report Methods for Developing Spacecraft Water Exposure Guidelines in 2000. A second report, Spacecraft Water Exposure Guidelines for Selected Contaminants, Volume 1 (2004a), used these guidelines to set exposure levels for nine chemicals: chloroform, dichloromethane, di-*n*-butyl phthalate, di(2-ethylhexyl) phthalate, 2-mercaptobenzothiazole, nickel, phenol, N-phenyl-beta-napthylamine, and silver.

Spacecraft water exposure guidelines (SWEGs) are to be established for exposures of 1, 10, 100, and 1,000 d. The 1-d SWEG is a concentration of a substance in water that is judged to be acceptable for the performance of specific tasks during rare emergency conditions lasting

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for periods up to 24 hours (h). The 1-d SWEG is intended to prevent irreversible harm and degradation in crew performance. Temporary discomfort is permissible provided there is no effect on judgment, performance, or ability to respond to an emergency. Longer-term SWEGs are intended to prevent adverse health effects (either immediate or delayed) and degradation in crew performance that could result from continuous exposure in closed spacecraft for as long as 1,000 d. In contrast with the 1-d SWEG, longer-term SWEGs are intended to provide guidance for exposure under the expected normal operating conditions in spacecraft.

WATER CONTAMINANTS

Water used in NASA's space missions must be carried from Earth or generated by fuel cells. The water is used for drinking, food reconstitution, oral hygiene, hygienic uses (handwashing, showers, urine flushing), and oxygen generation. Because of plans for longer spaceflights and habitation of the ISS, water reclamation, treatment, and recycling is required. Water for long spaceflights can be reclaimed from several onboard sources, including humidity condensate from the cabin, hygiene water (shower and wash water), and urine. Each of those sources will have a variety of contaminants. Humidity condensate will have contaminants released into the cabin from crew activities (for example, byproducts of crew metabolism, food preparation, and hygiene activities) from routine operation of the air-revitalization system, from off-gassing of materials and hardware, from payload experiments, and from routine in-flight use of the crew health care system. Wash water will include detergents and other personal hygiene products. Urine contains electrolytes, small-molecular-weight proteins, and metabolites of nutrients and drugs. It is chemically treated and distilled before recycling, which causes a variety of by-products to be formed. Other sources of chemical contaminants include mechanical leaks, microbial metabolites, payload chemicals, biocidal agents added to the water to retard bacterial growth (such as silver and iodine), fouling of the filtration system, and incomplete processing of the water.

It is also possible that contaminants in the spacecraft atmosphere will end up as toxic substances in the water system. The air and water systems of the ISS constitute a single life-support system, and the use of condensate from inside the cabin as a source of drinking water could introduce some unwanted substances into the water system.

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SUMMARY OF THE REPORT ON METHODS FOR DEVELOPING SWEGs

Data

In developing SWEGs, several types of data should be evaluated, including data on (1) the physical and chemical characteristics of the contaminant, (2) in vitro toxicity studies, (3) toxicokinetic studies, (4) animal toxicity studies conducted over a range of exposure durations, (5) genotoxicity studies, (6) carcinogenicity bioassays, (7) human clinical and epidemiology studies, and (8) mechanistic studies. All observed toxic effects should be considered, including mortality, morbidity, functional impairment, specific organ system toxicities (such as renal, hepatic, and endocrine), neurotoxicity, immunotoxicity, reproductive toxicity, genotoxicity, and carcinogenicity. Taste and odor thresholds are also relevant end points for setting SWEGs.

Data from oral exposure studies should be used—particularly drinking water and feed studies in which the duration of exposure approximates anticipated human exposure times. Gavage studies can also be used, but they should be interpreted carefully because they involve the bolus administration of a substance directly to the stomach within a brief period of time. Such exposure could result in blood concentrations of contaminants and attendant effects that might not be observed if the administration were spread out over several smaller doses, as would be expected with the normal pattern of water consumption. Dermal absorption and inhalation studies should also be evaluated, because exposure from those routes occur when water is used for hygiene purposes.

There are several important determinants for deriving a SWEG, including identifying the most sensitive target organ or body system affected; the nature of the effect on the target tissue; dose-response relationships for the target tissue; the rate of recovery; the nature and severity of the injury; cumulative effects; toxicokinetic data; interactions with other chemicals; and the effects of microgravity.

Risk Assessment

There are several risk assessment methods that can be used to derive SWEGs. Risk assessments for exposure to noncarcinogenic substances traditionally have been based on the premise that an adverse

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health effect will not occur below a specific threshold exposure. Given this assumption, an exposure guidance level can be established by dividing the no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL) by an appropriate set of uncertainty factors. This method requires making judgements about the critical toxicity end point relevant to a human in space, the appropriate study for selecting a NOAEL or LOAEL, and the magnitudes of the uncertainty factors used in the process.

For carcinogenic effects known to result from direct mutagenic events, no threshold dose would be assumed. However, when carcinogenesis results from nongenotoxic mechanisms, a threshold dose can be considered. Estimation of carcinogenic risk involves fitting mathematical models to experimental data and extrapolating to predict risks at doses that are usually well below the experimental range. The multistage model of Armitage and Doll (1960) 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 several biologic events (for example, mutations) that must occur in a specific order. There also is a two-stage model that explicitly provides for tissue growth and cell kinetics.

An alternative to the traditional NOAEL and LOAEL risk assessment methods that are used to set carcinogenic and noncarcinogenic concentrations is the benchmark dose (BMD) approach. The BMD is the dose associated with a specified low level of excess health risk, generally in the risk range of 1-10% ($BMDL_{01}$ and $BMDL_{10}$), that can be estimated from modeled data with little or no extrapolation outside the experimental dose range. The $BMDL_{01}$ and $BMDL_{10}$ are defined as the statistical lower confidence limits of doses that correspond to excess risks of 1% and 10% above background concentrations, respectively. Use of the lower confidence limit provides a suitable method to incorporate experimental uncertainty. However, the use of a central estimate of the benchmark dose, with incorporation of an additional uncertainty factor to account for experimental variation, may be more appropriate for certain kinds of data. Like the NOAEL and LOAEL, the $BMDL_{01}$ and $BMDL_{10}$ are points of departure for establishing exposure guidelines and should be modified by appropriate exposure conversions and uncertainty factors.

Scientific judgment is often a critical, overriding factor in applying the methods described above. It is recommended that when sufficient dose-response data are available, the BMD approach be used to calculate exposure guidelines. However, in the absence of sufficient data, or when

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special circumstances dictate, the other, more traditional approaches should be used.

Special Considerations for NASA

When deriving SWEGs, either by the traditional or BMD approach, it will be necessary to use exposure conversions and uncertainty factors to adjust for weaknesses or uncertainties about the data. When adequate data are available, exposure conversions that NASA should use include those to adjust for target tissue dose, differences in exposure duration, species differences, and differences in routes of exposure.[1] Uncertainty factors should also be used to extrapolate animal exposure data to humans, when human exposure data are unavailable or inadequate; to extrapolate data from subchronic studies to chronic exposure; to account for using $BMDL_{10}$ instead of $BMDL_{01}$ (or a LOAEL instead of a NOAEL); to account for experimental variation; and to adjust for spaceflight factors that could alter the toxicity of water contaminants. The latter factors are used to account for uncertainties associated with microgravity, radiation, and stress. Some of the ways astronauts can be physically, physiologically, and psychologically compromised include decreased muscle mass, decreased bone mass, decreased red blood cell mass, depressed immune systems, altered nutritional requirements, behavioral changes, shift of body fluids, altered blood flow, altered hormonal status, altered enzyme concentrations, increased sensitization to cardiac arrhythmias, and altered drug metabolism. There is generally little information to permit a quantitative conversion that would reflect altered toxicity resulting from spaceflight environmental factors. Thus, spaceflight uncertainty factors should be used when available information on a substance indicates that it could compound one or more aspects of an astronaut's condition that might already be compromised in space.

Another commonly used uncertainty factor is one that accounts for variable susceptibilities in the human population. That uncertainty factor is used to protect sensitive members of the general population, including young children, pregnant women, and the immune compromised. Because the astronaut population is typically composed of healthy nonpreg-

[1]Two liters per day was used as the default for drinking water consumption, although this quantity may not be applicable in all situations.

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nant adults, the committee believes that an uncertainty factor for intraspecies differences should only be used if there is evidence that some individuals could be especially susceptible to the contaminant. These differences could be observed among astronauts who possess genetic polymorphisms for well-established genes.

Exposure Guidelines Set by Other Organizations

Several regulatory agencies have established exposure guidance levels for some of the contaminants of concern to NASA. Those guidance levels should be reviewed before SWEGs are established. The purpose of this comparison would not be simply to mimic the regulatory guidelines set elsewhere, but to determine how and why other exposure guidelines might differ from those of NASA and to assess whether those differences are reasonable in light of NASA's special needs.

REVIEW OF SWEG REPORTS

NASA is responsible for selecting the water contaminants for which SWEGs will be established and for developing documentation on how SWEG values were determined. As described above, the procedure for developing SWEGs involves identifying toxicity effects relevant to astronauts and calculating exposure concentrations on the basis of those end points. The lowest exposure concentration is selected as the SWEG, because the lowest value would be expected to protect astronauts from manifesting other effects as well.

To ensure that the SWEGs are developed in accordance with the NRC guidelines (2000b), NASA requested that the NRC committee independently review NASA's draft SWEGs documents. NASA's draft documents summarize data relevant to assessing risk from exposure to individual contaminants in water only; they are not comprehensive reviews of the available literature on specific contaminants. Furthermore, although the committee is mindful that contaminants will be present as mixtures in drinking water and the potential exists for interactions, the committee was asked to consider each chemical on an individual basis. The committee reviews NASA's SWEG documents and provides comments and recommendations in a series of interim reports (see NRC 2000c,d,e; 2001; 2002; 2003; 2004b,c; 2005). The committee reviews

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NASA's documents as many times as necessary until it is satisfied that the SWEGs are scientifically justified.

Because of the enormous amount of data presented in the SWEG reports, the NRC committee cannot verify all the data used by NASA. The NRC committee relies on NASA for the accuracy and completeness of the toxicity data cited in the SWEG reports.

This report is the second volume in the series Spacecraft Water Exposure Guidelines for Selected Chemicals. SWEG reports for acetone, alkylamines, ammonia, barium, cadmium, caprolactam, formate, formaldehyde, manganese, total organic carbon, and zinc are included in the appendix of this report. The committee concludes that the SWEGs developed in those documents are scientifically valid values based on the data reviewed by NASA and are consistent with the NRC (2000b) guideline report. SWEG reports for additional chemicals will be presented in subsequent volumes.

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Appendixes

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1

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 parts per million [ppm]) and excellent solvent properties. It forms explosive mixtures with air or oxygen (see Table 1-1).

OCCURRENCE AND USE

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 (7.0-14.0 micromoles per liter [μmol/L] = 0.4-0.8 micrograms per milliliter [μg/mL]) and urine (4.0-35.0 μmol/L = 0.2-2.0 μg/mL) (Rowe and Wolf 1963; Wang et al. 1994; de Oliveira and Pereira Bastos de Siqueira 2004). Endogenous concentrations of acetone in the blood have been reported up to 10 μg/mL, and concentrations during diabetic ketoacidosis have ranged from 100 to 700 μg/mL (Gamis and Wasserman 1988). Data from a National Institute for Occupational Safety and Health (NIOSH) report (Stewart et al. 1975) on acetone suggest that normal blood acetone concentrations in women (1.8-4.2 mg% = 18-42 μg/dL) may be two to three times higher than in men (0.5-1.4 mg% = 5-14 μg/mL), but no other reports could be found to confirm this. High acetone concentrations in serum and breath are often indicative of altered metabolic states including diabetes, vitamin E deficiency, and fasting (NTP 1991).

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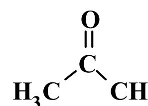
TABLE 1-1 Physical and Chemical Properties of Acetone[a]

Formula	C3H6O
Chemical Name	Acetone
Synonyms	Propanone, 2-propanone, dimethylketone, dimethyl formaldehyde, dimethylketal, ketone propane, beta-ketopropane, methyl ketone, pyroacetic acid, pyroacetic ether
CAS registry no	67641 
Molecular weight	58.09
Boiling point	56.48°C
Melting point	−94.6°C
Density	0.7972 g/cc (at 15°C)
Vapor Pressure	400 mm at 39.5°C, 200 mm at 25°C
Vapor density	2 (air = 1)
Solubility	Infinitely soluble in water; miscible with alcohol, dimethylformamide, chloroform, most oils, and ether
Lower Explosive Limit	2% (in air)
Upper explosive Limit	13% (in air)
Odor Threshold (in air)	13 ppm; 47 mg/m3
Odor Threshold (in water)	20 ppm; 20 mg/L

[a]Data from HSDB 2006.

Acetone is not routinely used in spacecraft during flight but may be part of in-flight scientific experiments. Acetone is found in the spacecraft atmosphere on almost every mission at concentrations up to 8 ppm in Skylab (Liebich et al. 1975) and up to 1.2 ppm during shorter Shuttle missions—probably from crew metabolism and offgassing.

TOXICOKINETICS AND METABOLISM

Much of the data in the literature regarding acetone toxicokinetics and metabolism involves exposure by inhalation, but because of the general distribution of acetone in body water and its relatively slow metabolism, as described below, the data should hold true for acetone exposures by ingestion as well.

Absorption

Acetone is rapidly and almost totally absorbed from the stomach and is also absorbed by inhalation, by mucous membranes, and, to some

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degree, through the skin. The rate of absorption of ingested acetone depends on the amount of food in the stomach. In one subject, peak blood levels of acetone were seen 10 min after ingestion of acetone on an empty stomach, while acetone ingested about 10 min after a meal was more slowly absorbed, with lower peak levels achieved at 48-59 min after ingestion (Widmark 1919).

Distribution

No studies were found on the distribution of acetone after ingestion.

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 the exhalation of acetone was strictly monoexponential, indicating that it does not distribute into a "deep compartment"—that is, one from which it is released only slowly. Also, acetone is water soluble and will not accumulate in adipose tissue.

Mice exposed to 2-[^{14}C]-acetone vapor (500 ppm) for periods of 1 h to 5 days (d) were examined for the tissue distribution of radioactivity (Löf et al. 1980; Wigaeus et al. 1982). The amount of radioactivity in tissues increased as the exposure time increased from 1 to 6 h but increased only slightly or not at all in all tissues except adipose tissue at exposure times greater than 6 h (12 h, 24 h, and 5 d) (Wigaeus et al. 1982). 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 inhalation exposure (6 h/d) to 500 ppm 2-[^{14}C]-acetone vapor, the radioactive concentration in mouse tissues was highest in brown adipose tissue, followed by liver and pancreas (Wigaeus et al. 1982). Only about 10% of the radioactivity in the liver was unchanged acetone.

Metabolism

Ramu et al. (1978) in a case report involving the ingestion of nail polish remover by an alcoholic estimated that humans can metabolize acetone at a rate probably not exceeding 1 g/h, but none of the metabolites were identified. On a gram per kilogram basis, the rate of acetone metabolism in humans has been reported to be about half that the rat

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(Haggard et al. 1944), with metabolism being nonlinear and saturable. Haggard et al. documented a zero-order elimination rate in rats of 13 milligrams per kilograms per hour (mg/kg/h) at a blood acetone concentration of 0.2 g/deciliter (dL). In a 10-d study of female rats, tolerance was reported to develop whereby the effects of inhaled acetone on the inhibition of avoidance behavior and escape response in rats became weaker upon repeated administration (Goldberg et al. 1964), probably because of an induction of metabolic enzymes in the liver.

Several studies in rats have shown that acetone can be metabolized by three separate gluconeogenic pathways, with the first step in all cases being the hydroxylation of one of the methyl groups by acetone monooxygenase to form acetol (NTP 1991). One of the intermediates in the metabolism of acetone to carbon dioxide is formate, which in humans, is metabolized more slowly than in rodents.

Elimination

The main route of excretion of acetone is via the lungs—regardless of the route of exposure—with very little excreted in the urine (Ramu et al. 1978; Wigaeus et al. 1981; Gamis and Wasserman 1988). About half of the acetone is exhaled unchanged in humans, and the other half is exhaled as carbon dioxide produced from the metabolism of acetone (Wigaeus et al. 1982). Several different estimates of the half-life of acetone in blood have been reported, with the reported half-life increasing with the dose of acetone (DiVincenzo et al. 1973; Ramu et al. 1978; Wigaeus et al. 1981; Gamis and Wasserman 1988). In acute intoxications in adult humans, the half-life of acetone in plasma has been estimated at approximately 31 h and is consistent with a first-order elimination process (Ramu et al. 1978). A more recent estimate of the elimination plasma half-life of acetone in humans is 18 h (Sakata et al. 1989). Jones reported half-lives for acetone in the blood and urine ranging from 3-27 h, but some of the measurements were from ingestion of isopropanol or denatured alcohol rather than acetone itself (Jones 2000). A half-life of only 3.9 h was estimated for volunteers inhaling acetone at 250 ppm for 4-h and assuming first-order kinetics (Dick et al. 1988). A case report of a 42-year-old man who intentionally swallowed 800 mL of acetone reported acetone concentrations of 2,000 mg/L in serum and 2,300 mg/L in urine and an elimination half-life of 11 h with sequelae-free survival after aggressive treatments including multiple gastric lavages, hyperventilation, hemofiltration, forced diuresis and hydration (Zettinig et al.

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1997). Two studies on inhaled acetone (Matsushita et al. 1969b and Di-Vincenzo et al. 1973, as cited in OSHA 1989) suggest that chronic intermittent exposure to high enough doses of acetone on a daily basis can lead to the bioaccumulation of acetone. Based on Ramu et al.'s (1978) estimate of the maximum metabolism rate in humans (1 g/h), accumulation in the blood should be seen for dose rates exceeding about 24 g/d. On a milligram per kilogram basis, Haggard et al.'s (1944) estimate of the metabolic rate of acetone in rats implies that dose rates exceeding about 11 g/d in humans could lead to an accumulation of acetone in a 70-kg person.

TOXICITY SUMMARY

Systemic effects reported in humans and animals after oral or inhalation exposure to acetone are described below and in Table 1-2.

At high doses, whether by inhalation or ingestion, manifestations of acute acetone toxicity in humans primarily involve central nervous system (CNS) depression that ranges from lethargy, slurred speech, and ataxia to stupor, coma, and respiratory depression (Ross 1973; Ramu et al. 1978; Gamis and Wasserman 1988). Other adverse effects of high doses include vomiting, hematemesis, excessive thirst, polyuria, hyperglycemia, and occasionally, metabolic acidosis (probably because of the metabolism of acetone to formate) (Ross 1973; Ramu et al. 1978; Gamis and Wasserman 1988). One Soviet investigator reported that four individuals acutely exposed (one by inhalation and three orally) to unspecified concentrations and amounts of acetone developed liver lesions and, in two of the orally intoxicated individuals, mild renal lesions (Mirchev 1977). The quality of this case study report was not evaluated, because it was written in Bulgarian with only the abstract translated into English; thus, it could not be determined if the four patients had also been exposed to other agents such as alcohol or may have had pre-existing lesions of the liver or kidneys.

Acute and Short-Term Exposures

General

Humans who ingest up to 20 mL of acetone do not show any adverse effects (Gosselin et al. 1984). Ingestion of 200 mL, however, can

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TABLE 1-2 Toxicity Summary

Concentration	Exposure Duration	Species	Effects	Reference
Effects in Humans				
~200 mL pure acetone (~2,241 mg/kg)	Bolus	Human	Deep coma; red and swollen throat and erosion in soft palate and entrance to esophagus; disturbance of gait 6 d after ingestion; diabetes-like condition 4 wk after ingestion with hyperglycemia, polyuria, and excessive thirst	Gitelson et al. 1966
Unknown amount of acetone; presumed ingestion by an alcoholic woman	1 wk?	Human	Lethargy, minimal responsiveness; blood acetone concentration = 0.25 g/dL; pharynx was not red or swollen, nor were there erosions of the soft palate; recovery was gradual over 3-4 d; acetone half-life = 31 h	Ramu et al. 1978
Estimated 6 oz of nail polish remover (65% acetone, 10% isopropanol)	Bolus	Human (30 mo old)	Sedated and nonresponsive; no reflexes; generalized tonic-clonic seizure; serum acetone concentration = 445 mg/dL; gradual recovery over 4 d with no adverse sequelae; acetone half-life = 19 h initially, then 13 h in the later stages of recovery	Gamis and Wasserman 1988
>12,000 ppm in air in a pit	2 min-4 hr	Human (n = 8)	Throat and eye irritation, leg weakness, chest tightness, headache, dizziness, confusion, unconsciousness, vomiting; gradual, dose-dependent recovery	Ross 1973

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Effects in Animals				
100,000 ppm in drinking water Rats male: 6.9 g/kg/d female: 8.6 g/kg/d Mice male: 10.3 g/kg/d female: 12.7 g/kg/d	14 d	F344/N rats, male and female; B6C3F1 mice, male and female	LOAEL for bone marrow hypoplasia in male rats; LOAEL for emaciated appearance in rats; LOAEL for decreased weight gain in male mice; decreased water consumption and weight gain in rats and male mice; liver hypertrophy in male and female mice	Dietz et al. 1991; NTP 1991
50,000 ppm in drinking water Rats male: 4.3 g/kg/d female: 4.4 g/kg/d Mice male: 6.3 g/kg/d female: 8.8 g/kg/d	14 d	F344/N rats, male and female; B6C3F1 mice, male and female	LOAEL for liver hypertrophy in female mice; NOAEL for bone marrow hypoplasia in males; LOAEL for decreased water consumption in rats and mice; LOAEL for decreased weight gain in rats; increased kidney weights in rats	Dietz et al. 1991; NTP 1991
20,000 ppm in drinking water male: 2.6 g/kg/d female: 2.3 g/kg/d	14 d	F344/N rats, male and female	LOAEL for increased relative kidney weight in female rats; LOAEL for increased relative liver weight in male and female rats	Dietz et al. 1991; NTP 1991
20,000 ppm in drinking water (3.9-5.5 g/kg/d)	14 d	B6C3F1 mice, male and female	LOAEL for liver hypertrophy in male mice and NOAEL for liver hypertrophy in female mice	Dietz et al. 1991; NTP 1991

(Continued)

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TABLE 1-2 Continued

Concentration	Exposure Duration	Species	Effects	Reference
10,000 ppm in drinking water Rats male: 1.6 g/kg/d female: 1.5 g/kg/d Mice male: 1.6 g/kg/d female: 3.0 g/kg/d	14 d	F344/N rats, male and female; B6C3F1 mice, male and female	NOAEL for liver hypertrophy	Dietz et al. 1991; NTP 1991
5,000 ppm in drinking water Mice male: 1.0 g/kg/d female: 1.6 g/kg/d	14 d	B6C3F1 mice, male and female	LOAEL for increased liver weight	Dietz et al. 1991; NTP 1991
50,000 ppm in drinking water (3.1-3.4 g/kg/d)	13 wk	F344/N rats, male and female	LOAEL for increased relative kidney weight in male rats; LOAEL for mild spermatogenic toxicity and increased relative weight of testis; LOAEL for mild leukocytosis in females; decreased water consumption, but no dehydration; mild macrocytic normochromic anemia in male rats; LOAEL for decreased body weight in rats; LOAEL for decreased water consumption in rats	Dietz et al. 1991; NTP 1991
50,000 ppm in drinking water (11 g/kg/d)	13 wk	B6C3F1 mice, female	LOAEL for increased liver and decreased spleen weights in female mice; increased kidney weights; LOAEL for decreased water consumption in mice	Dietz et al. 1991; NTP 1991

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20,000 ppm in drinking water (1.6-1.7 g/kg/d)	13 wk	F344/N rats, male and female	Decreased erythrocyte counts and hemoglobin concentrations in males; LOAEL for increased relative weight of liver in males and females; LOAEL for increased relative weight of kidney in females; LOAEL for increased severity of nephropathy in males; LOAEL for minimal-to-mild splenic hemosiderosis in males; LOAEL for mild leukocytosis in males	Dietz et al. 1991; NTP 1991
20,000 ppm in drinking water (5.9 g/kg/d)	13 wk	B6C3F1 mice, female	NOAEL for increased liver weight in female mice	Dietz et al. 1991; NTP 1991
10,000 ppm in drinking water (0.9 g/kg/d)	13 wk	F344/N rats, male	NOAEL for mild nephropathy and splenic hemosiderin deposits	Dietz et al. 1991; NTP 1991
5,000 ppm in drinking water (0.4 g/kg/d)	13 wk	F344/N rats, male	LOAEL for decreased reticulocyte counts (macrocytic anemia?)	Dietz et al. 1991; NTP 1991
5,000 ppm in drinking water (1.4 g/kg/d)	13 wk	B6C3F1 mice, male	LOAEL for increased liver weight in males	Dietz et al. 1991; NTP 1991
2,500 ppm in drinking water (0.2 g/kg/d)	13 wk	F344/N rats, male	LOAEL for marginally increased mean corpuscular hemoglobin and mean cell volume (indicative of folate deficiency?), with no change in mean corpuscular hemoglobin concentration (indicative of mild macrocytic normochromic anemia); these effects were not seen in female rats at concentrations below 50,000 ppm	Dietz et al. 1991; NTP 1991

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result in hyperglycemia, restlessness, throat irritation, vomiting that progresses to hematemesis, and progressive nervous system depression as indicated by stupor and shallow respiration (Krasavage et al. 1982; ACGIH 1986; Arena and Drew 1986). The toxic range of acetone has been estimated to be 200-300 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 concentrations up to 33 μg/mL, exposed by either ingestion or inhalation (Gamis and Wasserman 1988). The highest blood concentrations of acetone reported (445 μg/mL) produced stupor, respiratory depression, and convulsions in a 30-month (mo)-old boy who ingested about 8 ounces (oz) (26 mL/kg) of finger nail polish remover composed 65% of acetone, 10% of isopropanol, and 25% not reported. The anesthetic potency of acetone 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).

Inhalation of acetone at >1,000 ppm produces effects on the CNS, gastrointestinal tract, and kidneys in animals and humans. The following signs have been reported: CNS depression indicated by an initial stimulatory and 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; and 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; ACGIH 1986; Arena and Drew 1986; Grant 1986).

Hepatotoxicity

Acetone induces hepatocellular hypertrophy and dose-related increases in liver weight. It also induces microsomal enzymes that metabolize other chemicals, thereby potentially altering the toxicity of xenobiotics. These effects, however, are considered adaptive rather than adverse. The National Toxicology Program (NTP) conducted 2-week (wk) and 13-wk toxicity studies in rats and mice ingesting acetone in drinking water at concentrations of 0, 5,000, 10,000, 20,000, 50,000, and 100,000 ppm (Dietz et al. 1991; NTP 1991). The only histopathologic change as-

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sociated with acetone exposure in both the 2-wk and the 13-wk studies was centrilobular hepatocellular hypertrophy. Male and female mice exposed to acetone at 20,000 or 50,000 ppm developed minimal-to-mild centrilobular hepatocellular hypertrophy, and male mice developed moderate centrilobular hepatocellular hypertrophy at 100,000 ppm. There were no treatment-related clinical signs of toxicity during these studies.

Hematologic Effects

Species and gender differences exist in the hematologic effects of acetone in animals. Bone marrow hypoplasia was reported in five of five male, but no female rats exposed to acetone in their drinking water for 14 d at 100,000 ppm (6,942 mg/kg/d) (Dietz et al. 1991; NTP 1991). In mice treated for 14 d, it was not clear whether bone marrow was examined, but in the 13-wk study, no hematologic effects or histologically observable lesions in hematopoietic tissues were reported in mice (Dietz et al. 1991; NTP 1991).

CNS Toxicity

CNS effects in humans after the ingestion of acetone have been reported in cases of ingestion of large, but rarely quantified, amounts of acetone. No reports were found of CNS effects after ingestion of low doses of acetone in either humans or animals, but the following reports describe effects in humans and rats after inhalation exposure.

Dick et al. (1988, 1989) exposed 22 human volunteers to acetone at 250 ppm by inhalation for 4 h and found small but statistically significant differences from the controls in two measures of the auditory tone discrimination task ($p<0.05$) (a 7-14% increase in response time to detect a 760 hertz [Hz] tone in a series of 750 Hz tones and a 25% increase in false positives but no difference in the percent of correct hits) and on the anger-hostility scale (males only) of the profile of mood states (POMS) test ($p<0.001$). While these results are statistically significant, the small magnitude of the effects and the uncertain biologic relevance of the end points argue against using these results for the purposes of setting spacecraft water exposure guidelines (SWEGs). No other significant effects were seen in three other psychomotor tests (choice reaction time, visual vigilance, and memory scanning), one sensorimotor test (postural sway),

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and the remaining portions of the POMS psychological test. A no-observed-adverse-effects level (NOAEL) of acetone at 125 ppm (10.4 ± 2.4 μg/mL in blood) was reported for all measured effects when the subjects were simultaneously exposed to acetone at 125 ppm and methyl ethyl ketone at 100 ppm. Measurement of acetone concentrations in venous blood indicated that the concentrations at 1.5 h postexposure (11.9 ± 2.6 μg/mL) were about 78% of the concentrations at 4 h (15.3 ± 2.9 μg/mL), and by 20 h postexposure, the acetone concentration in the blood (1.5 ± 1.0 μg/mL) had returned to pre-exposure concentrations of 2 ± 2 μg/mL. (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 minutes (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 longer than 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 3,000, 6,000, 12,000, or 16,000 ppm for 4 h/d, 5 d/wk for 10 d. Body weight and growth were not affected at any dose, but escape behavior was suppressed, and ataxia was noted on day 1 in the 12,000 and 16,000 ppm groups. Avoidance behavior was inhibited in groups exposed at 6,000 ppm, 12,000 ppm, and 16,000 ppm. Tolerance to acetone developed, as evidenced by decreases in all the reported neurobehavioral effects.

Ataxia and Narcosis

Narcotic effects (lethargy, coma) have been described in several case reports of patients who had ingested acetone, but doses were unknown or only estimated (Ramu et al. 1978; Gamis and Wasserman 1988; Sakata et al. 1989). A marked disturbance of gait was observed in one patient 6 d after apparent recovery from coma caused by intentional ingestion of 200 mL of acetone (2,241 mg/kg) (Gitelson et al. 1966).

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Bruckner and Peterson (1981) found that 4-wk-old rats and mice exposed by inhalation to acetone at 12,600-50,600 ppm for up to 3 h were slightly more sensitive to its narcotic effects than were 8- to 12-wk-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 to 19,000 ppm, and 21 h were required to recover from a 3-h exposure at 25,300 ppm (Bruckner and Peterson 1981).

Diabetic Effects

In the case described above of the man who survived ingestion of 200 mL of pure acetone (2241 mg/kg), a diabetes-like condition was reported, including hyperglycemia (2.5 mo after ingestion), polyuria, and excessive thirst (4 wk after ingestion) (Gitelson et al. 1966). According to Gitelson et al., hyperglycemia and glycosuria are commonly seen in cases of acetone poisoning. In humans, Gitelson et al. (1966) note that acetone-induced hyperglycemia appears to be consistently reversible, but after various durations of persistence.

Subchronic and Chronic Toxicity

NTP conducted 2-wk and 13-wk toxicity studies in rats and mice receiving acetone in drinking water at concentrations of 0, 5,000, 10,000, 20,000, 50,000, and 100,000 ppm for 2 wk and 0, 1,250 (male mice only), 2,500, 5,000, 10,000, 20,000, and 50,000 (rats and female mice only) ppm for 13 wk (Dietz et al. 1991; NTP 1991). Decreased water consumption was seen at ≥50,000 ppm in rats and female mice. There were no treatment-related clinical signs of toxicity during these studies.

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Neurologic Effects

No clinical or histologic evidence of neurotoxicity was observed in rats and mice ingesting acetone in drinking water at concentrations of 0, 2,500, 5,000, 10,000, 20,000, and 50,000 ppm for 13 wk (Dietz et al. 1991; NTP 1991).

Nephrotoxicity

The incidence and severity of nephropathy in male, but not female, rats (histologically identical to the chronic progressive nephropathy of aging rats) increased with increasing doses of acetone, particularly at 20,000 or 50,000 ppm in rats exposed for 13 wk (Dietz et al. 1991; NTP 1991). Significantly increased relative kidney weights were seen in female rats exposed to acetone for 13 wk to 20,000 or 50,000 ppm, but in male rats, such increases were significant only at 50,000 ppm. The kidney-weight changes were associated with nephropathy.

Splenic Effects

Minimal-to-mild splenic pigmentation (hemosiderin) was seen in the splenic pulp of male rats ingesting acetone at 20,000 and 50,000 ppm (1,700 and 3,400 mg/kg/d, respectively) in drinking water for 13 wk (Dietz et al. 1991; NTP 1991).

Liver Effects

Minimal hepatocellular hypertrophy occurred in two of 10 female mice exposed to acetone at 50,000 ppm for 13 wk (Dietz et al. 1991; NTP 1991). Increased relative liver weights were seen in female mice given acetone in drinking water for 13 wk at 50,000 ppm and in both sexes of rats at ≥20,000 ppm (Dietz et al. 1991; NTP 1991). In the absence of treatment-related clinical signs of toxicity, these effects are considered adaptive rather than adverse.

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Immunologic and Hematologic Effects

No peer-reviewed reports on the immunotoxicity of acetone were found. A study that is currently available only as a conference abstract reported that acetone administration via drinking water for 28 d in accordance with the U.S. Environmental Protection Agency (EPA) Immunotoxicology Test Guideline did not produce immunotoxicity in CD-1 mice at doses as high as 1,144 mg/kg/d (acetone concentrations up to 6,000 ppm) (Anderson et al. 2004). Body weights and hematologic parameters showed no treatment-related effects because of acetone consumption.

Evidence of macrocytic anemia was seen in male rats exposed to acetone in drinking water for 13 wk (Dietz et al. 1991; NTP 1991) with a lowest-observed-adverse-effect level (LOAEL) of 400 mg/kg/d (5,000 ppm) and a NOAEL of 200 mg/kg/d (2,500 ppm). The evidence consisted of significantly decreased hemoglobin concentration, increased mean corpuscular hemoglobin and mean corpuscular volume, decreased erythrocyte counts, decreased reticulocyte counts and platelets, and splenic hemosiderosis. In another study, increased hemoglobin, hematocrit, and mean cell volume were seen in male but not female rats treated by gavage at 2,500 mg/kg/d for 46 d, and in both males and females at this dose for 13 wk (American Biogenics Corp. 1986).

Carcinogenicity

There are no published studies that have assessed whether exposure to acetone is associated with an increased incidence of cancer in humans.

Genotoxicity

In studies from the NTP, acetone was not mutagenic in Salmonella typhimurium strains TA97, TA98, TA100, TA1535, or TA1537, with or without metabolic activation (NTP 1991). Acetone did not induce sister chromatid exchanges or chromosome 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 5,000-20,000 ppm in drinking water for 13 wk (NTP 1991).

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Reproductive Effects in Humans

Stewart et al. (1975) exposed 10 female volunteers to acetone vapors at 0 or 1,000 ppm for 1 h, 3 h, or 7.5 h/d for 1 wk. Three of the four female subjects exposed for 7.5 h/d experienced early menstrual periods after 4 d of exposure at 1,000 ppm.

Reproductive and Developmental Toxicity in Animals

In male rats, ingestion of acetone at a concentration of 50,000 ppm in drinking water for 13 wk resulted in depressed sperm motility, caudal epididymal weight, and epididymal weight; no significant effects were seen at 10,000 ppm (Dietz et al. 1991; NTP 1991). The relative, but not the absolute, testis weight was increased at 50,000 ppm (Dietz et al. 1991; NTP 1991). Typically, testicular toxicants decrease testes weights, so the biologic significance of these results is unclear. Acetone concentrations of 50,000 ppm produced a statistically significant increased incidence of abnormal sperm (NTP 1991).

Mast et al. (1989) reported mild developmental toxicity and mild maternal toxicity in rats exposed by inhalation to acetone at 11,000 ppm for 6 h/d, 7 d/wk during days 6-19 of gestation, but no effects were seen at 2,200 ppm. In the same study, mice exposed to acetone at 6,600 ppm for 6 h/d, 7 d/wk during days 6-17 of gestation had significant increases in resorptions and significant decreases in fetal weights. The effects on maternal weight were weak. At 2,200 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 EC25 [effective concentration producing malformation in 25% of test embroys]) at 1.0% but not at 0.9%.

Interaction with Other Chemicals

Hepatotoxicity induced by chloroform and other haloalkanes is potentiated by previous administration of ketonic solvents, including acetone, to mice or rats (Hewitt et al. 1980). Cytochrome P4502E1 (CYP2E1) activity was reported to be induced more than 10-fold in Kupffer cells isolated from rats given acetone at 1% volume per volume

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(v/v) in their drinking water for 7 d (Koop et al. 1991). Acetone induces hepatic cytochrome CYP2E1, which potentiated 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). In male rat kidney, acetone treatment induced CYP2E1 apoprotein sixfold (Ronis et al. 1998). Lee et al. (1998) reported that rats given acetone at 5% in drinking water for 7 d had sevenfold increased activities of CYP2E1 in the liver but no such increases in the lung. CYP2E1 is involved in the metabolism of a wide variety of low molecular weight hydrocarbons and halocarbons. While the toxicity of the CYP2E1 metabolites of most compounds is lower than that of the parent compound, the metabolites of some compounds are cytotoxic, potentially mutagenic or carcinogenic.

An investigation of the pharmacologic and metabolic interactions between ethanol and several ketones, including acetone at doses of 10, 20, and 40 mmol/kg, found that acetone doses of 20 and 40 mmol/kg reduced the rate of metabolism and elimination of ethanol from the blood in male CD-1 mice and prolonged the ethanol-induced loss of righting reflex (Cunningham et al. 1989). The acetone, dissolved in corn oil, was injected intraperitoneally (ip) 30 min before the ip injection of ethanol at 4 g/kg. The mean elimination rate of ethanol from the blood was found to be markedly reduced in mice treated with acetone at 40 mmol/kg, which is thought to be related to acetone's reduction of alcohol dehydrogenase activity.

ATSDR's MRL Calculations and Rationale

The intermediate duration MRL was based on a NOAEL value of 200 mg/kg/d (2,500 ppm in drinking water) for macrocytic anemia in rats in the 13-wk drinking water study (see Table 1-3). The NOAEL was divided by an uncertainty factor (UF) of 100 (10 for extrapolation from animals to humans and 10 for human variability). SWEG calculations, however, do not include an intraspecies factor (for interindividual human variability in sensitivity) because the astronaut population consists of

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healthy adults, so protection of very young or sick persons is not required.

EPA's RfD Calculations and Rationale

The EPA's reference dose (RfD) (see Table 1-3) was based on mild nephropathy as the critical effect, as seen only in male rats in the NTP (1991) study: "The data were analyzed using the NOAEL/LOAEL approach using a point of departure of 900 mg/kg-day based on an increased incidence of mild nephropathy. The following UFs are applied to the effect level: 10 for consideration of intraspecies variation (UFH; human variability) [not used in SWEG calculations], 3 ($\sqrt{10}$) for extrapolation for interspecies differences (UFA; animal to human), 3 ($\sqrt{10}$) to account for extrapolation from subchronic studies (UFs; subchronic to chronic), and 10 to account for a deficient database (UFD). The total UF $= 10 \times \sqrt{10} \times \sqrt{10} \times 10 = 1000$" (EPA 2003).

RATIONALE FOR ACs

Acceptable Concentrations (ACs) can be set for the following adverse effects of acetone exposure discussed above: hematologic toxicity (bone marrow hypoplasia and macrocytic anemia) and splenic hemosid-

TABLE 1-3 Exposure Limits Set by Other Organizations

Organization	Exposure Limit (mg/kg/d)	Reference	Equivalent Drinking Water Concentration (mg/L)[a]
ATSDR	2 (intermediate duration MRL)	ATSDR 1994	50
EPA	0.9 (chronic RfD)	EPA 2003	2.5

[a]The equivalent concentration of acetone in water that would yield the indicated mg/kg/d dose (MRL or RfD) for a 70-kg person drinking 2.8 L of water.
Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; EPA, U.S. Environmental Protection Agency; MRL, minimal risk level—an estimated daily dose likely to be without risk of deleterious noncancer effects for a specified duration of exposure of the human population; RfD, reference dose—an estimated daily dose likely to be without risk of deleterious effects for a lifetime exposure of the human population.

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TABLE 1-4 Spacecraft Water Exposure Guidelines

Duration	Concentration (ppm or mg/L)	Target Toxicity
1 d	3,500	Bone marrow hypoplasia
10 d	3,500	Bone marrow hypoplasia
100 d	150	Macrocytic anemia
1,000 d	15	Macrocytic anemia

erin deposits. ACs that protect against these effects will also protect against more-severe effects such as ataxia, immediate CNS depression, and death. Calculation using the guidelines established by the National Research Council's (NRC's) Committee on Toxicology (2000) to determine the highest AC for each major end point and exposure duration is documented below. If exposure data were available for both the concentration of acetone in drinking water and the daily dose (in mg/kg/d) of acetone, the ACs were calculated based on the daily dose. A SWEG value (see Table 1-4) is set for each exposure duration on the basis of the end point that yielded the lowest AC at that exposure duration. The resulting ACs for the various end points are compiled in Table 1-5 and compared.

1-d and 10-d ACs

One-d and 10-d ACs can be based on the 4,300 mg/kg/d (50,000 ppm) NOAEL for bone marrow hypoplasia in male rats after 14 d of exposure (Dietz et al. 1991; NTP 1991). Benchmark dose analysis was not appropriate for the bone marrow hypoplasia data because of the dose-response behavior; that is, the only dose at which hypoplasia was seen was the highest dose (100,000 ppm), at which 100% of test animals had hypoplasia. An interspecies UF of 10 is applied to extrapolate from rats or mice to humans. The AC is not adjusted higher for the shorter-exposure durations (1 or 10 d versus 14 d). Thus, for bone marrow hypoplasia, the AC is calculated as follows:

human dose rate = 4,300 mg/kg/d ÷ 10 (species) = 430 mg/kg/d.

Because microgravity induces a reduction in red cells that would be exacerbated by bone marrow hypoplasia, the AC will be reduced by a spaceflight factor of 3. To achieve a dose rate of 430 mg/kg/d for a 70-kg

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TABLE 1-5 Acceptable Concentrations (ACs)

			UFs					ACs (mg/L)			
End Point	Exposure Data	Species and Reference	Inter-individual	To NOAEL	Inter-species	Exposure Time	Space-flight	1 d	10 d	100 d	1,000 d
NOAEL for bone marrow hypoplasia	4,312 mg/kg/d (50,000 ppm); 2 wk; drinking water	Rat, male (NTP 1991)	1	1	10	1	3	3,500	3,500	—	—
NOAEL for macrocytic anemia	200 mg/kg/d (2,500 ppm); 13 wk; drinking water	Rat, male (NTP 1991)	1	1	10	0.9	3	—	—	150	15
NOAEL for mild nephropathy and splenic hemosiderin	1,700 mg/kg/d (10,000 ppm); 13 wk; drinking water	Rat, male (NTP 1991)	1	1	10	0.9	1	—	—	2,000	200
SWEG								3,500	3,500	150	15

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astronaut consuming 2.8 L of water per d, the concentration of acetone in the drinking water would need to be as follows:

$$\text{1-d and 10-d ACs} = (430 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \div 3 \text{ [spaceflight]}) = 3{,}500 \text{ mg/L (rounded)}.$$

100-d ACs

Mild macrocytic anemia was reported in male rats receiving acetone for 13 wk at doses above 200 mg/kg/d (acetone concentrations >2,500 ppm) (Dietz et al. 1991; NTP 1991). Above 900 mg/kg/d (10,000 ppm), mild nephropathy and splenic hemosiderosis were reported in male rats. Hepatocellular hypertrophy is considered adaptive. Therefore, ACs were calculated based on NOAELs for anemia, nephropathy, and hemosiderosis. For macrocytic anemia, the AC is calculated as follows:

$$\text{100-d ACs} = 200 \text{ mg/kg/d} \div 10 \text{ (species)} = 20 \text{ mg/kg/d}.$$

Because microgravity induces a reduction in the number of red cells that would be exacerbated by bone macrocytic anemia, the AC will be reduced by a spaceflight factor of 3. For a 70-kg astronaut consuming 2.8 L of water per d, the concentration of acetone in the drinking water needed to achieve a dose rate of 20 mg/kg/d would be

$$(20 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times 3) = 170 \text{ mg/L (rounded)}.$$

A factor of 90 d/100 d = 0.9 was used to adjust the NOAEL-based AC for the difference in exposure durations between the 90-d rodent data and the 100-d astronaut exposure durations. Thus, for macrocytic anemia, the AC is calculated as follows:

$$\text{AC} = 0.9 \times 170 \text{ mg/L} = 150 \text{ mg/L}.$$

For mild nephropathy and splenic hemosiderin, the AC is calculated as follows:

$$\text{100-d ACs} = 900 \text{ mg/kg/d} \div 10 \text{ (species)} = 90 \text{ mg/kg/d}.$$

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For a 70-kg astronaut consuming 2.8 L of water per d, the concentration of acetone in the drinking water needed to achieve a dose rate of 90 mg/kg/d would be as follows:

$$(90 \text{ mg/kg/d} \times 70 \text{ kg}) \div 2.8 \text{ L/d} = 2{,}300 \text{ mg/L (rounded)}.$$

A factor of 90 d/100 d = 0.9 was used to adjust the NOAEL-based AC for the difference in exposure durations between the 90-d rodent data and the 100-d astronaut exposure durations. Thus, for mild nephropathy and splenic hemosiderin, the AC is calculated as follows:

$$100\text{-d AC} = 0.9 \times 2{,}300 \text{ mg/L} = 2{,}000 \text{ mg/L (rounded)}.$$

The odor and taste of acetone at 4,000 mg/L in drinking water is distinctly noticeable (mild) but not objectionable to all of six male test subjects (H. Garcia, National Aeronautics and Space Administration, Houston, TX, unpublished material, 2004).

1,000-d ACs

ACs for 1,000-d exposures were derived by dividing the ACs for 100-d exposures by a factor of 10 for longer-exposure duration. For macrocytic anemia, the AC is calculated as follows:

$$\begin{aligned} 1{,}000\text{-d ACs} &= 0.1 \times 100\text{-d ACs} \\ &= 0.1 \times 150 \text{ mg/L} \\ &= 15 \text{ mg/L}. \end{aligned}$$

For mild nephropathy and splenic hemosiderin,

$$\begin{aligned} 1{,}000\text{-d ACs} &= 0.1 \times 100\text{-d ACs} \\ &= 0.1 \times 2{,}000 \text{ mg/L} \\ &= 200 \text{ mg/L}. \end{aligned}$$

The odor and taste of acetone at 400 mg/L in drinking water were undetectable to all of six male test subjects (H. Garcia, National Aeronautics and Space Administration, Houston, TX, unpublished material, 2004).

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Reproductive Effects

After 4 d of exposure to acetone vapors at 1,000 ppm for 7 h/d, female volunteers experienced premature menstrual periods (Stewart et al. 1975). Other than transient irritation, no other adverse effects were reported (Stewart et al. 1975). The National Aeronautics and Space Administration (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

Microgravity is known to cause "space anemia," a decrease in total blood volume and total number of red blood cells but no decrease in the concentration of red blood cells. The mechanism has been shown to involve increased excretion of fluid from plasma into urine and a decrease in the production of erythropoietin and, therefore, decreased production of red blood cells. However, microgravity does not appear to cause any increase in the rate of destruction of red blood cells.

Of the effects induced by exposure to acetone, only bone marrow hypoplasia and macrocytic anemia could potentially be exacerbated by known effects of launch, microgravity, or re-entry. Because the mechanisms involved in the control of space anemia are not well known enough to rule out the possibility of additive or synergistic effects with bone marrow hypoplasia and macrocytic anemia caused by acetone exposures, a spaceflight factor of 3 was used to adjust the ACs.

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2

Ammonia

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

Formula	NH_3
Chemical name	Ammonia
CAS registry no.	7664-41-7
Synonyms	Anhydrous ammonia, AM-FOL, liquid ammonia
Molecular weight	17.03
Melting point	−77.7°C
Boiling point	−33.35°C
Water solubility	31% (at 25°C)
Odor	Sharp and irritating

[a]Data are from Merck Index 1989.

OCCURRENCE AND USE

Ammonia (see Table 2-1) is not routinely measured in air samples from space vehicles; however, ground-based data from simulated space habitats show that under nominal conditions, concentrations of ammonia remain less than 0.2 milligrams per cubic meter (mg/m^3); however, unexpected sources can boost the concentration to 0.9 mg/m^3 or more (James et al. 2002). During the 90-day (d) phase of the Lunar Mars Life Support Test (LMLST), the venting of headspace gases from the bioreactor and wastewater containers caused an increase in the airborne ammonia. The lower concentration (0.2 mg/m^3) represents a steady-state condition between the release of ammonia, primarily from human metabolism, and the removal of ammonia by charcoal filters and humidity conden-

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sate. The typical concentrations of ammonia found in humidity condensate and processed water from space habitats are shown in Table 2-2.

The U.S. Laboratory Module of the International Space Station (ISS) uses anhydrous ammonia in the external loop of the heat exchanger, and there is a remote chance that some of this ammonia could enter the internal heat-exchange loop containing water and then reach the inhabited portion of the module. Because there are many liters of anhydrous ammonia in the external loop, such a series of leaks could result in a catastrophe. If such a leak were suspected, the water-recovery systems would be stopped until the atmospheric ammonia concentration returned to nominal levels. In addition, some payload proponents want to use ammonia in the cooling of their hardware.

TOXICOKINETICS

Ingested ammonia, like endogenously produced ammonia, is readily absorbed from the gastrointestinal (GI) tract, metabolized to urea primarily in the liver, and then excreted (as urea) mostly by the kidney. A healthy person has a high capacity for metabolizing ingested ammonia.

Absorption

Bacteria in the digestive tract produce ammonium ions (NH_4^+) from the metabolism of nitrogen-containing compounds that come from in-

TABLE 2-2 Ammonia in Water Samples[a]

Vehicle or Habitat	Type of sample	Typical Concentration	Reference
Mir space station	Humidity condensate	3-15 mg/L	Pierre et al. 1996
	Processed water	Not detected	
LMLST	Consumed water	0-0.01 mg/L	Pierre et al. 2002
ISS Expeditions 4-5	Potable water	<0.002-0.13 mg/L	Plumlee et al. 2003
	Stored water	<0.002-0.04 mg/L	
ISS Flight 7A	Humidity condensate	20.0-42.0 mg/L	Wyle Report 2002

[a]NH_3 as nitrogen.
Abbreviations: ISS, International Space Station; LMLST, Lunar Mars Life Support Test; mg/L, milligram per liter.

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gested food. The total amount of ammonia produced is about 4,000 mg/d, and about 99% of that is absorbed from the colon (Summerskill and Wolpert 1978). Using a method free of significant interference from glutamine-containing compounds, Brown et al. (1957) found that the range of plasma concentrations of ammonia as nitrogen in 10 healthy young males was 0.30-0.55 mg per liter (L). Thus, the body normally has a high capacity to deal with ammonia and allows very little of it to remain unchanged in the general circulation.

An ingested dose of ammonia appears to be readily absorbed and metabolized to urea in the liver (Conn 1972). In 20 healthy human subjects given ammonium chloride (NH_4Cl) tablets, as done in ammonia tolerance tests (dose of 20.0 mg per pound [mg/lb] of body weight, with a maximum of 3 g administered), the arterial blood concentration of ammonia showed a small transient increase. The peak, which was small, occurred in most subjects at 15 minutes (min), but in a few subjects, the peak occurred after 30 min. However, in a group of cirrhotic patients, the increase in the concentration of ammonia in the blood was much greater and was slower to return to baseline levels. These findings suggest that in healthy subjects, ammonia is readily absorbed from the GI tract and that the liver removes it from the blood (Conn 1972).

In a study of four human subjects who had received surgical colon bypass for chronic hepatic encephalopathy, solutions of different pH and NH_4Cl concentrations were infused. Approximately half as much ammonia was absorbed under acidic conditions (pH = 5), where there is little ammonia, unlike under basic conditions (pH = 9), where a much higher portion of ammonia compared with ammonium ions is present (Castell and Moore 1971).

In people with normal renal function, an increase in dietary protein causes a decrease in the retention of ammonium hydroxide (^{15}N) from NH_4Cl (Richards et al. 1975). Subjects were dosed with ^{15}N-labeled NH_4Cl in five equal doses, each given at 4-hour (h) intervals, with total doses between 9 and 17 mg per kilograms (mg/kg) body weight. About 30% of the isotope was retained in the first 7 d in people on a normal diet; however, those restricted to a low-protein diet for 3 weeks (wk) before the dosing, retained about 70% of the isotope in 6 d (Richards et al. 1975).

Distribution

Ammonium ions that are absorbed from the gut into the portal circulation are converted to urea in the liver and excreted in the urine. Un-

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ionized ammonia diffuses readily into cells, whereas ammonium ions hardly reach the intracellular compartment (Stabenau et al. 1958). Because of the chemical equilibrium between dissolved ammonia and ammonium ions, the latter can be absorbed indirectly. Ammonium compounds that reach the circulatory system can penetrate into cells in the rest of the body as ammonia, where it can be incorporated into proteins.

Metabolism

Ammonia and ammonium ions are metabolized to urea and glutamine in the process shown in Figure 2-1. At physiologic pH (7.25), only 1% of the ammonia is in the form of ammonia (pK_a = 9.25). Bacteria in the gut decompose nitrogenous compounds into ammonia, which is absorbed into the portal circulation and then converted into urea in the liver. Conversely, urea passed from the liver to the GI tract is converted to ammonia by bacterial urease (Powers-Lee and Meister 1988). In the liver, ammonia is also converted to glutamine, which is the storage and transport form of ammonia. Many other enzymatic reactions in the liver produce significant amounts of ammonia (Powers-Lee and Meister 1988); however, a treatise on those is beyond the scope of this document.

Elimination

Ingested ammonia is metabolized to urea and primarily excreted in the urine; however, lesser amounts are excreted in the feces, sweat, and exhaled breath (Richards et al. 1975; Utell et al. 1989). As one would expect, this pattern is similar to that for elimination of endogenously produced ammonia.

TOXICITY SUMMARY

A small number of animal toxicity studies conducted mostly on rodents, suggest that ingested ammonia causes little, if any, systemic toxicity. Exposures at 40 mg/kg/d and above seem necessary to cause any adverse effects, and this dosage should be considered a lowest-observed-adverse-effect level (LOAEL). Exposures of about 20 mg/kg/d appear to be a no-observed-adverse-effect level (NOAEL) (ATSDR 2002). Some

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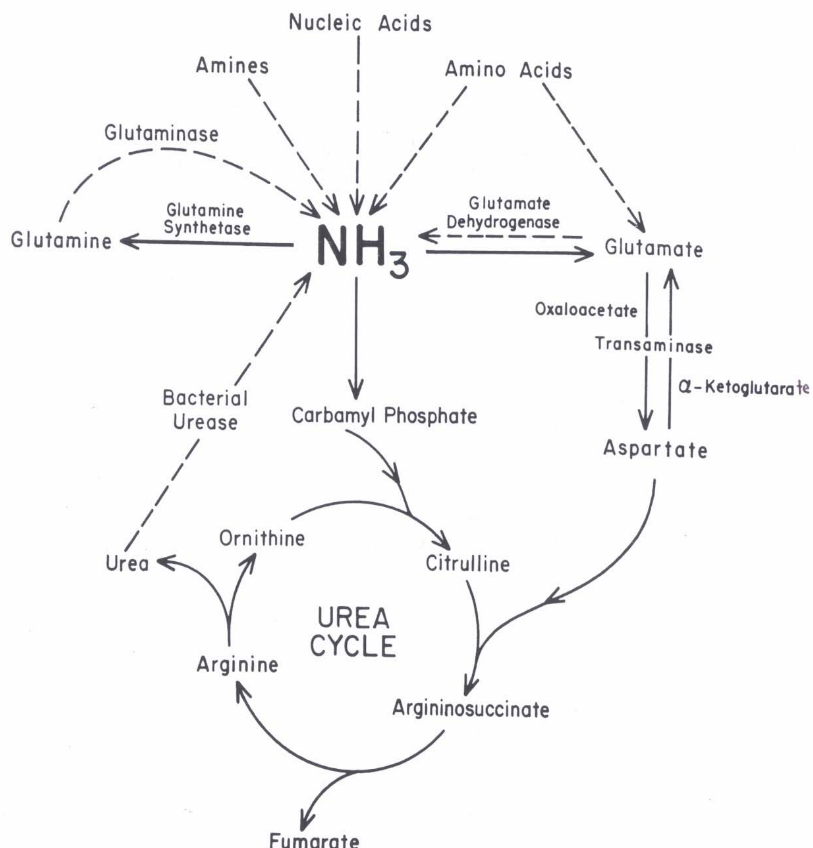

FIGURE 2-1 Metabolism of endogenous ammonia. Source: Powers-Lee and Meister 1988. Reprinted with permission; copyright 1988, Raven Press, New York.

studies are targeted at understanding the neurologic effects of high concentrations of ammonia in the blood as seen in liver failure; however, massive oral doses are necessary to increase and sustain these concentrations in healthy test animals. Effects on neuroreceptor binding and bone formation have been reported. Because only a few studies involve ammonia ingestion, they will not be grouped in the traditional exposure-time intervals. The toxicity database is insufficient to support a discussion of the comparative toxicity of different forms of ingested ammonia;

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furthermore, the form present in potable water is not known a priori. For the ISS, it is likely to be NH_4Cl, but that could change in future systems.

Esophageal lesions and edema were reported in five persons after single ingestions of household ammonia (ammonium hydroxide [^{15}NH]) (Christesen 1995). Ammonia solutions in excess of 4% are generally considered caustic. In adults, only those with symptoms after ingesting strong alkali compounds and other caustic material are at risk for complications, the most common of which is esophageal injury. Thus, systemic injury from ingested ammonia is unlikely for one-time, large aqueous doses.

In a study designed to assess the effect of NH_4Cl on the hypercalcemic effect of parathyroid hormone, one group of male Sprague-Dawley rats was given only NH_4Cl. The dose was a 1.5% (15,000 mg/L) concentration of NH_4Cl in their drinking water for 5-6 d. At this time, the average serum calcium in rats ingesting NH_4Cl was 0.6 mg/dL higher than in rats drinking regular distilled water (Barzel 1975). In an earlier study with the same protocol except that the rats drank the spiked water for 330 d, the investigators found that, compared with the bones of controls, the exposed animals had reduced calcium and less fat-free solid (Barzel and Jowsey 1969). NH_4Cl-induced osteoporosis in growing dogs was reported as part of a study assessing the effects of this model on serum phosphatase (Bodansky et al. 1932).

Effects on neuroreceptor function have been studied in models of hyperammonemia. In one study, Wistar rats were exposed through the dam to ammonia administered as ammonium acetate (20% concentration by weight) from day 1 of pregnancy through weaning (21 d postnatal) (Minana et al. 1995). They demonstrated a long-lasting impairment of *N*-methyl-D-aspartate (NMDA) receptor function in cerebellar neurons placed in culture and tested for binding to [^{3}H]MK-801, which labels open NMDA channels. Body-weight increases in rats held for 140 d and exposed to ammonia either early in life (gestational day 1 to 21 d after birth) or during their entire life were lower than in the controls (Minana et al. 1995). Using another hyperammonemia model in which Wistar rats were given ammonium acetate (20% concentrate by weight in their diet) and 5 millimolar (mM) ammonium acetate in their drinking water for 3, 7, or 15 d, Boyano-Adanez et al. (1996) found a decrease in the equilibrium measures of somatostatin binding to synaptosomes from the frontoparietal cortex and hippocampus. The effect was statistically significant only in the groups exposed for 7 or 15 d.

Renal effects, which are probably adaptive rather than adverse, can be induced by sustained oral doses of NH_4Cl, which result in chronic

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acidosis (Benyajati and Goldstein 1975). Repeated administration of NH_4Cl (5 millimoles per kg [mmol/kg]) twice daily for 3 d to rats about 10 d old resulted in increased ammonia excretion and increased phosphate-dependent glutaminase activity in kidney homogenates. Following cessation of the treatments, the ammonia excretion and enzyme activity fell rapidly to normal levels. The authors considered these reversible changes to be adaptive.

A 90-d administration of ammonium sulfamate to groups of 20 albino rats of various ages at doses of 0, 100, 250, and 500 mg/kg resulted in a NOAEL (Gupta et al. 1979), although the highest-dose group of adult females failed to gain as much weight as the other groups. Clinical pathology results, gross necropsy findings, and histopathology results were not changed by the dose of ammonium sulfamate to the animals.

The most important biologic property of ammonia for our purposes is its odor. The odor threshold for ammonia in air is 5.2 ± 2.0 parts per million (ppm), and it is 1.5 ppm when dissolved in water (Amoore and Hautala 1983). These concentrations are well below any that might induce toxic effects even with prolonged exposure; however, the ingestion of water having an obvious odor would not be tolerated by astronauts for very long.

Cancer

Ammonia alone does not seem to cause cancer in rodents; however, when it is given in conjunction with known carcinogens, it can increase the tumorigenic response. Toth (1972) gave 0.1%, 0.2%, and 0.3% concentrations of ^{15}NH to Swiss mice for their entire lifetimes after they were 5-6 wk old. Similarly, inbred C3H mice were given the lowest dose for their lifetimes. In the Swiss mice, there was no apparent increase in tumor incidence with increasing doses or in comparison to control mice, and the dose given to the C3H mice did not increase the incidence of spontaneous breast tumors.

In a much more limited study, Uzvolgyi and Bojan (1980, 1985) found that neither ammonia alone nor diethyl pyrocarbonate alone caused an increase in lung tumors when given to female Kid: CFLP mice in gavage doses twice per wk for 4 wk, but when the two were given together, more than half the mice showed lung tumors. The authors suggest that the tumors could have resulted from the formation of urethane as a reaction between the two compounds.

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Ammonia may promote tumors induced by *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG) in male Sprague-Dawley rats given 83 mg/L/d of the carcinogen for 24 wk in their drinking water (Tsujii et al. 1995). After the administration of the MNNG, rats were given a 0.01% solution of ammonia or tap water for an additional 24 wk. The number of gastric tumors was approximately double in the MNNG-treated animals receiving the ammonia water compared with those receiving tap water.

Genotoxicity

The database on genotoxicity is scanty at best, and many of the standard assays have never been done. There is one report that 22 workers exposed to ammonia gas at a fertilizer factory had an increase in chromosomal aberrations and sister chromatid exchanges (Yadav and Kaushik 1997). Other studies show mixed results of the potential for ammonia to cause genotoxicity; however, they were carried out before the development of standard assays and consensus-control procedures for such assays (summarized in ATSDR 2002).

Reproductive Toxicity

No studies of the reproductive effects of ingested ammonia were found.

Developmental Toxicity

Except for the study by Minana et al. (1995) described above, no reports of the developmental effects of ingested ammonia were found. Minana et al. reported that exposure to ammonia during gestation and lactation results in offspring with long-lasting NMDA-receptor impairment and decreased body-weight gains. The dose was given in feed as ammonium acetate at 20% of the food by mass. This study did not follow standard protocol for the detection of developmental toxicants and involved a large dose of ammonium acetate, so its relevance to the risk assessment of ammonia in drinking water is uncertain.

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Spaceflight Effects

Bone resorption during spaceflight constitutes one of the more serious, progressive effects of being in microgravity for long periods (Schneider et al. 1994). Even with exercise countermeasures, some bone-mineral-density losses were noted in specific bones. Massive doses of NH_4Cl (about 1.5% in drinking water) that cause metabolic acidosis result in bone resorption and bone deformities in rabbits and dogs (Seegal 1927; Bodansky et al. 1932; Barzel and Jowsey 1969). Given the doses of NH_4Cl necessary to affect bone metabolism, it is extremely unlikely that astronauts could ingest enough ammonia to increase their propensity for bone loss in space.

Synergistic Effects

Synergistic effects of ammonium salts with other chemicals in the body have not been reported except as noted in the cancer section above.

LIMITS SET BY OTHER ORGANIZATIONS

The U.S. Environmental Protection Agency (EPA) has not set a maximum contaminant level (MCL) for ammonia in drinking water, nor is there a reference dose (RfD) for chronic oral exposure. The lifetime health advisory level (HAL) set by EPA (2000) for ammonia is 30 mg/L as nitrogen. A HAL is an estimate of the concentration of a chemical in drinking water that is not expected to cause any adverse noncancer effects for a lifetime of exposure. The World Health Organization (WHO) noted the water odor threshold of 1.5 mg/L and the taste threshold at 35 mg/L and declined to propose a health-based water standard for ammonia (WHO 2002 as cited in the ATSDR 2002). No other drinking water standards were found in a literature search.

The most recent Agency for Toxic Substances and Disease Registry (ATSDR 2004) toxicologic profile for ammonia does not list an oral minimal risk level (MRL).

The Food and Drug Administration (FDA 1973) concluded that ammonia and ammonium salts in food are not a health concern; however, limits were placed on a number of products. Most relevant for the purposes here is the limit set on nonalcoholic beverages of 0.003%, or 30 ppm, for dibasic ammonium phosphate.

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RATIONALE FOR SWEGS

In setting a spacecraft water exposure guideline (SWEG) for ammonia, it is essential that potable water remain free of any factors that might discourage crew water consumption. Although crew members are expected to consume water at a rate of 2,000 mL/d, the amount typically consumed is from 2,000 to less than 1,000 mL/d (Lane and Smith 1999). Urine production is correspondingly reduced from 2,800 mL/d to less than 500 mL/d. Thirst sensation appears to be reduced during spaceflight. The NOAEL in studies of various lengths is approximately 20 mg/kg/d, which for a 70 kg person drinking 2.8 L of water per day leads to a water concentration of 500 mg/L. This is an ingestion of 1,400 mg/d, or about one-third of the amount produced in the adult GI tract (Summerskill and Wolpert 1978). From a strictly toxicologic perspective, that amount would seem to be safe; however, the average odor threshold for ammonia in water is only 1.5 mg/L (Amoore and Hautla 1983). This threshold is based on only two reports with quite different thresholds reported (standard error = 2). The SWEGs were set to ensure that, at least for long periods of time, the crew drinks ample water.

1-d SWEG = 5 mg/L
10-d SWEG = 1 mg/L
100-d SWEG = 1 mg/L
1,000-d SWEG = 1 mg/L

The rationale for the 1-d SWEG is that the crew could drink water with a slight odor for 1 d by mixing something with it that masks the smell. For the longer-term SWEGs (10-1,000 d), there should be little chance that the crew would ever detect an odor from their drinking water and thus drink less. All of these limits are well below any that might be expected to cause adverse health effects. If an ammonia-like odor was coming from the water, the crew would also be expected to adapt and become less sensitive to it.

Finally, these limits are consistent with those accepted for alkylamines (see Chapter 4). For monoalkylamines, the limits for 1, 10, 100, and 1,000 d are 4, 2, 2, and 2 mg/L, respectively. Limits for di- and trialkylamines are somewhat lower than those for monoalkylamines (see Chapter 4). These SWEGs were based on the odor thresholds for these compounds.

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3

Barium and Barium Salts

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PHYSICAL AND CHEMICAL PROPERTIES

Barium (Ba) salts have a range of solubilities in water. Ba metal is insoluble in water but soluble in alcohol. The three least soluble Ba salts are the sulfate, the carbonate, and the sulfide. The acetate, the cyanide, the chloride, and the nitrates and alkaline salts, such as the oxides and hydroxide, are highly soluble in water (see Table 3-1), some even at 0°C. In general, the acid-soluble Ba salts are very toxic when compared with the least soluble salts, such as Ba sulfate ($BaSO_4$).

OCCURRENCE AND USE

Ba is one of the alkaline earth metals. It occurs in nature as a free metal and as salts. It is also produced for various industrial uses. The Ba salt most commonly found in the earth's crust is $BaSO_4$, which is found in limestone (barite), shales, and rocky sediments. In a crushed form, it is the source for several other Ba compounds.

The major use of $BaSO_4$ is in the oil and gas industry to make lubricant muds for drilling. Ba in salt forms has been reported to be present in almost all surface waters (at 2-340 micrograms per liter [µg/L]) (Kopp and Kroner 1967). The release of Ba compounds from Ba manufacturing and processing plants and from sedimentary rocks by leaching in certain areas might be the reason for its presence in surface waters. The finished water of public systems frequently contains Ba at 1-172 µg/L (Tuovinen 1980). A mean concentration of 43 µg/L and a maximum of 380 µg/L were reported in the largest U.S. cities (NRC 1977, pp. 229-230)

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TABLE 3-1 Physical and Chemical Properties of Ba and Ba Compounds

Compound	Form	Molecular Weight	Solubility	Comment
Ba	Ba	137.3	Insoluble in water; soluble in alcohol	
Ba chloride	$BaCl_2$	208.2	375 g/L at 26°C	65.95% Ba
Ba chloride dehydrate	$BaCl_2 \cdot 2H_2O$	244.3	375 g/L at 26°C	56.2% Ba
Ba acetate	$Ba(CH_3COO)_2$	255.5	1 g/1.5 mL	53.77% Ba
Ba sulfate	$BaSO_4$	233.4	1.6 mg/L at 20°C	Also known as "barite"; 58.84% Ba
Ba carbonate	$BaCO_3$	197.37	20 mg/L at 20°C	69.58% Ba
Ba sulfide	BaS	169.42	1.1 mg/L	81.08% Ba
Ba nitrate	$Ba(NO_3)_2$	261.38	Freely soluble	52.55% Ba

Source: Data from Merck 1989.

McCabe et al. (1970) and Calabrese (1977) reported that Ba was present in about 2,600 analyzed drinking water samples. It was found at about 1.5 milligrams (mg) per L in samples from areas of northern Illinois and northern Iowa. Because the solubility of Ba depends on the concentrations of total sulfate in the medium and because sufficient concentrations of sulfates are in the natural waters, it is difficult to maintain more than 1.5 mg/L in water (EPA 1985). The World Health Organization (WHO) has reported that the range of daily dietary intake of Ba is 300-1,770 μg per day (4-25 μg per kilogram body weight per day). WHO (1990) also reported that the concentrations of Ba measured in U.S. drinking waters are 1-20 μg/L. Several other reports indicated that the concentrations are much higher (Kopp 1969; Calabrese 1977).

Ba compounds are used to make not only drilling lubricants but also paints and pigments, textile dyes, greases, bricks, tile, glass, and rubber. Ba nitrate ($Ba(NO_3)_2$) is used in pyrotechnics. Because of its high radiopacity, $BaSO_4$ has been used by doctors for taking x-rays of the stomach, intestines, and respiratory and urinary tract systems and in bronchography. A high-density suspension of Ba sulfate—usually 340 g suspended in 150 mL of water—is administered orally for radiologic evaluation of the small bowel. This represents a dose of 4.89 g per kilogram (kg).

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Humans might be exposed to Ba at high concentrations in occupational settings or in areas proximate to Ba mining and processing.

PHARMACOKINETICS AND METABOLISM

Absorption

Only very scanty human data are available on the absorption of Ba and its salts from the gastrointestinal (GI) tract. Lisk et al. (1988) studied the absorption and excretion of selenium and Ba in humans who consumed Brazil nuts, which contain both. The nuts they used contained Ba at 1,953 parts per million (ppm). As part of the study, one male subject (68 kg) ingested nut meat equivalent to Ba at 179.2 mg (2.64 mg/kg) in a single dose. Total urine and feces for each day was collected for 15 days (d) after the dose. From the total amount of Ba excreted in feces and urine, which was only 10% of the ingested dose, it appears that 90% of the dose was absorbed. This is the highest percentage ever reported for Ba absorption. It is not clear what form of Ba is contained in Brazil nuts or how other materials, including fat, would have facilitated such a high absorption.

The absorption rate was estimated to be 5% in the adult (ICRP 1973) on the basis of results from two human volunteers. It has also been suggested that infants might have significantly greater absorption rates than adults (Lengemann 1959). For radiologic examination of the small bowel, a solution of Baricon, a highly insoluble suspension of $BaSO_4$ at about 4.89 g/kg, or Ba at 2.88 g/kg, is usually administered to patients. In clinical practice, the amount given to adults is not based on body weight; the commercial preparation of preweighed $BaSO_4$ (340 g) is suspended in 150 mL of water. According to the *Merck Index* (Merck 1989), $BaSO_4$ is practically insoluble in water (1 g in 400,000 parts), dilute acid, and alcohol. It is soluble only in hot, concentrated sulfuric acid. In the above example, the 150 mL of water dissolves $BaSO_4$ at only 0.375 mg (Ba at 0.003 mg/kg). Neither the pH of stomach acid nor the alkalinity of the intestinal contents contributes to the solubility of ingested $BaSO_4$ used in the clinical setting. Dallas and Williams (2001) cited a study from a student thesis (Bligh 1960) in which isotopic Ba was given intravenously (iv) or given in orange juice as an oral dose to cancer patients. Feces and urine samples were collected for 7-10 d, and radioactivity was deter-

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mined. Bligh 1960, as cited in Dallas and Williams (2001), also performed equivalent experiments in 15-month- (mo) old female rats to compare Ba metabolism in humans with that in animals. The study concluded that an average of 9% of radioactive Ba was absorbed in humans and about 10% in rats.

In laboratory animals, absorption varies significantly with species, age, and composition of the diet. For example, Taylor et al. (1962) reported that although older rats absorbed 7% of ingested Ba chloride ($BaCl_2$), the amount absorbed by rats 22 d old or younger was at least 10 times higher. Fasted adult rats absorbed about 20%. GI absorption in dogs has been calculated to be about 7% (Cuddihy and Griffith 1972).

Measurements of Ba in the serum of dogs indicate that peak absorption from the GI system occurs within 1 h (Chou and Chin 1943). McCauley and Washington (1983) examined the effect of oral intubation of various anions of the Ba salt on the relative rates of uptake and tissue deposition in rats. Male Sprague-Dawley rats weighing 250-300 g were maintained on a diet of Ba at less than 1 mg/kg of food for at least 1 mo before the experiment. They were orally administered ^{131}Ba as sulfate (SO_4), chloride (Cl), or carbonate (CO_3) at pH 7.0 (0.5 mL/100 g body weight of a 10 mg/L solution). Animals sacrificed at 2, 5, 10, 20, 30, 60, and 120 minutes (min) and 24 hours (h) after intubation. Amount of radioactivity was measured in the blood and various tissues at various intervals. One group of animals was also intubated with ^{131}Ba as the chloride anion ($BaCl_2$) after the 24-h fast and studied at various times thereafter.

With $^{131}BaCl_2$, in the nonfasted rats, the maximum concentrations of ^{131}Ba in blood was reached in 60 min, and at 24 h, the activity was about 90% of the maximum value, indicating a very slow rate of elimination. However, in the fasted rats, the peak radioactivity was measured at 15 min, and the radioactivity after 4 h was only 50% of the peak activity, indicating that fasting can affect both the rate of absorption and elimination. In the nonfasted rats, when ^{131}Ba was intubated as the sulfate of the carbonate anion, the peak radioactivity in the blood was reached at 60 min, similar to the nonfasted $BaCl_2$ group. The authors stated that when large oral doses of BaSo4 are used in radiopaque x-ray diagnosis, only a very small fraction is absorbed. No overt toxic effects have been reported from "Ba swallows" ($BaSO_4$ administered orally) during diagnostic procedures.

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Distribution

From the available studies on the distribution of Ba in the human body, it appears that Ba distributes mostly (over 93% of the body burden) in the bones and teeth and to a small extent, in the eye, lungs, skin, and adipose tissue in humans at less than 1% of total body weight (Schroeder et al. 1972; ATSDR 1992).

From autopsy data, Sowden and Stitch (1957) reported that the Ba concentrations in human bones ranged from 7 ppm in children (0-3 y) to 8.5 ppm in adults (33-74 y), indicating no age-related accumulation. In mice iv injected with $^{131}BaCl_2$, the radioactivity was localized primarily in the bones, although distribution into other tissues was also observed (Dencker et al. 1976). Twenty-four hours after ^{131}Ba (as $^{131}BaCl_2$) intubation, the ^{131}Ba concentration measured in five tissues (activity per g tissue weight) was in the order: heart > eye > skeletal > muscle > kidney > liver. The concentration in the cardiac muscle was at least threefold higher than that of the eye. In addition, the accumulation of radioactivity in the eye from the $BaSO_4$ and the $BaCl_2$ groups were similar in the $BaCO_3$-intubated rats; it was 50% of that of the $BaCl_2$-treated group (McCauley and Washington 1983). The radioactivity in the bones was not reported.

Excretion

In several studies, radioactive Ba was iv injected for the evaluation of the kinetics profile in urine and feces. In humans, the main excretion route for ingested Ba is the feces (about 72%); about 3% is excreted in the urine (Tipton et al. 1966). Harrison et al. (1967) reported that when ^{133}Ba was iv injected to one healthy 60-y-old man, 9% of the injected dose was excreted in the urine and 84% was excreted in the feces. In another study by the same authors, cumulatively 20% of the injected dose was excreted in urine and feces in 24 h, 70% in 3 d, and about 85% after 10 d (Tipton et al. 1966). The excretion half-life was about 35 h. According to the National Research Council (NRC) (1977), 20% of ingested Ba is excreted in the feces and 7% in the urine within 24 h. In dogs that received Ba by gavage, most of the Ba was excreted within a few days (Cuddihy and Griffith 1972). Syed et al. (1981) reported that 10-15% of a radioactive dose of Ba iv injected was excreted in the feces within the first 24 h after dosing; the report noted very similar rates in rats and mice. In one study, it was reported that rats and humans excreted Ba in

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comparable ways, and fecal excretion of Ba was found to be greater than urinary excretion (Bligh 1960, as cited in Dallas and Williams 2001). Approximately 0.5% of the Ba dose was excreted into bile within 2 h in bile-duct-cannulated Sprague-Dawley rats iv injected with $^{133}BaCl_2$ at 1.8 µg. In parallel to plasma concentrations, biliary Ba concentrations reached their peak in the first 15 min after administration and rapidly declined thereafter; Ba was not concentrated in the liver. Biliary excretion is not important for elimination when Ba is ingested (Edel et al. 1981). The kinetics of Ba elimination appear to have three phases. Rundo et al. (1967) estimated that the half-times of ^{133}Ba for these phases were 3, 6, 34.2, and 1,033 d.

TOXICITY SUMMARY

A review of the literature on pharmacokinetics (absorption, distribution, and elimination) and toxicity (described in the sections below) indicates that there are several complexities in those areas. For example, although $BaSO_4$ is considered extremely insoluble in water, some studies comparing uptake kinetics could not differentiate $BaSO_4$ from highly soluble Ba compounds. There are wide variations in the amounts reported for Ba absorption as a function of dose that have not been described clearly in the literature. Although several studies have described the cardiovascular effects of Ba, $BaSO_4$ is used extensively in everyday diagnostic radiology without any serious adverse consequences that might cause it to be discontinued.

Acute Toxicity (<1 d)

Ba, like other alkaline earth metals, is a bone-seeking element (Bauer et al. 1957), but its function in bone is not known. Ba can replace calcium and can carry out several calcium-mediated effects, such as the release of adrenal hormones and of neurotransmitters from adrenergic synapses (Douglass and Rubin 1964). Ba can also release catecholamines in the absence of acetylcholine. The most studied effects of Ba toxicity are vasoconstriction, hypertension, and those that occur in the muscular system. These end points have been the focus of several investigations of chronic low-dose exposure.

Several studies conducted in the late 1960s and early 1970s suggest that electrocardiogram (ECG) abnormalities were encountered during Ba

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enema x-rays of older patients and patients with heart diseases. ECG abnormalities were pronounced in patients over the age of 60. Minor ECG abnormalities occurred in the lower age range in patients over 60 y of age (Berman et al. 1965; Eastwood 1972). In 1975, Roeske et al. studied patients over 60 and reported potentially serious effects such as incidence of arrhythmias and ST-segment changes during Ba enema x-ray studies. In 58 unselected, consecutive patients (ages 60-98 y old) undergoing routine Ba enema examinations, 12-lead ECG and 100-cycle cardiac rhythm recordings were performed, in addition to systemic arterial pressure measurements in the supine and upright positions. These tests were done before, during, and after the enema study. Subsequent ST-segment changes were also analyzed from the ECG recordings. In 27 of the 58 patients, abnormal ECG recordings (positive alterations as defined by stated criteria) were noted. Twenty-three patients developed significant arrhythmias during the Ba enema, four exhibited new ST-segment depressions, and 31 patients had negative results. The significant observations were that patients who were taking digitalis (an anti-arrhythmia medication) did not exhibit positive ECG alterations, whereas patients who were not receiving digitalis exhibited positive results. Furthermore, randomly chosen patients who received an iv dose of glucagon to prevent intestinal spasms did not respond differently from those who did not receive glucagon. This indicated that atrial arrhythmias and ST-segment changes might not be because of Ba-induced GI spasms.

Blakeborough et al. (1997) reported the results of a 1992-1994 survey, including questionnaires completed by U.K. consultant radiologists, of complications of Ba enema examinations in 750,000 patients. An overall mortality of about 1 in 57,000 was reported. A total of 82 complications (contraindications) were reported. Of 16 patients observed to have cardiac arrhythmias, 9 died. Seven of the nine patients who died were over the age of 75. Only a few of the other patients had a previous history of cardiac problems. $BaSO_4$, the insoluble salt of Ba, was used. There were no reported cases of such effects from Ba swallows during radiologic examinations. Thus, Ba-induced cardiac arrhythmias seem to occur only during enemas in extremely few cases, and patients over age 70 appear to be more susceptible if they have a prior documented history of cardiac disease. Because these patients had a health condition that warranted such a diagnostic procedure, application of these results to normal healthy populations could be limited.

In general, the toxicity of soluble Ba salts is far greater than that of insoluble forms (NRC 1977) when administered orally. Profound changes in cardiac, skeletal, and smooth-muscle functions resulting from

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acute and accidental ingestion of soluble Ba salts have been reported. Several investigators (Roza and Berman 1971; Talwar and Sharma 1979; Wetherill et al. 1981) have reported cases of hypertension, paralysis of skeletal muscle, and cardiac arrests due to ingested soluble Ba salts. Infusion by iv of $BaCl_2$ to anesthetized dogs or guinea pigs resulted in increased blood pressure and cardiac arrhythmia (Roza and Berman 1971; Hicks et al. 1986). The study also reported skeletal muscle flaccidity and paralysis in dogs (Roza and Berman 1971). Although extremely rare, anomalies of cardiac rhythm during the Apollo and Skylab missions (Leguay and Seigneuric 1981) and episodes of ventricular tachycardia during long-duration missions (Fritsch-Yelle et al. 1998, see also readers' notes in Ellestad 1999) have been reported. On the basis of those findings, there is concern that ingestion of Ba could potentiate such effects during short- and long-duration missions.

There have been numerous case reports of humans exposed to Ba through accidental or intentional oral ingestion. Death occurred in six cases of accidental or intentional ingestion of Ba salts. Two deaths were attributed to cardiac arrest (one from severe GI hemorrhage), and in three cases, the specific cause was not determined (Ogen et al. 1967; Das and Singh 1970; Talwar and Sharma 1979). Several cases of food poisoning from Ba carbonate were reported by Lewi and Bar-Khavim (1964) and Diengott et al. (1964). Symptoms of gastroenteritis, a feeling of numbness, diarrhea, vomiting, muscular twitching, and paralysis resulted from ingesting meat contaminated with $BaCO_3$. Of all cases, 5-10% were fatal within the first 48 h. In two patients, flaccid paralysis was found to be because of hypokalemia caused by low-serum potassium, and ECG recordings showed typical hypokalemic changes. Administration of potassium changed the clinical course or accelerated recovery from Ba poisoning, indicating that at least some of the effects are the result of hypokalemia.

The acute oral LD_{50} (dose lethal to 50% of subjects) for Ba varies with species, compound, and age. For example, in the rat and guinea pig, the LD_{50} for $BaCl_2$ is 118 and 76 mg/kg, respectively (Sax 1984). In humans, the lowest dose that caused death (LD_{LO}) for $BaCl_2$ is 11.4 mg/kg, while it is 70, 170, and 90 mg/kg in the mouse, rabbit, and dog, respectively (Sax 1984). Mortality has been observed in experimental animals following acute and chronic oral exposures to $BaCl_2$ and Ba acetate ($Ba(CH_3COO)_2$) (Schroeder and Mitchener 1975; Tardiff et al. 1980; Borzelleca et al. 1988). The acute oral LD_{50} values for female and male rats were determined to be 269 and 277 mg/kg, respectively (Borzelleca et al. 1988), after the rats were gavaged with $BaCl_2$ in water at doses

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ranging from 60 to 960 mg/kg (Ba at 40-640 mg/kg). Primary necropsy indicated hemorrhagic areas in the stomach and inflammation of the intestines. Tardiff et al. (1980) initially conducted an acute oral toxicity study of $BaCl_2$ in adult (60-70 d old) and weanling (21-25 d old) male and female rats to determine the LD_{50} for each group. The acute oral LD_{50} values were calculated to be 132 mg/kg in the adult rats and 220 mg/kg in the weanling rats.

Syed and Hosain (1972) reported LD_{50} values for different Ba salts administered via iv to two strains of mice (ICR and Swiss-Webster). For the chloride, nitrate, and acetate, the LD_{50} values were in the ranges of 8.12-11.32 mg/kg for the Swiss-Webster mice and 19.2-23.31 mg/kg for the ICR mice. The values for $BaCl_2$, $Ba(NO_3)_2$, and $Ba(CH_3COO)_2$ did not vary appreciably, although $BaCl_2$ was the most toxic. Because of the wide variation in the absorption of Ba salts, these values cannot be extrapolated to oral doses.

In a 1-d exposure study, Borzelleca et al. (1988) gavaged rats with $BaCl_2$ at 30, 100, and 300 mg/kg after a 24-h fast. A decrease in body weight, liver-to-brain weight ratios, and increases in kidney weight as a percentage of body weight were noted only at the highest dose. It appears that 100 mg/kg is a no-observed-adverse-effect level (NOAEL) for 1 d. Changes that occurred in the clinical chemistry were not dose related. At necropsy, male rats that received 300 mg/kg showed ocular discharge, fluid in the trachea, and darkened liver. In addition, inflammation of both the small and large intestines was seen in both the male and female rats at 300 mg/kg. On the basis of these findings, $BaCl_2$ at 100 mg/kg (equivalent to elemental Ba at 66 mg/kg) was identified as a NOAEL.

Infusion via iv of $BaCl_2$ to anesthetized dogs (Roza and Berman 1971) or guinea pigs resulted in increased blood pressure and cardiac arrhythmia (Hicks et al. 1986). Roza and Berman (1971) conducted a study on intact, anesthetized mongrel male and female dogs to elucidate the mechanisms of Ba-induced hypokalemia and hypertension and the interaction of Ba and potassium in the hearts of dogs in vivo. The interesting observations in this study were hypertension, a decrease in plasma potassium and an increase in red-cell potassium (shift in the potassium from extracellular to intracellular water) that caused hypokalemia, and myocardial toxicity resulting from hypokalemia. Increased mean corpuscular-red-cell volume (23% increase) leading to a substantial increase in hematocrit was also observed. In part of the study, seven dogs were iv infused with $BaCl_2$ at two rates of infusion (one dog at 1 micromole [μmol]/kg/min and six more dogs at 2 μmol/kg/min). ECG changes were tracked. The cessation of infusion was determined by the appearance of

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an abnormal ECG. Although an increase in blood pressure was invariably seen during the first 5-10 min of infusion, it subsided 30-40 min after the infusion was finished. Although this was an iv-infusion study, one can attempt to extrapolate to an oral dose using an oral-absorption factor. There is no NOAEL, but the appearance of an abnormal ECG is considered a lowest-observed-adverse-effect level (LOAEL).

Short-Term Toxicity (2-10 d)

In a separate study by Borzelleca et al. (1988), male and female Sprague-Dawley rats (22-30 d old, 10 per group) were gavaged with $BaCl_2$ in deionized water at 100, 145, 209, or 300 mg/kg for 10 d (doses of Ba at 66, 96,138, or 198 mg/kg/d). Mortality of the female rats in the 198 mg/kg group increased, and one male rat from the 209 mg/kg group died. No consistent pathologic findings were noted. Decreased body weights and decreased ovary-to-brain weight ratios were noted in female rats. Decreased blood urea nitrogen (BUN) was observed in females in all treated groups but was observed in males only at the highest dose. Male rats showed a decrease in leukocytes at 209 mg/kg but not at the higher dose. The significant differences in BUN at all doses in female rats indicate that female rats are more sensitive to the short-term toxic effects of $BaCl_2$. A dose of Ba at 66 mg/kg appears to be a LOAEL for changes in BUN. The clinical significance of the extent of the change observed in this study is questionable.

Subchronic Toxicity (11-100 d)

In a human study conducted by Wones et al. (1990), 11 male volunteers (ranging in age from 27 to 61 y of age) with no history of hypertension, diabetes, or any cardiovascular disease participated in a 10-week (wk) protocol. The subjects consumed plain drinking water at 1.5 L/d for the first 2 wk. That was followed by 4 wk of consuming 1.5 L of water containing Ba as $BaCl_2$ at 5 ppm/d; and that was followed by 4 wk of consuming 1.5 L/d of water containing Ba as $BaCl_2$ at 10 ppm. The subjects were on a controlled basal diet (dietary contribution of Ba was assumed to be 0.75 mg/d). ECGs and 24-h continuous ECG monitoring were performed. The treatments did not result in any apparent changes in blood pressure, cholesterol, triglyceride, glucose, or potassium concentrations. According to the findings of the Wones et al. (1990) study, a

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dose of 10 mg/L (equivalent to a dose of Ba at 0.21 mg/kg/d and water consumption at 1.5 L/d) can be considered a NOAEL for adverse effects of Ba in humans exposed for 4 wk.

Tardiff et al. (1980) exposed male and female Charles River rats to Ba (as $BaCl_2$) at 0, 10, 50, or 250 ppm in drinking water for 4, 8, or 13 wk. The estimated doses ranged from 1.7-45.7 mg/kg/d. For all dose levels, the intake of Ba decreased because of reduced water intake, and at termination of the study, the dosage rate was at half of the initial dose. No changes related to Ba ingestion were observed in clinical signs, hematologic parameters, or serum chemistry. The only change noted was a statistically significant decrease in water consumption in the highest-dose group. A slight decrease of adrenal weights in treated animals was noted. The increase in dose, not the increase in duration, produced increased concentrations of tissue Ba; the highest concentration was found in bone. Blood pressure was not measured in this study. A concentration of 50 ppm was identified as a NOAEL for change in water consumption.

The National Toxicology Program (NTP 1994) conducted a 13-wk study in which male and female F344/N rats were exposed to $BaCl_2$ dihydrate at 0, 125, 500, 1,000, 2,000, and 4,000 ppm (Ba at 0, 10, 65, 110, and 200 mg/kg/d for males and 0, 10, 35, 65, 115, and 180 mg/kg/d for females). In the highest-dose group, three males and one female died. The cause of death could not be determined. Significant decreases in water consumption were noted in the 4,000 ppm group. Relative and absolute organ-weight changes were also noted in the 2,000 and 4,000 ppm groups. Mild focal and multifocal areas of dilation were seen in the renal proximal tubules of the 4,000 ppm group. In addition to these effects, significant decreases in the magnitude of undifferentiated motor activity were observed in both sexes of rats receiving the 4,000 ppm dose, whereas the decreases were marginal at other doses. No other changes pertaining to neurobehavioral assessments were noted. Also, no changes were seen in cardiovascular measurements such as heart rate, systolic blood pressure, or ECG. The three notable adverse effects in the 13-wk NTP drinking water study are the effects on the renal proximal convoluted tubules (accompanied by significantly increased relative kidney weights), the effects on motor activity, and the significant decreases in water consumption. For renal effects, a LOAEL of 2,000 ppm (Ba at 180-200 mg/kg) and a NOAEL of 1,000 ppm (Ba at 110-115 mg/kg) were identified.

In another subchronic-to-chronic exposure study by Perry et al. (1989), female weanling Long Evans rats (45 g body weight) were pro-

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vided drinking water containing Ba (salt not specified) at 1, 10, or 100 ppm. Water intake, systolic pressure, hematocrit, plasma catecholamine, and plasma concentrations of inorganic ions and others were measured at 1, 2, 4, 5, 12, and 16 mo. The average systolic pressure increased significantly after exposure at 100 ppm for 1 mo or longer. Thus, a dose of 10 ppm can be identified as a NOAEL using changes in systolic pressure as the toxicity end point. It is not known if weanling rats are more sensitive than adult rats to $BaCl_2$-induced hypertension. The study used a rye-based diet deficient in some required minerals, which limits the findings.

Chronic Toxicity (>101 d)

A health survey of workers at a Sherwin-Williams plant concluded that workers exposed by inhalation to Ba ores and $BaCO_3$ during grinding and mixing operations for at least 5 y had a significantly higher incidence of hypertension (7/12 or 58%) compared with workers who never worked in Ba processes (5/25 or 20%) (NIOSH 1982).

Data on chronic toxicity of oral ingestion of Ba^{+2} come from human epidemiologic studies and animal studies. In a retrospective human epidemiologic study, Brenniman and Levy (1985) collected data from two communities in northern Illinois that had markedly different concentrations of Ba^{+2} in their drinking water. In one community, the mean concentration of Ba^{+2} in the drinking water was 0.1 mg/L (0-0.2 mg/L); in the other community, the mean concentration was 7.3 mg/L (2-10 mg/L). The study report had two parts: the first compared the mortality in these two communities (Brenniman et al. 1979), and the second (Brenniman et al. 1981) reported the results of blood pressure and health questionnaire data from a randomly selected pool of subjects. The pool included residents 18-75 y old who had lived in the community for more than 10 y. Data analysis was also performed on a subpopulation that did not have water softeners in their homes. The authors reported that the water concentration of other minerals, socioeconomic conditions, and demographic characteristics were comparable in the two communities.

In the first part, age- and gender-adjusted cardiovascular mortalities in the two communities were compared. Male and female mortalities from all “cardiovascular diseases” and “heart disease (arteriosclerosis)” in the high-exposure community were significantly higher than those in the low-exposure community. Generally, the group of people age 65 and older accounted for the largest clinical differences (Brenniman et al.

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1979). Population mobility was greater in the high-exposure community, and confounding factors, such as the use of water softeners, were not accounted for.

In the second part of the study, three blood pressure measurements were taken within a span of 20 min, and responses to a questionnaire were collected from test subjects. The health questionnaire included questions about subjects' medications and documented heart diseases, stroke and renal diseases, age, gender, smoking habits, and family history. No significant differences in mean systolic or diastolic blood pressures or in rates of hypertension, heart disease, stroke, or kidney disease were found between the men and women of the community with increased Ba concentrations and those of the community with low Ba concentrations. In a later analysis of the data, criteria were used for the number of years of residency, use of high blood pressure medications, and use of water softeners. No significant differences in mean systolic and diastolic pressure were found between the two communities in either males or females (Brenniman et al. 1981). When males and females 18-75 y of age who did not use water softeners or take high blood pressure medications and who had lived in the community more than 10 y were considered, data from only 85 males and 116 females from one community and 71 males and 93 females from the other community could be used. Study results were not affected when these criteria were included in the analysis. In addition, the prevalence of hypertension, stroke, heart disease, and kidney disease was not significantly different in males and females in these two communities. These results have been summarized by Brenniman and Levy (1985).

Perry et al. (1989) administered $BaCl_2$ to female weanling Long Evans rats in drinking water at 0, 1, 10, or 100 ppm (estimated doses of Ba at 0, 0.07, 0.7, or 7.1 mg/kg/d) for 1, 4, or 16 mo (the 1-mo study was described above). Ba doses in the 16-mo study were 0, 0.054, 0.54, or 5.4 mg/kg/d. Over the 16 mo of the experiment period, the average intake of water varied from 16 to 28 mL/d, and food intake ranged from 15 to 23 g/d per rat. The rats were fed a diet low in trace metals. Systolic blood pressure was measured at 1, 2, 4, 8, 12, and 16 mo. In the 4-mo study, significant increases in blood pressure were observed in rats in the highest-dose group; no change in blood pressure was observed in rats treated with either of the lower doses. Significant increases in blood pressure also were noted in the 16-mo study in rats treated with 10 ppm and 100 ppm doses (0.54 and 5.4 mg/kg/d); no changes were observed in the 1-ppm group. Thus, the systolic blood pressure progressively increased with time in the 100 ppm group; it was 17 millimeters of mercury (mm

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Hg) higher than in the corresponding controls. Functional and biochemical studies of the heart after 16 mo of exposure to Ba at 100 ppm indicated significantly decreased contractile element-shortening velocity (decreased cardiac-contraction rates and depressed excitability). These studies identified a NOAEL of 1 ppm (0.17 mg/kg/d) for hypertension.

In another chronic exposure study (McCauley et al. 1985), groups of male and female rats received drinking water containing Ba (as $BaCl_2$) at 1, 10, 100, or 250 ppm—female rats for 36 wk and male rats for 46 and 68 wk. Histologic examinations of many tissues did not reveal any significant changes. It was reported that there were no significant changes in food or water consumption. In an extension of the study during which ECG recordings were made, Sprague-Dawley-derived CD rats were administered $BaCl_2$ in drinking water for 5 mo at 0 or 250 ppm (estimated intakes of 1 and 38.5 mg/kg/d due to the 1 mg/kg/d diet contribution). In this study, Ba induced a significant enhancement of 1-norepinephrine-induced bradycardia compared with controls 4 min after norepinephrine administration. The Ba-treated rats showed normal heart rates by 60 min, whereas controls had depressed heart rates.

In the chronic portion of the 1994 NTP study, male and female F344/N rats were exposed to $BaCl_2$ in drinking water for 2 y at 0, 500, 1,250, and 2,500 ppm (calculated doses of Ba at 0, 15, 30, and 60 mg/kg/d for males and 0, 15, 45, and 75 mg/kg/d for females). No significant increases in mortality occurred in the Ba-exposed groups. Only small reductions were noted in body weights in the 2,500 ppm group. Dose-related decreases in water consumption of about 23% were observed in male and female rats. The only chemical-related sign of kidney toxicity was an increase in the relative and absolute kidney weights of females at 2,500 ppm, an effect seen even at the 15-mo interim evaluation.

NTP (1994) also examined the subchronic and chronic toxicity of Ba in mice. In these studies, groups of male and female $B6C3F_1$ mice received Ba as $BaCl_2$ dihydrate in drinking water. They received 0, 125, 500, 1,000, 2,000, or 4,000 ppm for 13 wk or 0, 500, 1,250, or 2,500 ppm for 2 y. The animals were fed an NIH-07 diet; Ba content was not reported. Increased mortality was observed in the subchronic and chronic toxicity studies at the highest doses tested (4,000 and 2,500 ppm, respectively). Tubule dilatation, renal tubule atrophy, tubule cell regeneration, and the presence of crystals primarily in the lumen of the renal tubules were observed, indicating renal toxicity. The mice also exhibited elevated BUN, another indicator of renal toxicity. For renal effects, NOAELs were identified at 2,000 ppm for the subchronic exposure dura-

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tion (205 and 200 mg/kg/d for male mice and female mice, respectively) and 1,250 ppm for the chronic exposure duration (75 and 90 mg/kg/d for male and female mice, respectively).

Schroeder and Mitchner (1975a, b) exposed groups of male and female Long Evans rats and Charles River CD mice to Ba (as $Ba(CH_3COO)_2$) at 0 or 5 ppm in drinking water. They reported proteinuria—significant compared with that in controls—in male rats exposed to Ba in water for 152 d. An increase in serum cholesterol in females and an alteration in serum glucose concentrations in males were also observed. No adverse alterations in life span, growth, or histopathology of the heart, lungs, kidneys, liver, or spleen were observed in either species. Thus, these studies identify a LOAEL of 5 ppm (Ba at 0.61 mg/kg/d) for renal glomerular damage, evidenced as proteinuria, in male rats maintained on low-mineral diets. A NOAEL of 5 ppm (Ba at 1.2 mg/kg/d) was identified for similarly exposed mice.

Genotoxicity and Mutagenesis

No data on in vivo genotoxic effects of Ba were available. Most in vitro studies in prokaryotic test systems revealed that Ba was not mutagenic. For example, $BaCl_2$ and $Ba(NO_3)_2$ were negative in the Ames assays with *Salmonella typhimurium* strains TA1535, TA1538, TA1537, TA97, TA98, and TA100 with or without metabolic activation (Monaco et al. 1991). $BaCl_2$ did not inhibit growth in wild and recdeficient *Bacillus subtilis* strains (Nishioka 1975). Negative results have also been observed for $Ba(NO_3)_2$ in the rec assay using *B. subtilis* strains H17 and H45 (Kanematsu et al. 1980). The NTP (1994) report describes a detailed battery of in vitro genetic toxicologic assays conducted on $BaCl_2$ (NTP 1994). $BaCl_2$ induced gene mutations in L5178Y mouse lymphoma cells with metabolic activation but not in the absence of metabolic activation (NTP 1994). Tests on the fidelity of DNA replication by avian myeloblastosis virus DNA polymerase did not indicate any effects of $Ba(CH_3COO)_2$ or $BaCl_2$ (Sirover and Loeb 1976). In mammalian cells, $BaCl_2$ did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells, with or without activation (NTP 1994).

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Carcinogenicity

None of the reported epidemiologic studies indicated any Ba-related cancer in humans. Under the U.S. Environmental Protection Agency's (EPA's) proposed *Guidelines for Carcinogenic Risk Assessment* (EPA 1996), Ba would be classified as a Group D ("not classifiable as a carcinogen to humans"). Rats and mice were exposed to Ba at 5 ppm (equivalent to 0.7 mg/kg/d for rats and 0.95 mg/kg/d for mice) as $Ba(CH_3COO)_2$ in drinking water for their lifetime (about 540 d) (Schroeder and Mitchener 1975a, b). Gross and microscopic examinations of heart, lungs, liver, kidneys, and spleen did not reveal differences in the incidence of tumors between Ba-acetate-treated animals and vehicle controls in either the Long-Evans study or in the Swiss-Webster mice study. Only one exposure dose was used in this study. In the Tardiff et al. (1980) study, no histopathologic abnormalities of the liver, kidneys, spleen, heart, brain, skeletal muscle, femur, or adrenals were found in rats exposed to Ba at up to 250 ppm in drinking water for 90 d. In the McCauley et al. (1985) $BaCl_2$ drinking water study, neoplasms were observed in several tissues, but they were not chemical related.

In a chronic exposure study conducted by the NTP (1994), male and female $B6C3F_1$ mice (60 animals of each gender per dose group) received $BaCl_2$ dihydrate in drinking water at concentrations of 0, 500, 1,250, or 2,500 ppm for 2 y. From water consumption and body weight data, the authors estimated the daily doses for the treated groups to be 30, 75, and 160 mg/kg/d for males and 40, 90, and 200 mg/kg/d for females. The animals continued in the study until they were moribund or died naturally, or they were sacrificed at the end of the study. Necropsy and complete histopathologic examinations were performed on all animals. The incidence of neoplasms in the Ba-exposed mice was not significantly higher than in control mice.

In the same chronic exposure study (NTP 1994), male and female F344/N rats (60 animals per gender per dose group) received drinking water containing $BaCl_2$ dihydrate at 0, 500, 1,250, or 2,500 ppm for 2 y. Using measured water consumption and body weights, the authors estimated daily Ba doses for the treated groups at 15, 30, and 60 mg/kg/d for males and 15, 45, and 75 mg/kg/d for females. No chemical-related, noncarcinogenic histologic changes were observed in any organs or tissues. No statistically significant increases in the incidence of neoplasms were observed in the Ba-exposed rats. Although benign and malignant pheochromocytoma of the adrenal medulla (combined) and mononuclear cell leukemia were noted in male rats, mammary gland neoplasms (fibroade-

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noma, adenoma, or carcinoma) were observed in female rats, these changes appeared to decrease with increasing dose. These studies strongly indicate that Ba (as Ba salts) administered orally is noncarcinogenic to animals (see Table 3-2 for a summary of the studies).

Reproductive Toxicity

No data were located regarding reproductive effects of orally ingested Ba in humans. However, some data from animal studies have been reported. Borzelleca et al. (1988) reported that in rats gavaged with $BaCl_2$ at 98 mg/kg/d, no changes in testicular weight and no gross lesions of the ovaries or testes were observed; however, at that dose, decreased ovary weight and decreased ovary-to-brain-weight ratio were noted. Intermediate and chronic oral exposure of rats to nominal concentrations of Ba (37.5 or 15 mg/kg/d, respectively) in drinking water was not associated with any gross or histopathologic lesions of the uterus, ovaries, or testes (McCauley et al. 1985). There are no reports on the effects of Ba (orally ingested) on reproductive function, even though inhalation of $BaCO_3$ dust has been reported to induce disturbances in spermatogenesis in male animals and shortened estrous cycle and morphologic changes of ovaries in females (Tarasenko et al. 1977).

Developmental Toxicity

Little information is available on the developmental effects of Ba after oral exposure. Morton et al. (1976) reported that when Ba concentrations in drinking water increased, the rate of congenital central nervous system malformations decreased. Increased mortality, increased leukocyte count, disturbances in liver function, and increased urinary excretion of hippuric acid were observed in offspring of female rats administered $BaCO_3$ at 18.3 mg/kg/d orally during pregnancy (Tarasenko et al. 1977).

RATIONALE

Acceptable concentration (AC) values were determined following the guidelines of the NRC (2000). For each exposure duration, the

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TABLE 3-2 Toxicity Summary for Ba Salts Administered Orally

Ba Compound	Dose and Route of Exposure	Exposure Duration	Species	Effects	Reference
$BaCO_3$	Acute exposure—accidental poisoning	<1 d	Human	Diarrhea, vomiting, weakness in limb to paralysis, muscular twitching, GI hemorrhage,	Lewi and Bar-Khayim 1964
$BaCO_3$	Food poisoning	<1 d	Human (one male and one female)	Death, severe weakness in limbs, high blood pressure, abnormal ECG, tingling of the extremities	Diengott et al. 1964
$BaCl_2$	Abnormal ECG; 48-21 mg/kg	<1 d	Dogs (iv infusion study)	Hypokalemic and cardiovascular effects; a combination of ectopic ventricular contractions, ventricular tachycardia, etc.; abnormal ECG	Roza and Berman 1971
$BaCl_2$	Oral gavage; range of doses from 34 to 540 mg/kg to find median lethal dose	1 d	Rats, male and female	95% of effects occurred within 5 h after the dose; medial lethal doses for male and female rats were 419 and 408 mg/kg, respectively	Borzelleca et al. 1988
$BaCl_2$	Oral gavage; 20, 66, and 198 mg/kg	1 d	Rats (fasted overnight), male and female	8 of 10 males and 7 of 10 females died within 24 h in the 198 mg/kg groups; fluid in the trachea, darkened liver, inflammation of the intestines in both sexes of rats at the highest-dose group, as well as both sexes showing decreased liver-to-brain and kidney-to-body weight ratios; changes were not dose consistent; insignificant histopathology	Borzelleca et al. 1988

(Continued)

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TABLE 3-2 Continued

Ba Compound	Dose and Route of Exposure	Exposure Duration	Species	Effects	Reference
$BaCl_2$	Oral gavage; 66, 96,138, and 198 mg/kg	10 d (1/d)	Rats, male and female	At the max dose, lower survival of female rats; decreased body weight; decreased ovaries-to-brain weight ratios; decreased BUN in all doses in females and only at the highest dose in males	Borzelleca et al. 1988
$BaCl_2$	Drinking water; 2 wk no Ba, then 4 wk of 0.12 mg/kg, then 4 wk of 0.21 mg/kg	10-wk split study ($BaCl_2$ only for 8 wk)	Human, males	None of the following parameters showed any change: systolic, diastolic BP, plasma total cholesterol and triglycerides, serum and urinary albumin, serum and urinary concentrations of sodium and potassium; no significant arrhythmia	Wones et al. 1990
$BaCl_2$	Drinking water; 0.0643, 0.643, and 6.43 mg/kg	1, 2, 4,12, and 16 mo	Rats, female weanling	At the end of 1 mo, systolic BP increased at highest dose and continued during the entire study; the high-dose group at 16 mo showed depressed cardiac contractility and conduction	Kopp et al. 1985; Perry et al. 1989
$BaCl_2$	Drinking water; 0, 2.75, 13.7, and 66.25 mg/kg	4, 8, and 13 wk	Rats, male and female	No hematologic or serum chemistry changes; decreased water consumption in the high-dose group	Tardiff et al. 1980

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$BaCl_2$	Drinking water; 0, 1, 10, 100, and 250 ppm (1, 1.15, 2.5, 16, and38.5 mg/kg/d, respectively), for histopathology study; 0 or 250 ppm for ECG study; and 0.3, 10, or 100 ppm for blood pressure studies; 10 ppm was equal to Ba^{+2} at 1.5 mg/kg	Females: 36 wk; males: 46 and 68 wk	Rats, male and female	Three studies: histopathology, ECG, and BP studies; heart muscle sensitization at 5 mo (enhancement of NE-induced bradycardia); renal gross morphology (ultrastructural changes in the glomeruli; no histopathologic lesions in the kidney	McCauley et al. 1985
$BaCl_2.2\ H_2O$	Drinking water; 0, 10, 65, and 200 mg/kg for males; 0, 10, 35, 65, 115, and 180 mg/kg for females	Chronic and subchronic study (13 wk and 2 y)	Rats, male and female; mice, male and female	Chemical-related deaths in females; significant decreases in water consumption; increased absolute and relative organ weight changes; minimal to minor dilation of renal proximal tubes; decreased motor activity in both sexes at the highest dose; no cardiovascular-related parameters changed	NTP 1994
Ba $(CH_3.COO)_2$	Drinking water; 0 or 0.61 mg/kg for mice and 0.25 mg/kg for rats	Life-term study	Rats, male and female; mice, male and female	A significant reduction in longevity; proteinurea in male rats at 5 mo; increased serum cholesterol in females	Schroeder and Michner 1979

Abbreviations: BP, blood pressure; BUN, blood urea nitrogen; ECG, electrocardiogram; GI, gastrointestinal; iv, intravenous; NE, norepinephrine.

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TABLE 3-3 Spacecraft Water Exposure Guidelines for Soluble Ba (Salts)

Duration	Concentration (mg/L)	Toxicity End Point	Data Source
1 d	21	Cardiotoxicity	Roza and Berman 1971
10 d	21	Cardiotoxicity	Roza and Berman 1971
100 d	10	Decreased water consumption	Tardiff et al. 1990; NTP 1994
1,000 d	10	Decreased water consumption	NTP 1994

spacecraft water exposure guideline (SWEG) value (see Table 3-3) was set on the basis of the lowest value among the ACs for all the significant adverse effects at that exposure duration. ACs were calculated assuming a nominal potable water use of 2.8 L/d (including 0.8 L of water used for the reconstitution of food), in contrast to EPA's reference volume of 2 L/d of water. A value of 70 kg was used as the nominal adult body weight. Changes in body weights and organ weights alone were not considered biologically relevant adverse effects.

Spaceflight is known to reduce the volume of blood and the number of peripheral blood cells; therefore, spaceflight might have an additive effect to those of chemicals that produce adverse hematologic effects. Due to shifts in body fluids, astronauts tend to feel less thirsty and may become dehydrated; therefore, chemicals that affect water consumption could be very detrimental to spaceflight crew. Hence, a factor of 3 was applied during the AC calculations to reduce the risk of these effects. Astronauts are physically challenged, resulting in potentially increased sensitization to cardiac arrhythmia. The National Aeronautics and Space Association (NASA) uses a factor of 5 to modify the ACs of chemicals that can increase sensitization to cardiac effects. In the case of Ba, such a factor has been applied when the end point is cardiac toxicity (see 1-d AC and 10-d AC).

Inorganic chemical analysis of humidity condensate samples and the recycled-processed water samples from several missions in Mir indicated that in the humidity condensates, the ionic Ba concentrations occurred at a maximum of 113.92 mg/L. The average concentration in all humidity condensate samples was 0.64 mg/L. Analysis of Ba in the recycled-processed water from humidity condensates indicated the presence

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of Ba^{+2} at a maximum of 2.44 mg/L and an average of 0.19 mg/L. It was found in 18 of 22 samples analyzed. The maximum concentration exceeded the NASA-Russian interim water quality specifications of 1 mg/L and the EPA maximum contaminant level (MCL) of 2.0 mg/L. (See Table 3-4 for summary of guidelines set by government organizations.) Hence, it was decided to derive SWEGs for different durations of exposure though drinking water (Pierre et al. 1999). The NRC (1982) calculated a recommended concentration of 4.7 mg/L. It was originally derived from the American Conference of Governmental Industrial Hygienists (ACGIH) 8-h time-weighted average (TWA) using an absorption factor of 20% and a safety factor of 2.

The Agency for Toxic Substances and Disease Registry (ATSDR) (1992) did not derive minimal risk levels (MRLs) for Ba ingested orally for acute, intermediate, or chronic durations, because human case studies did not provide adequate dose characterization related to adverse effects, and the animal studies did not provide sufficient data to identify a target organ. Even though a LOAEL and a NOAEL can be derived from the blood pressure studies of Perry et al. (1985, 1989), the resulting MRL would have been 20 times lower than the WHO estimated intake of Ba from dietary and other sources.

EPA derived an oral reference dose (RfD) of 0.07 mg/kg/d on the basis of a NOAEL of 7.3 mg/L obtained from the epidemiologic study of Ba exposure from Illinois drinking water supplies (Brenniman et al. 1984). An uncertainty factor (UF) of 3 for intra-individual sensitivities was used, and the human reference body weight of 70 kg was assumed, as well as a water intake rate of 2 L/d. The values were substantiated by the Wones et al. (1990) experimental study in humans. The study did not report any changes, including in ECGs, and thus, may serve as a good lower-bound risk estimate for adverse effects on the cardiovascular system.

Dallas and Williams (2001) reviewed the principal and supporting studies in which hypertension was identified as the critical effect or end point that were used by EPA in 1998 for the oral RfD (McCauley and Washington 1983; Brenniman and Levy 1985; McCauley et al. 1985; Wones et al. 1990; NTP 1994). EPA also considered increases in kidney weights reported in the NTP (1994) subchronic and chronic exposure rat and mice studies to be an adverse renal effect. According to Dallas and Williams, the NTP (1994) study should be used as the principal study, and renal effects should be considered the most appropriate end point for deriving an RfD. Hence, these authors assert that EPA should revise the

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TABLE 3-4 Current Water Regulations and Guidelines Set by Other Organizations

Organization	Standard	Value
EPA (EPA 2002)	MCLG	2 mg/L
	MCL	2 mg/L
	HA	
	1-d HA (child)	0.7 mg/L
	10-d HA (child)	0.7 mg/L
	RfD[a]	0.07 mg/kg/d
	Lifetime HA	2 mg/L
	DWEL	2 mg/L
	Cancer Group	D
ATSDR	Acute oral MRL	None derived
	Intermediate oral MRL	None derived
	Chronic oral MRL	None derived (see Rationale section for explanation)
State of Alabama	Drinking water	1.0 mg/L
	Groundwater	1.0 mg/L
District of Columbia	Public water supply	1.0 mg/L
State of Florida	Surface and potable water	1.0 mg/L
State of Texas	Community water system	1.0 mg/L

[a]While this document was in the final stages of going to the press, NASA learned that EPA has revised the RfD for barium and compounds. According to the revised RfD assessment that was available in July 2005, it has been established that the oral RfD is 0.2 mg/kg/d due to the selection of a new principal study and critical effect, the 2-y NTP drinking water study and nephropathy, respectively, and using benchmark dose modeling. EPA used only one model, the multistage model, that they considered the best fitted model. EPA used $BMDL_{05}$ and factors of 10 for animal to human extrapolation, 10 for interindividual sensitivities, and 3 for data deficiencies, (a total UF of 300) (IRIS 2005). One might note that NASA also used the same end point from the same study and used benchmark dose modeling. However, no further discussions on the revised RfD value from EPA and its change in approaches are included in this document.

NOTE: The values for the air are provided here because initially the safe drinking water levels were calculated by using the 0.5 mg/m^3 air data and applying an absorption factor of 20% for extrapolation to oral ingestion; see NAS 1977 and NAS 1982).

Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; EPA, U.S. Environmental Protection Agency; HA, Health Advisory; MCL, maximum contamination level; MCLG, maximum contamination level goal; MRL, minimal risk level; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; TLV-TWA, Threshold Limit Value–time-weighted average.

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RfD for Ba on the basis of renal effects, such as the renal tubular dilatation, multifocal to diffuse nephropathy, and kidney lesions observed in the NTP (1994) study. This is also supported by the McCauley et al. (1985) study in which signs of inefficient glomerular filtration and hypertension were reported. Dallas and Williams (2001) identified NOAELs for renal effects from the NTP studies (60 mg/kg/d for male rats, 75 mg/kg/d for female rats, 75 mg/kg/d for male mice, and 90 mg/kg/d for female mice) and, using the most sensitive NOAEL (male rats appeared to be more sensitive), calculated an RfD. Modifying factors proposed by Doursen (1994) were used in the derivation of the proposed RfD:

$$(60 \text{ mg/kg/d}) \div (10 \times 3 \times 1 \times 3 \times 1 \times 1)$$
$$= 0.66 \text{ mg/kg/d (rounded to 0.6 mg/kg/d)},$$ [1]

where

60 mg/kg/d = NOAEL;

10 = human variability (intraspecies variability);[2]

3 = interspecies extrapolation factor;

1 = subchronic to chronic factor;

3 = insufficiency in the database (uncertainty whether this dose will protect against effects such as developmental or reproductive toxicity);

1 = LOAEL to NOAEL (because NOAEL is already known) extrapolation factor; and

1 = outstanding uncertainties adequately addressed.

1-d AC

No human acute study data were available to derive a 1-d AC. The available data are from accidental oral poisoning with Ba salts at uncertain doses yielding very serious adverse effects. For example, the case report from Talwar and Sharma (1979) describes the cardiotoxic effects

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[1]Note this value is about an order of magnitude over the existing EPA RfD value of 0.07 mg/kg/d. Using the EPA default variables of 70 kg body weight and 2 L/d, this RfD proposed by Dallas and Williams can be calculated to yield a DWEL (Drinking Water Equivalent Level) of 23 mg/L (0.66 mg/kg/d × 70 kg ÷ 2 L/d).

[2]NASA will not use a human variability factor of 10 for calculating the ACs for barium; a species factor of 10 will be used.

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of $BaCl_2$ following an accidental ingestion of rat poison. Although the changes were suggestive of acute myocardial injury, there were no data on the dose. There are a number of such reports.

Using mongrel dogs, Roza and Berman (1971) studied the hypokalemic and cardiovascular effects, such as hypertension, resulting from iv infused $BaCl_2$. In these experiments, an increase in blood pressure invariably was observed, but that toxicity end point cannot be used for humans, because the association between blood pressure and Ba intake has not been well established. The iv infusion of $BaCl_2$ was done for various periods of time at various infusion concentrations until the dogs showed evidence of abnormal ECGs. In addition, the investigators observed generalized muscle twitching and skeletal muscle contractions. In another part of the investigation, the authors observed changes in the mean corpuscular volume (23% increase) and a decrease in plasma potassium with a concomitant increase in red-cell potassium. A 1-d AC was calculated on the basis of cardiotoxicity.

The dogs were infused with 1 or 2 µmol/kg/min. The cessation of infusion was determined by the appearance of abnormal ECG. The total dose was calculated on the basis of that time and the dose of Ba was converted to µg/kg/min. Multiplying the dose and time yields the cumulative dose. That was set as the LOAEL. It was calculated for each dog, and a mean of the doses was taken. One value was rejected as an outlier. An absorption factor to reflect differences between the iv and oral routes was used, and a value of 10% was used in this case. The iv LOAEL was multiplied by a factor of 10 to get the oral-equivalent LOAEL dose. Even though the infusion was carried out for a duration shorter than 100 min, the dose is considered to be a 1-d dose and was not extrapolated for time. To obtain a NOAEL, a factor of only 2 was applied because this NOAEL can be considered sufficiently lower than the lower-bound estimate of the LOAEL. The detailed 1-d AC calculations are as follows, as recommended by the committee (the oral-equivalent LOAEL in this study for abnormal ECG was 84 mg/kg/d):

$$(84 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2 \times 10 \times 2.8 \text{ L/d} \times 5)$$
$$= 20 \text{ mg/L (rounded from 21 mg/L)},$$

where

2 = LOAEL-to-NOAEL extrapolation factor;

10 = species extrapolation factor; and

5 = spaceflight factor and for cardiotoxicity and other effects on muscle contraction.

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The following factors were considered when using iv data to derive exposure concentrations for the oral route. Split oral doses over the period of a day would not result in a consistent blood level (steady-state concentration) that would be sustained over as long a period of time as an infusion and cause such abnormal responses in cardiograms. The built-in safety factor is that the total amount of Ba will not be consumed at one time but will be ingested in several boluses over active crew time (about 16 h).

Two 1-d ingestion studies on Ba salts were initially designed to derive an LD_{50} in animals (Tardiff et al. 1980; Borzelleca et al. 1988). Borzelleca et al. (1988) also studied the adverse effects of ingestion of Ba salts at doses below the LD_{50}. Rats were gavaged with $BaCl_2$ at 30, 100, or 300 mg/kg (equivalent to Ba at 20, 66, and 198 mg/kg) in water. Twenty-four hours later, decreases in body weight and liver-to-brain-weight ratios, increases in kidney weight as a percentage of body weight (at the highest dose), and observed changes in hematocrit and clinical chemistry were not dose related. At necropsy, male rats receiving $BaCl_2$ at 300 mg/kg showed ocular discharge, fluid in the trachea, and darkened liver. In addition, inflammation of the small and large intestines was observed in both male and female rats at 300 mg/kg. The rats were fasted for 24 h prior to the gavage dose. There was a very high mortality at this dose, but there were no mortalities at 100 mg/kg, which is only three times lower. The mortality dose-response curve appears to be steep. In the subsequent 10-d study, when the same high dose of 300 mg/kg was administered by gavage to nonfasted rats, only 3 of 10 females died even after 10 doses. Subsequently, a dose of $BaCl_2$ at 100 mg/kg (equivalent to Ba at 66 mg/kg) can be identified as a NOAEL for these effects on the GI tract and the lungs. From these data for GI inflammatory effects, a 1-d AC can be calculated as follows:

$$(66 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 165 \text{ mg/L},$$

where

70 kg = nominal body weight;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption.

10-d AC

Wones et al. (1990) administered $BaCl_2$ in drinking water to 11 human male volunteers in a 10-wk protocol. In the first 2 wk, no Ba was

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administered. In the next 4 wk, the subjects were exposed to Ba at 5 ppm (0.11 mg/kg/d). That was followed by 4 wk of exposure to Ba at 10 ppm (0.21 mg/kg/d). This study was evaluated for deriving a 10-d AC. No significant changes in systolic blood pressure or in any variables related to hypertension (plasma total cholesterol, triglycerides, low-density lipoprotein [LDL] or high-density lipoprotein [HDL], serum albumin, and potassium concentrations) were noted. This study indicated that Ba at 0.21 mg/kg/d did not induce any abnormal ECG. If an AC were calculated on the basis of that value, it would be overly conservative. These data were from a human study, but it is interesting to note that in the 2-y NTP (1994) $BaCl_2$ subchronic and chronic exposure study, there was no evidence of changes in cardiovascular measurements, which included systolic blood pressure, heart rate, and ECGs from each of the rats at the end of 15 mo of exposure to $BaCl_2$ through drinking water.

In an NTP (1994) study, male and female F344/N rats were administered concentrations of $BaCl_2$ dihydrate in drinking water at 0, 125, 250, 500, 1,000, or 2,000 ppm for 15 d (estimated delivered doses of Ba at 10, 15, 35, 60, or 110 mg/kg). Water consumption by male and female rats that received Ba at 110 mg/kg was about 16% lower than that of the controls during week 2. A NOAEL for Ba of 60 mg/kg/d was identified.

A 10-day AC for Ba for decreased water consumption can be calculated as follows:

$$(60 \text{ mg/kg} \times 70) \div (10 \times 2.8 \text{ L/d} \times 3) = 50 \text{ mg/L (rounded)},$$

where

60 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
3 = spaceflight factor for dehydration.

The 10-d short-term toxicity study by Borzelleca et al. (1988) was also considered for deriving an AC for 10 d. In this protocol, male and female Sprague-Dawley-derived CD rats were gavaged a water solution of $BaCl_2$ at doses of 100, 145, 209, and 300 mg/kg (equivalent to Ba at doses of 66, 96, 138, and 198 mg/kg/d). Death of female rats occurred at the 138 and 198 mg/kg doses of Ba. Although no compound-related body-weight or relative-organ-weight changes were noted, decreases in BUN, a variable usually of clinical importance in the diagnosis of renal toxicity, decreased with all four doses in the female rats and at 198 mg/kg doses in the male rats. The changes, although statistically signifi-

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cant, were small and may not be of clinical significance. In addition, the decrease did not appear to be dose related. Hence, the data were not used for AC derivation. In the NTP (1994) $BaCl_2$ drinking water study, BUN was not altered in male or female rats.

The study by Perry and associates (Kopp et al. 1985; Perry et al. 1989) was also evaluated for deriving a 10-d AC. Weanling female Long-Evans hooded rats were provided Ba as $BaCl_2$ in drinking water at 1, 10, and 100 ppm (Ba at 0.071, 0.71, and 7.1 mg/kg/d) and studied for 1-16 mo. Indirect measurement of systolic pressure of unanesthetized rats indicated that after exposure to Ba at 100 ppm (7.1 mg/kg/d), the average systolic pressure increased significantly (12 mm Hg higher than the mean of controls) for 1 mo or longer. From this study, Ba at 100 ppm (7.1 mg/kg/d) appears to be a LOAEL for blood pressure changes. The study was not used for AC calculations because the rats received a rye-based diet, which is low in trace metal content compared with standard lab chow, which includes calcium and potassium. It was noted that the calcium content of the diet is below minimum requirements, and because calcium and Ba act as agonists, the animals may have been more sensitive to Ba administration. Hence, the data were not used because of the two key confounding factors of calcium and potassium content in the diet.

A 10-d AC was also obtained from the 1-d acute-cardiotoxicity study carried out in dogs by Roza and Berman (1971) described in the 1-d AC section. Once Ba is absorbed into the systemic circulation, it is rapidly removed from blood and deposited in the bone. Because cardiotoxicity and effects on skeletal muscle activity depend on circulating Ba concentrations, it is doubtful whether the blood concentrations of Ba will accumulate if ingested daily at this dose (21 mg/L). Furthermore, in the NTP (1994) studies in rats that received Ba at up to 200 mg/kg/d (as $BaCl_2{\cdot}2H_2O$) in drinking water for 13 wk, there were no changes in heart rate or blood pressure, nor did that result in abnormal ECGs. So, the 1-d AC was extended for use also as a 10-d AC, all safety factors still being applicable:

10-d AC for cardiac toxicity = 20 mg/L (rounded from 21 mg/L).

100-d AC

To derive a 100-d AC, the NTP 13-wk subchronic drinking water exposure study (NTP 1994), and studies by Tardiff et al. (1980) and Perry et al. (1989) were considered.

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In the Tardiff study, groups of young adult rats (4 wk old) of both sexes were provided $BaCl_2$ in drinking water for 4, 8, and 13 wk at concentrations of 0, 10, 50, and 250 mg/L. (The average Ba dose range was 1.7-45.7 mg/kg/d, based on the cumulative 13 wk dose given by the authors.) There were no compound-related adverse effects on clinical signs, hematologic measurements, serum enzyme activities, serum ions, gross pathology, or histopathology. The only significant observation was the reduction in water consumption in the high-dose group in male and female rats. Since dehydration is an important factor for spaceflight crews, a 100-d AC was derived for this parameter. A LOAEL for Ba of 45.7 mg/kg/d and a NOAEL of 9.7 mg/kg/d were identified.

Thus, a 100-d AC for Ba for reduction in water consumption can be calculated as follows:

$$(9.7 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times 3 \times [100 \text{ d}/91 \text{ d}])$$
$$= 7 \text{ mg/L (rounded)},$$

where

9.7 mg/kg /d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption;
3 = spaceflight factor for dehydration; and
100 d/91 d = time extrapolation factor.

A benchmark dose (BMD) calculation could not be carried out, because standard deviations for individual doses and corresponding water consumption data were not available.

The Perry et al. (1989) study could not be used because of reasons mentioned under the 10-d AC derivation section: the rats were on a low-mineral diet, which may have increased the sensitivity of rats to Ba-induced hypertension.

In an NTP (1994) 13-wk subchronic exposure study, groups of male and female F344/N rats were exposed to $BaCl_2$ dihydrate in drinking water at 0, 125, 500, 1,000, 2,000, or 4,000 ppm (0, 10, 65, 110, and 200 mg/kg/d for males and 0, 10, 35, 65, 115, and 180 mg/kg/d for females). In at least 30% of both male and female rats exposed to the highest dose, minimal-to-mild focal and multifocal areas of dilation of the renal proximal convoluted tubules were observed. These renal histopathologic lesions were not severe. Similar effects were observed in the glomerulus by McCauley et al. (1985) in rats given Ba^{+2} at 1,000 ppm in

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their drinking water; thus, the NTP observation is considered consistent and significant. Because this effect was also accompanied by an increase in absolute and relative kidney weights at the 2,000 ppm dose, a LOAEL of 2,000 ppm and a NOAEL of 1,000 ppm (Ba at 65 mg/kg/d for both sexes) for renal effects were identified. Another important observation in this NTP study (1994) is that there was a substantial reduction in the calcium concentrations in the upper levels of femoral bone in male and female rats receiving $BaCl_2$ in drinking water at 65 mg/k/d measured at 15 mo after the start of the dose. Ba is a bone-seeking element and is known to replace calcium from the bone. Bone demineralization and kidney stone formation are important concerns for short- and long-duration spaceflights; this renal effect may be exacerbated by Ba exposure. Hence, a factor of 3 for space-related effects will also be applied.

A 100-d AC for renal effects can thus be calculated as follows:

$$(65 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L} \times [100 \text{ d}/91 \text{ d}] \times 3)$$
$$= 50 \text{ mg/L (rounded)},$$

where

65 mg/kg/d = NOAEL;
10 = species extrapolation factor;
70 kg = nominal body weight;
2.8 L/d = nominal water consumption;
100 d/91 d = time factor for extrapolation; and
3 = spaceflight factor to further protect against renal effects and bone demineralization.

In the 13-wk subchronic toxicity NTP study (1994), the other toxicologic end point observed was the neurobehavioral decrement measured as the magnitude of undifferentiated motor activity in both sexes of rats and mice receiving $BaCl_2$ dihydrate administered in drinking water at 4,000 ppm for 13 wk (Dietz et al. 1992; NTP 1994). In the female mice, the forelimb grip strength was also significantly lower than that of controls at 13 wk. In the above NTP subchronic exposure rat study, a LOAEL for a decrease in motor activity was identified at a concentration of 4,000 ppm (Ba at 200 mg/kg/d). A dose of 110 mg/kg/d was identified as a NOAEL.

Thus, the 100-d AC for neurobehavioral effects can be derived as follows:

$$(110 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times [100 \text{ d}/91 \text{ d}])$$
$$= 250 \text{ mg/L (rounded)},$$

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where
110 mg/kg/d = NOAEL;
10 = species extrapolation factor;
70 kg = nominal body weight;
2.8 L/d = nominal water consumption; and
100 d/91 d = time extrapolation factor.

A BMD calculation was carried out for the above data. A $BMDL_{01}$ was determined for male and female rats using the data in Tables 3-5 and 3-6: Ba at 41 mg/kg/d for male rats and 32.5 mg/kg/d for female rats, where $BMDL_{01}$ is the 95% lower-confidence limit of a BMD corresponding to a 1% effect. Female rats appear to be more sensitive than males.

A 100-d AC for neurobehavioral effects can be calculated as follows:

$$(BMDL_{01} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times [100 \text{ d}/91 \text{ d}]) = 75 \text{ mg/L},$$

where
32.5 mg/kg/d = $BMDL_{01}$;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
100 d/91 d = time extrapolation factor.

TABLE 3-5 Undifferentiated Motor Activity of Male Rats after a 13-wk $BaCl_2 \cdot 2H_2O$ Exposure in Drinking Water

Concentrations of $BaCl_2$ (ppm)	Dose (mg/kg/d)	Activity (min) ± SD
0	0	21.08 ± 2.85
125	10	18.25 ± 3.16
500	30	18.77 ± 1.61
1,000	65	19.47 ± 1.17
2,000	110	18.78 ± 2.47
4,000	200	17.13 ± 3.83

Abbreviations: SD, standard deviation.
Source: NTP 1994.

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TABLE 3-6 Undifferentiated Motor Activity of Female Rats after a 13-wk Exposure to $BaCl_2 \cdot 2H_2O$ in Drinking Water

Concentrations of Ba (ppm)	Dose (mg/kg/d)	Activity (min) ± SD
0	0	24.44 ± 3.67
125	15	20.42 ± 3.26
500	35	20.87 ± 2.78
1,000	65	22.79 ± 4.49
2,000	115	19.84 ± 3.42
4,000	180	16.88 ± 6.64

Abbreviations: SD, standard deviation.
Source: NTP 1994.

1,000-d AC

Four chronic toxicity studies were considered to derive a 1,000-d AC for Ba in drinking water. The human retrospective epidemiologic study conducted by Brenniman and coworkers in 1979, 1981, and 1985 and was considered for the 1,000-d AC derivation. In short, the data consisted of results from a study on two communities with widely differing concentrations of Ba in the drinking water (mean concentrations of Ba at 0.1 mg/L in one community and 7.3 mg/L in the other community). A high correlation between age-adjusted mortality from cardiovascular diseases and concentrations of Ba was noted.

The analysis did not take into consideration a few confounding factors applicable to the high-Ba community, such as change in the population dynamics (an increase of 70% in the population of this community), use of water softeners (because the water source for this community was hard), and use of blood pressure medications. In addition, the noted increase in the mortality was higher in subjects who lived in the community 10 y or less, meaning that the increase was concentrated in those new populations who migrated into this community. No prior medical history of these individuals before they migrated into this region is known.

In a follow-up study, no significant differences were observed in the mean systolic and diastolic blood pressures of men or women between the two communities. When the above-mentioned additional criteria were incorporated into the data analysis, the prevalence rates for hypertension, stroke, heart disease, and kidney disease in males and females were not significantly different between these two communities.

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Hypertension as the toxicity end point and mortality from cardiovascular diseases in the Brenniman and Levy (1985) study were not used for deriving the 1,000-d AC for the following reasons: (1) blood pressure measurements were taken only once during the data-collection session, and (2) the mortality was not robust enough to be used to set an AC.

EPA used that study to derive an oral RfD for soluble Ba salts. The value of 7.3 mg/L (the average Ba concentration in the high-Ba community) was used to derive an adjusted NOAEL by using a UF of 3 because the study had a large number of test subjects of both sexes in a broad age range.

Thus, the EPA oral RfD of 0.07 mg/kg/d was calculated as follows:

$$(7.3 \text{ mg/L} \times 2 \text{ L/d}) \div (70 \text{ kg} \times 3) = 0.07 \text{ mg/kg/d},$$

where 2 L/d is the nominal water consumption, 70 kg is the nominal body weight, and 3 is the factor to reduce the NOAEL.

Dallas and Williams (2001) considered the Brenniman and Levy (1985) study on two Illinois communities with 70-fold differences in Ba concentrations in water in which it was concluded that there were no significant differences in blood pressure, heart diseases, or kidney diseases associated with Ba and from which EPA chose a NOAEL without a LOAEL. According to Dallas and Williams (2001), cardiovascular effects occur at doses well above those reported for renal effects, and therefore, renal effects should be the end points used for oral RfD. Using the data from the NTP (1994) $BaCl_2$ drinking water study discussed in the earlier sections, these authors proposed an RfD of 0.6 mg/kg/d using a NOAEL for Ba of 60 mg/kg/d and a modifying factor of 90 (as described earlier in this section).

Three animal studies were considered for AC calculations. In the Schroeder and Mitchener (1975a, b) studies, rats and mice exposed for life to Ba as Ba acetate at 5 ppm in drinking water showed protein-urea after 5 mo, indicating renal toxicity. Unfortunately, only one Ba concentration was used, and proteinuria was tested only semi-quantitatively. Therefore, the observation was not used for AC calculations, despite data from the NTP 2-y study and the Dietz et al. (1992) study on mice using $BaCl_2$ in drinking water, which demonstrated renal toxicity. In the second study, Perry et al (1989) reported Ba-induced hypertension when weanling rats were orally exposed to $BaCl_2$ at 0, 1, 10, and 100 ppm in water for 1, 4, and 16 mo (Ba at 0, 0.071, 0.71, or 7.1 mg/kg/d). Increased systolic pressure was noted in rats exposed to 7.1 mg/kg/d for 16

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mo. The study was not used because of reasons outlined in the 10-d AC section.

In the third study, a chronic toxicity study by NTP (1994), both male and female rats were exposed to $BaCl_2$ in their drinking water for 2 y at concentrations of 0, 500, 1,250, and 2,500 ppm (Ba at 0, 15, 30, and 60 mg/kg/d for males and 0, 15, 45, and 75 mg/kg/d for females). Three toxicologically significant observations were noted in this report. Water consumption decreased in a dose-dependent manner; an approximate 23% reduction compared with control values occurred at 2,500 ppm. Evidence of renal injury was noted in female mice: BUN increased, and the kidneys had abnormal pigmentation. Renal nephropathy and crystal formation in the renal tubules were observed in mice that received $BaCl_2$ at 2,500 ppm in the diet for 2 y. The chemical-related renal toxicity was not seen in rats. These effects were used to derive 1,000-d ACs as follows.

A dose-related decrease in water consumption was seen as early as 5 wk in the 60 mg/kg/d group. First, a 1,000-d AC was derived based on reduction of water consumption in rats (not in mice). Although at the highest dose (60 mg/kg/d) the decreased water consumption was pronounced, water consumption was also found to be lower in the 30 mg/kg/d group. A NOAEL was identified as 15 mg/kg/d for Ba. A 1,000-d AC for a decrease in water consumption is calculated as follows:

$$(15 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L} \times [1{,}000 \text{ d}/730 \text{ d}] \times 3) = 9 \text{ mg/L},$$

where

15 mg/kg/d = LOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption;
1,000 d/730 d = time factor; and
3 = spaceflight factor for dehydration.

The 1,000-d AC for reduction in water consumption is 9 mg/L based on the NTP study (1994).

A 1,000-d AC was also calculated using data on the renal toxicity end points (nephropathy and renal crystal formation) from male and female mice after 2 y of exposure to $BaCl_2$ in their drinking water). These data have been summarized in Table 3-7. A summary of the NOAEL and $BMDL_{01}$ values are shown in Table 3-8.

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TABLE 3-7 Incidence of Non-Neoplastic Lesions in Kidneys of Mice Exposed to $BaCl_2$ in Drinking Water for 2 Years

	Male Mice			Female Mice		
$BaCl_2$ (ppm)	Dose (mg/kg/d)	Renal Crystals	Nephropathy	Dose (mg/kg/d)	Renal Crystals	Nephropathy
0	0	0/50	1/50	0	0/50	0/50
500	30	0/50	0/50	40	0/53	2/53
1,250	75	1/48	2/48	90	0/50	1/50
2,500	160	21/50	19/50	200	36/54	37/54

Source: NTP 1994.

TABLE 3-8 Benchmark Dose ($BMDL_{01}$) and NOAELs for $BaCl_2$-Induced Renal Lesions in Mice

Gender	Parameter[a]	For Renal Crystals (Dose mg/kg/d)	For Nephropathy (Dose mg/kg/d)
Male	$BMDL_{01}$	40	32
Male	NOAEL	75	75
Female	$BMDL_{01}$	75	62
Female	NOAEL	90	90

[a]$BMDL_{01}$ is defined as the 95% lower confidence limit estimate of a benchmark dose corresponding to an excess risk of 1%. Bench Mark Dose Software version 1.3.2, developed by EPA, was used.

Using both the NOAEL and the $BMDL_{01}$ values listed in Table 3-8, ACs were calculated for both renal toxicity end points as follows:

$$(\text{NOAEL or } BMDL_{01} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times [1{,}000 \text{ d}/730 \text{ d}] \times 3),$$

where
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption;
1,000 d/730 d = time extrapolation factor; and
3 = spaceflight safety factor for renal effects risk and bone demineralization risks.

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Male mice seem to be more susceptible to renal crystal formation as well as nephropathy than the female mice, and the values based on the BMD approach are conservatively lower than the NOAEL method (see Table 3-9). The most conservative of AC values for renal crystals (24 mg/L) and nephropathy (19 mg/L) in male mice, derived using the BMD method, were chosen as the 1,000-d AC. These values are entered in the AC summary table (Table 3-10).

TABLE 3-9 Summary of 1,000-d ACs Based on Renal Lesions

Species and Sex	ACs Based On[a]	AC for Renal Crystals[b] (mg/L)	AC for Nephropathy[b] (mg/L)
Mice, male	$BMDL_{01}$	*24*	*19*
Mice, male	NOAEL	46	45
Mice, female	$BMDL_{01}$	46	37
Mice, female	NOAEL	55	54

[a]The following common factors were used for both: 70 kg = nominal body weight; 2.8 L/d = nominal water consumption; 10 = species extrapolation factor; 1,000 d/730 d = time extrapolation factor; and 3 = spaceflight factor for renal-effects risk and bone-demineralization risks.
[b]The italicized values represent the most conservative value.

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TABLE 3-10 Acceptable Concentrations (ACs) for Ba in Drinking Water

			UFs				ACs (mg/L of water)				
Toxicity End Points	Exposure	Species	To NOAELs	Species Factor	Time Factor	Space-flight Factor	1-d	10-d	100-d	1,000-d	Reference
Fluid in the trachea, darkened liver, inflammation of intestine	NOAEL = 66 mg/kg; 1 d; gavage	Rats, male and female (fasted)	1	10	1	1	165		—	—	Borzelleca et al. 1988
Abnormal ECG	LOAEL = 84 mg/kg/d; iv infusion	Dogs, male and female	2	10	1	5	21	21	—	—	Roza and Berman 1971
Reduced water consumption	NOAEL = 60 mg/kg; 15-d data	Rats, male and female	1	10	1	3	—	50	—	—	NTP 1994
Reduced water consumption	LOAEL = 45.7 mg/kg; NOAEL = 9.7 mg/kg	Rats, 13 wk	1	10	(100/91)	3	—	—	7	—	Tardiff et al. 1980
Renal (histo-pathologic lesions); thymic and spleen atrophy in male and female mice	NOAEL = 65; 13 wk; drinking water	Rats, male and female	1	10	(100/91)	3	—	—	50	—	NTP 1994
Neurobehavioral effects (motor activity)	NOAEL = 110; 13 wk; drinking water	Rats, male and female	1	10	(100/91)	1	—	—	250	—	NTP 1994

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Neurobehavioral effects (motor activity)	$BMDL_{01}$ = 32.5 (BMD method); 2 y; $BaCl_2$ in drinking water	Rats, female	1	10	1	1	—	—	75	—	NTP 1994
Reduced water consumption	NOAEL = 15; 2 y; drinking water	Rats	1	10	1.37[a]	3	—	—	—	9	NTP 1994
Renal Crystal formation	$BMDL_{01}$ = 40; 2 y; $BaCl_2$ in drinking water	Mice, male	1	10	1.37[a]	3	—	—	—	24	NTP 1994
Nephropathy	$BMDL_{01}$ = 32; 2 y; $BaCl_2$ in drinking water	Mice, male	1	10	1.37[a]	3	—	—	—	19	NTP 1994
SWEG[b]							21	21	10[b]	10[b]	

[a]Factor for time extrapolation from 730-d to 1,000-d.
[b]These values were rounded to 10 for both 100- and 1,000-d ACs.

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4

C1-C4 Mono-, Di-, and Trialkylamines

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PROPERTIES AND OCCURRENCE

Background

Alkylamines are colorless, flammable gases or liquids that emit "fishy" or ammonia-like odors. The alkylamines share common properties, including fat solubility, high alkalinity, and, for those amines with boiling points less than 100˚C (Table 4-1), considerable volatility. These properties account for their irritation to skin and mucous membranes and for their classification as hazardous chemicals in the workplace. Amines are produced endogenously by microbial breakdown of food constituents choline and lecithin (Asatoor and Simenhoff 1965), which are found in many common foods (such as eggs and liver) (Spellacy and Watts 1979). Methylamine is biosynthesized by degradation of muscle sarcosine and creatine (Davis and DeRopp 1961). Methylamine, dimethylamine, and trimethylamine are found in human gastric fluid and in the saliva and blood of humans and other mammalian species, including rats, ferrets, and dogs (Zeisel et al. 1988). Amines are also normal constituents of mammalian wastes, including urine, feces, and exhaled air. Humans excrete approximately 1 millimole (mmol) each of methylamine, dimethylamine, and trimethylamine in urine per day following consumption of a normal diet (Ziesel et al. 1983). Following consumption of 27 mmol of choline chloride, six healthy human subjects excreted 2 mmol of methylamine, 2 mmol of dimethylamine, and >17 mmol of trimethylamine in urine per day.

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TABLE 4-1 Physical and Chemical Properties of the Alkylamines

Alkylamine	CAS Registry No.	Formula	Molecular Weight	Synonyms	Boiling Point (°C)	Melting Point (°C)	Vapor Pressure (torr at 25°C)	Density (°C)	Solubility
Methyl	74-89-5	CH_5N	31.07	Aminomethane Carbinomine Methanamine	6.3	−93.5	2,650	0.662 (25)	Water Alcohol Ether
Ethyl	75-04-7	C_2H_7N	45.1	Ethanamine Aminoethane Monoethylamine	16.6	−81.2	1,048	0.689(15)	Water Alcohol Ether
Propyl	107-10-8	C_3H_9N	59.13	1-Aminopropane Propanamine *n*-Propylamine	48-49	−83	310	0.719 (20)	Water Alcohol Ether
Isopropyl	75-31-0	C_3H_9N	59.13	2-Aminopropane	33-34	−101	579.6	0.694	Water
n-Butyl	109-73-9	$C_4H_{11}N$	73.14	1-Butanamine 1-Aminobutane Mono-*n*-butylamine Methanamine	78	−50	92.9	0.741 (20)	Water Alcohol
Dimethyl	124-40-3	C_2H_7N	45.03	*N*-Methyl-ethanamine	6.8	−92.2	1520	0.684 (0)	Water
Diethyl	109-89-7	$C_4H_{11}N$	73.14	*N*-Ethyl-1-butanamine	55.6	−50	237	0.707 (20)	Water
Di-*n*-butyl	111-92-2	$C_8H_{19}N$	129.24	*n*-Butyl-	160	−60	2.59	0.760 (20)	Water Alcohol

(Continued)

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TABLE 4-1 Continued

Alkylamine	CAS Registry No.	Formula	Molecular Weight	Synonyms	Boiling Point (°C)	Melting Point (°C)	Vapor Pressure (torr at 25°C)	Density (°C)	Solubility
Trimethyl	75-50-3	C_3H_9N	59.13	TMA *N*,*N*-Dimethyl-methanamine Amine, trimethyl	2.87	−117	1,610	0.636 (20)	Water Alcohol Ether
Triethyl	121-44-8	$C_6H_{15}N$	101.22	(Diethylamino)-ethane *N*,*N*-Diethylethan-amine TEN	89.5	−115	57.1	0.726 (25)	Water Alcohol Ether
Tripropyl	102-82-9	$C_9H_{21}N$	143.31	*N*,*N*-Di-propyl-1-propanamine Tri-*n*-propylamine Propyldi-*n*-propylamine	156	−93	2.25	0.75 (25)	Water
Tributyl	102-69-2	$C_{12}H_{27}N$	185.4	Tri-*n*-butylamine Tris-*n*-butylamine *N*,*N*-dibutyl-1-butanamine	216-217	<−70	0.29	0.778 (20)	Water Alcohol Ether

Source: Data from HSDB 2001.

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Amines occur naturally in various plants, such as tobacco, algae, and celery, as well as in other consumables, such as cheese, brown breads, boiled beef, rice, wine, and coffee (Benya and Harbison 1994). They are used in industry as solvents, rubber accelerators, ion-exchange resins, and catalysts.

Detection in Spacecraft

The Water and Food Analytical Laboratory (WAFAL) at Johnson Space Center has evaluated eight alkylamines found to be contaminants in water samples from spacecraft. Those amines—methylamine, ethylamine, propylamine, di-*n*-butylamine, trimethylamine, triethylamine, tripropylamine, and tributylamine—are illustrated with other target alkylamines in Figure 4-1. Twenty-eight raw humidity-condensate samples, 29 recycled-water samples from the condensate-recovery system galley dispenser, and eight stored-water samples were collected in 2-4-week (wk) intervals during Mir space station missions from 1995 to 1998 (Pierre and Schultz 1999). Mean concentrations of detected amines in raw humidity condensate included methylamine at 0.092 milligrams per liter (mg/L) (8 of 28 samples), di-*n*-butylamine at 0.003 mg/L (6 of 28 samples), and triethylamine at 0.002 mg/L (6 of 28 samples). Triethylamine, the only alkylamine reported at above the detection limit in recycled galley samples, was detected in 1 of 27 galley samples at 0.012 micrograms per liter (µg/L). Amines were not detected in stored water samples.

In-flight galley and low iodine residual system (LIRS [see list of abbreviations used in this chapter]) cartridge effluent samples were collected on Space Transportation System (STS)-95 after a malfunction of the LIRS water reclamation system (Schultz 1998). The crew reported an objectionable taste and smell of galley water, which was later attributed to the presence of amines—specifically trimethylamine, tributylamine, and tripropylamine at 60 mg/L, 176 mg/L, and 793 mg/L, respectively. WAFAL studies determined that the most likely source of the amines in the water samples was the decomposition of iodosorb and microbial check valve resins during normal storage and/or gamma irradiation sterilization. Amines were not detected in samples taken from the STS-95 ACTEX (activated carbon/ion exchange) water reclamation system.

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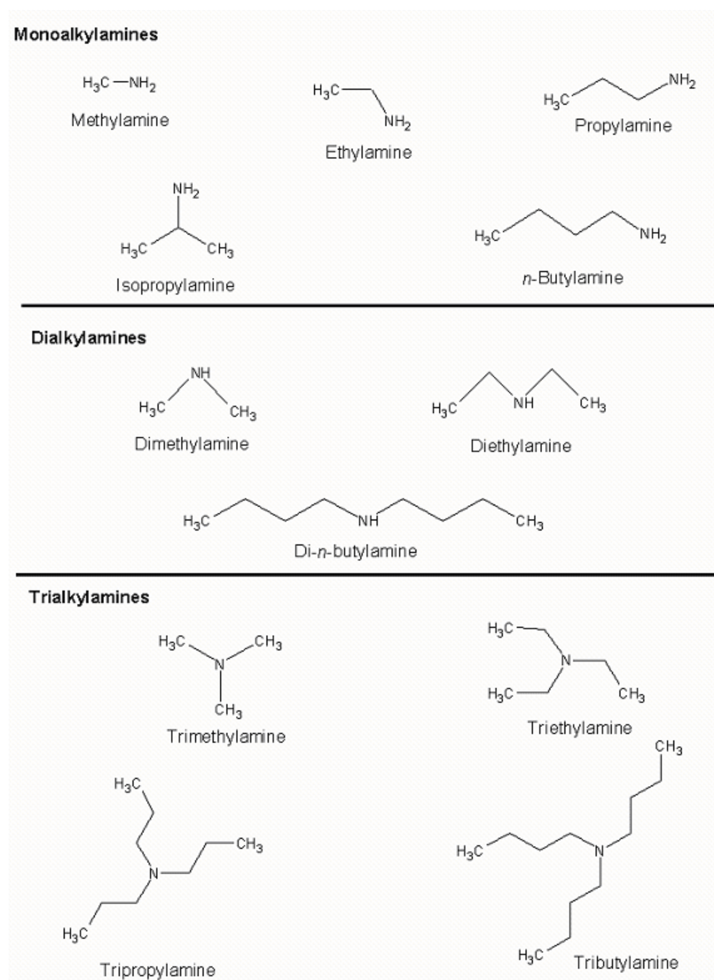

FIGURE 4-1 Alkylamine groups relevant to spacecraft water exposure guidelines. Source: Adapted from the National Library of Medicine 2006.

PHARMACOKINETICS AND METABOLISM

In general, amines are well absorbed from the gut and respiratory tract (Benya and Harbison 1994). Lower aliphatic amines are primarily metabolized to corresponding carboxylic acids and urea; aldehydes and ammonia are intermediate compounds. Monoamine oxidases (MAOs), flavin-containing monooxygenases (FMOs), and cytochrome P-450 enzymes are all purportedly involved in amine biotransformation and have

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Abbreviations Used in Text

3-MeAde	3-methyladenine
ACTEX	activated carbon/ion exchange
BA	*n*-butylamine
BCPN	*N*-butyl-*n*-(3-carboxypropyl)nitrosamine
BHA	butylated hydroxyanisole
CCV	clear cytoplasmic vacuoles
DBA	di-*n*-butylamine
DEA	diethylamine
DMA	dimethylamine
EA	ethylamine
FMO3	flavin-containing monooxygenase (isomer 3)
IPA	isopropylamine
LIRS	low iodine residual system
MA	methylamine
MDA	malondialdehyde
NDBA	*N*-nitrosodi-*n*-butylamine
PA	propylamine
SSAO	semicarbazide-sensitive amine oxidase
TBA	tributylamine
TEA	triethylamine
TEAO	triethylamine-*N*-oxide
TMA HCl	trimethylamine hydrochloride
TMA	trimethylamine
TMAO	trimethylamine-*N*-oxide
TPA	tripropylamine

overlapping substrate specificities. Biotransformation of amines might involve oxidative deamination or de-alkylation, as well as N-oxidation. Available data on the metabolism of alkylamines relevant to spacecraft water exposure guidelines (SWEGs) are presented below.

Amine Biotransformation Enzymes

Two classes of flavoproteins, MAOs and FMOs, are implicated in the metabolism of amines. MAO A and MAO B are involved in the deamination of both biogenic and xenobiotic amines (Cashman 1997). MAO A preferentially oxidizes biogenic amines, such as serotonin and epinephrine, whereas MAO B oxidizes noncatecholamines, such as phenylethylamine and benzylamine. MAOs oxidatively deaminate pri-

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mary, secondary, and tertiary amines; however, amine substrates that have a methyl group on the alpha carbon (with respect to the nitrogen atom of the substrate) are resistant to oxidation by MAO (Benedetti and Dostert 1994). Methylamine, ethylamine, and propylamine are established as poor substrates for MAO (Benya and Harbison 1994).

FMOs are a family of enzymes that detoxify organic nitrogen- and sulfur-containing nucleophilic xenobiotics by converting the lipophilic heteroatom-containing compounds to polar, oxygenated metabolites that are readily excreted (Cashman 1997). Nucleophilic tertiary amines, sulfides, thiones, and phosphines are converted to their corresponding *N*-, *S*-, and *P*-oxides. Dietary xenobiotics have been demonstrated to influence the expression of rat liver FMO. Ziegler (1984) proposed that FMO is induced by one or more organic nitrogen- or sulfur-containing xenobiotics present in food. A defect in the FMO isomer FMO3 causes the disorder trimethylaminuria. Affected individuals cannot effectively metabolize trimethylamine (TMA) to its *N*-oxide, TMAO (Ayesh et al. 1993). Missense and nonsense mutations in the FMO3 gene have been identified as causes of the more persistent “primary” trimethylaminuria (Treacy et al. 1998; Dolphin et al. 2000). Several other common genetic polymorphisms have been shown to cause partial attenuation of FMO3 N-oxidation capacity. Genetic polymorphisms distributed in Canadian and Australian white populations have been suggested to modulate the activity of human FMO3 (Cashman et al. 2000). Affected individuals excrete large amounts of unchanged malodorous TMA in their skin, breath, sweat, and urine. These excretions impart a fishy smell to the individual, and the disease is hence referred to as the “fish-odor syndrome.” Normally, healthy individuals excrete 50 mg of TMA per day (Ayesh and Smith 1992). That amount might vary with diet. Greater than 90% of excreted TMA is in the *N*-oxide form, TMAO.

Formation of triethylamine-*N*-oxide (TEAO) from ingested or inhaled triethylamine (TEA) conceivably is dependent on an FMO (Akesson et al. 1988). As in the case of other aliphatic trialkylamines, TEA purportedly is metabolized by addition of oxygen to its nucleophilic nitrogen (Ziegler 1984). N-oxygenation of trialkylamines is a significant route for the oxidative metabolism of a large number of medicinal amines and naturally occurring alkaloids that have basic side chains. The formation of aliphatic *N*-oxide is representative of FMO metabolism of xenobiotic amines.

The semicarbazide-sensitive amine oxidase (SSAO) is an enzyme (or a family of enzymes) found in vascular smooth muscle cells, in cartilage, and in microvessels of the retina and brain (Yu 1998). This amine

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oxidase, which is inhibited by semicarbazide and pargylamine, oxidizes various endogenous and exogenous amine substrates (Yu 1990). Methylamine and ethylamine are readily oxidized by male Wistar rat aorta SSAO (Yu 1990). Substrate affinity for SSAO was observed in C1-C18 aliphatic amines, with affinity increasing with carbon-chain length. Aldehydes are products of SSAO deamination of aliphatic amines.

Methylamine

Methylamine (MA) in humans might be derived from metabolic reactions, including the deamination of adrenaline (Schayer et al. 1952) and the metabolism of sarcosine and creatine (Dar et al. 1985). MA might also be ingested from food and drink or via inhalation of tobacco smoke. Asatoor and Simenhoff (1965) have proposed that methylamine enters one of two metabolic pathways: (1) metabolism to carbon dioxide and ammonia, or (2) transmethylation to dimethylamine (DMA) using methionine as the methyl donor (Figure 4-2). In vivo studies conducted by Dar et al. (1985) concluded that MAO does not play a major role in the metabolism of MA. In vitro studies have shown that MA is deaminated by a SSAO to hydrogen peroxide and formaldehyde (Yu and Zuo 1996). Other in vitro studies have demonstrated oxidative deamination of methylamine by SSAO (Boor et al. 1992; Yu 1998; Yu and Zuo 1996; Yu and Zuo 1993). Formaldehyde was shown to be a metabolic product when rat aortic homogenate supernatant or purified porcine SSAO preparations were incubated with 1 millimolar (mM) of MA (Boor et al. 1992). Formaldehyde was measured by high-performance liquid chromatography (HPLC) detection of 2,4-dinitrophenylhydrazine-formaldehyde adducts. In this same study, formaldehyde production was inhibited by semicarbazide, an SSAO inhibitor, but not by pargyline, an MAO inhibitor, when MA rat aorta homogenates or porcine SSAO were incubated with MA.

MA was demonstrated to be an endogenous substrate for SSAO (Precious et al. 1988). MA metabolism by amine oxidase activities was studied in rat aorta and human umbilical artery preparations using ^{14}C radiochemical assays or spectrophotometric determination of hydrogen peroxide (H_2O_2) formation resulting from MA metabolism. Deamination of MA was completely inhibited by the SSAO-selective inhibitors semicarbazide and propargylamine. Deamination of MA was not inhibited by MAO-selective inhibitors clorgyline and pargylamine.

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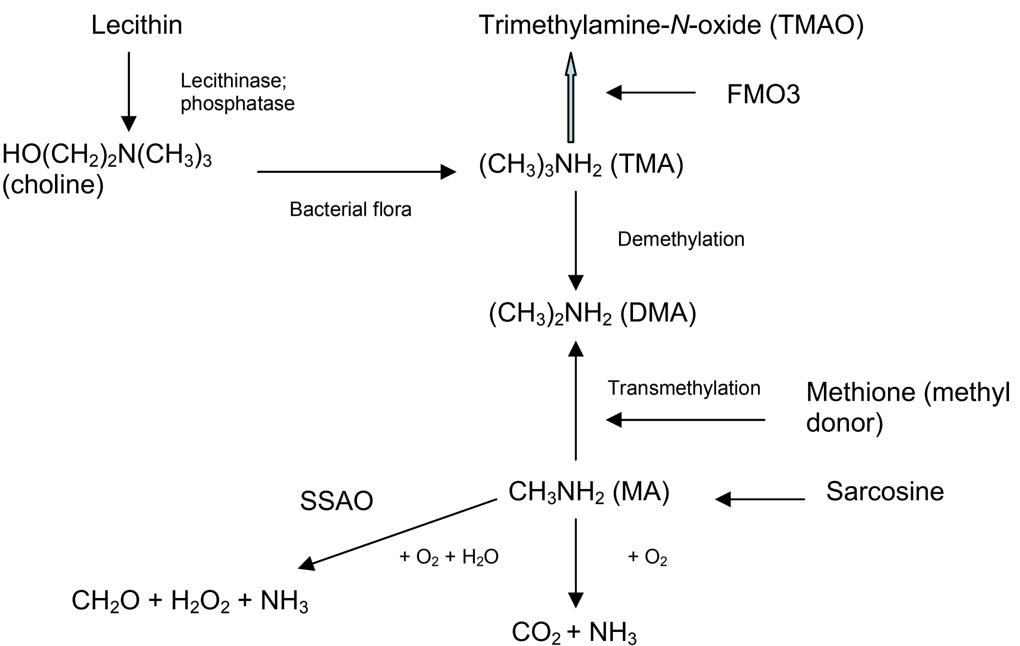

FIGURE 4-2 Metabolic pathways of methylamines as proposed by Asatoor and Simenhoff (1965), Boor et al. (1992), Ayesh et al. (1993), and Yu (1998). Abbreviations: DMA, dimethylamine; FMO3, flavin-containing monooxygenase isomer 3; MA, methylamine; SSAO, semicarbazide-sensitive amine oxidase; TMA, trimethylamine.

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Lyles and McDougall (1989) proposed that MA is metabolized in vivo by SSAO. When male albino Sprague-Dawley rats were treated with semicarbazide (100 mg per kilogram [kg]), a chemical that irreversibly inhibits SSAOs, urinary excretion of MA increased threefold (24 hours [h]) to sixfold (48 h) for two daily periods following dosing. The authors conclude that the results do not prove that decreased degradation of MA occurs specifically because of semicarbazide; however, the results support the argument that MA is metabolized to formaldehyde via SSAO. In a separate study, when SSAO enzyme preparations from rat aorta were treated with semicarbazide, deamination of MA was completely inhibited (Yu 1990).

Trimethylamine

Radioactive [^{14}C]-TMA hydrochloride was administered orally in aqueous solution at 15 mg/kg to three female adult rats (150-200 g body weight) of each of seven different strains, Wistar, Lewis, Fischer, A/GUS, PVG, DA, and BN (Al-waiz and Mitchell 1991). There were no statistically significant differences in results among the strains investigated. TMA, its metabolite TMAO, and DMA were detected by thin layer chromatography. Greater than 75% of the administered dose of TMA was excreted in the urine within 24 h after dosing. Amounts up to 9% were detected in the feces. Rats excreted 45% of the administered TMA dose as TMAO, 3% as DMA, and the remaining dose as unchanged TMA. Results from this study suggest N-oxidation as the major metabolic path and demethylation as the secondary path for TMA biotransformation.

TMA is a common constituent of urine and is produced by the action of normally present intestinal bacteria on dietary choline. TMA is proposed as an intermediate in the production of endogenous DMA (Asatoor and Simenhoff 1965). Male albino rats (number not given) received 100 mg of TMA hydrochloride by gastric intubation. A second group of rats received 50 mg of intraperitoneally (ip) administered TMA hydrochloride. Urinary excretion of DMA in treated animals was measured prior to dosing. Both groups of rats were observed to have marked increases in urinary excretion of DMA following TMA hydrochloride dosing. Orally dosed rats excreted 40 µg of DMA per milligram of creatinine prior to dosing and 185 µg of DMA per milligram of creatinine after dosing. Rats ip dosed excreted 45.6 µg of DMA per milligram of creatinine prior to dosing and 110 µg of DMA per milligram

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creatinine after dosing. These results suggest that TMA is physiologically demethylated to DMA.

TMA commonly occurs in mammalian urine and is produced by normal digestive and intestinal microbial breakdown of dietary lecithin and choline (Al-waiz et al. 1987a). In humans, the major precursor of TMA is dietary choline (Humbert et al. 1970). TMA is metabolized partly by N-demethylation to form urea and formaldehyde and partly by oxidation to TMAO (Parke 1968). TMA is also reported to be an intermediate in the metabolism of choline to DMA (Asatoor and Simenhoff 1965). Four healthy men received single oral doses of TMA by gelatin capsule at 300 and 600 mg per capsule (Al-waiz et al. 1987a). Analysis of urine prior to TMA administration demonstrated TMA, TMAO, and DMA at concentrations of 10, 311, and 98 micromoles (µmol) per 8-h urine sample, respectively, presumably resulting from normal metabolism of dietary sources. Eight hours following ingestion of the 300 mg (5 mmol) TMA capsule, urinary TMA, TMAO, and DMA increased 11-, 10.2-, and 1.4-fold, respectively. Urinary concentrations of TMA increased 39-fold (390 µmol) and concentrations of TMAO increased 24-fold (7,464 µmol) after ingestion of the 600 mg capsule. A minimal 1.8-fold increase in DMA (175 µmol) was noted. For both doses, TMAO composed 93% of the excreted TMA, suggesting that N-oxidation is a primary metabolic pathway for orally ingested TMA. A fish-like odor was imparted to urine samples after dosing at both concentrations.

Trimethylaminuria

Trimethylaminuria, also referred to as "fish-odor syndrome," is marked by the inability of affected individuals to effectively metabolize TMA to its relatively odorless *N*-oxide metabolite, TMAO (Al-waiz et al. 1987c). This condition is biochemically characterized by excretion of abnormally large amounts of unoxidized TMA, which consequently imparts a fish-like odor to the affected individual. Primary trimethylaminuria is the malodorous phenotypical expression in individuals who are homozygous for the recessive allele that results in impairment of the TMA N-oxidation mechanism. Individuals with primary trimethylaminuria are deficient in hepatic FMO3 activity, which catalyzes the N-oxidation of dietary TMA. However, trimethylaminuria may present as sequelae to other underlying disorders, such as liver cirrhosis or uremia, in which individuals with chronic kidney failure have increased total body burden of TMA as a result of bacterial overgrowth and decreased

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renal clearance. Trimethylaminuria may also occur in cases of vaginitis or cervical cancer (Brewster and Schedewie 1983).

Genetic Predisposition

Al-waiz et al. (1987c) conducted an experiment to examine the genetic derivation of trimethylaminuria by measuring urinary excretion of TMA and TMAO in two families that each had one member affected by fish-odor syndrome. The two affected family members excreted only 22% and 10%, respectively, of a normal dietary load of TMA as the oxidized metabolite TMAO. Following oral administration of TMA at 300 mg, the affected people excreted only 18% and 5% TMAO, respectively, and were observed to have a prominent fish odor. Healthy individuals given an oral dose of TMA at 600 mg excreted 93% of the dose as TMAO. Parents of the affected family members excreted 95% of a 300 mg TMA dose as TMAO, but when given TMA at 600 mg, TMAO excretion dropped to 79% of the dose and TMA excretion was increased. Urinary excretions of TMAO were normal in other tested family members who did not have the defect but were decreased in a sibling who apparently was also affected.

The occurrence of trimethylaminuria in the general population was investigated in a random population study in which 169 individuals were screened for TMA N-oxidation (Al-waiz et al. 1987c). Two test subjects were observed to be deficient in N-oxidation, and upon challenge with TMA at 600 mg (oral route assumed), those subjects were determined to be carriers of the N-oxidation impairment allele. Based on this evaluation, approximately 1% of the population may be carriers of the genetic defect purportedly associated with trimethylaminuria. Several genetic polymorphisms have been shown to modulate the activity of human FMO3 (Akerman et al. 1999a, b; Cashman et al. 2000). Modulated FMO3 activity might result in less severe symptoms of trimethylaminuria. There is also evidence that heterozygotes are subject to N-oxidation saturation when challenged with increased loads (for example, 600 mg) of TMA (Ayesh and Smith 1992).

Psychosocial Effects

Trimethylaminuria is not harmful, but affected persons might experience serious psychosocial problems as a direct result of the disorder

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(Al-waiz et al. 1987c). The highly objectionable body odor associated with trimethylaminuria can be destructive to the personal, social, and work life of affected individuals. Those individuals undergo personal distresses, including social isolation, ridicule, and rejection, and medical or psychological misdiagnosis (Ayesh et al. 1993).

Relevance to Crew Operations

Expression of trimethylaminuria should be revealed in the physical and clinical evaluation of potential spacecraft crew. Mild presentations of the anomaly might exist in some crew members but should not adversely impact flight operations or the performance of affected crew, provided that crew health is otherwise adequate. Malodor can be managed by reduction or elimination of foods that contain TMA or its precursors (Ayesh et al. 1993).

Triethylamine

TEA is metabolized into TEAO by N-oxidation resulting from intestinal bacteria activity (Akesson et al. 1989). Akesson et al. (1989) established that humans given oral doses of TEA at 25 mg (248 µmol) produced DEA at less than 0.5% of the dose in urine samples obtained 8-14 h after administration. The authors concluded that DEA was generated via microbial activity within the gastrointestinal (GI) tract, absorbed, and then excreted in the urine. TEAO in urine samples averaged 33% of the orally administered TEA dose.

Role of Bacteria in the Production of Endogenous Amines

TMA urinary excretions in male Sprague-Dawley rats (125 g body weight) fed a choline-deficient diet for 1 wk decreased only slightly compared with those of animals fed choline-supplemented diets (control) (Ziesel et al. 1985). Urinary excretion of TMA in choline-deficient rats was 2 µmol/kg per 24 h, whereas excretion was 2.5 µmol/kg per 24 h in control rats. MA urinary excretions in choline-deficient animals were comparable to those in choline-supplemented animals. These results suggest that TMA and MA are formed endogenously from sources other

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than dietary choline. In this same study, rats with no bacterial colonization of the bowel (germ-free) exhibited markedly decreased urinary excretion of TMA and MA in a 24-h collection period compared with control animals. MA urinary excretion decreased from 40 µmol/kg per 24 h (controls) to 16 µmol/kg per 24 h (germ-free animals). TMA excretion decreased from 5 µmol/kg per 24 h (controls) to 0.7 µmol/kg per 24 h. These results suggest that both TMA and MA are formed via action of bacteria within the gut.

Absorption

Triethylamine

Human volunteers who received oral doses of TEA demonstrated absorption of >90% from the GI tract (Akesson et al. 1989). Four men received a single oral dose of TEA at 25 mg (248 µmol) dissolved in 15 milliliters (mL) of 0.1 M hydrochloric acid and adjusted to a pH of 7.0. Greater than 90% (90-97%) of orally administered TEA was recovered as TEA and its metabolite TEAO in the urine. TEAO recovered in the urine corresponded to an average of 33% of the dose.

Trimethylamine

Radioactive [^{14}C]-TMA hydrochloride was administered to female adult rats as a single oral dose at 15 mg/kg (Al-waiz and Mitchell 1991). This study demonstrated GI absorption of orally ingested TMA followed by rapid removal of the dose via urinary excretion. More than 75% of the administered radioactivity was excreted in the urine within 24 h after dosing. Up to 9% of radioactivity was detected in the feces.

Distribution

The apparent volume of distribution (during the terminal elimination phase) following a single oral dose of TEA at 25 mg in four male human volunteers was calculated to be 196 L assuming 100% bioavailability (Akesson et al. 1989).

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Excretion

Amines are normal constituents of mammalian wastes, including urine, feces, and exhaled air. Sources of normal urinary and fecal aliphatic amine levels include both dietary intake and endogenous production of amines. Aliphatic amines normally excreted in urine include MA, ethylamine (EA), DMA, and TMA; DMA is the major constituent (Asatoor and Simenhoff 1965). Ziesel et al. (1983) report that humans excrete approximately 1 mmol each of MA, DMA, and TMA per day in their urine following consumption of a normal diet; however, amine excretion varies with diet.

Methylamine and Trimethylamine

MA and TMA were excreted in the urine of five healthy subjects (one male, four female), ages 23 to 35, after ingestion of restricted diets containing known amounts of the amines (Zeisel and deCosta 1986). Subjects were given diets without fish for 2 d (control diet), diets containing fish for 1 d, and the control diet for the final day. In urine samples collected in 24-h blocks, excreted MA amounts were comparable throughout the experiment regardless of dietary content. TMA excretion in urine following the fish diet was nine times greater than urinary excretion following the control diet (1.6 µmol per 24 h per kilogram of body weight versus 0.17µmol per 24 h per kilogram of body weight). TMA urinary excretion remained elevated at 0.86 µmol per 24 h per kilogram of body weight on the day after the fish was eaten (five times greater than that on control days). Zeisel and deCosta (1986) suggest that TMA in the fish was absorbed from the intestine into the blood and then excreted in urine. TMA was not detected in the diet that did not contain fish.

Rabbits were maintained on a diet of carrots and given TMA hydrochloride dissolved in water intragastrically at unspecified intervals. TMA hydrochloride dosing concentrations were 0.3-0.6 g per rabbit (Langley 1929). Between 4% to 20% of ingested TMA was excreted unchanged, and 80-96% was excreted as other substances, including varying amounts of ammonia and urea and insignificant amounts of DMA.

Three male volunteers were administered a single oral dose of 62 mg of [^{14}C]-TMA hydrochloride dissolved in water (Al-waiz et al. 1987b). Excretion patterns demonstrated rapid absorption. Urine was the

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primary route of excretion. Within 6 h of treatment, 84% of the administered dose was recovered, and 95% was recovered by 24 h. TMAO was the major metabolite of the administered TMA, accounting for greater than 95% of the given dose in the excreted urine.

[^{14}C]-TMA administered orally in aqueous solution to adult female rats at 15 mg/kg was excreted primarily in the urine (Al-waiz and Mitchell 1991). Over 75% of the dose was excreted in the urine within 24 h of dosing. Up to 9% of the dose was detected in the feces. Approximately 45% of the TMA dose was excreted as TMAO, 3% as DMA, and the remaining dose as unchanged TMA.

Triethylamine

Following an oral dose of TEA at 248 µmol in four healthy male humans, the plasma half-life of TEA ranged from 2.4 to 3.5 h and the urine half-life ranged from 2.5 to 3.0 h (Akesson et al. 1989). The plasma clearance following oral TEA administration ranged from 41.2 to 50.7 L/h. Nonrenal clearance ranged from 23.3 to 31.6 L/h. Renal clearance following oral exposure to TEA averaged 28.6 L/h.

Methylamine, Dimethylamine, and Trimethylamine

Fourteen healthy human subjects were found to excrete approximately 1 mmol each of MA, DMA, and TMA per day after ingesting a normal diet (Zeisel et al. 1983). Following a single oral dose of 27 mmol (3.5 g) of choline chloride (in orange juice), human subjects (n = 6) excreted MA and DMA at 2 mmol/d and TMA at 17 mmol/d. After consumption of 27 mmol (10.2 g) of choline stearate in orange juice, six subjects excreted DMA at 1.2 mmol/d and TMA at 9.3 mmol/d. Five subjects who ingested 27 mmol (20 g) of lecithin excreted DMA at 1.7 mmol/d and TMA at 3.8 mmol/d; There was no increase in MA excretion. In this same study, Sprague-Dawley rats were maintained on choline-free diets. Each rat received a single orogastric dose of either choline chloride or lecithin; each dose delivered 200 mg of free choline base per kilogram of body weight. TMA urinary excretion in dosed animals was more than 200% of that in controls.

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TOXICITY SUMMARY

There are no studies available on human oral toxicity for the targeted group of alkylamines. Limited animal toxicity studies have been conducted with MA, propylamine (PA), *n*-butylamine (BA), diethylamine (DEA), DMA, di-*n*-butylamine (DBA), TMA, TEA, and tributylamine (TBA). The studies discussed in this document, some of which were conducted prior to general acceptance of good laboratory practice guidelines, lacked descriptive information on experimental proceedings, such as methods of oral administration and designation of animal strain and physical condition. At least one study that did not achieve peer-reviewed publication is included, but it presents an investigation of mammalian toxicity employing the preferred oral route of administration and the targeted compounds. Also, because of the rapid absorption characteristics of alkylamines, studies employing ip amine administration to test subjects are discussed.

On the basis of existing data, mammalian toxicity to alkylamines appears to consists of adverse central nervous system (CNS) effects, including tremors, convulsions, and lethargy. Other responses include reproductive or developmental toxicity, statistically significant variations in hepatic enzymes, and amine-induced leucopenia. It is difficult to interpret results of studies that are poorly presented or that reveal adverse health conditions or husbandry of test animals. There are no data available on the oral toxicity of ethylamine (EA), isopropylamine (IPA), or tripropylamine (TPA) beyond the LD_{50}s (concentrations lethal to 50% of subjects). A toxicity summary is presented in Table 4-2.

Formation of *N*-Nitroso Compounds

The in vivo formation of *N*-nitroso compounds from reaction of amines and nitrite has been investigated relative to the toxicologic importance of nitrosamines in the development of human cancer. In vivo formation of nitrosamines may occur in the stomach or in other parts of the GI tract. Salivary nitrites react with various amines in the stomach (L'hirondel 1999), where favorable acid conditions (pH of 2-4) for the formation of nitrosamines prevail (Klaassen 1996). In vivo formation of nitrosamines is obviously affected by the dietary intake and the pharmacokinetics of both amines and nitrates (or nitrites). Dietary sources of

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TABLE 4-2 Toxicity Summary

Dose of Alkylamine	Treatment Time	Species	Toxic Effects	Reference
Acute Exposures (1-5 d)				
100 mg/kg (MA)	Single dose	Rat	LD_{50}	Kinney et al. 1990
400 mg/kg (EA)	Single dose	Rat	LD_{50}	Smyth et al. 1954
570 mg/kg (PA)	Single dose	Rat	LD_{50}	Smyth et al. 1962
820 mg/kg (IPA)	Single dose	Rat	LD_{50}	Smyth et al. 1951
3,200 mg/kg (IPA)	Single dose	Rabbit	LD_{50}	Lewis 1996
2,700 mg/kg (IPA)	Single dose	Guinea pig	LD_{50}	Lewis 1996
500 mg/kg (BA)	Single dose	Rat	LD_{50}	Smyth and Carpenter 1944
371 mg/kg (BA)	Single dose	SD rat	LD_{50}	Cheever et al. 1982
430 mg/kg (BA)	Single dose	Mouse	LD_{50}	Lewis 1996
430 mg/kg (BA)	Single dose	Guinea pig	LD_{50}	Lewis 1996
698 mg/kg (DMA)	Single dose	Rat	LD_{50}	Dzhanashvili 1967
316 mg/kg (DMA)	Single dose	Mouse	LD_{50}	Dzhanashvili 1967
240 mg/kg (DMA)	Single dose	Guinea pig and rabbit	LD_{50}	Dzhanashvili 1967
540 mg/kg (DEA)	Single dose	Rat	LD_{50}	Smyth et al. 1951
500-600 mg/kg (DEA)	Single dose	Mouse	LD_{50}	Patel et al. 1985
550 mg/kg (DBA)	Single dose	Mouse	LD_{50}	Smyth et al. 1954
290 mg/kg (DBA)	Single dose	Mouse	LD_{50}	Trubko 1975
230 mg/kg (DBA)	Single dose	Guinea pig	LD_{50}	Trubko 1975
500 mg/kg (TMA)	Single dose	Rat	LD_{50}	Kinney et al. 1990
546 mg/kg (TMA)	Single dose	Mouse	LD_{50}	Kinney et al. 1990

(Continued)

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TABLE 4-2 Continued

Dose of Alkylamine	Treatment Time	Species	Toxic Effects	Reference
460 mg/kg (TEA)	Single dose	Rat	LD_{50}	Smyth et al. 1951
546 mg/kg (TEA)	Single dose	Mouse	LD_{50}	Kagan 1965
96 mg/kg (TPA)	Single dose	Rat	LD_{50}	Smyth et al. 1969
340 mg/kg (TBA)	Single dose	Rat	LD_{50}	Monsanto Co. 1992
455 mg/kg (TBA)	Single dose	Rat	LD_{50}	Le Din Min 1976
114 mg/kg (TBA)	Single dose	Mouse	LD_{50}	Le Din Min 1976
615 mg/kg (TBA)	Single dose	Rabbit	LD_{50}	Le Din Min 1976
350 mg/kg (TBA)	Single dose	Guinea pig	LD_{50}	Le Din Min 1976
0.25-4 mL/kg (PA)	Single dose	Wistar rat, male, 3- to 4-wk-old	No observed effects at 0.25-0.5 mL/kg; death at 1-4 mL/kg; piloerection, eye opacity, fur discoloration, emaciation for one survivor of five dosed at 1 mL/kg	Union Carbide 1977
290-300 mg/kg (DBA)	Single dose	Mouse and rat	GI fatty infiltration and local irritation	Trubko 1975
230 mg/kg (DBA), intragastric	Single dose	Guinea pig	GI fatty infiltration and local irritation	Trubko 1975
100, 160, 250, 400, or 500 mg/kg (DBA), gavage	Single dose	ICR mouse (five per gender per group)	Lethargy at 100-400 mg/kg; convulsions at 500 mg/kg; death in two of five females at 250 mg/kg, five of five females at 400 and 500 mg/kg, and five of five males at 500 mg/kg	Putnam et al. 1995
0.7-2 cm^3/kg (DBA), intragastric	Single dose	Rabbit (n = 6)	Death at 1-2 cc/kg; increased reflex, convulsions at 0.7 cc/kg	Hanzlik 1922

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10-20 mg (TEA), intragastric	Single dose (1 per day for 3 d)	Rabbit (n = 6), 3.3-5 kg	Decreased formation of normal blastocysts	Chang 1964
500-600 mg/kg (BA), gavage	Single dose	Sprague-Dawley rat, male and female	Ataxia, convulsions	Cheever et al. 1982
50 mg/kg (DEA), intragastric	Single dose	Mice (n = 15), male	Hepatic nodules in 5 of 15	Rijhsinghani et al. 1982
Short-Term Exposures (6-30 d)				
7.8, 31, 78, 155 mg/kg (MA); 15, 59, 150, 295, mg/kg (TMA); intraperitoneal injection	Daily for 17 d	CD-1 mouse (n = 6-11)	Abdominal contractions lasting 3-10 min at maximal doses; TMA induced transient ataxia and tremors at 150 and 295 mg/kg; TMA at 295 mg/kg increased dead fetuses, caused death in 5 of 11 mice	Guest and Varma 1991
443 mg/kg (TMA), intraperitoneal injection	Daily for 10 d	CD-1 mouse (n = 5)	Decreased progeny brain DNA; decreased serum testosterone; adverse male postnatal growth	Guest and Varma 1993
Subchronic Exposures (30-180 d)				
54, 105, 210 mg/kg/d (TMA), diet	90 d	Sprague-Dawley rat, (n = 5-6), male, 4-wk-old	33-50% reduction in seminal vesicle weight at highest dose	Amoore et al. 1978
Chronic Exposures (0.5-3 y)				
400 mg/kg/d (DMA), diet	9 mo	Wistar rat (n = 30), male	Increased lipid peroxidation as indicated by higher MDA production, 19% over controls; 60% increase in lysosomal enzymes	Darad et al. 1983

(Continued)

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TABLE 4-2 Continued

Dose of Alkylamine	Treatment Time	Species	Toxic Effects	Reference
In Vitro Studies				
Culture at 0-2 mM (MA, DMA, and TMA)	48 h	CD-1 mouse embryos	Decreased yolk-sac diameter, head and crown rump length, embryonic DNA, RNA, protein content; decreased fetal survival	Guest and Varma 1991
Culture at 0.75 mM (TMA)	42 h	CD-1 mouse embryos	Embryonic growth inhibition (70% of controls); neural tube defects in 73% of embryos; 60% decrease in DNA and RNA content	Guest and Varma 1992

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nitrates and nitrites include vegetables, drinking water, and cured meats. Nitrates are also produced endogenously when extracellular nitrogen monoxide combines with oxygen to form nitrates, nitrites, and nitrosamines; production is increased by various physiologic and pathologic conditions (L'hirondel 1999). Dietary sources of amines include fish, an array of edible plants, dairy products, boiled beef, and a variety of beverages. Nitrosamines are found in foods such as cured meats and oils (Lijinsky 1976; Lijinsky 1980).

Human urinary excretion of nitrates is rapid and first-order; renal clearance occurs within 24 h of ingestion (Green et al. 1981). Nitrate ions that reach the stomach intact (that is, that escape bacterial conversion to nitrites) are not involved in nitrosamine formation (L'hirondel 1999). It is the nitrite that is the source of the "nitroso" group for nitrosamines formed in vivo from secondary amines (Klaassen 1996). Approximately 5% of ingested nitrate is converted to nitrite (Vermeer et al.1998). Nitrites in saliva arise from the reduction of nitrate in food or water by bacteria in the mouth (Lijinsky 1976; Tenovuo 1986). Salivary nitrites, upon swallowing, react with various amines in the stomach to form nitrosamines (L'hirondel 1999).

Endogenous formation of *N*-nitrosodi-*n*-butylamine (NDBA) was studied to examine butylated hydroxyanisole inhibition of DBA nitrosation in rats (Airoldi et al. 1987). Rats given three doses of DBA at 50 mg/kg perorally at 8-h intervals along with one 1.4 mg dose of sodium nitrate ($NaNO_3$) reportedly excreted 0.085 nanomoles (nmol) of the NDBA metabolite *N*-butyl-*n*-(3-carboxypropyl)nitrosamine (BCPN) in a 24-h post-dose urine sample. A second set of rats given DBA at 10 μmol and $NaNO_2$ at 20 μmol in 1 mL of DMSO twice at 12-h intervals was observed to excrete 0.213 nmol of BCPN in a 24-h postdose urine sample. Administration of DBA alone did not result in urinary BCPN excretion. Animals in this study are assumed to have received standard dietary feedings in addition to dose chemicals. In vitro nitrosation of DBA was tested utilizing an incubation medium containing DBA at 10 mM and $NaNO_2$ at 20 mM (alone or together with varying concentrations of BHA). The percentage of in vitro nitrosated DBA was 22.5%; in vivo nitrosation of DBA was measured at 0.005% in the animal experiments. The authors concluded that the comparatively smaller amount of DBA nitrosation in the in vivo study might be attributed to the limited period of DBA and/or nitrite in the stomach. The authors also noted that observed nitrosation in dosed animals might have occurred in other parts of the gastric tract.

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Relative to amine-induced tumor formation, DEA and TMAO fed to rats in combination with nitrite for 1-2 years (y) did not induce excess tumor formation (Lijinsky 1980; Lijinsky and Taylor 1977). Fifteen male and 15 female Sprague- Dawley rats were given 0.1% amine (average) and 0.2% $NaNO_2$ in 60 mL of water 5 d/wk for 2 y. Results of this study indicate that insufficient nitroso compounds were formed to induce tumors, although it is possible that nitroso formation occurred. To date, there are no studies that suggest that the target alkylamines are carcinogenic by reaction of ingested amines and nitrates or nitrites.

Human volunteers (eight males), ages 22-50, who were nonsmokers and free of medications were evaluated for urinary excretion of 3-methyladenine (3-MeAde), a biomarker used to assess endogenous formation of NDBA or other methylating agents that induce genetic damage by alkylation of DNA (Fay et al. 1997). Urinary levels of 3-MeAde varied, ranging from 8 to 150 nmol/d when volunteers consumed free-choice diets. Basal concentrations of 3-MeAde at 2-3 nmol/d were recorded when subjects consumed a controlled liquid diet. Urinary concentrations of 3-MeAde increased slightly when subjects consumed fresh or frozen fish; however, that increase was attributed to preformed 3-MeAde in the fish itself. When subjects consumed frozen fish, which contained a quantity of DMA 50-fold higher than that in fresh fish, urinary concentrations of 3-MeAde ranged from 3 to 4 nmol/d. When subjects consumed frozen fish and 325 mg of $NaNO_3$, urinary concentrations of 3-MeAde ranged from 2 to 3 nmol/d. From these results, the authors suggest that endogenous formation of *N*-nitrosodimethylamine (NDMA) from DMA is insignificant. The authors concluded that exposure to endogenously formed NDMA from dietary DMA in frozen fish appears negligible when compared with exposure from preformed dietary *N*-nitroso compounds. The authors also suggested that their findings indicate minimal health risk concerns in humans consuming high concentrations of DMA in various foods.

Acceptable daily intake (ADI) guidelines for nitrate and nitrite have been set by the World Health Organization (WHO) at 3.67 mg/kg and 0.13 mg/kg, respectively (Vermeer et al. 1998). Twenty-five healthy women (nonsmokers and medication free) who had an average weight of 60 kg were evaluated for nitrosamine formation (Vermeer et al. 1998). During control weeks, subjects received low-nitrate diets. During test weeks, subjects received an ADI nitrate load (220 mg/d) combined with meals of fish (high amounts of amines), including cod, salmon, shrimp, and pollack. NDMA and *N*-nitrosopiperidine (NPIP) were detected in multiple 24-h urine samples. The mean NDMA urinary excretion during

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control weeks was 287 nanograms (ng) per 24-h sample, and the mean during test weeks was 871 ng per 24-h sample. This study concluded that nitrosamine formation increases during nitrate intake in combination with meals of fish. The authors of this study did not evaluate preformed nitrosamine concentrations in the test meals consumed by the subjects.

A separate study (Lakritz et al. 1982) performed with human volunteers fed test meals containing nitrate and fish concluded that nitrosamine concentrations in urine, blood, and gastric juices were not significantly affected by ingestion of nitrosamine precursors. Yamamoto et al. (1980) measured blood nitrosamines in eight human subjects fed a Japanese diet rich in nitrate and amines. There were no observed increases in nitrosamine concentrations in the blood samples of test subjects.

In vivo formation of nitrosamines in humans was observed following subjects' ingestion of a bacon, lettuce, and tomato sandwich and beer (Fine et al. 1977). NMDA concentrations increased from 2 μg before the meal (in a blood volume of 6 L) to 4.4 μg 35 minutes (min) after the meal. NMDA concentrations in blood prior to the meal indicate the presence of background concentrations of nitrosamines. Fine et al. (1977) also observed that when volunteers maintained diets low in nitrite and nitrate and high in ascorbate for 24 h prior to the meal, NMDA could not be detected in their blood.

L'hirondel (1999) asserts that the amounts of nitrosamines formed in the stomach during the metabolism of nitrates are miniscule and are not capable of increasing the incidence of cancer in humans. There are no studies (in rats or mice) that have proved that sustained intake of nitrates results in an increased incidence of cancer. The author maintains that the amount of nitrosamines formed in the stomach during the metabolism of nitrates is several tens of thousands times smaller than the potential toxic concentration.

Prediction of in vivo formation of nitrosamines is difficult because of affecting factors, including reaction conditions in the stomach, nitrite and amine concentrations, and types of amines available (Lijinsky 1980). However, because formation of nitrosamines has been demonstrated in humans and because it is impossible to establish a "safe" dose for carcinogens (Lijinsky 1976), prudence in dietary consumption of foods containing nitrates or nitrites is recommended for spacecraft crew. On the basis of the above observations and deductions, it can be reasonably argued that there is minimal risk of amine nitrate- or nitrite-induced cancer for astronauts relative to that posed by orally ingested amines. Preformed dietary nitrosamines would be of greater concern, but it has been determined that the concentrations of direct dietary nitrosamine intake in a

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normal diet is several hundreds of times lower than potentially toxic levels (i.e., the no-observed-adverse-effect concentrations [NOAELs] for ingested nitrosamines in animals) (L'hirondel 1999).

Amine Induction of Cytosolic Alterations

Tertiary amines have been observed to induce cytoplasmic vacuoles in cultured rat urinary carcinoma or rabbit aorta muscle cells (Rorig et al. 1987). Aliphatic diamines and the bis-tertiary amines were both potent inducers of clear cytoplasmic vacuoles (CCV). The cationic moiety (the highly basic or protonated amine component with hydrophilic properties) of amphiphilic amine drugs and chemicals employed in a study by Ruben et al. (1993) was determined to be responsible for the induction of CCV. Ruben suggests a complimentary link between the basicity of the cationic moiety of amphiphilic amines and the storage of such drugs in cytoplasmic acidic vesicular compartments. Entrapped protonated amines cause an osmotic swelling of lysosomes, resulting in the morphologic manifestation of CCV (De Duve et al. 1974). A moiety of an anti-arrhythmic cationic amphiphilic bis-tertiary amine drug that had pKa's for each amine of >8.0 was determined to be a highly potent vacuole inducer (Rorig et al. 1987). Most of the target alkylamines of this document have pKa's of 10 or greater at 25ºC (see Table 4-1). These amines will be positively charged at a pH of less than 10. On the basis of chemical equilibrium considerations, at a physiologic pH of 7.4 there is a fraction of amine that has no charge that can pass through membranes, although the charged species cannot. In principle, alkylamines can employ this equilibrium behavior to distribute between acidic vesicles and areas of the cell that have a higher pH.

Chemical induction of CCV, as in the case of cationic amphiphilic amine drugs, indicates intracellular storage (Ruben 1987; Ruben et al. 1989; Burmester et al. 1990). Vacuolated cells did not show evidence of cell degeneration, such as dysplasia, atrophy, functional impairment, release of lactic dehydrogenase, or other ultra-structural cellular alterations, whether in whole animal or cultured mammalian cells. From these studies, it can be concluded that vacuolar induction is a sign of intracellular storage, is within the physiologic limits of cells, and is not associated with overt toxicity. There are no data describing vacuolar induction or other cellular alterations by the target alkylamines.

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Acute Toxicity (1-5 d)

Lethality and General Toxicity

Several independent studies have demonstrated the lethality of intragastrically administered alkylamines.

Propylamine (PA)

In toxicity studies conducted by the Union Carbide Corporation in 1977, PA was delivered intragastrically to 3- to 4-wk-old male Wistar rats (Union Carbide 1977). Three of three rats that received a 4 mL/kg dose died within the hour. Five of five rats dosed with 2 mL/kg died within 1 d. One of five rats survived a dose of 1 mL/kg. Exposure symptoms of the survivor included piloerection, bloody urine, emaciation, discoloration of fur, and bilateral lens opacity to complete eye opacity. Pathology of animals receiving lethal doses included petechial to diffuse hemorrhages of the lungs, stomach and intestinal distention, intestinal discoloration, and prominent acini of the liver. Five rats per group dosed with 0.5 mL/kg or 0.25 mL/kg exhibited no symptoms of toxicology, although one death occurred in the 0.5 mL/kg group.

Di-n-butylamine (DBA)

DBA was administered in a single dose by oral gavage to 6- to 8-wk-old male and female ICR mice weighing between 24 and 38 g (Putnam et al. 1995). In the toxicity assay of this study, mice received doses ranging from 100 to 500 mg/kg. Mortality, which occurred within 2 d of dosing, was observed in female and male mice receiving 250 mg/kg and in all dosed animals receiving 400 mg/kg or greater. Clinical manifestations included lethargy and, at the highest dose of 500 mg/kg, convulsions in both male and female mice.

Six rabbits received intragastric dosages of DBA at 1-2 cubic centimeters (cm^3) per kilogram body weight (Hanzlik 1922). Death occurred in all dosed animals within 6 min of dosing. Clinical symptoms associated with dosing included increased reflex excitability and increased

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pulse and respirations followed by convulsions, coma, and eventual death.

Hepatotoxicity

Thirty $C_{57}BL \times C_3H$ F1 male mice were administered a single intragastric dose of DEA at 50 mg/kg dissolved in distilled water to assay induction of hepatic nodules (Rijhsinghani et al. 1982). Mice were sacrificed between 70 and 110 wk after dosing. Five of 15 examined mice developed hepatic nodules; three adenomatous and two trabecular lesions were observed. Two of 17 control mice (treated with distilled water) developed trabecular lesions.

GI Toxicity

Pathomorphologic examination of the internal organs of animals that were administered intragastric doses of DBA at 290-300 mg/kg (mice and rats) and 230 mg/kg (guinea pigs) revealed fatty infiltration, changes indicating local GI irritation, and signs of necrotic liver damage (Trubko 1975). Further experimental methods were not stated.

Reproductive Toxicity

Rabbits (six) were intragastrically fed TEA at 10 or 20 mg 1, 2, and 3 d following artificial insemination to examine the effectiveness of mammalian antifertility agents (Chang 1964). Animals were sacrificed on day 6 of the experiment, and calculation of normal blastocysts was based on the number of corpora lutea. Dosing at 10 and 20 mg produced uterine blastocysts totaling 29% and 12% of corpora lutea. Normal blastocysts in untreated animals were 89% of total corpora lutea. Results indicate that TEA is effective in preventing the development of rabbit ova into normal blastocysts. Chang (1964) suggests that TEA inhibits the normal mechanism of blastocyst implantation into the endometrium; however, data presented in this study do not verify that.

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CNS Toxicity

Di-n-*butylamine*

Five male and five female ICR mice were dosed with DBA at 100, 160, 250, 400, or 500 mg/kg dissolved in 20 mL corn oil; dosing was by a single oral gavage (Putnam et al. 1995). Lethargy occurred in both male and female mice at 100, 160, 250, and 400 mg/kg. Convulsions were observed in male and female mice at the 500 mg/kg dose. Additional testing to assess the clastogenic potential of DBA by increased incidence of micronucleated polychromatic erythrocytes in bone marrow produced negative results.

n-*Butylamine*

BA was administered by gavage to male and female weanling Sprague-Dawley rats at 100, 200, 300, 400, 500, and 600 mg/kg in corn oil (Cheever et al. 1982). Animals were observed for mortality and toxicity for 14 d following dosing. Signs of toxicity included sedation, ataxia, nasal discharge, gasping, and salivation. Convulsions and death were observed at higher doses (amount not specified). Further description of toxic response was not presented. An LD_{50} of 371 mg/kg was calculated for BA.

Other Adverse Acute Effects

Methylamine-Induced Leucopenia

Five $B6C3F_1$ female mice were dosed daily with MA at 122, 489, or 978 mg/kg in Hanks balanced salt solution via intraperitoneal injection for 5 d to assess the immunologic effects of the decomposition products of the agricultural pesticide sodium methyldithiocarbamate (Keil et al. 1996). Suppression of immune parameters in dosed animals was assayed to include decreases in natural killer cell activity, spleen and thymus weight, lymphocytes, and specific thymocytes. White blood cell counts in MA-dosed animals decreased by 27%, 60%, and 55%, respectively, compared with control animals. All decreases were statistically significant. White blood cell differentials revealed no change in proportional

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leukocyte cell types resulting from this study. No further adverse immunologic effects measured in this study were induced by MA.

Enzyme Alterations

Di-*n*-butylamine Doses of one-fifth and one-twentieth the LD_{50} (58 mg/kg and 14.5 mg/kg) administered to white mice caused decreased hepatic diaminoxidase activity (Trubko 1975). The route of administration in this study was not clarified.

Dimethylamine Male Swiss albino CD-1 mice and Sprague-Dawley rats were treated with DMA at 25 or 50 mg/kg by a single ip injection or for 3 consecutive days (Galli et al. 1993). Monooxygenase activities, including ethoxyresorufin *O*-deethylase (EROD), pentoxyresorufin *O*-dealkylase (PROD), *p*-nitrophenol hydroxylase (pNPH), and aminopyrine *n*-demethylase (APD), were monitored for amine effects on hepatic oxidative enzymes. Significant inhibitory effects were induced by DMA. Reductions in EROD, PROD, and *p*NPH resulted in levels that ranged from 54% to 86% of controls for the single ip injections at 50 mg/kg. For 3-d repeated dosing, enzyme reductions resulted in levels ranging from 36% to 75% of controls. Single injections at 25 mg/kg induced a 17-47% reduction in activity of the hepatic enzymes PROD, EROD, and pNPH. Multiple injections at 25 mg/kg induced a 28-57% reduction. There were no significant alterations in the expression of ADP enzymes.

Short-Term Toxicity (6-30 d)

CNS Toxicity

Trimethylamine

TMA administered to CD-1 mice intraperitoneally at 150 or 295 mg/kg once per day from day 1 to day 17 of gestation caused ataxia, shallow and rapid breathing, nasal discharge, and tremors (Guest and Varma 1991). Those effects persisted for 10 min; complete recovery was observed within 20 min.

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Triethylamine

In short-term experiments using white mice perorally administered TEA (doses not given), marked CNS effects were observed (Kagan 1965). Physical signs of CNS toxicity included increased excitability replaced by inhibition, disturbances in the coordination of movements, and clonic spasms. Further experimental details were not presented.

In a non-peer-reviewed study, 3-month (mo)-old albino rats grouped in tens (five male and five female) were administered TEA intragastrically at 0, 5, 15, 30, and 60 mg three times per week for 6 wk (Davison et al. 1965). TEA doses were 14, 43, 86, and 172 mg/kg, respectively. Female rats receiving TEA at 172 mg/kg were observed to have red blood cell counts at 63% of those in controls. Treated groups of rats averaged 9% lower red blood cell counts than controls. This decrease was attributed to hemorrhaging of the stomach lining, which was revealed upon necropsy. White blood cell count, differential cell counts, and sodium and potassium values were within control levels. At 30 mg (86 mg/kg), rats exhibited convulsions and abnormal balance and tail wringing when disturbed. These symptoms disappeared 6-8 h after dosing. Rats given TEA at 60 mg (172 mg/kg) exhibited marked convulsions. Symptoms included extended periods of hunching, odd posturing, lethargy, violent head shaking, tail wringing, and tremors. Convulsions were most severe in females, and death occurred only in females. An evaluation of the significance of observed changes in dosed rats was not presented. Additional pathology upon examination of lung tissue and kidneys revealed the presence of pneumonia, to which the author attributes the excess animal mortality occurring in the study. The results of this study are difficult to interpret because of the adverse health status of test animals.

Subchronic Toxicity (30-180 d)

Reproductive Toxicity

Groups of five or six male Sprague-Dawley rats were fed daily doses of TMA at 27, 54, 105, or 210 mg/kg for 90 d (Amoore et al. 1978). The maximum concentration of TMA administered in the diet that was not incompatible with normal food consumption and growth was 54 mg/kg. That dose was hence affirmed to be the no-adverse-effect level

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(NAEL) for TMA for this study. Rats administered the highest dose of 210 mg/kg (four times the NAEL level) were observed to have 33-50% reductions in seminal vesicle weights compared with controls. No data specific to pathologic or physiologic changes were presented for animals that received the 105-mg/kg dose.

Enzyme Alterations

Rabbits dosed with DBA at 7.6 mg/kg for 6 wk exhibited a 47% increase in serum glutamic oxalate and a 36% increase in glutamic pyruvic transaminase (Trubko 1975). The maximal response was observed in week 14 of the experiment; a return to normal enzymatic activity levels was observed by week 21 of observation. The route of administration was not clarified. Other data defining the quality of experimental methods are not presented in the study.

Chronic Toxicity (0.5-3 y)

Hepatotoxicity

In vitro microsomal lipoperoxidation and hepatic lysosomal enzyme activity were studied for toxic response in male Wistar rats administered DMA (Darad et al. 1983). DMA was administered to 30 rats at a concentration of 0.2% in drinking water for 9 mo. The DMA dose per rat was 400 mg/kg/d. Hepatic microsomal lipoperoxidation was assayed by malondialdehyde formation. DMA caused significantly higher peroxidation in rat liver microsomes, eliciting 19% greater malondialdehyde formation than in controls. DMA increased free activities of cathepsin by 32% over controls and of acid phosphatase by 11% over controls. Free activity of both enzymes increased significantly, by 60%, when measured as a fraction of total activity.

Enzyme Alterations

Rabbits and rats orally administered TBA at 6.1 and 4.5 mg/kg, respectively, for 6 mo were observed to have significantly decreased hepatic diamine oxidase activity when compared with control animals (Le Din Min 1976). Diamine oxidase activity in dosed animals was measured

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at 48% of the activity in control animals. In addition, serum amine oxidase activity was observed to decrease to below 30% of the baseline value. Values of other measured parameters, including differential cell counts, were stated as not significantly distinct from those of control animals. It is unclear whether the author conducted this study or results were obtained from other studies.

Genotoxicity

The evaluated alkylamines (MA, EA, PA, IPA, BA, DMA, DEA, DBA, TMA, TEA, TBA) were negative for induction of point mutations with and without hamster or rat Aroclor-induced S9 fraction in *Salmonella typhimurium* mutagenic assays (Mortelmans et al. 1986; Zeiger et al. 1987). Tested alkylamines were negative in other mutagenicity tests that employed prokaryotic systems (Table 4-3).

DMA was positive for induction of a dose-dependent increase in mitotic gene conversion and point reverse mutation in the mutagenic assay employing the D7 strain of *Saccharomyces cerevisiae* (Galli et al. 1993). The authors attributed the positive results, which occurred in the presence of S9 fraction to formaldehyde, to the primary DMA metabolite.

Mammalian genotoxicity assays of DBA yielded both positive and negative results. DBA was positive for induction of sister chromatid exchanges in Chinese hamster cells (Abe and Sasaki 1977). Cell type or other experimental data for this study are not presented. When tested for chromosomal aberrations using Chinese hamster lung fibroblasts, DBA was evaluated as negative for mutagenicity, although it was judged “suspicious” in accordance with the authors’ experimental criteria of 5-5.9% greater occurrence of chromosomal aberrations than controls (<3%) (Ishidate and Odashima 1977). In an unpublished study, male and female ICR mice (five per gender per dose) were given a single oral gavage dose of DBA at 100, 160, 250, 400, or 500 mg/kg dissolved in 20 mL corn oil (Putnam et al. 1995). The results of an assessment of the clastogenic potential of DBA by induction of increased incidence of micronucleated polychromatic erythrocytes in bone marrow were concluded to be negative. DBA was also negative for chromosomal aberrations in human embryo and hamster lung fibroblasts and in rat bone marrow cells in genotoxicity assays conducted in separate studies (Kawachi et al. 1980).

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TABLE 4-3 Summary of Amine Genotoxicity Studies

Test	Metabolic Activation	Concentration (Mutation Frequency)[a]	Exposure Duration	Cytotoxicty Test and Lethal Dose	Genotoxicity Evaluation	Reference
Methylamine						
L5178Y MOLY forward mutation assay	None	Distilled water (38) (average of three cultures) 0.65, 1.3, 1.95, 2.6[b] mM (negative) 3.9 mM (57) 5.2 mM (57)	4 h	3.9 mM	Positive	Caspary and Myhr 1986
Salmonella mutagenicity (TA 98, 100, 1535, 1537)	None and 10% RLI or 10% HLI Aroclor 1254 S9 fraction	0 (water as solvent) 33, 100, 333, 1,000, 3,333, 10,000 µg/plate (negative)	48 h	No toxicity	Negative	Mortelmans et al. 1986
Salmonella mutagenicity (TA 98, 100, 104)	(5%, 15%, 30%) S9 fraction Aroclor 1254	0 (DMSO as solvent) 0.0368, 0.368, 0.736, 14.72, 29.44 mg/plate (negative)	48 h	29.4 mg/plate	Negative	Meshram et al. 1992
Ethylamine						
Paper-disk *E. coli* reversion to streptomycin independence	None	0.01-0.025 mL solution or small crystals	Not given	No data	Negative	Syzbalski 1958

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Salmonella mutagenicity (TA 98, 100, 1535, 1537)	None and 10% RLI or 10% HRL Aroclor 1254 S9 fraction	0 (water as solvent), 100, 333, 1,000, 3,333, 10,000 µg/plate (negative)	48 h	No toxicity	Negative	Mortelmans et al. 1986
Propylamine						
Salmonella mutagenicity (TA 98 and 100)	None and Aroclor Hamster S9	10 µL in DMSO per plate (negative)	48 h	No toxicity	Negative	Speck et al. 1982
Isopropylamine						
Salmonella mutagenicity (TA 98, 100, 1535, 1537)	None and 10% RLI or 10% LHI Aroclor 1254 S9 fraction	0 (water as solvent), 10, 33, 100, 333, 1,000, 3,333, 10,000 µg/plate (negative)	48 h	No toxicity	Negative	Zeiger et al. 1987
n-Butylamine						
Salmonella mutagenicity (TA 98, 100, 1535, 1537)	None and 10% RLI or 10% HRL Aroclor 1254 S9 fraction	0 (water as solvent), 3.3, 10, 33, 100, 333, 1,000, 3,333 µg/plate (negative)	48 h	No toxicity	Negative	Zeiger et al. 1987

(Continued)

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TABLE 4-3 Continued

Test	Metabolic Activation	Concentration (Mutation Frequency)[a]	Exposure Duration	Cytotoxicty Test and Lethal Dose	Genotoxicity Evaluation	Reference
Dimethylamine						
Saccharomyces cerevisiae D7; mitotic genconversion and point reverse mutation	None and S9 rat	0 (1.03 convertants/10^5; 0.40/10^6 revertants) 0.5, 1 mM (negative) 2 mM[b] (3.8-fold increase convertants; 3.5-fold increase revertants) 3 mM (3.8-fold increase convertants; 3.5-fold increase revertants) 4 mM (3.8-fold increase convertants; 3.5-fold increase revertants)	2 h	Cell survival 25% of controls at 4 mM	−S9, negative +S9, positive Genotoxicity results of DMA metabolites	Galli et al. 1993
Salmonella mutagenicity (TA 98, 100, 1535, 1357)	None and 10% RLI 10% HLI Aroclor 1254 S9 fraction	0 (water as solvent) 33, 100, 333, 1,000, 2,000, 3,333, 4,000, 4,500 µg/plate (negative)	48 h	Toxicity at 3,333 µg/plate	Negative	Zeiger et al. 1987
Diethylamine						
Salmonella mutagenicity (TA 98, 100, 1535, 1357)	None or 10% RLI 10% HLI Aroclor 1254 S9 fraction	0 (water as solvent) 33, 100, 333, 1,000, 3,333 µg/plate (negative)	48 h	No toxicity	Negative	Zeiger et al. 1987

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Di-*n*-butylamine						
Chinese hamster ABS	None	0 (ethanol) (0.2 breaks per cell) 10^{-6}-10^{-3} M (in ethanol)	26 h	No data	Negative	Abe and Sasaki 1977
Chinese hamster SCE	None	0 (ethanol) (3.36 SCEs per cell) 10^{-6}-10^{-3} M (in ethanol) 5×10^{-4} M^b (5.83 SCEs per cell)	26 h	No data	Positive	Abe and Sasaki 1977
Paper-disk *E. coli* reversion to streptomycin independence	None	0.01-0.025 mL solution or small crystals	Not given	No data	Negative	Syzbalski 1958
Salmonella mutagenicity (TA 98, 100); *Bacillus subtilis*; hamster lung fibroblast and rat bone marrow ABS/SCE; silkworm mutation	None	Not given	Not given	No data	Negative	Kawachi et al. 1980

(Continued)

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TABLE 4-3 Continued

Test	Metabolic Activation	Concentration (Mutation Frequency)[a]	Exposure Duration	Cytotoxicity Test and Lethal Dose	Genotoxicity Evaluation	Reference
Salmonella mutagenicity (TA 98, 100, 1535, 1537)	None or 10% RLI or 10% HLI Aroclor 1254 S9 fraction	0 (water as solvent), 100, 333, 1,000, 3,333, 10,000 µg/plate	48 h	No toxicity	Negative	Mortelmans et al. 1986
Salmonella mutagenicity	-S9/+S9	Not given	Not given	No data	Negative	Sugimura et al. 1977
CHL ABS	None	0.2-15.5 mg/mL in ethanol	48 h	No data	Negative	Ishidate and Odashima 1977
Trimethylamine						
Salmonella mutagenicity (TA 98, 199, 1535, 1537)	None or 10% RLI or 10% HLI Aroclor 1254 S9 fraction	0 (water as solvent) 10, 33, 100, 333, 1,000 µg/plate (negative)	48 h	No toxicity	Negative	Mortelmans et al. 1986
Triethylamine						
Salmonella mutagenicity (TA 98, 100, 1535, 1357)	None or 10% RLI 10% HLI Aroclor 1254 S9 fraction	0 (ethanol as solvent) 100, 333, 1,000, 3,333, 10,000 µg/plate (negative)	48 h	No toxicity	Negative	Zeiger et al. 1987

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Tributylamine						
Salmonella mutagenicity (TA 98, 100, 1535, 1537)	None 10% RLI or 10% LHI Aroclor 1254 S9 fraction	0 (ethanol as solvent) 33, 100, 333, 1,000, 3,333 µg/plate	48 h	No toxicity	Negative	Zeiger et. al. 1987

[a]Relative to controls.
[b]Lowest effective dose.
Abbreviations: ABS, aberrations; DMSO, dimethylsulfoxide; HLI, hamster liver induced; MLA, mouse lymphoma forward mutation assay; RHI, rat liver induced; SCE, sister chromatid exchange.

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MA was positive in the mouse lymphoma forward mutation assay employing L5178Y mouse lymphoma cells (Caspary and Myhr 1986). The two highest effective doses, 3.9 and 5.6 mM, produced lethality in the cells. There are no data available on TPA mutagenicity testing and no further data available for other target alkylamines.

The target alkylamines for this document can be considered nonmutagenic on the basis of the results of Ames *S. typhimurium* mutagenicity assays, although there are limited data from mutagenicity tests that employed eukaryotic or mammalian cell systems that produced positive results for amine-induced genotoxicity. The potential genotoxicity of the target alkylamines is considered negligible when related to the levels of possible ingestion by crew members over designated periods of exposure. Derived exposures guidelines for target alkylamines should protect against potential genotoxicity resulting from alkylamine ingestion during spaceflight.

Developmental Toxicity

Methylamine and Trimethylamine

MA and TMA were administered to CD-1 mice by ip injection once per day from day 1 to day 7 of gestation (day 0 = day of mating) (Guest and Varma 1991). The objective of this study was to determine if amines adversely affect fetal development. Amine doses were 7.8, 31, 78, 155 mg/kg, for MA and 15, 59, 150, and 295 mg/kg for TMA. Amines were dissolved in 0.9% saline for injections. TMA at 150 and 295 mg/kg and MA at 155 mg/kg caused abdominal contractions at the injection site that lasted from 3 to 10 min. The same doses of TMA caused ataxia, shallow and rapid breathing, nasal discharge, and tremors. These effects persisted for 10 min; complete recovery was observed within 20 min. The TMA dose of 295 mg/kg (maximum dose) produced death in 5 of 11 pregnant mice and 6 of 6 virgin mice. TMA at the maximum dose adversely affected fetal development by significantly increasing the number of dead fetuses per litter (0.83 dead fetuses per litter for controls and 1.63 dead fetuses per litter for TMA-exposed animals); however, that dose was lethal to almost half the dams.

Both MA and TMA produced concentration-dependent adverse effects in cultured CD-1 mice embryos (Guest and Varma 1991). Mice were killed on day 8 of gestation and embryos were scored for development on the basis of an adaptation of the developmental scoring assess-

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ment described by Brown and Fabro (1981). Head length was most adversely affected by both MA and TMA at concentrations of 0.5-2 mmol (maximum doses). Decreases in yolk-sac diameter, crown-rump length, and fetal survival were also observed at 0.5-2 mmol. TMA was the more toxic of the amines. TMA and MA induced significantly decreased embryonic DNA and RNA and protein content at 0.25-2 mmol (TMA) and 0.5-2 mmol (MA).

Trimethylamine

Intraperitoneal injections of TMA at 7.5 mmol (443 mg/kg) administered to Swiss CD-1 mice (n = 5) from gestation day 6 to gestation day 15 caused a 15% decrease in brain DNA and a 10% decrease in brain protein in male and female offspring at 3 wk after birth (Guest and Varma 1993). In male offspring, these decreases persisted through 8 wk after birth; treated animals had 636 µg total brain DNA and 32 mg total brain protein compared with 748 µg total brain DNA and 35 mg total brain protein in controls. Serum testosterone in 8-wk-old male offspring was 5.6 nmol/L compared with 16 nmol/L in controls, demonstrating a 65% decrease. Other growth-related variables measured in offspring that showed 6-20% decreases compared with controls included body, brain, kidney, and seminal vesicle weights. The authors hypothesize that TMA adversely affected postnatal growth of male offspring by decreasing testosterone production, which consequently reduced brain DNA and protein.

TMA interferes with histiotrophic nutrition in the developing CD-1 mouse embryo by inhibiting receptor-mediated protein uptake by the yolk sac of CD-1 mice (Guest et al. 1994). The authors concluded that this inhibition causes a reduction in macromolecular synthesis in embryos and consequently causes reductions in growth and abnormal development. TMA at a concentration of 0.75 mM inhibited the uptake of [^{125}I]-BSA in 15-d isolated yolk sacs. Uptake in TMA-treated yolk sacs was 75% of the uptake in controls after a 5-h culture period. In this same study, yolk-sac uptake of [^{3}H]-leucine in TMA-treated embryos (28.7 femtomoles [fmol]) was 47% of that in controls (61.2 fmol) in 8-d mouse embryos cultured for 16 h with TMA at 0.75 mM. Incorporation of radioactivity in TMA-treated embryo protein (8.9 fmol) was 44% of that in controls (20.3 fmol).

In an earlier study (Guest and Varma 1992), TMA at 0.75 mM inhibited the growth and morphologic development of treated CD-1 mouse

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embryos, caused neural-tube defects, and decreased macromolecular synthesis. Treated embryos grew to only 70% of controls. Neural tube defects, characterized by a split-head appearance, occurred in 73% of TMA-treated embryos. Data from this study suggest that TMA-induced developmental inhibition is temporally related to decreased DNA and RNA synthesis, because DNA and RNA embryonic content decreased by as much as 60% in comparison with controls.

Mechanistic Toxicology

Methylamine

MA has been shown to inhibit DNA synthesis stimulated by epidermal growth factor (EGF), insulin, and serum (King et al. 1981). Inhibition of DNA synthesis was determined by cell incorporation of [^{3}H]-thymidine. MA had no effect on resting-level DNA synthesis in cultured human fibroblasts. However, MA inhibited EGF-induced mitogenesis in confluent monolayers of human fibroblasts incubated with MA at 0-20 mM; the maximal inhibition was observed at MA concentrations of 10-20 mM. A 50-75% inhibition of cell incorporation of ^{3}H was demonstrated in 3T3 cells when stimulated by insulin at 5-20 µg/mL in the presence of MA at 10 mM. Human fibroblasts incubated in either 1% or 10% serum without MA or with MA at concentrations up to 20 mM for 23 h demonstrated a 50% decrease in DNA synthesis at 10 mM.

Trimethylamine

Embryos of CD-1 mice treated with TMA at 0.75 mM exhibited decreased macromolecular synthesis (Guest and Varma 1992). DNA content of TMA-treated embryos was decreased significantly (10 µg per embryo) compared with that of controls (25 µg per embryo) beginning 16 h into the 42-h culture period. [^{3}H]-thymidine incorporation into DNA in TMA-treated embryos also was significantly decreased when compared with controls beginning 8 h into the culture period. RNA content of TMA-treated embryos was significantly decreased compared with controls at 16 h into the culture period and remained so throughout the experiment. Uptake of [^{3}H]-uridine into TMA-treated embryos was decreased significantly beginning at 8 h into the culture period. TMA decreased the incorporation of [^{3}H]-leucine into embryonic protein during

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the entirety of a 24-h culture period; the decrease became significant beginning at 8 h into the culture period.

Triethylamine

TEA inhibits rat liver hydrosteroid sulfotransferase activity (Matsui et al. 1993). Hepatic cytosolic fractions obtained from adult male and female Wistar rats were incubated with the biochemically prepared substrate 3'-phosphoadenosine 5'-phosphosulfate (PAPS) and TEA at 100 or 300 μM to assess inhibition of sulfotransferase activity. Under normal physiologic conditions, sulfotransferase catalyzes the transfer of a sulfate group from PAPS to a variety of xenobiotic and endogenous compounds, including androgens. In female rats, sulfotransferase activity toward the androgens androsterone and dehydroepiandrosterone by TEA at 100 μM ranged from 16% to 47% of controls. Sulfotransferase activity toward the androgens by TEA at 300 μM ranged from 15% to 49% of controls. These results indicate that TEA is an inhibitor of hydroxysteroid sulfotransferase. In this same study, multiple amines at 300 μM each were examined for inhibitory actions on sulfotransferase activity toward the androgen dehydroepiandrosterone obtained from male and female hepatic cytosols. DBA, TEA, TPA, and TBA decreased sulfotransferase activity to 40-60% of that of the controls. Other tested amines, MA, EA, PA, and TMA, resulted in 87%, 79%, 96%, and 99% of control sulfotransferase activity. The author notes that the tertiary amines with three to four carbons had greater effect on sulfotransferase activity than did the primary or secondary amines, with the exception of DBA. There are currently no data on whether TEA can inhibit human androgen sulfotransferase activity.

Odor and Taste Aversion

The chemical senses of taste and smell are critical to nutrition and serve to alert individuals to potentially hazardous exposures. Smell is more sensitive than taste, and only a minute quantity of an airborne stimulating agent is required to elicit olfactory sensation (Moncrieff 1967; Guyton and Hall 2000). Humans have an innate ability to detect bad, aversive smells and to use smell as a warning device. The relationship between taste (gustation) and smell (olfaction) is illustrated during episodes of nasal congestion when the sense of taste becomes dulled. The

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tastes of foods encountered in daily life are strongly affected by their smell (Kaneda et al. 2000). It is proposed that the flavor of food is ostensibly affected by its aroma. Volatile food molecules reach the nasal cavity prior to ingestion, and other molecules travel via the retronasal route, through the pharynx, to reach olfactory neurons (Freedman 1993).

Olfaction and gustation are closely related. Neuronal inputs from both olfactory and gustatory receptors travel in several common areas of the brain. The olfactory and gustatory neural pathways are known to converge in various parts of the brain, although those pathways have not been fully elucidated. The insular cortex, the orbital-frontal cortex, and the amygdala are candidate sites that might serve as a central point of intermodal convergence that contains neurons responsive to the combined inputs of olfactory and gustatory chemosensation (Dalton et al. 2000). In an investigation of central neural integration of simultaneous chemosensation that used benzaldehyde and saccharin as odor and taste stimuli, respectively, at (individual) sub-threshold levels, Dalton et al. (2000) concluded that benzaldehyde thresholds were 28% lower with saccharin in the mouth than the thresholds obtained with benzaldehyde alone. In effect, sensitivity to benzaldehyde was significantly increased by the presence of subthreshold concentrations of saccharin in the mouth. This finding supports the functional significance of neural response to combinations of odor and taste stimuli (the sense of smell working in conjunction with the sense of taste to create a flavor perception). It also supports the existence of central neural integration loci that respond to commonly conveyed chemosensory events.

Odor and taste perceptibility are primary concerns for potable water consumed by the space crew. Aversion to odor or taste of drinking water supplies can result in or exaggerate crew dehydration and, consequently, may contribute to diminished crew performance in spaceflight. Alkylamines have a distinctly unpleasant odor characterized as “fishy” or ammoniacal. Amines are nasal irritants; however, symptoms of irritation do not occur from chronic exposure to amine concentrations less than 10 parts per million (ppm) (Benya and Harbison 1994). Aversion to the taste or odor of amine-contaminated water occurred during the operative phase of the LIRS water reclamation assembly wherein concentrations were 60 mg/L, 176 mg/L, and 793 mg/L for TMA, TBA, and TPA, respectively, in a single galley sample.

Geometric means of air and water-dilution odor thresholds are presented for MA, EA, BA, IPA, DMA, DEA, TEA, and TMA in Table 4-4 as compiled by Amoore and Hautala (1983). Thresholds are based on

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TABLE 4-4 Water Odor and Taste Perceptibility Thresholds for the Alkylamines

Alkylamine	Water-Dilution Odor Threshold (mg/L)	Taste Perceptibility (water mg/L)
MA	*2.4*[a] 3.3[b]	NA
EA	*4.3*[a]	NA
PA	NA	NA
BA	*6.0*[c]	3.5-4.0[c]
IPA	*4.9*[a]	NA
DBA	*2.0*[c]	3.5-4.0[c]
DEA	*0.47*[a] 10[d]	8[d]
DMA	*0.29*[a] 23.2[b]	NA
TMA	0.0002[a] *1.7*[b]	NA
TEA	*0.42*[a] 4.0[d]	3.0[d]
TPA	NA	NA
TBA	*0.8*[e]	NA

[a]Amoore and Hautala 1983 (presented as geometric means).
[b]Baker 1963.
[c]Trubko 1975.
[d]Kagan 1965.
[e]Le Din Min 1976.
Note: Italicized numbers were used in the calculation of ACs for odor aversion.
Abbreviations: BA, *n*-butylamine; DBA, dibutylamine; DEA, diethylamine; DMA, dimethylamine; EA, ethylamine; IPA, isopropylamine; MA, methylamine; NA, data not available; PA, propylamine; TBA, tributylamine; TEA, triethylamine; TMA, trimethylamine; TPA, tripropylamine.

human studies conducted to assess odor perception of amines in air or water. Water-dilution odor thresholds represent the concentrations in water that generate odor thresholds in headspace vapors. Water odor and taste perceptibility thresholds for amines offered by other investigators are included in the table. There are no data available on thresholds for PA or TPA. Several amines have at least two reported threshold concentrations.

Limited data are available on taste perceptibility of alkylamines in potable water. Taste perceptibility concentrations for BA, DBA, DEA,

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and TEA were roughly comparable to respective amine water-dilution odor threshold concentrations reported by the same authors (Kagan 1965; Trubko 1975; Amoore and Hautala 1983). In addition, taste perceptibility concentrations for DBA, DEA, and TEA are similar to their respective recommended maximal permissible concentrations. The recommended maximal permissible concentration for TBA (0.3 mg/L reported by Le Din Min [1976]) is comparable to its reported water-dilution odor threshold.

REGULATORY STANDARDS

There are no regulatory standards set for alkylamines in potable water relevant to SWEGs.

RATIONALE

No toxicity studies were found in which humans were orally administered target SWEG alkylamines. Oral toxicity data on EA, IPA, and TPA were not found. Limited oral toxicity data in nonhuman mammalian species were reported for MA, PA, BA, DMA, DEA, DBA, TMA, TEA, and TBA. The majority of those studies do not present findings that reliably indicate amine induction of toxicity, but they present physiologic changes or irregularities that could be biologically significant. One such study, Davison et al. (1965), cannot be effectively interpreted because of adverse health conditions in the majority of the study animals. SWEGs are not set to protect against developmental toxicity. (The SWEGs are presented in Table 4-5.)

Alkylamines are involved in comparable physiologic disposition, including rapid absorption, N-oxidation, and elimination. Chemosensory thresholds for the amines also are comparable. Therefore, the derived SWEG values apply broadly to each alkylamine group (the mono-, di-, and trialkylamines discussed in this document). Because odor perception is a more sensitive end point than taste perception (Moncrieff 1967; Guyton and Hall 2000), water-dilution odor thresholds were the preferred chemosensory stimuli used to establish the SWEG values for the alkylamines. Odor perception is presented as a nontoxic chemosensory end point that will prevent or minimize flight-crew dehydration. It is expected that odor perception would adversely impact crew willingness to drink amine-contaminated potable water supplies. Smell aversion to

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TABLE 4-5 Spacecraft Water Exposure Guidelines for the Alkylamine Groups[a]

Duration	Concentration (mg/L)	End Point
Monoalkylamines		
1 d	2.0	Odor perception
10 d	2.0	Odor perception
100 d	2.0	Odor perception
1,000 d	2.0	Odor perception
Dialkylamines		
1 d	0.3	Odor perception
10 d	0.3	Odor perception
100 d	0.3	Odor perception
1,000 d	0.3	Odor perception
Trialkylamines		
1 d	0.4	Odor perception
10 d	0.4	Odor perception
100 d	0.4	Odor perception
1,000 d	0.4	Odor perception

[a]Data from Amoore and Hautala 1983; Le Din Min 1976; Trubko 1975; Baker 1963.

malodorous water supplies could cause or increase the possibility of crew dehydration, which could adversely affect crew performance and could increase both crew health risks and mission flight risks. Therefore, the SWEG values based on odor perception are intended to support optimal flight-crew consumption of potable water supplies, thus minimizing crew dehydration.

Acceptable concentrations (ACs) (see Table 4-6) were established for toxic or adverse health end points—for the alkylamines, those include reproductive toxicity and odor perception. ACs were determined on the basis of guidelines established by the National Research Council (NRC 2000). By convention, each crew member is assumed to ingest 2.8 L of water per day of flight and to have an average body weight of 70 kg. Interspecies and lowest-observed-adverse-effect level (LOAEL)-to-NOAEL factors of 10 each were employed as needed. ACs for alkylamine groups were based on odor perception, because developmental toxicity is not considered relevant for space crew. Odor thresholds (Table 4-4) used to establish ACs were selected on the basis of the most recently conducted experimental assessments and/or on the consistency among alkylamine groups. The lowest of the odor thresholds was

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TABLE 4-6 Acceptable Concentrations (ACs) for Relevant Mono-, Di-, and Trialkylamines[a]

			Uncertainty Factors				ACs (mg/L)			
End Point	Exposure Data	Species and Reference	To NOAEL	Interspecies	Exposure Time	Space-flight	1 d	10 d	100 d	1,000 d
Reproductive toxicity	NOAEL = 54 mg/kg	Amoore et al. 1978	1	10	1.1	1	—	—	120	—
Odor perception	Water dilution odor threshold	Humans (Amoore and Hautala 1983; Trubko 1975; Le Din Min 1976; Baker 1963)								
	Monoalklyamines		—	—	—	—	2	2	2	2
	Dialkylamines		—	—	—	—	0.3	0.3	0.3	0.3
	Trialkylamines		—	—	—	—	0.4	0.4	0.4	0.4
SWEGs for monoalkylamines							2	2	2	2
SWEGs for dialkylamines							0.3	0.3	0.3	0.3
SWEGs for trialkylamines							0.4	0.4	0.4	0.4

[a]Includes methylamine, ethylamine, propylamine, isopropylamine, *n*-butylamine, dimethylamine, diethylamine, di-*n*-butylamine, triethylamine, tripropylamine, and tributylamine.

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selected for alkylamines that had multiple reported thresholds, with the exception of that selected for TMA, because the lowest threshold reported for that chemical is presumed to be an experimental outlier. It is reasonable to expect that crew members would tolerate consumption of malodorous water for a short period of time (1 d). One-day ACs were set as the mean water-dilution odor threshold for each amine group. Ten-day ACs were set at the lowest odor threshold for each amine group, as were long term ACs (100- and 1,000-d).

1-d ACs

Amoore and Hautala (1983), Baker (1963), Trubko (1975), and Le Din Min (1976) reported water-dilution odor threshold concentrations for monoalkylamines MA, EA, IPA, and BA at 2.4, 4.3, 4.9, and 6.0 mg/L, respectively (Table 4-4). Respective thresholds for dialkylamines DMA, DEA, and DBA were reported at 0.29, 0.47, and 2.0 mg/L. For trialkylamines TEA, TMA, and TBA, water-dilution odor thresholds were reported at 0.42 mg/L, 1.7mg/L, and 0.8 mg/L, respectively. The lowest of the reported odor threshold values for each group of alkylamines, with exception of a presumed outlier value for TMA (0.0002 mg/L) that was not used, was selected as the AC. The 1-d ACs for the alkylamine groups were established as follows:

1-d AC for monoalkylamines = 2 mg/L,
1-d AC for dialkylamines = 0.3 mg/L,
1-d AC for trialkylamines = 0.4 mg/L.

10-d ACs

Amoore and Hautala (1983), Baker (1963), Trubko (1975), and Le Din Min (1976) reported water-dilution odor threshold concentrations for monoalkylamines, dialkylamines, and trialkylamines (Table 4-4). The lowest of the reported odor threshold values for each group of alkylamines, with the exception of a presumed outlier value for TMA (0.0002 mg/L) that was not used, was selected as the AC. The 10-d ACs for the alkylamine groups were established as follows:

10-d AC for monoalkylamines = 2mg/L,
10-d AC for dialkylamines = 0.3mg/L,
10-d AC for trialkylamines = 0.4mg/L.

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100-d ACs

Reproductive Toxicity

Amoore et al. (1978) reported that among rats receiving TMA at 27, 54, 105, and 210 mg/kg daily for 90 d, rats dosed at 210 mg/kg demonstrated a 33-50% reduction in seminal vesicle weight upon examination. No adverse effects were seen in rats receiving 54 mg/kg, and that was considered a NOAEL. The AC calculation assumed a 70 kg body weight and water consumption of 2.8 L/d. A factor of 10 was applied for species extrapolation, and a factor of 1.1 was applied for time extrapolation. The 100-d AC from the data of Amoore et al. (1978) was calculated as follows:

(54 mg/kg/d [NOAEL] × 70 kg) ÷ (10 [species extrapolation] × 2.8 L/d × 1.1 [time extrapolation]) = 120 mg/L (rounded).

This calculated dose, 120 mg/L, approaches or exceeds detected measures of trialkylamines presumed to be responsible for the objectionable odor and taste of in-flight galley water on shuttle flight STS 95.

A benchmark dose calculation (by benchmark dose software from the U.S. Environmental Protection Agency NCEA, version 1.3.1) for this study was executed using the above animal dose and physiologic data. A benchmark dose calculation at the 95% confidence interval and at a benchmark response of 1% ($BMDL_1$) resulted in a 223 mg/L AC for reproductive toxicity.

(98 mg/kg [$BMDL_1$ or NOAEL] × 70 kg) ÷ (10 [species extrapolation] × 2.8 L/d × 1.1 [time extrapolation]) = 223 mg/L (rounded).

This calculated dose, 223 mg/L, approaches or exceeds detected measures of trialkylamines presumed to be responsible for the objectionable odor and taste of in-flight galley water on shuttle flight STS 95. Moreover, because of scanty dose-response data presented in this study, the author elected to forego consideration of derived benchmark dose calculations.

Water-Dilution Odor Thresholds

Amoore and Hautala (1983), Baker (1963), Trubko (1975), and Le Din Min (1976) reported water-dilution odor threshold concentrations for monoalkylamines, dialkylamines, and trialkylamines. The lowest of the

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reported odor threshold values for each group of alkylamines, with exception of a presumed outlier value for TMA (0.0002 mg/L) that was not used, was selected as the AC. The 100-d ACs for the alkylamine groups were established as follows:

100-d AC for monoalkylamines = 2mg/L,
100-d AC for dialkylamines = 0.3mg/L,
100-d AC for trialkylamines = 0.4mg/L.

1,000-d ACs

Amoore and Hautala (1983), Baker (1963), Trubko (1975), and Le Din Min (1976) reported water-dilution odor threshold concentrations for monoalkylamines, dialkylamines, and trialkylamines. The lowest of the reported odor threshold values for each group of alkylamines, with exception of a presumed outlier value for TMA (0.0002 mg/L) that was not used, was selected as the AC. The 1,000-d ACs for the alkylamine groups were established as follows:

1,000-d AC for monoalkylamines = 2mg/L,
1,000-d AC for dialkylamines = 0.3mg/L,
1,000-d AC for trialkylamines = 0.4mg/L.

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5

Cadmium (Inorganic Salts)

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OCCURRENCE AND USE

Cadmium (Cd) occurs naturally in the earth's crust. In the environment, it exists as oxides or salts such as cadmium chloride, cadmium sulfate, or cadmium sulfide (see Table 5-1). It also exists as a complex with lead, zinc, and copper ores. In the industrial setting, cadmium is used extensively in smelting, batteries, plastics and metal plating, welding, fabric dyes, glass glazes, and the burning of fossil fuels. In the general population, the sources for cadmium exposure are food and water. There have been numerous reports of the contribution of cadmium to the indoor environment from cigarette smoke, the most important single source of exposure to the general population. Cigarettes and cadmium have been the subject of several investigations, especially using pregnant women who smoke. Depending upon the source of tobacco, a single cigarette may contain from 1 to 2 micrograms (µg) of cadmium (FDA 1993). The average daily intake of 10-20 µg per day (d) of cadmium by adults in the United States has been reported (Reeves and Chaney 2002). There are reports that among food sources, certain vegetables, rice, and meat (kidney and liver) contribute significant concentrations of cadmium. In an FDA survey of fresh clams and oysters from U.S. coastal areas, it was reported that they contain significant amounts of cadmium (Caper and Yess 1996). It has also been stated that the diets in which shellfish constitutes a significant portion contain twice as much cadmium as a more mixed diet. Usually the concentration of cadmium in drinking water in the United States is about 2 µg per liter (L) (below the regulatory level of 5 µg/L). At hazardous waste sites, where cadmium contamination of soil

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TABLE 5-1 Physical Properties of Inorganic Cadmium and Cadmium Salts

Formula	Cd[a]	$CdCl_2$[b]	$CdSO_4$[c]	$CdCO_3$[d]	CdO[e]	CdS[f]
Chemical name	Cadmium	Cadmium chloride	Cadmium sulfate	Cadmium carbonate	Cadmium oxide	Cadmium sulfide
Synonyms	Cadmium colloidal	Cadmium dichloride	Cadmium sulfate	Otavite, cadmium monocarbonate	Cadmium fume; cadmium monoxide	Cadmium yellow; greenockite
CAS registry no.	7440-43-9	10108-64-2	10124-36-4	513-78-0	1306-19-0	1306-23-6
Molecular weight	112.41	183.32	208.47	172.42	128.41	144.47
Cadmium (mole %)	100%	61.2%	53.8%	65%	87.5%	77%
Solubility	Insoluble	140 g/dL	75.5 g/dL	Insoluble	Insoluble	Soluble at 1.3 mg/L

[a]Data are from HSDB 2006a.
[b]Data are from HSDB 2006b.
[c]Data are from HSDB 2006c.
[d]Data are from HSDB 2006d.
[e]Data are from HSDB 2006e.
[f]Data are from HSDB 2006f.

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is very high because of the disposal of batteries, sewage sludge, and use of phosphate fertilizers, cadmium has the potential to contaminate the water supplies (Elinder 1985). Cadmium can also leach from copper piping in plumbing with nonlead-based solder joints into residential drinking water. A recent survey, the National Human Exposure Assessment Survey in Maryland (NHEXAS-MD), in which 381 water samples were taken from 73 households, indicated only 12 of the households had concentrations of cadmium higher than the method detection limit of 0.1 μg/L (Ryan et al. 2000).

Cadmium has been found in the humidity condensate samples collected from several Mir missions at a mean concentration of 27 μg/L, with a maximum concentration of 240 μg/L. In the water processed from humidity condensates, cadmium was not found above 1 μg/L. Once, up to 36 μg/L of cadmium was reported in the crew drinking water collected from the International Space Station (ISS) water-use port in the galley. The problem was isolated. Because cadmium is the most common ingredient in zinc coatings and because of the well-known toxicity of soluble cadmium, the National Aeronautics and Space Administration (NASA) needed to determine the spacecraft water exposure guidelines (SWEGs) for cadmium in case there is a malfunction of the multifiltration beds or other components of the ISS water reclamation system leading to the contamination of ISS crew potable water.

TOXICOKINETICS AND METABOLISM

The absorption, distribution, and elimination of cadmium have been extensively studied in animals. The route of exposure can greatly affect these parameters and thus the toxic effects. Our discussions will be limited to studies that describe exposure by ingestion of soluble cadmium salts through food and drinking water or gavages only. The data from inhalation exposures, or from intravenous (iv), subcutaneous (sc), or intraperitoneal (ip) injections, may be mentioned but will not be discussed in any detail. Almost all the studies pertaining to ingestion of cadmium have used cadmium chloride ($CdCl_2$). Very few studies have used cadmium acetate, cadmium sulfate, or cadmium iodide.

Absorption

Whole-body counting after a single oral dose of radiolabeled cadmium has been used for the quantification of whole-body retention and

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the estimation of intestinal cadmium uptake in humans (Newton et al. 1984). The intestinal absorption seems to take place in two steps: first, the trapping of cadmium by the mucosal epithelial cells—this is not considered true absorption; and second, clearance of this trapped cadmium into blood. A large portion of the ingested cadmium is not taken up by the mucosal cells and thus passes through the gastrointestinal (GI) tract without being absorbed, although some is trapped in the intestinal mucosa (Kjellström et al. 1978; Foulkes 1986). Most of the studies on the GI absorption of cadmium have compared the dose given with the amount retained shortly after the dose was administered. This has been measured as whole-body retention by counting a radioactive tracer (^{109}Cd or ^{115}Cd). Because excretion of absorbed cadmium is very slow, whole-body cadmium retention may not be an underestimation of cadmium absorption (at least in the short term). The total retention of cadmium in the bodies of humans has been measured after ingestion of radioactive cadmium. About 25% of a dose of cadmium administered mixed with food to five healthy adults was retained after 3-5 d, but retention decreased to about 6% after 20 d (Rahola et al. 1973 as cited in ATSDR 1999). Similar results were obtained with 14 healthy adults who after 1-2 weeks (wk) retained an average of 4.6% of a dose of $CdCl_2$ in water taken with a meal (McLellan et al. 1978). In a related study, Flanagan et al. (1978) estimated that the average intestinal net uptake of cadmium was 2.6% in males and 7.5% in females. Foulkes (1991) inferred from studies using intestinal segments that mucosal cadmium uptake is a saturable process (see Lehman and Klaassen 1986). Once taken up by the mucosal cells, cadmium is generally believed to be partly bound to intestinal metallothionein (MT).

A number of studies indicate that the retention or overall absorption of $CdCl_2$, cadmium nitrate, or cadmium sulfate given orally is 1-2%. Rats (Decker et al. 1958; Moore et al. 1973a, b) and mice (Cotzias et al. 1961; Nordberg 1972; Ogawa et al. 1972; Valberg et al. 1976) exposed to a single oral dose of radioactive $CdCl_2$ retained about 1-2% of it. Nordberg et al. (1985) reported that in monkeys (*Saimiri sciureus*), the retention of $^{115}CdCl_2$ administered by stomach tube depended on the dose: about 1% from cadmium at 1 μg per kilogram (kg) and 2.9-3.2% from cadmium at 0.17-1.7 mg/kg. Suzuki and Taguchi (1980), who administered ^{109}Cd by stomach tube to *Macaca irus* monkeys, observed that after 19 d, 12% of the dose was retained and 50% of this was still in the GI mucosa. They reported a final retention of 6% of administered cadmium in the monkeys. The results of several studies on absorption of cadmium from single oral bolus studies show that monkeys and humans seem to absorb

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(retain) more cadmium than rodents. Animal studies indicate that absorption of cadmium is complex and depends on the dose rate. Engstrom and Nordberg (1979a) observed that in mice, the absorption was 0.5% from a 1 µg/kg dose, 1.4% from 15 to 750 µg/kg, and 3.25% from 37.5 mg/kg (the highest dose tested). The highest dose caused morphologic changes in the GI mucosa.

In a study designed to determine the GI absorption of cadmium, rats were given one oral dose of cadmium (mixed with ^{109}Cd) as $CdCl_2$ (1 or 10,000 µg/kg) and the cadmium content was determined in organs 3 hours (h) later (Lehman and Klaassen 1986). The percent of the dose that was absorbed was dose-dependent (0.35 and 1% at 1 and 10,000 µg/kg, respectively). However, at 3 h, liver cadmium increased 80,000 times between the 1 and 10,000 µg/kg doses. Similar data were obtained for kidney, blood, pancreas, and bone. At the 1 µg/kg dose, 60% of cadmium in intestinal cytosol was bound to MT, whereas at the 10,000 µg/kg dose, 50% of the cadmium was bound to MT. It is also interesting to note that even when cadmium was iv injected into rats (at 0.1, 0.3, 1.0, or 3.0 mg/kg), the biliary excretion percentage of the injected dose measured 2 h after injection was dose dependent, increasing as the dose increased (Gregus and Klaassen 1986).

To evaluate the precise nature of cadmium absorption by the intestine and the role of MT in the intestinal absorption of cadmium in rats, a study was conducted by Goon and Klaassen (1989) using an isolated intestinal loop preparation in situ, which allowed direct measurement of intestinal absorption under nearly physiologic conditions. Cadmium (0.1, 10, 100, 1,000, or 10,000 µg/kg) was injected intraluminally into the isolated intestinal loop in situ and all mesenteric venous (portal) blood exiting from the loop was collected for 90 minutes (min). Absorption of cadmium into the portal circulation was low at all doses studied. At low doses (0.1 and 10 µg/kg), little difference was noted in the fractional absorption of cadmium (0.09% and 0.14% of the dose, respectively). However, in rats administered cadmium at 100 µg/kg, the fractional absorption of cadmium was 10-fold greater (1.1% of the dosage). Administration of higher doses of cadmium (1,000 and 10,000 µg/kg) further increased the percentage of the dose absorbed, a nonlinear absorption kinetics (1.8% and 3.4%, respectively) (Goon and Klaassen 1989). This dose-dependent increase in fractional absorption is not because of saturation of intestinal MT at high doses, because this phenomenon was seen even when the intestinal level of MT was experimentally increased (MT induction) by pretreatment of the rats with zinc injections (Goon and Klaassen 1989). In an experiment conducted by Liu et al. (2001) to clar-

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ify the role of MT in Cd absorption and tissue distribution, single oral doses of ^{109}Cd (1 or 300 micromol/kg) were administered to MT-null mice and their parental wild-type mice. Four hours after Cd administration about 0.15% of the lowest dose (1 micromol/kg) and 0.75% of the highest dose (300 micromol/kg) in the liver, and similarly about 0.05% of the lowest dose and 0.15% of the highest dose in kidneys were found in both MT-null and wild-type mice. This experiment indicated that the absorption and initial distribution of orally administered Cd was dose dependent but was not influenced by MT.

Differences between the rodent and human diets may be responsible for the reported differences in absorption rates. Rabar and Kostial (1981) found that retention was at least four times greater when rats were fed bread, meat, or milk that humans consume.

Cadmium absorption from the GI tract can be influenced by several factors (for instance, age and dietary constituents such as iron, calcium, zinc, and dietary fiber content [see Wing 1993]). When human volunteers consumed crabmeat into which cadmium was incorporated, the whole-body retention ranged from 1.2% to 7.6% with a mean of 2.7% (Newton et al. 1984). This was only slightly lower than the values of 4.6-6.0% when cadmium salt was ingested as a solution (McLellan et al. 1978). This seems to indicate that complexation of cadmium in the food did not influence the estimated absorption significantly.

Cadmium Absorption and Age

Consistent with data on absorption of other metals, several animal studies have indicated that in general, absorption of cadmium is higher at younger ages. This appears also partly because of slower excretion from younger animals (Kello and Kostial 1977; Kostial 1984). For example, Engstrom and Nordberg (1979b) reported that absorption from an oral dose of ^{109}Cd in mice of different ages decreased from 5.2% in 1-month (mo)-old mice to 2.9% in 3-mo-olds and 2.1% in 6-mo-old mice. Similar data are not found for rats. No human data could be found. After a single oral administration of ^{115}Cd to albino rats 1-26 wk old, the whole-body cadmium retention (measured 1 or 2 wk after dosing) was higher in sucklings than in weaned rats, and retention of cadmium in the kidneys was 5-7 times higher in the sucklings than in older rats (Kostial 1984).

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Cadmium and Iron

The body store of iron influences cadmium absorption. Subjects with low iron stores (assessed by serum ferritin concentrations) had an average absorption of 8.9%, and those with adequate iron stores had an average absorption of 2.3% (Flanagan et al. 1978). In 10 human subjects with low body iron stores (serum ferritin less than 20 nanogram (ng)/mL), the average absorption of cadmium labeled with ^{115m}cadmium at 25 µg from a test meal was 8.9 ± 2.0% (mean ± SD), whereas, it was 2.3 ± 0.3% in 12 subjects with normal iron stores (serum ferritin greater than 23 ng/mL). The biologic half-life of ^{115m}cadmium in three of the subjects ranged from 90 to 202 d. Thus, iron deficiency in both experimental animals and human subjects lead to the increased absorption of cadmium (Flanagan et al. 1978). Some of the gender differences in absorption arise from differences in iron status between men and women. Females may absorb a larger fraction of dietary cadmium than men because they have lower body stores of iron (Berglund et al. 1994; Vahter et al. 1996; Choudhury et al. 2001). Increased tissue cadmium concentration in iron deficient rats by increased intestinal absorption and 10-fold higher retention in the body has also been documented (Park et al. 2002). It was proposed that iron depletion upregulates the expression of divalent metal transporter protein (DMT1) in the intestine leading to the increased absorption and transport of cadmium.

Cadmium and Calcium

Low calcium intake has been shown to increase the whole-body retention of cadmium. Correspondingly, the uptake of calcium decreases when the ingestion of cadmium is high. Female rats on low-calcium diets exposed to cadmium as $CdCl_2$ at 25 mg/L in drinking water for 1 or 2 mo had accumulated about 50% more cadmium than the rats on a high-calcium diet (Larsson and Piscator 1971). Washko and Cousins (1977) showed that when rats were exposed to cadmium as $CdCl_2$ at 25 ppm in drinking water for 8 wk, the groups that were fed a low-calcium diet (0.1%) had enhanced cadmium retention and cadmium toxicity (for example, depressed packed-cell volume and the highest concentrations in lung, liver, and kidneys) compared with groups fed a high-calcium diet (0.6%). In another study, rats on a low-calcium diet or normal-calcium diet were given rice containing cadmium at 0.1-0.6 mg/kg for 74 wk. Liver and kidney cadmium concentrations in the low-calcium-diet group

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were 1.5-5 times greater than in the normal-calcium-diet group (Kobayashi et al. 1971). Similarly, liver and kidney cadmium concentrations in rats on a low-calcium diet exposed to cadmium at 0-10 mg/L for 1 year (y), were at least two times higher than those on a normal-calcium diet (Piscator and Larsson 1972, as cited in Nordberg et al. 1985). Similar results have been reported by several investigators (Hamilton and Smith 1978). According to Brzoska and Moniuszko-Jakoniuk (1998), large intakes of calcium can protect against absorption, accumulation, and toxicity of cadmium.

Cadmium and Zinc

Several reports in the literature seem to clearly indicate that zinc plays a critical role in cadmium-induced toxicity and even carcinogenesis (see, for example, Petering et al. 1971; Goering and Klaassen 1984; Waalkes et al. 1989, 1992). Dietary zinc intake has an important effect on the absorption, accumulation, and toxicity of cadmium. Increased zinc supply reduced cadmium absorption. It is beyond the scope of this document to discuss the mechanism of competition between cadmium and zinc. Several studies have been carried out using in vitro systems and should be interpreted carefully because the result can vary depending on whether intraluminally perfused rat intestinal preparations were used or the vascularly perfused system was used. It also depends on the relative concentrations of zinc and cadmium in the perfusion medium (Foulkes and Voner 1981; Hoadley and Cousins 1985; Jaeger 1990).

Marginal nutritional status (not a deficient status) with respect to the minerals zinc, iron, or calcium can result from consuming one staple grain such as rice, wheat, sunflower kernels, or maize as a large proportion of the diet. To evaluate the effect of such a diet on the interactions of these minerals with cadmium, Reeves and Chaney (2002) conducted a study in female rats (SAS:VAF [SD] Charles River rats) that were fed a diet containing a high proportion of milled rice that was formulated to give only marginal concentrations of these metals. The diet was supplemented with cadmium (0.25 mg/kg diet), and retention of ^{109}Cd (intrinsically labeled $CdCl_2$) was measured. The rats consumed the rice diet, with or without cadmium, for 5 wk and then were fed 1 g of radiolabeled-cadmium diet for 2 wk. The results were compared with control whose diet contained adequate amounts of these previously mentioned minerals. The authors reported that whole-body retention of cadmium in rats consuming diets low in zinc, iron, or calcium was significantly more than

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rats consuming a diet adequate in all these minerals. There was a significant interaction of zinc and iron with cadmium; thus, marginal zinc along with marginal iron resulted in greater whole-body retention of (radioactive) cadmium than it did in rats fed adequate zinc and marginal iron. Cadmium concentration in the duodenum was also 10-fold higher in rats fed diets marginally deficient in zinc and iron compared with rats fed diets containing adequate amounts of these minerals. Marginal intake of zinc led to higher concentrations of cadmium in the liver, without any change in concentrations of cadmium in the kidney (Reeves and Chaney 2002).

Cadmium and Low Dietary Protein

Suzuki et al. (1969) reported that in mice given a low-protein diet 24 h before an oral dose of $^{115}CdCl_2$, the kidney and liver and the whole body had considerably higher concentrations of cadmium than in mice given a high-protein diet. Whole-body retention of cadmium was 9% (mean of 5-14%) in mice fed a low-protein diet and 4.5% (mean of 3-10%) in mice fed a high-protein diet. On the other hand, a refined diet high in fat and protein increased cadmium absorption in mice, partially because of increased GI-passage time (Schafer et al. 1986).

Cadmium and Fasting

The acute oral toxicity of cadmium (as $CdCl_2$) was enhanced in rats fasted 24 h, as shown by a markedly decreased LD_{50} (the dose lethal to 50% of test subjects). Rats were administered cadmium at 75 mg/kg orally 24 h after fasting and euthanized after a further 4 or 24 h for various assays (Shimizu and Morita 1990). Cadmium uptake by the liver (both concentration and content) 24 h after cadmium treatment was higher in fasted rats than in fed rats. Fasting enhanced the focal degenerative and necrotic changes from the midlobular to the pericentral region in the livers. The authors also found changes and shifts in the distribution of liver glutathione (GSH) in the fasted and cadmium-treated rats different from that seen in the control group (Shimizu and Morita 1990, 1992).

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Metabolism and Transport

Evidence from studies with rats and mice has led investigators to propose a complex mechanism for the transport of cadmium in the intestinal epithelial cells. The mechanism involves the binding of cadmium to membrane proteins involved in the transport of essential metals, such as calcium, iron, and zinc, and endocytosis of the membrane proteins bound to cadmium (Cd-MT) by enterocytes in the small intestine. The transport proteins are DMT1, iron transporter protein (MTP1), and zinc transporter protein (ZTL1). These processes have been described in a recent review by Zalups and Ahmad (2003). Competition by Cd for essential metal transporters could explain the greater gastrointestinal Cd absorption when iron, zinc, and calcium dietary levels are low. Cadmium exists mostly as the Cd^{2+} ion and is not known to undergo any direct oxidation, reduction, or alkylation. It can avidly bind to sulfhydryl groups in proteins (especially albumin and MT) and other molecules (Nordberg et al. 1978, 1985). In plasma, cadmium is initially and predominantly bound to protein of high molecular weight (albumin) a short time after exposure (in the case of parenteral administration) or 1 d after single oral administration. The cadmium-protein complexes are taken up by the liver. After 2-3 d, when plasma cadmium concentrations are low, plasma cadmium is also bound to a low-molecular-weight protein, MT (Norberg et al. 1985; Foulkes and Blanck 1990). MT is very rich in cysteine and capable of binding as many as seven cadmium atoms per molecule. MT is inducible in most tissues by exposure to cadmium among other metals and agents (Waalkes and Goering 1990). Initially the albumin-bound cadmium is taken up by the liver. In the liver, cadmium induces the synthesis of MT, and a few days after exposure, MT-bound cadmium appears in the blood plasma. MT is ultrafiltrable and taken up by the tubules (Nordberg 1992). Thus, the binding is of interest in relation to kidney function, especially with respect to glomerular filtration and tubular reabsorption of MT-bound cadmium. MT is thought to be a carrier of cadmium from liver to kidney. MT concentrations in urine and plasma of cadmium-exposed workers and in urine samples from cadmium-exposed Japanese farmers and *itai-itai* disease (a disease resulting from consuming cadmium-contaminated rice in Japan) patients have been extensively reported (Chang et al. 1980; Nordberg et al. 1982; Tohyama et al. 1982; Falck et al. 1983).

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Distribution

Since we will restrict ourselves to discussing the oral route of cadmium exposure, the distribution of cadmium administered via iv, ip, or sc injection will not be discussed here. Upon ingestion of cadmium, a considerable quantity is retained in the intestinal walls. Once transported to blood, it is transported to all the tissues, with a large portion in liver, kidneys, muscle, and bones. The distribution depends on the exposure regimens and the amount of time after exposure. Initially most of the absorbed cadmium will go to the liver, with small amounts going to the kidneys. With increasing time after exposure, cadmium will be redistributed and concentrations in the kidneys will increase. The time-dependent changes in the distribution to kidneys and liver have been the subject of numerous investigations because these organs are considered storage organs (Kotsonis and Klaassen 1978; Weigel et al. 1984; Andersen et al. 1988; Jonah and Bhattacharya 1989). In the liver and kidneys of cadmium-exposed animals, more than 80% of the cadmium is bound to MT.

The concentration of cadmium is higher in the kidney cortex than in the medulla. Renal damage initially occurs in the proximal tubules located in the renal cortex. Livingston (1972) analyzed the concentrations in serial sections of the cortex and found that cadmium decreased from the outer layer to the inner medulla. A ratio of 2:1 between the outer cortex and inner medulla has been reported. In humans, 65-75% of the whole kidney is cortex and 25-35% is the medullar collection system, and this ratio is usually taken into consideration when calculating the cadmium concentration in cortex and the whole kidney concentration (Kjellström and Nordberg 1978; Kjellström et al. 1984; Svartengren et al. 1986). A number of studies have confirmed the selection distribution of cadmium to the cortex of the kidney in preference to the medulla for different routes of exposure, exposure types, and animal species. The cadmium concentration in kidney and liver tissues is very low at birth but markedly increases with age. In humans with normal exposures, liver accumulation reaches near steady-state at age 30, and kidney accumulation reaches a maximum at 40-50 y of age (Bernard and Lauwerys 1986). In muscles, accumulation of cadmium continues throughout life (Elinder 1985; Satarug et al. 2000).

Decker et al. (1958) gave ^{115}Cd nitrate to rats (cadmium at 6.6 mg/kg) by gavage. After 8 h, the largest total amount was in the liver, although the highest concentration was in the kidney. The maximum concentration in both tissues was reached at 72 h after exposure. Kotsonis and Klaassen (1977) administered a single oral bolus dose of

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radioactive cadmium chloride at 0, 25, 50, 100, and 150 mg/kg and reported that at the end of 2 d, the tissue cadmium concentration was highest in the liver, which contained most of the body burden. This was followed by the intestine > kidney > pancreas > spleen > heart > lung > muscle > other organs. After 2 wk, most tissue concentrations were decreased by 50%, but concentrations in the liver remained unchanged at the maximum, and in the kidney, the concentrations had increased three- to four-fold. In addition, in the kidney, the MT concentration was also increased several-fold at 2 wk, whereas in the liver, it remained at the 2-d level. Dorian et al. (1995) have shown in several investigations that cadmium eventually binds to the proximal tubules, where it is distributed equally to the convoluted and straight segments. While there are many studies of the distribution of cadmium after a single bolus dose or a range of doses, there are few reports on the distribution of cadmium after repeated administration of minimum amounts roughly equal to typical human exposure.

Ando et al. (1998) studied the pattern of accumulation of cadmium in the liver and kidney in female rats given a diet containing $CdCl_2$ at 8, 40, 200, or 600 ppm for 2, 4, or 8 mo. The authors reported that although the tissue cadmium concentrations increased as a function of dose, there was a plateau in the kidney at a concentration of 250 μg/g. The highest-dose group reached the plateau at 2 mo and remained the same at 4 and 8 mo while the 200 ppm group took 8 mo. However, in the liver, it did not reach a plateau even with cadmium at 200 and 600 ppm. The decrease in the ratio of kidney to liver as the dose increased indicated that at low doses, cadmium accumulated preferentially in the kidney and that the converse is true for the high dose. Hiratsuka et al. (1999), from the same research group, reported the tissue distribution of cadmium in female rats given minimum amounts of cadmium-polluted rice or $CdCl_2$ for 8 mo, indicated the absence of any hepatic or renal lesions at the end of the experiment regimen even at 40 ppm. In this study, $CdCl_2$ was given at 5.08, 19.8, and 40.0 ppm in the diet (doses of 360, 1,438, or 2,883 μg/kg). After 1, 4, and 8 mo, the concentration of cadmium in the liver and kidneys had increased as a function of dose. The concentration of MT in the liver, kidney, serum, and urine had increased with dose at 4 and 8 mo. However, the distribution rates of cadmium (percent of administered dose) to the liver and kidney changed with the dose. In liver, it increased from 40% at the lower dose to 60% at the highest dose, whereas in the kidney, it decreased from 20% at lower dose to 10% in the high-dose group.

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Liu et al. (2000) reported data from several studies examining the role of MT in cadmium absorption, tissue distribution, and elimination. Using MT-I/II Null mice and wild-type mice, the author and his colleagues also evaluated whether MT can protect against cadmium-induced renal toxicity. In wild-type mice, chronic oral consumption of cadmium via drinking water (30, 100, or 300 ppm) or feed (100 ppm cadmium) had no effect on body weight over the 6-mo exposure period (Liu et al. 2000). Immunohistochemical staining for MT showed that renal MT concentrations increased several-fold over those of controls. Kidney-to-body weight ratios were unaffected by up to 100 ppm either in water or in feed, although in the 300 ppm water group they were increased. Renal cadmium (μg/g kidney) increased with dose; at both highest doses (300 ppm water and 100 ppm diet), the tissue cadmium concentrations were 50 μg/g. Renal MT was induced in both the cytoplasm and nucleus. Consumption of cadmium in water (30, 100, or 300 ppm) or in feed (100 ppm) did not increase urinary excretion of gamma-glutamyl transferase (GGT), *N*-acetyl-β-D-glucosaminidase (a marker of renal tubular dysfunction), glucose, or protein, indicating a lack of nephrotoxic effects. Similarly, blood urea nitrogen (BUN), an indicator of renal dysfunction, was not increased in cadmium-treated groups. However, renal morphology in the 300 ppm water group showed degenerated proximal tubules and glomerular swelling. Mice exposed to cadmium in their feed (100 ppm) had glomerular swelling and severe tubular degeneration (Liu et al. 2000). Hepatotoxicity was not indicated, as measured by the activity of serum alanine aminotransferase under the conditions of this study. In this study the authors also documented that the lack of MT rendered the MT-null mice vulnerable to severe renal toxicity, even though the cadmium concentrations in the kidney of MT-null mice were much lower than in wild-type mice. MT protects against cadmium-induced liver injury as it increased MT binds cadmium in the cytosol, reducing cadmium content in the organelles. Thus, MT-null mice are highly susceptible to cadmium toxicity (Liu et al. 2000).

Excretion and Elimination

Most orally administered cadmium is not absorbed systemilcally and is therefore excreted in the feces. The amount that is truly absorbed, which is very low after oral exposures, is excreted very slowly, urinary and fecal excretion being equal (Kjellström and Nordberg 1978). After repeated exposures, excretion increases over time, and there is an asso-

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ciation between body burden and urinary cadmium excretion. Especially after the kidney has accumulated excess cadmium and renal damage has set in, urinary excretion of cadmium increases significantly. There have been a number of studies designed to examine cadmium excretion rates after sc injections in rats, mice, and rabbits, but they will not be discussed here.

The normal rate of urinary cadmium excretion in humans is about 1 μg/d, and urinary cadmium concentration increases with age. In individuals exposed to cadmium, urinary cadmium excretion and concentration are signs of renal dysfunction. Animal experiments have shown that fecal excretion is contributed to by both direct excretion from the mucosa and by biliary excretion; intestinal excretion contributes the most.

Using isolated rat jejunal segments or everted jejunal sacs, Foulkes (1979, 1985, 2000) has extensively studied possible mechanisms of cadmium absorption, transfer, and transport, and their determinants. Andersen et al. (1988) conducted an experiment in which 7- to 8-wk-old mice were given a single oral bolus of various doses of $CdCl_2$ (5, 70, 270, or 790 μmoles/kg or 15.7, 30.4, 59.6, and 88.8 mg/kg/d). The doses were labeled with ^{109}Cd. Whole-body counting was done at 15 min and 1, 2, 3, 4, 7, and 10 d after dosing. On d 10, all animals were killed, and tissues, including the GI tract, were removed and counted (Andersen et al. 1988). It was estimated that in rats, the whole-body elimination of cadmium from a single oral bolus is biphasic, with a rapid phase in which about 95% of the dose is eliminated within 4 d (mainly fecal elimination) and another, much slower, phase representing loss from the rest of the body. In long-term studies in mice, the latter phase has been reported to be about 200 d. This latter phase seems to vary with the strain of mice between 66 and 245 d (Engstrom and Nordberg 1979a, b). These studies also indicate that the half-life depends on the dose. Kjellström and Nordberg (1986) estimated from their kinetic model, developed after collating the literature data, that the half-life of cadmium in liver is between 6 and 38 y, and for kidney it is about 4 to 19 y (see discussion below).

Recently, Liu and Klaassen (1996) reported that MT does not play a role in the initial distribution of cadmium to tissues but does play a major role in the retention of cadmium, especially from liver, kidney, and pancreas. They conclude that the persistence of cadmium in the body is at least partially because of cadmium binding to MT in tissues. These investigators studied the role of MT in the tissue distribution and retention of cadmium using MT-I and MT-II null (MT-null) mice. The elimination of cadmium was much faster in MT-null mice than in control mice. In control mice, about 40% of the cadmium administered was found in the

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liver 24 h after administration, and the majority was bound to MT. In contrast, only 20% of the administered cadmium was found in the liver of MT-null mice. Cadmium concentrations in kidney, pancreas, and spleen were also lower in MT-null than in control mice 1 wk after administration. The cadmium concentration in kidneys of control mice continued to increase with time, but in MT-null mice, it did not increase, indicating that the binding of cadmium by MT is an important source of cadmium in the kidney.

Using experimental and epidemiologic studies, Kjellström and Nordberg (1986) developed an eight-compartment kinetic model (human cadmium toxicokinetic model) to describe absorption, distribution, and biotransformation of cadmium. From this model, half-lives of cadmium in the whole body of mice, rats, rabbits, and monkeys have been calculated to be from several months to several years. Half-life in the slowest phase was from 20% to 50% of the maximum life span of the animal (Kjellström and Nordberg 1986). In the human body, the main portion of the cadmium body burden is found in the liver and kidney and in other tissues (particularly muscle, skin, and bone). From their model, Kjellström and Nordberg estimated that half-time for elimination from the human kidney would be between 6 and 38 y, and for the human liver, between 4 and 19 y. Using assumptions that urinary excretion depends on blood concentration and kidney concentration and total excretion is assumed to equal daily intake at steady state, Kjellström and Nordberg (1978, 1986) estimated daily fecal and urinary excretion to be 0.007% and 0.009% of body burden, respectively.

Recently, Choudhury et al. (2001) developed the Cadmium Dietary Exposure Model (CDEM), basing it on modifications to the cadmium biokinetic model of Kjellström and Nordberg (KNM). The model predicts a mean peak kidney cadmium burden of about 3.5 mg in males (range of 2.2-5.1 mg) corresponding to a peak renal cortex concentration of 15 µg/g wet cortex (range of 10-22 µg/g). Predicted kidney cadmium concentrations of females were higher than those of males: 5.1 mg (3.3-7.6 mg) total kidney and 29 µg/g (19-43 µg/g) wet cortex. According to the authors, the predicted and observed urinary cadmium excretion rates in males and females agreed with empirical estimates based on the National Health and Nutritional Evaluation Survey, 1988-1994 (NHANES III), with females predicted and observed to excrete about twice as much cadmium in urine as males. The authors suggested that females may also absorb a larger fraction of ingested dietary cadmium than males, and this difference may be the result of lower body iron stores in females than in males.

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Urinary cadmium excretion (cadmium μg/g creatinine) in the United States as predicted by nonsmoking, non-occupationally exposed population subset of the NHANES III report indicated that for all age categories, mean urinary cadmium excretion was higher for females than males if only nonsmokers are taken into account. Urine cadmium (U-Cd), expressed either as uncorrected μg/L or creatinine corrected (μg/g creatinine) increased with age and with smoking. The CDEM results indicated that predicted and observed urinary cadmium in males and females agreed with empirical estimates based on NHANES III, with females predicted and observed to excrete approximately twice the amount of cadmium in urine as in males (Choudhury et al. 2001).

TOXICITY SUMMARY

A number of toxic effects in humans and animals resulting from cadmium exposures have been reported. This has been the subject of great interest and concern due to cadmium-induced *itai-itai* disease in Japan among people who were exposed to rice contaminated with cadmium. *Itai-itai* or "ouch-ouch" disease was a serious health problem in a rural area in north central Japan. Severe osteoporosis and osteomalacia (soft bone) with tubular changes in the kidney were considered to be associated with excess intake of cadmium. It is a painful disease that includes kidney damage and bone demineralization. The disease primarily affected elderly Japanese women who had multiple child deliveries and who were exposed to water and rice contaminated with cadmium.

In the cadmium toxicology literature, although renal dysfunction has been well documented as the primary effect in humans, reports of several animal studies have attributed death, GI distress, hepatic injury, testicular atrophy, renal dysfunction, hypertension, anemia, CNS effects, reproductive and developmental effects, and possible cancers of the prostate and testes to exposure to cadmium via water or food. Numerous reports exist, mostly from studies conducted in mice, on the effects on immune system parameters that result from exposure to cadmium through the oral route. Some of these effects have also been occasionally evaluated in humans occupationally exposed to cadmium (WHO 1984) where exposure to other metals such as nickel and arsenic cannot be ruled out. Cadmium-associated changes with confounding factors of smoking, drinking, and age have been difficult to delineate.

Cadmium ions have a high affinity for tissue thiols, induce the synthesis of a carrier cysteine-rich polypeptide called MT, and impair pro-

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teoglycan metabolism. Significant renal effects include tubular nephropathy manifested by proteinuria, aminoaciduria, glucosuria, phosphaturia, and calcium wastage. Chronic sequelae include a decrease in the glomerular filtration rate and an increased risk of kidney stone disease. Biologic monitoring of cadmium absorption includes determination of U-Cd and of low-molecular-weight marker proteins, such as β-2-microglobulin (β-2m) (Nogawa and Kido 1993) and retinol binding protein, the tubular reabsorption of which is impaired before a frank proteinuria.

Acute Exposures

Death has been reported in two cases of humans who used cadmium in suicide attempts. In these cases, massive fluid loss, edema, and widespread organ destruction were noted. In one case, the death was because of the ingestion of cadmium iodide, and the dose was estimated to be 25 mg/kg as cadmium. Death occurred in 7 d (Wisniewska-Knypl et al. 1971). In another case, death occurred in 33 h because of the ingestion of $CdCl_2$, and the cadmium dose was estimated to be 1,840 mg/kg (Buckler et al. 1986).

There have been several single oral bolus studies in mice and rats from which the LD_{50} has been derived. A short summary is presented in Table 5-2.

Specific Short-Term Oral Exposure Studies

Although no surveys of environmentally exposed populations have reported GI symptoms (Roels et al. 1981), numerous human and animal studies indicate that oral exposure to cadmium in high concentrations causes severe irritation to the GI epithelium, resulting in nausea, vomiting, salivation, abdominal pain, cramps, and diarrhea (Buckler et al. 1986; Andersen et al. 1988; Nordberg et al. 1973). GI symptoms have been reported in children who drank soft drinks containing cadmium. The estimated concentration was 16 mg/L (Nordberg et al. 1973). Nordberg et al. (1973) calculated the dose that induced vomiting as 0.07 mg/kg (assuming ingestion of 150 mL and body weight of 35 kg for the child). The emetic threshold doses for cadmium have been estimated to be 3-90 mg and concentrations of soluble cadmium salt solutions exceeding 15 mg/L (CEC 1978). In humans, if vomiting occurs, the risk of con-

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TABLE 5-2 Oral LD_{50} Values for Few Inorganic Cadmium Compounds, as Stated in the Registry of Toxic Effects of Chemical Substances (RTECS)

Compound	Species	LD_{50} (mg/kg)	Cause of Death
Cadmium chloride	Rat	88	Gastrointestinal hypermotility, diarrhea
	Mouse	60	Weight loss
	Guinea pig	63	Not reported
Cadmium iodide	Rat	222	Not reported
	Mouse	166	Not reported
Cadmium nitrate	Rat	300	Not reported
	Mouse	47	Not reported
Cadmium acetate	Rat	333	Not reported

Source: Data from CCOHS 2006.

tinuous exposure to a higher dose is minimized. Frant and Kleeman (1941) reported on four outbreaks of cadmium poisoning. Three members of a family, two children and one adult, drank lemonade made from a yellow crystalline powder and became ill with nausea, abdominal cramps, and vomiting in 15 min. The drink had 300 ppm of cadmium. The authors also reported an incident in which seven subjects (five adults and two children) drank a punch containing cadmium at about 85 ppm and experienced GI symptoms as mentioned above. They also reported two other cases, one of five subjects consuming a gelatin dessert that contained cadmium at 530 ppm, and the other of eight subjects consuming tea that contained cadmium at 160 ppm. The authors also cited the cases from the department of health record of 29 children who had violent nausea after eating frozen ice pops. Each ice pop contained cadmium at 13-15 ppm. Although the exact total amount consumed that caused the GI effects was not reported, it appears that a concentration of 13 mg/L can cause these effects.

Single gavage doses of $CdCl_2$ to mice induced toxic gastroenteritis and hepatic and renal lesions. Experiments were performed with 7- to 8-wk-old CBA/Bom mice, and the acute toxicity of $CdCl_2$ was studied after single gavage doses of 0, 0.6, 3.9, 7.9, 15.7, 30.3, 59.6, and 88.8 mg/kg (Andersen et al. 1988). On day 10, all animals were sacrificed. In addition to determining mortality, tissue damage to gastric epithelium, intestinal epithelium, liver, kidney, and testes was also scored on groups dosed with cadmium at 30.3 mg/kg and above. The authors observed decreased peristalsis at doses of cadmium at 59.6 and 88.8 mg/kg and intes-

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tinal atony leading to higher fractional absorption at these doses. This may have led to the systemic toxicity. At the 88.8 mg/kg dose, very severe necrosis of the stomach was observed. The damage to intestinal epithelial tissue was more pronounced in the proximal parts of the tract (Andersen et al. 1988). Tissue damage to stomach and duodenum were observed even at 30.3 mg/kg/d. Because histopathology was not done on mice treated at doses below 30.3 mg/kg, a no-observed-adverse-effect level (NOAEL) cannot be identified. Similar results were found by Basinger et al. (1988) who reported that a single oral gavage dose of cadmium as $CdCl_2$ at 1 mmole/kg (112 mg/kg) given to mice induced gastric epithelial tissue damage and hepatic damage.

Kotsonis and Klaassen (1977) reported that in male Sprague-Dawley rats given single doses (gavage) of radioactive $CdCl_2$ (cadmium at 0, 25, 50, 100, or 150 mg/kg), liver hexobarbital oxidase activity measured after 2 d was lower in 100 and 150 mg/kg dose groups, without any change in the levels of hepatic cytochrome P-450 or aniline hydroxylase activity. Testicular function, measured after 14 d in rats of the 100 and 150 mg/kg groups, was decreased. Protein excretion and urine flow was decreased for the first 2 d in these dose groups but returned to normal values. Daily motor activity was determined in a five-tier residential maze to measure locomotion. For 2-3 d, this activity (both nocturnal and diurnal) was lower in 50, 100, or 150 mg/kg groups, but after 3 d, the motor activity was comparable to that of untreated controls (Kotsonis and Klaassen 1977). Because normal motor activity is critical for spaceflight-related activities, these data can be used for a 1-d acceptable concentration (AC). A dose of cadmium at 50 mg/kg will be a lowest-observed-adverse-effect level (LOAEL), and 25 mg/kg will be a NOAEL.

Borzelleca et al. (1989) conducted short-term (a 1-d and a 10-d) gavage and drinking water toxicity studies. They will be described separately because the doses and concentrations are different.

For the gavage experiment protocol, male and female Sprague-Dawley-derived Wistar rats received $CdCl_2$ solution in water as a gavage at 25, 51, 107, or 225 mg/kg/d (cadmium at 15, 31, 65, or 137 mg/kg/d) for 1-10 consecutive days. Several measurements were made that included mortality; organ and body weight and changes in their relative ratios; several clinical chemistry parameters such as serum enzymes, glucose, proteins, BUN, electrolytes, and hematologic parameters; and urinary parameters such as ketones, glucose, and protein. Gross pathologic examinations were also performed at necropsy (Borzelleca et al. 1989).

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In the 1-d gavage dosage study, no significant effects on hematologic parameters, serum chemistries, or urinalysis parameters were reported. Three of 10 male rats died within 1 d in the two highest-dose groups, whereas among females, only 2/10 died at the highest dose. One day after the dose was given, the lung-to-body weight ratio in the highest-dose group was significantly higher only in males. However, necropsy of animals that died did not reveal any gross histopathologic lesions (Borzelleca et al. 1989).

In the 10-d gavage study, dose-dependent mortality was observed. All male and female rats of the highest-dose group died by day 7. Even at the lowest dose ($CdCl_2$ at 25 mg/d), two animals died. Weights and organ-to-body weight ratios were decreased for most of the organs, including the testes in the males. In general, organ weights (as the percentage of body weight) decreased in all dosed groups. Testicular necrosis was noted in male rats receiving $CdCl_2$ at 107 and 225 mg/kg. Atrophy or loss of spermatogenic elements were also noted in these groups. In the high-dose group of male rats, focal necrosis of hepatocytes was noted. In addition, in both male and female rats, mild focal necrotic changes in kidney tubular epithelium were observed occurring in all dosed groups in a dose-dependent manner (Borzelleca et al. 1989). Because of high mortality at the high dose and significant levels of mortality at other doses, it was decided not to use the data for AC calculations. In addition, the authors had also carried out a 10-d drinking water study (Borzelleca et al. 1989).

In the short-term drinking water exposure study (Borzelleca et al. 1989), male and female Sprague-Dawley-derived Wistar rats (10 each per dose) were exposed for 10 consecutive days to $CdCl_2$, which was added in the drinking water at concentrations to give theoretical doses of $CdCl_2$ at 2.5, 25, and 51 mg/kg/d. The authors observed decreases in water consumption as a function of concentration of cadmium in water. Exposure-dose calculations based on actual water consumption were reported as $CdCl_2$ at 1.8, 12.8, and 18.2 mg/kg/d for males, and 1.8, 13.3, and 22.6 mg/kg/d for females, which corresponded to a mean for both males and females to cadmium at 1.1, 7.8, and 11.1 mg/kg/d. Except for some decreases in body and organ weight, no compound-related histopathologic effects were noted at the end of the study. Among several clinical chemistry parameter measures, only decreases (about 45%) in serum alkaline phosphatase (ALP), and serum protein were seen both in male and female rats, but the clinical relevance of decreases in serum is not easily interpretable. An increase in serum BUN was seen at the highest dose in male rats. Qualitative urine analysis (using only reagent strips)

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indicated that a dose of cadmium at 7.8 mg/kg/d can be identified as a LOAEL and a dose of 1.1 mg/kg/d as a NOAEL for increased protein excretion in urine. Increased BUN in the 11.1 mg/kg/d group supports these data.

Exposure Duration > 10 d

Kotsonis and Klaassen (1978) conducted a study in which male Sprague-Dawley rats were exposed to cadmium via drinking water at concentrations of 10, 30, and 100 ppm for 24 wk. Several measurements that included hemoglobin, hematocrit, blood pressure, testicular function, drug-metabolizing enzyme activities, histopathology, and others were made after 3, 6, 12, and 24 wk. Food and water intake, urine flow, protein excretion, and motor activity were measured weekly. In the 30 ppm groups, the water intake was lower than that of controls only during weeks 1, 2, 8, 14, and 15; whereas in the 100 ppm groups, it was significantly lower throughout the study. From the mean water consumption data, the authors calculated the mean amount of cadmium to be 0.41, 1.09, and 2.82 mg/d for the 10, 30, and 100 ppm groups, respectively, resulting in exposure doses of 1.15, 2.92, and 8.51 mg/kg/d. In the 30 and 100 ppm groups, increased concentrations of protein in urine were observed after 6 wk of exposure, indicating nephrotoxicity. A slight focal tubular necrosis was also observed by week 24 (Kotsonis and Klaassen 1978).

In addition, for several weeks starting at week 3 and decreasing with time, daily motor activity in the 30 and 100 ppm groups was significantly lower than that of controls (Kotsonis and Klaassen 1978). Because changes in water consumption and motor activity were seen as early as 3 wk of exposure to cadmium, these data will be used for 10-d AC derivation.

In a rat study, a significant decrease in hemoglobin and a 50% decrease in water intake within 2 wk were reported after young male and female rats were exposed to cadmium at 50 ppm in drinking water (Decker et al. 1958). In this study, 34-d-old albino Sprague-Dawley rats (male and female) weighing about 100 g were given drinking water containing cadmium as $CdCl_2$ at 0.1, 0.5, 2.5, 5.0, 10.0, or 50.0 ppm. The study was conducted for 1 y except that the 50 ppm group was terminated at 90 d. Based on the water consumption data recorded 2 wk after dosing, the estimated dose for 10 ppm was 0.7 mg/kg/d, and the dose for 50 ppm was 3 mg/kg/d. Microscopic observations indicated many micro-

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cytic, hypochromic red blood cells and polychromasia (change of colors) with 8-10 nucleated red blood cells per 100 white blood cells. The authors reported that in groups treated with cadmium at 0.1-10.0 ppm in water for 1 y, changes in body weight gain and water and food intake were not different from those of controls, nor were any other pathologic changes noted. A NOAEL of 10 ppm (about 0.7 mg/kg/d) was thus identified.

However, the authors also stated that mortality because of respiratory infection occurred in all the groups, accompanied by pleuritis and emphysema. Because of the lack of confidence in the quality of the animal maintenance, the study will not be considered in the AC derivation for any duration.

Although data from the 3 mo of observations of the 50 ppm group can be used for a 10-d AC for hematologic changes and for a 100-d AC for both hematologic and water consumption effects, we have low confidence in the observations. Also, the results are not consistent with the reports of Kotsonis and Klaassen (1978), who found no change in hemoglobin and hematocrit concentrations in male Sprague-Dawley rats given cadmium at 10, 30, and 100 ppm in drinking water for 24 wk (6 mo).

Sakata et al. (1988) reported that oral administration of cadmium to Wistar rats at 100 mg/L in drinking water (estimated dose rate of cadmium at 12 mg/kg/d) for 12, 26, 50, and 100 d resulted in iron-deficiency anemia characterized by microcytic hypochromic red blood cells and in decreased plasma iron. In the bone marrow, the density of late erythroid progenitors steadily increased as plasma iron decreased because of cadmium administration, which reached a plateau after 50 d. Cadmium treatment inhibited the in vitro growth of erythroid progenitor cells in a dose-dependent manner. Although there were no changes in the red blood cell count, hemoglobin decreased as early as 26 d and hematocrit decreased as early as 12 d. This study was not used for AC calculation because of the lack of dose-response data, and a NOAEL could not be identified.

Anemia was reported in rats fed diets containing cadmium as $CdCl_2$ at 30 mg/kg diet (cadmium at 2.4 mg/kg/d) for 4 wk. The test diets contained cadmium at 30 mg/kg either as $CdCl_2$ or as cadmium incorporated in pigs' livers; the control group was fed a diet containing liver from a pig not treated with cadmium. cadmium-treated groups showed increased plasma aspartate and alanine aminotransferase activities. In addition, their spleens showed decreased extramedullary hematopoiesis (Groten et al. 1990). In groups fed $CdCl_2$, all of these effects were more pronounced than for the group fed cadmium incorporated in liver; the cadmium con-

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centration in the livers paralleled this. However, there were no differences in kidney cadmium concentrations between groups fed the cadmium-incorporated meat or $CdCl_2$ in the diet. This study lacked dose-response data, and Kotsonis and Klaassen (1978), who used even higher doses in drinking water than used in this study in diet, did not observe any such effects. Hence, the data from this study were also not considered for AC derivation.

Horiguchi et al. (1996) proposed that cadmium-induced anemia is caused by the low production of erythropoietin in the kidneys following renal injury. Thus, anemia may be secondary to cadmium-induced renal injury.

Ogoshi et al. (1989) conducted a study on the effect of cadmium ingestion on mechanical strength of the bone using female young, adult, and older rats. Rats received cadmium as $CdCl_2.2½H_2O$ in drinking water for 4 wk. Young rats (21 d old) received cadmium at 0, 5, and 10 ppm, adult rats (24 wk old) received cadmium at 0, 10, 20, 40, 80, and 160 ppm, and old rats (1.5 y old) received cadmium at 0, 80, and 160 ppm. Rat strain was not specified. Compression strength of the metaphysis and diaphysis and the bending strength of the diaphysis of the femur were measured. Cadmium content of the kidney, liver, and femur were measured. At the end of 4 wk, although a decrease in the mechanical strength of the bone of young rats was noted even at a dose of cadmium at 5 ppm, no changes were noted in the adult and old rats at cadmium doses up to 160 ppm. Young rats accumulated more cadmium, especially in the bones, than adult and old rats. In the young rats, the strength was negatively correlated with bone cadmium content but not correlated with kidney cadmium content. This may be because young rats absorb more cadmium than adult and older rats. Based on the results from adult rats, for 4 wk, a NOAEL of 160 ppm (or a dose rate of cadmium at 22 mg/kg/d) for bone effects can be identified.

In a related study, Gur et al. (1995) studied the influence of cadmium administered through drinking water on the repair of injured bone. Rats were given cadmium at 20 ppm and 200 ppm in water for 5 wk. Significant reductions (43%) in ALP and tartrate-resistant acid phosphatase (46% reduction) were seen in samples taken from the bone. No cadmium-related liver or kidney abnormal histology was seen. Calcium accumulation in the newly formed repair tissue at the site of injury was also significantly reduced (53%) in the cadmium-treated (200 ppm) rats in comparison to control rats. The authors concluded that exposure to 200 ppm of cadmium in water for 5 wk affects the bone-repair process (reduction in ALP in osteoblastic cells). Because the bone was already

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damaged and these findings reflect bone repair, these data could not be used for AC derivation.

Young male Long Evans rats (40-50 g) were exposed to cadmium at 20 ppm and 40 ppm as $CdCl_2$ (estimated dose rate of cadmium at 2.9 and 5.8 mg/kg/d) in drinking water for 14 wk (Pleasants et al. 1992). The body weight of the 20-ppm group was not different from that of the untreated controls; also, the relative kidney, liver, and testes weights were not affected. However, the 40-ppm groups showed increased kidney and testes weights, although body weights showed a significant decrease starting at 7 wk. The femurs of this group revealed an increase in water and a decrease in mineral content (ash) with cadmium exposure. These findings were considered symptomatic of osteoporosis. Although no changes in hematocrit or peripheral red blood cell counts were noted, rats exposed to cadmium at 40 ppm for 14 wk showed evidence of erythrocyte hypochromia with intracellular non-heme iron inclusions (Pleasants et al. 1992). In a follow-up study, the authors (Pleasants et al. 1993) exposed 40-50 g male weanling Long Evans rats to cadmium at 80 ppm as $CdCl_2$ (11.6 mg/kg/d) in their drinking water for 14 wk. Cadmium-treated rats displayed smaller body weight gains and larger relative kidney and testis weights than controls. Exposure of rats to cadmium significantly depressed the hematocrit and erythrocyte counts. A dose rate of cadmium at 2.9 mg/kg/d appears to be a NOAEL for all these effects.

A study of adult male rats exposed to cadmium in the diet (100 ppm/kg diet or cadmium at 9 mg/kg body weight) for 60 d and then tested in a Digiscan activity monitor indicated that cadmium decreased movement and increased rest time (Nation et al. 1990). Kotsonis and Klaassen (1977, 1978) reported that both single-bolus (LOAEL of cadmium at 50 mg/kg) and intermediate-duration exposures (NOAEL of 1.1 and a LOAEL of 2.82 mg/kg/d) to cadmium affect neurologic behavior. Nation et al. (1984) also showed that intermediate-duration exposure to cadmium (5 mg/kg/d) induced anxiety in animals, as manifested by increased passive avoidance behavior; cadmium at 1 mg/kg/d was without any effect and thus is a NOAEL for 60 d.

The effect of cadmium on hypertension is equivocal. Studies in which a hypertensive effect of cadmium has been reported have had several drawbacks. Fowler et al. (1975) exposed rats to cadmium in drinking water at concentrations ranging from 0.2 to 200.0 mg/L for 12 wk. After 6 wk, the authors noticed morphologic changes in the vascular system of the kidneys in all treated rats. There was a thickening of small and median arteries and dilation of the large ones. Blood pressure was not measured in this study. Eakin et al. (1980) conducted a study on

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weanling rats to assess effects of cadmium on hypertension. Rats (16 male OSU Brown rats) were fed a diet containing cadmium as cadmium acetate at 150 ppm for 16 wk. Body weight, blood pressure, and plasma renin were measured at week 2, 4, 8, and 12, and terminally at week 16. While the blood pressure of control rats increased with age during the experiment, the blood pressure of the cadmium group decreased from week 8 to week 16. There was no change in plasma renin concentrations. Eakin et al. (1980) also conducted a long-term experiment in which cadmium was administered in drinking water at 0, 10, and 20 ppm for 64 wk. Blood pressure was measured every 4th wk for 24 wk and then once every 8 wk until the 64th wk. No significant changes were observed in blood pressure.

Loeser and Lorke (1977b) conducted a study in which cadmium in the form of $CdCl_2$ was administered with the feed in concentrations of 0, 1, 3, 10, and 30 ppm over a period of 3 mo to groups of 2 male and 2 female beagle dogs. The systolic and diastolic blood pressure of the treated animals of all groups up to 30 ppm was within the normal range.

Although cadmium accumulated in the liver and kidneys, liver function (as measured by glutamate dehydrogenase and lactate dehydrogenase [LDH]) and kidney function (as measured by para-aminohippuric acid and inulin clearance) were unaffected at the end of 3 mo. Hematology parameters measured at 1 and 3 mo (erythrocyte sedimentation rate, hemoglobin, hematocrit, and erythrocyte and leucocyte count) did not indicate any clinically significant results (Loeser and Lorke 1977b).

Loeser and Lorke (1977a) conducted very similar subchronic studies in male rats (SPF Wistar, n = 5/dose) ingesting a diet containing cadmium (1, 3, 10, or 30 ppm) for 3 mo. A vast array of hematologic (including hemoglobin, hematocrit, erythrocyte and leukocyte counts, and differential counts), liver function (serum ALP, aspartate transaminase, alanine transaminase, bilirubin, and serum proteins detected by electrophoresis), and renal function parameters (sugar, albumin, bile pigments, urinary proteins detected by electrophoresis, urinary leucine aminopeptidase (LAP), and aspartate transaminase)) were measured. In addition, blood pressure, blood sugar, and cholesterol were evaluated, as well as cadmium concentrations in liver and kidney for all the doses. The authors did not find any significant adverse effects as judged by the above parameters.

The National Academy of Sciences calculated a 7-d suggested no-adverse-response level (SNARL) based on the Loeser and Lorke study (1977a) where rats were fed $CdCl_2$ in their diets at 1 to 30 ppm of cadmium for 3 mo. With the assumption that rats consumed 20 g of diet per

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d and their body weight was 250 g, the exposure dose was calculated to be cadmium at 2.4 mg/kg. For a 70 kg human consuming 2 L/d, after applying a factor of 1,000, the 7-d SNARL was calculated to be 0.08 mg/L (NAS 1980) as follows:

$$(2.4\ \text{mg/kg} \times 70\ \text{kg}) \div (1{,}000 \times 2\ \text{L}) = 0.08\ \text{mg/L}.$$

Exposure Duration > 100 d

Several epidemiologic studies in Japan have provided models for dose-response relationships for adverse health effects from exposure to cadmium. One such study was performed on inhabitants of the Kakehashi River basin in Ishikawa Prefecture in Japan (878 cadmium-exposed males and 972 cadmium-exposed females) (Nogawa et al. 1989; Kido and Nogawa 1993; Nakagawa et al. 1993; Nishijo et al. 1995; Nakashima et al. 1997). The subjects were followed for 9 y. Controls for this study were 133 males and 161 females without exposure to cadmium. β-2m in urine was used as an index of the effect of cadmium on health, and the average cadmium concentration in locally produced rice was used as an indicator of cadmium exposure. The range of length of residence of the subjects was 1-70 y (Nogawa et al. 1989), and the mean residence time in the polluted area was 57 y in the case of males and 53 y in the case of females. Cadmium exposure was found to affect health in a dose-related manner when the subjects were classified according to the average cadmium concentration in their village's rice and their length of residence in the polluted area. Abnormal β-2m was defined as >1,000 µg/L or 1,000 µg/g urine creatinine. The concentration of cadmium in the rice from 22 hamlets varied from 0.63 to 0.87 µg/g in heavily polluted areas and from 0.48 to 0.72 µg/g in moderately polluted areas. From the available data, the total cadmium intake that produced an adverse effect on health was calculated as 2,000 mg for both men and women. The authors calculated that the daily cadmium intake corresponding to a 2,000 mg total dose for 50 y is 110 µg/d, or 0.0021 mg/kg/d, for men and women. This will be considered a LOAEL.

Buchet et al. (1990) conducted a cross-sectional population study from 1985 through 1989 in Belgium to assess environmental exposure to cadmium and renal dysfunction. Eligible subjects (n = 1,699) aged 20-80 y were studied as a random sample of four areas of Belgium with varying degrees of cadmium pollution, primarily from contaminated water and food. After standardization for several possible confounding factors, five

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variables (urinary excretion of retinol-binding protein, *N*-acetyl-β-glucosaminidase, β-2m, amino acids, and calcium) were significantly associated with U-Cd (which was used as a marker of cadmium body burden), suggesting the presence of tubular dysfunction. There was a 10% probability of the values of these variables being abnormal when cadmium excretion exceeded 2-4 µg/24 h. There was also evidence from this study that diabetic subjects may be more susceptible than normal subjects to the toxic effect of cadmium on the renal proximal tubule. The subjects excreting cadmium in urine at more than 2 µg in 24 h were predominantly women. Using cadmium at 2 µg/24 h as an estimated threshold for adverse concentrations, and assuming oral absorption of 5% and a daily excretion rate of 0.005% of body burden, Buchet et al. (1990) concluded that about 50 ppm of oral intake of 1 µg/kg/d would be retained in the renal cortex for 50 y. Using a toxicokinetic model, Clewell et al. (1997) arrived at an LOAEL of 0.84 µg/kg/d if the entire intake is via the oral route, using a factor of 3 to get a minimal LOAEL and not applying any other factor. A minimal risk level (MRL) for the Agency for Toxic Substances and Disease Registry (ATSDR) was calculated as 0.84 µg/kg/d ÷ 3 = 0.30 µg/kg/d or 0.0003 mg/kg/d.

Animal Studies

Itokawa et al. (1974) administered cadmium as $CdCl_2$ in drinking water (50 mg/L or 5.6 mg/kg/d) to male Wistar rats fed a diet sufficient or deficient in calcium and studied the renal and skeleton lesions by histology and biochemical measurements 120 d after exposure to cadmium. According to the authors, the daily mean cadmium intake was 0.9 mg/d. Only the effects of cadmium in rats fed a calcium-sufficient diet will be reported here. Significant reductions in the number of erythrocytes (about 30%), hematocrit (about 30%), and amounts of hemoglobin (about 25%) were observed. Light microscopic examination of the kidneys revealed considerable desquamation and vacuolization of the tubular epithelium; other remarkable changes noted were marked degeneration and necrosis of the glomeruli and hypertrophy of the kidneys. Histology indicated thinning of bone cortex in treated rats. Significant increases in serum urea and ALP were also noted. As the levels of hepatotoxic marker enzymes such as serum aspartate transaminase and serum alanine transaminase were not affected, the changes in the serum urea and ALP seem to be related to a dysfunction in bone metabolism. Because of a lack of dose-response data, a NOAEL could not be identified.

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In a study designed to compare early signs of cadmium toxicity via oral and inhalation exposures, Prigge et al. (1978) conducted a study in female Wistar rats (170-190 g) that were exposed to cadmium in drinking water at 25, 50, and 100 ppm (estimated doses of 4, 8, and 16 mg/kg) for 90 d. There was a significant decrease in serum iron as a function of dose and a decrease in serum ALP. Although ALP is present in several tissues, the major contribution to serum ALP seems to come from bone. The reduction of ALP in this study is not completely interpretable with respect to the effect of cadmium on the bone, although a reduction in serum bone-specific ALP may reflect a reduced bone-repair process or osteoblastic process. For example, Itokawa et al. (1974) observed an increase in serum ALP along with skeletal changes in rats exposed to cadmium at 50 ppm in drinking water for 120 d (see above). Proteinuria is the most significant observation in this study. Although there was a 20% increase in proteinuria in the 4 mg/kg group, it was statistically significant only in groups dosed with cadmium at 8 mg/kg and higher. A NOAEL of 4 mg/kg/d and a LOAEL of 8 mg/kg/d were identified for nephrotoxic effects in this study.

Male Sprague-Dawley rats were exposed to cadmium in drinking water at 10, 30, or 100 ppm (1.15, 2.92, or 8.51 mg/kg/d) for 24 wk (Kotsonis and Klaassen 1978). Central nervous system function was assessed by motor activity, and the "hourly nocturnal" and "daily motor activities" had decreased with time for the 30 ppm and 100 ppm groups by week 24. Also, renal injury was evident 6 wk after dosing as judged by the increased protein in the urine in these groups. There was also a slight focal tubular necrosis. A LOAEL for these effects seems to be 30 ppm, and 10 ppm (1.15 mg/kg) seems to be a NOAEL for 24 wk. The daily water intake was significantly less in the 100 ppm group throughout the study. No significant changes were noted in hemoglobin or hematocrit concentrations. Histopathologic examination of several tissues at 3, 6, 12, and 24 wk indicated that the only significant change was slight focal tubular atrophy at week 24.

Several investigators have studied the effect of oral administration of cadmium on the respiratory system, but results are equivocal. Petering et al. (1979) reported a reduced static compliance and lung lesions (not specified) in male Sprague-Dawley rats exposed to cadmium at 1.2 mg/kg/d in water for 200 d. Rats exposed to cadmium as $CdCl_2$ at 3.62 mg/kg/d in drinking water for 120 d developed emphysema (Petering et al. 1979). On the contrary, no respiratory system effects were observed in female SPF Wistar rats after 90 d of exposure to cadmium in drinking water at 16 mg/kg/d (Prigge 1978). Also, no histopathologic lesions of

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the lung were found in male Sprague-Dawley rats after 24 wk of exposure to cadmium in drinking water at a maximum dose of 2.82 mg/kg/d (Kotsonis and Klaassen 1978). In the study by Prigge (1978), findings inconsistent with those of Petering et al. (1979) might be because of the insensitivity of the particular rat strain used to study emphysema and/or the short duration for which the rats were observed.

Sutou et al. (1980a) administered $CdCl_2$ by gavage for 9 wk to male and female Sprague-Dawley rats at daily doses of 0, 0.1, 1.0, and 10.0 mg/kg. This study was a part of a fertility study, and the sample size of the nonpregnant female group was very low; hence, female rat data will not be discussed. In the male rats, a 9% increase seen in the number of red cells in the male 10 mg/kg group, without any observed change in hemoglobin and hematocrit concentrations, was not interpretable. Activities of hepatotoxicity marker enzymes (glutamic-pyruvic transaminase [GPT] and glutamic-oxaloacetic transaminase [GOT]) in serum were not altered even at the highest dose. Serum clinical chemistry indicated that a significant increase (about 10%) occurred in serum creatinine at 1 mg/kg and above. In general, increased serum creatinine is clinically used as an indicator of decreased kidney function (glomerular filtration). However, the authors reported decreased food intake and water intake at the higher doses; the importance of this increase is questionable. No change was observed in the BUN even at 10 mg/kg. No nephrotoxic markers were measured in the urine. Other serum parameters measured, ALP, LDH urea nitrogen, bilirubin remained at concentrations comparable to controls. A NOAEL of cadmium at 10 mg/kg for hepatotoxicity for male rats can be identified (Sutou et al. 1980a). The authors did not carry out any histopathology of the organs of the male rats. However, they reported focal necrosis of the liver in five out of eight nonpregnant female rats and slight atrophy of glomeruli in two of eight of these female rats (Sutou et al. 1980a).

In a subchronic study (Ogoshi et al. 1992), young female rats were exposed to cadmium at 0, 5, and 10 ppm in drinking water for 20 wk. Old rats (18 mo) received only 0 and 40 ppm in drinking water but were exposed for 7 mo. In this study, the authors observed decreased compression strength of the femur bone (metaphysis) in the 10 ppm group of the young rats and the 40 ppm group of the older rats. The bone cadmium content (ng/g dry weight of femur) of the older rats was about twice that of young rats (10 ppm group). Because of variation in the duration, direct comparison of the data is difficult. Conservatively, cadmium at 5 ppm can be identified as a NOAEL. Because the concentration of cadmium in the kidney was below the critical concentration and there was no indica-

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tion of kidney damage at the times when the effects on bone were noted, it seems that the effects on bone are not secondary to any renal effects that might be present.

Female Sprague-Dawley rats were given cadmium at 200 ppm (30 mg/kg/d) in drinking water for 11 mo (Bernard et al. 1981). From the 8th mo of treatment, the cadmium concentration in the kidney cortex leveled off at a value of 250 μg/g wet weight, and this phenomenon coincided with the occurrence of proteinuria. The proteinuria was characterized by an increased urinary excretion of high-molecular-weight (HMW) proteins, particularly γ globulins. Aminoaciduria also increased, which suggested the existence of a slight tubular dysfunction (Bernard et al. 1981). In a later study, female Sprague-Dawley rats exposed to cadmium in drinking water at 200 ppm (or 30 mg/kg) for 2-10 mo showed an increase in albuminuria and at 10 mo developed slight tubular damage, as evidenced by increased urinary excretion of β-2m and β-*N*-acetylglucosaminidase (NAG) (Bernard et al. 1988).

Enzymes from different cellular compartments of the nephron were measured in male and female weanling Wistar rats that received cadmium (as $CdCl_2$) in their diet at concentrations of 0, 10, 50, and 250 ppm [0.66, 3.33, and 16.66 mg/kg/d] for a total of 72 wk (Bomhard et al. 1999). At the end of the study period, histopathology of the kidneys was done. Concentrations up to and including 50 ppm did not induce any adverse effect (NOAEL). Beginning with week 13, in males and females receiving 250 ppm, increased excretion of cytosolic phosphohexose isomerase enzyme was seen. Several nephron-related enzymes were measured in 24-h urine-sample pools collected during study week 1, 4, 8, 13, 26, 32, 57, and 68. The brush border enzymes (GGT, ALP, and leucine arylamidase) were not changed in a time-dependent manner in female rats. GGT activity was lower during the entire study period in the 250 ppm male rats, and activities of ALP and LAP were significantly lower than the control values from week 1 to week 18. Excretion of the lysosomal enzymes aryl sulfatase A, β-galactosidase, and NAG seemed to be unaffected. In the kidneys of 250 ppm males and females, histopathology after 72 wk revealed chronic and acute degenerative changes.

Male Wistar rats were fed diets containing cadmium as $CdCl_2$ at 0.3, 3, 30, or 90 mg/kg for 10 mo (Groten et al. 1994). In the highest-dose group, an increase in urinary LDH activity, a sign of renal injury, was seen first at 4 mo of treatment at 90 mg/kg. After 8 and 10 mo, the renal effect became more pronounced and urinary enzyme activities of LDH, NAG, and ALP were all higher (Groten et al. 1994).

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A critical target organ for accumulation of cadmium was well demonstrated in an experiment by Mitsumori et al. (1998). Female Sprague-Dawley rats were fed a diet containing $CdCl_2$ at 0, 8, 40, 200, or 600 ppm (0, 0.33, 1.6, 8, or 24 mg/kg/d) for 2, 4, and 8 mo from 5 wk of age. Hepatotoxicity was observed after 2 mo in the groups treated with ≥200 ppm. By 4 mo, the rats in the 600 ppm group had developed periportal liver cell necrosis. At 4 mo, surviving rats given cadmium at 600 ppm showed anemia and decreased hematopoiesis in the bone marrow, in addition to reduction of cancellous bone in their femurs. Renal toxicity, characterized by vacuolar degeneration of proximal tubular epithelia, was apparent in the groups treated with ≥200 ppm from 2 mo, becoming more prominent in the high-dose rats at 4 mo. Hepatic accumulation of cadmium increased linearly with the duration of treatment. The concentration of cadmium in the renal cortex of rats reached a plateau concentration of 250 µg/g within the first 2 mo. The renal concentration of cadmium in the 200 ppm group when renal toxic lesions were first detected at 2 mo ranged from 104 to 244 µg/g. Although no renal lesions were observed in the 40 ppm (1.6 mg/kg/d) group even after 8 mo, despite the presence of 91-183 µg/g amounts of $CdCl_2$ for periods longer than 8 mo, accumulation of cadmium might gradually progress. Shibutani et al. (2000), in a later study from the same laboratory, used a concentration of cadmium lower than in the previous study (concentrations of 1.1, 5, 20, or 40 ppm/kg diet) for a longer time (up to 22 mo). The observed incidence or severity of nephropathy in the treated animals was not different from that in controls. No renal or hepatic lesions were noted in this long-term low-cadmium-dose study.

In CBA/H mice that drank water containing $CdCl_2$ at 300 mg/L for 12 mo (45 mg/kg/d), bone marrow hypoplasia was noted, characterized by significant reduction of the totipotent stem cells, granulocyte-monocyte progenitor cells (GM-CFUc), and erythroid progenitor cells (Hays and Margaretten 1985). The bone marrow cellularity and the proliferative capacity of GM-CFUc measured in vitro were decreased. Consistent with these changes, anemia with reticulocytopenia and neutropenia was also seen. Peripheral red blood cells were hypochromic, with diminished bone marrow iron. Because this study did not provide dose- or time-response data, it was not useful for AC derivation.

Bomhard et al. (1987) conducted a study to evaluate the chronic effects of single versus multiple oral cadmium administrations on the testes of mature male Wistar rats. Cadmium was administered as $CdCl_2$ in water in a single bolus dose of 50 mg/kg or in 10 weekly oral doses of 5 mg/kg. Some of the animals were necropsied after 12 and 18 mo, re-

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spectively; the remainders were kept in the study for up to 30 mo. In a supplementary study, male rats were treated once with 200 or 100 mg/kg po. This experiment was terminated after 6 mo. Animals having received 1 × 100 or 1 × 200 mg/kg po showed severe lesions of the entire testicular parenchyma with massive calcification. These testicular lesions were not seen in rats that received a single 50 mg/kg dose or in rats treated 10 times, once per wk for 10 wk at 5 mg/kg po.

In a study that evaluated the relationships of certain urinary enzymes and various anatomical areas of the kidney (Gatta et al. 1989), 40 Wistar rats were exposed to $CdCl_2$ at 16 ppm in drinking water for 4, 16, 40, or 60 wk. At the end of each period, all the cadmium-dosed rats and five controls were assessed for creatinine clearance, fractional excretion of GGṬ and α-glucosidase (indices of anatomical tubular damage), and fractional clearance of lysozyme (an index of functional tubular damage). Subsequently, the rats were sacrificed and their kidneys examined. No histologic impairment was seen for up to 16 wk of exposure according to light and electron microscopy. A widespread vesiculation of proximal tubular cells with mitochondrial and lysosomal alterations was found at 40 wk and was more evident at 60 wk. The brush border never showed any damage (normal excretion pattern of GGT, an enzyme situated in this structure). Urinary α-glucosidase was increased only at 60 wk and showed the most severe anatomic damage. Urinary lysozyme, an index of tubular function, was increased at 40 wk and 60 wk.

A pronounced increase in the mean duration of the estrous cycle, mainly because of the lengthening of diestrus, was noted in female rats that received an aqueous solution of $CdCl_2$ for 14 wk, 5 d/wk by gavage at doses of 0.04, 0.4, 4.0, and 40.0 mg/kg/d. This was seen 6 wk after treatment only in rats in the 40 mg dose group (Baranski and Sitarek 1987).

Loeser (1980) conducted a long-term 2-y feeding study on cadmium with Wistar rats (50 male and female) ingesting a diet containing $CdCl_2$ at 1, 3, 10, and 50 ppm. In this study, the author did not look at any parameters except food consumption, body weight changes, and extensive histopathology. Except for a significantly decreased growth rate in the 50 ppm dose group, the author did not find any cadmium dose-related change in benign or malignant neoplasia. In particular, there was not a single case of prostate tumors.

Ohta et al. (2000) conducted a study evaluating the relationship between renal dysfunction and bone metabolism (osteotoxicity) in 6-wk-old male rats after long-term oral cadmium administration of $CdCl_2$ by gavage, 6 consecutive d/wk for 60 wk (360 doses) at doses of 2, 5,10, 20,

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30, and 60 mg/kg for 60 wk. Hepatotoxicity was assessed by determining plasma hepatotoxic marker enzymes GOT (also called AST, L-aspartate aminotransferase), and GPT (also called ALT, alanine aminotransferase). Several urinary variables such as glucose, protein, creatinine, amino acids, and the excretion of urinary enzymes such as NAG, alanine aminopeptidase, and glutathione-s-transferase were measured as nephrotoxic indices. Nephrotoxicity was also confirmed by histopathologic examination of renal tissue especially by evaluating morphologic changes in the proximal tubular cells (Ohta et al. 2000). Bone metabolism and osteotoxicity were evaluated by measuring bone mineral density (by radiographic microdensitometry), urinary excretion of pyridinoline (Pyr, synonym pyridinium collagen crosslinks) and deoxypyridinoline (Dpyr), and plasma intact-osteocalcin. Usually Pyr and Dpyr provide rigidity and strength to the bone. During the bone resorption process, these are released into the circulation and are excreted unmetabolized in the urine (Ohta et al. 2000). Manifestations of enzymuria followed the dose and duration of exposure. From the 5th wk, both hepatotoxic and nephrotoxic markers were found to be higher in rats treated with cadmium at 30 and 60 mg/kg. In 10 mg/kg dose groups, GST increased as early as 5 wk after treatment. Proximal tubular regeneration, vacuolization, and eosinophilic bodies were mainly observed in the groups receiving 20, 30, and 60 mg/kg at this time. Such changes were noted at the 30th wk in rats administered 5 and 10 mg/kg and at the 60th wk in the lowest-dose group (2 mg/kg).

As far as osteotoxicity is concerned, dose groups of 5 and 10 mg/kg were studied only for 30 wk for bone mineral density and other markers of bone resorption. Bone mineral density measured at the midpoint of the femur decreased as the cadmium dose increased. In the high-dose groups (30 and 60 mg/kg), the decrease in bone mineral density was observed at about the same time or before increased excretion of aforementioned urinary enzymes were observed. No significant differences were noted in the 2 mg/kg group at the 5th wk. At 10 wk, bone mineral density was unaffected in both 5 and 10 mg/kg groups. The rats of the 10 mg/kg group showed changes in bone mineral density only at 30 wk. In rats of groups dosed with cadmium at 2-10 mg, a reduction in bone mineral density was seen after the onset of renal toxicity. Significant increases in Pyr and Dpyr—the bone resorption markers—were also observed in high-dose groups during the early part of the treatment regimen (Ohta et al. 2000). These data were not considered for AC derivations as the doses in this study were administered as single boluses (gavage) and data from drinking water studies were preferred.

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Cadmium and Immunotoxicity

Several studies on cadmium immunotoxicity have been extensively reviewed by Koller (1996, 1998) and others (Bernier et al. 1995). The reviews concluded that it can be demonstrated that cadmium, in vivo, can modulate certain immune responses with a tendency to induce various degrees of immunosuppression, although the results from numerous rodent studies on humoral immune responses and cell-mediated immune responses as a result of exposure to cadmium appear to be contradictory and human data from occupational settings (probably because of coexposure to other metals) are inconsistent. However, the complex nature of the results and the direction of effects make the data difficult to interpret so as to be quantitatively useful for deriving ACs with any confidence. Some of the studies have been described here.

A few studies have measured changes in parameters of immunotoxicity because of cadmium exposure (mostly by inhalation) in an occupational setting, but the results are somewhat ambiguous (see Bernier et al. 1995). There are no human epidemiologic data demonstrating any immunotoxic effects of cadmium on populations exposed via an oral route.

Although little information is available from human studies, the effects of cadmium on the immune system have been studied extensively in animals, particularly in rodents. A review of the experimental literature indicates that the immunotoxic effects of cadmium are dependent upon dose, time between cadmium administration and exposure to antigen, and mode and length of exposure. In some instances, species-, strain-, and age-specific effects have been reported (Koller 1980; Malave and de Ruffino 1984; Fujimaki 1985, 1987; Thomas et al. 1985; Borgman et al. 1986; Exon et al. 1986; Blakley 1988; Cifone et al. 1989; Schulte et al. 1994; Bernier et al. 1995; Fawl et al. 1996).

Cadmium-induced immunotoxicity has been studied using various approaches such as host-resistance challenge assays that assess increased susceptibility against bacterial and/or viral pathogens; primary and secondary immune response against specific antigens (sheep red blood cells [sRBC]); antibody titers; proliferative response of peripheral lymphocytes to a particular mitogen; and shifts in lymphocyte subpopulations. Due to a vast amount of literature from studies employing a variety of exposure routes (which likely do not extrapolate well to ingestion), only results from oral exposures will be discussed in this section.

The effects of cadmium on the immune system of rodents exposed either by drinking water or gavage have been thoroughly reviewed by Koller (1998).

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Host Resistance and Exposure to Cadmium

In studies in which different strains of mice were exposed orally to a soluble cadmium salt and subsequently challenged with an infectious viral or bacterial pathogen, effects on host resistance were highly variable and ranged from no effect (Ilback et al. 1994) to increased host survival time and tumor regression rate (Kerkvliet et al. 1979).

Thomas et al. (1985) also studied the effects of cadmium ($CdCl_2$) on host resistance against viral and bacterial agents. The investigators treated female $B_6C_3F_1$ mice with $CdCl_2$ at 10, 50, or 250 mg/L via drinking water for 90 d and then challenged on day 91 with various infectious agents such as influenza virus, herpes simplex virus type I (HSV-1) and type II (HSV-2), and *Listeria monocytogens*. Cadmium treatment did not alter the mortality or mean survival times (MST) following a primary infectious challenge or a secondary challenge (21 d after the primary challenge) of the survivors with HSV-1, influenza virus or *L. monocytogens* compared to controls. However, resistance to HSV-2 challenge in cadmium-treated animals decreased (not statistically significantly) as the dose of cadmium increased. Exon et al. (1986) reported that the incidence of viral-induced mortality was lower in male Swiss Webster mice exposed to cadmium sulfate, acetate, or chloride in the drinking water at 3, 30, or 300 mg/L for 10 wk followed by an inoculation of encephalomyocarditis virus (EMCV). When resistance to coxsackievirus B3 (CB3)-induced myocarditis in female Balb/c mice was investigated after a dose of cadmium at 2 millimolar (mM) (225 mg/L) in drinking water for 10 wk, no influence on mortality from the CB3 infection was found. The inflammatory and necrotic lesions in the myocardium were also not changed (Ilback et al. 1994). On the other hand, Kerkvliet et al. (1979) observed that male C57BL/6 mice exposed to cadmium as $CdCl_2$ at 0, 3, 30, or 300 ppm in their drinking water for 21 wk had significantly inhibited in vivo growth of MSB-induced tumors and had enhanced manifestations of cell-mediated cytotoxicity in the tumor-bearing hosts and that the latter was inversely correlated to the dose.

Recently, Seth et al. (2003) pretreated CD-1 mice (either sex) and 6-wk-old C57BL/6 mice with a single oral dose of cadmium as $CdCl_2$ by gavage at either 0.13 or 0.26 mg/kg. Subsequently, animals were inoculated with Venezuelan equine encephalitis virus (VEE), EMCV, or Semliki Forest virus (SFV). Increased severity of symptoms and mortality compared to untreated controls were noted in all cases. An early onset of virus infection was found in the brains of cadmium-treated animals. This indicated immunosuppression as a result of cadmium treatment.

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From the above results, the extent of immunotoxic effects of cadmium when ingested via the oral route cannot be conclusively determined. The concentrations used in most of these studies are several-fold higher than those shown to result in nephrotoxicity.

Humoral Immunity and Exposure to Cadmium

Humoral immunity is conferred by B lymphocytes, but maximum responses in most cases require interactions with both the innate and cell-mediated arms of the immune response. Although data concerning the effects of cadmium on humoral immunity are conflicting and depend upon rodent species, strain, dose, and duration of exposure, mechanisms important in maintaining humoral-mediated immunity (B lymphocyte proliferation, antibody formation/response, and antibody-complement interactions) appear sensitive to the immunotoxic effects of cadmium.

For example, Koller et al. (1975) observed a significant decrease in the plaque-forming cell (PFC) response (immunoglobulin g [IgG]) to injected sRBC in Swiss Webster mice given $CdCl_2$ orally at 3, 30, or 300 ppm for 10 wk. Although immunoglobulin M (IgM) was also depressed, it appeared to recover 14 d after the cessation of exposure. Blakley (1985) and Blakley and Tomar (1986) reported suppression of splenic antibody production against injected sRBC when 6-wk-old BDF1 or CD-1 female mice were exposed to $CdCl_2$ in their drinking water at 5,10, or 50 mg/L for 3 wk. Borgman et al. (1986) exposed young mice to cadmium at 50 ppm via drinking water for 3 wk, and mice were killed at 0, 3, and 6 wk after the cessation of the dose (that is, at 3, 6, and 9 wk of the experiment). When sRBCs were injected immediately at the end of the experiment and splenic PFC response enumerated 5 d after immunization, suppression was observed; after 7 d, PFC response was similar to controls. Injection of sRBCs 3 wk after experiment cessation revealed no effect on the PFC response. Results demonstrate that effects on humoral immunity observed immediately following cadmium exposure were no longer visible 3 wk following the cessation of exposure. Blakley (1986) reported that in female albino Swiss mice exposed to cadmium in drinking water for 280 d at doses of 0, 5, 10, or 50 mg/L, deaths that normally occur from spontaneous murine lymphocytic leukemia in this mouse strain were increased by 33% in the 10 and 50 mg/L groups, indicating immunosuppressive effects. According to the authors, this effect was possibly because of the suppression of the T-lymphocyte-dependent antibody response. Also, albino Swiss mice exposed to cadmium as $CdCl_2$ at

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30, 100, or 300 ppm in drinking water for 35 d revealed significant decreases in IgM and IgG titers against sRBC and IgG titer against bovine serum albumin (BSA) in groups treated with cadmium at 100 and 300 ppm (Dan et al. 2000).

In contrast to the above findings, Ohsawa et al. (1988) reported that spleen cells of ICR mice administered cadmium as $CdCl_2$ via drinking water at 3, 30, and 300 ppm for 10 wk showed enhanced antibody-forming ability in response to sRBC after exposure. In addition, Malave and de Ruffino (1984) observed that the PFC response to sRBC was moderately increased in C57BL/6 mice administered cadmium at 50 ppm in drinking water for either 3-4 wk or 9-11 wk; the antibody response to sRBC was depressed in the 300 ppm dose group exposed for 9-11 wk.

However, Thomas et al. (1985) did not find any effect on PFC numbers when B6C3F1 mice were administered cadmium as $CdCl_2$ at 10, 50, or 250 ppm for 90 d in drinking water. Similarly, when female Balb/c mice were given cadmium as $CdCl_2$ at 1, 10, or 100 ppm in drinking water for 7, 14, 21, 28, 60, or 90 d, the titer of antibodies in the blood (IgM and IgG) produced when immunized with the specific antigen dinitrophenyl-BSA were no different from the untreated control mice (Schulte et al. 1994).

Thus, the reports on antibody response to an administered antigen in rodents (particularly in mice) orally treated with cadmium vary from decreases, increases, and no changes reported.

Cell-Mediated Immunity

Several studies have measured cell-mediated immunity to cadmium exposure by determining splenic B and T lymphocyte blastogenesis in response to mitogens. T-cell function was measured using proliferative response to phytohemagglutinin (PHA) and concanavalin A (ConA) and B-cell mitogenic response to bacterial lipopolysaccharide (LPS) and, in some cases, to pokeweed mitogen (PWM). Mitogen stimulation assays per se are not a measure of immune function of these immunocyte cell populations (Koller 1998).

Cultured splenic cells from male Swiss Webster mice administered cadmium as $CdCl_2$ at 160 ppm in drinking water for 30 d showed decreased proliferative response to PWM and PHA (Gaworski and Sharma 1978), whereas Muller et al. (1979) reported increased stimulation to ConA and PHA in mice exposed to $CdCl_2$ orally at 30, 300, or 600 ppm for 10 wk. Malave and de Ruffino (1984) reported that in C57BL/6 mice

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administered cadmium at either 50 or 200 ppm in their drinking water for either 3-4 wk or 9-11 wk, proliferative responses to PHA and ConA were increased. Chopra et al. (1984a) reported that when $CdCl_2$ was given to male albino Wistar rats as a gavage (5 mg/kg/d) in water for 7 wk, cultured splenocytes showed an inhibition of proliferative response to ConA. However, cadmium increased the antibody-dependent, cell-mediated cytotoxic activity (ADCC) of the splenocytes (effector cells) against chicken red blood cells (cRBC) (target cells).

In another study, the authors (Chopra et al. 1984b) did not find any difference in stimulation by PHA, ConA, or PWM of peripheral lymphocytes recovered from rhesus monkeys (*Macaca mulata*) orally exposed to cadmium at 5 mg/kg (n = 3 to 4) for 2 and 6 mo. Based on the results, the authors suggested (albeit prematurely) that oral cadmium exposure does not produce immunosuppression. Given that only one cadmium dose was examined and that lymphocyte proliferation was the only end point examined, more studies are needed to support the author's conclusion.

Thomas et al. (1985) treated adult female $B_6C_3F_1$ mice with $CdCl_2$ at 10, 50, or 250 mg/L of via drinking water for 90 d and determined splenic lymphocyte blastogenesis of T- and B-lymphocytes. T-cell mitogenic response to PHA and ConA and B-cell mitogenic response to *Salmonella typhosa* lipopolysccharide were both found to be significantly decreased in the 10 and 250 mg/L group, but the decrease was not statistically significant in the 50 mg/L group; effects on lymphoproliferation occurred in the absence of any change in the viability of splenic cells isolated from all three treatment groups. In the same study, the authors noted that delayed-type hypersensitivity response was unaffected by cadmium treatment (Thomas et al. 1985). Alternatively, Blakley (1985) reported that ConA-induced T-lymphocyte proliferation was unaffected in mice treated with cadmium at 5-50 mg/L in their drinking water for 3 wk; in contrast, a dose-dependent enhancement of B-lymphocyte proliferation to *Escherichia coli* lipopolysaccharide (LPS) mitogen was noted. Stacey et al. (1988) studied the effects on T- and B-cell blastogenesis of in vivo exposure to cadmium administered as a gavage at 2.5, 25, or 250 μg/kg for up to 6 wk or via drinking water at 5 ppm (400 μg/kg/d) for 6 wk. An effect of cadmium exposure by gavage on mitogen-stimulated splenocyte proliferation was not statistically different from controls at the end of 2 wk even at the highest dose (250 μg/kg). At the end of 6 wk, the observed increase in response to both mitogens was statistically significant but only at the highest dose. In the drinking water study, ingestion of cadmium at 5 ppm (400 μg/kg/d) for 6 wk increased the splenocyte proliferative responsiveness to ConA, but not to LPS. Thus, the

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mode of oral exposure also makes a difference for cadmium responsiveness.

Ilback et al. (1994) reported that the observed increase in lymphoproliferation to LPS stimulation in BALB/c mice treated with cadmium at 2 mM (225 mg/L) in drinking water for 10 wk was not statistically significant compared to controls. Alternatively, delayed-type hypersensitivity (DTH) response to sRBC and splenic T-cell proliferative response to BSA were observed in albino Swiss mice exposed to $CdCl_2$ at 100 or 300 ppm in drinking water for 35 d but not in the 30 ppm group (Dan et al. 2000).

Cadmium and Innate Immune Function

Thomas et al. (1985) reported that peritoneal macrophage phagocytosis of opsonized chromium (^{51}Cr)-labeled cRBC in cadmium-treated groups was significantly increased in mice exposed to $CdCl_2$ at 10, 50, or 250 ppm in their drinking water for 90 d. The authors also reported that natural killer (NK) cell activity to lyse YAC-1 lymphoma cells showed a nonsignificant increase in treated animals (Thomas et al. 1985). Ohsawa et al. (1983) reported no change in the numbers of B- or T-lymphocytes in the blood or spleen of ICR mice orally exposed to cadmium for 10 wk at 3, 30, or 300 ppm in drinking water. Cifone et al. (1989) reported that rats exposed to $CdCl_2$ at 200 or 400 ppm in their drinking water for 6 mo demonstrated during the first month, a decrease in large granulocyte lymphocyte numbers (that is, NK cells) in the peripheral blood. This initial decrease was followed by a marked increase during the rest of the 5 mo of treatment. In a parallel manner, NK-cell cytotoxic activity of rat peripheral blood lymphocytes (PBL) or splenocytes against YAC-1 target cells (a A/Sn mouse T-lymphoma cell line) from treated groups was depressed during the first month of treatment followed by a persistent increase until the end of the treatment period (Cifone et al. 1989).

Stacey et al. (1988) studied the effects of in vivo exposure to cadmium as a gavage on NK cell and killer (K) cell functions at 2.5, 25, or 250 μg/kg for up to 6 wk or exposed to cadmium via drinking water at 5 ppm for 6 wk. Neither the NK- nor the K-cell activity was altered because of cadmium treatments by either exposure routes at the end of 2 or 6 wk. Ohsawa et al. (1988) reported induction of anti-nuclear antibodies (ANA) in ICR mice exposed to cadmium as $CdCl_2$ in drinking water at 3, 30, and 300 ppm, whereas, in inbred BALB/c mice, the changes in ANA was seen only at the 300 ppm dose, demonstrating species specificity and

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sensitivity. Recently, Leffel et al. (2003) reported that in New Zealand black-and-white F1 mice (a genetically predisposed model for spontaneous development of an autoimmune disease) exposed to cadmium at 0, 3, 30, 3,000, or 10,000 parts per billion (ppb) in tap water (much lower concentrations than those used in the Oshawa study) for 2, 4, 28, or 31 wk, there was an increased incidence of ANA and immune complex deposition in the kidney after 4 wk of exposure in all treated groups. Lafuente et al. (2003) observed that in adult male rats exposed for 1 mo to $CdCl_2$ at 0, 5, 10, 25, 50, and 100 ppm in drinking water, the B-lymphocytes increased with doses of $CdCl_2$ at 5 and 10 ppm in both spleen and thymus, although they decreased at doses greater than 25 ppm. In the high-dose groups, spleen-derived $CD4^+$ cells were decreased, and at the lower doses, $CD8^+$ cells were increased. The authors noted that although the concentration of cadmium in the spleen was lower than that in the thymus, the $CD4^+$ and $CD8^+$ T-cell subsets were altered only in the spleen and not in the thymus (Lafuente et al. 2003).

When female Balb/c mice were given cadmium as $CdCl_2$ at 1, 10, and 100 ppm in drinking water for 7, 14, 21, 28, 60, or 90 d, there was a significant loss of thymus and spleen weight in the 100 ppm group (Schulte et al. 1994). This is inconsistent with the observations of Cifone et al. (1989) who demonstrated that thymus weight was unchanged even after 6 mo of exposure of 2-mo-old female CD rats to cadmium in drinking water at 200 or 400 ppm.

The apparently conflicting and complex nature of results, as evidenced from the descriptions of the effects of cadmium on the immune response, do not allow the use of the extensive data for AC calculation for cadmium based on immunotoxicity.

Genotoxicity

It is beyond the scope of this document to describe all the genotoxic studies on cadmium. This document will, as far as possible, be restricted to describing studies that pertain to genotoxic effects resulting from oral ingestion of cadmium.

Cytogenetic studies conducted in human populations exposed to cadmium compounds have been recently reviewed by Verougstraete et al. (2002). Conflicting results of genotoxicity studies have been reported for humans exposed to cadmium. Evidence concerning chromosomal aberrations (CAs) in humans following oral exposure to cadmium is equivocal (Shiraishi and Yoshida 1972; Bui et al. 1975; Tang et al.

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1990). Shiraishi and Yoshida (1972) examined lymphocyte chromosomes in blood samples obtained from seven female patients suffering from *itai-itai* disease (exposed to cadmium through consumption of contaminated rice) and from a control group of seven similarly aged females unexposed to cadmium. The frequency of chromosomal abnormalities (various types) in the cells of the *itai-itai* patients was higher than that of controls. The mean percentage of cells with structural aberrations was 50.6% (range of 14-64%) in cadmium-exposed groups and 0.6% (range 0-2%) in the unexposed group. In addition, the frequency of aneuploid cells was higher in the exposed population than in controls. In an additional examination 3 y later (Shiraishi 1975) with 12 *itai-itai* patients and 9 controls, the author obtained similar results. However, Bui et al. (1975), who examined only four female *itai-itai* patients from the cadmium-contaminated area and four from a non-cadmium-contaminated area (three females and one male), did not find any difference in structural aberrations or prevalence of aneuploidy between these groups. Tang et al. (1990) examined cultured peripheral lymphocytes from 21 men and 19 women in China who had been environmentally exposed to cadmium (as judged by urinary cadmium and soil cadmium concentrations). Lymphocyte cultures from exposed subjects were significantly different with respect to chromosome aberration frequency from cultures from unexposed subjects. Furthermore, among cadmium-exposed subjects, ones with higher cadmium concentrations in their urine had higher chromosome-aberration frequencies and more severe aberration types (Tang et al. 1990), with a good correlation between U-Cd and the aberration frequency. In another study, Fu et al. (1999) reported a significant increase in the frequency of chromosome aberrations and micronuclei in peripheral lymphocytes from 56 people in China who were environmentally exposed to cadmium for up to 30 y. There was a significant correlation between the increased frequency of CA and MN and the levels of U-Cd.

Mutagenic effects of cadmium, as revealed by chromosome changes, have mainly been seen in cells exposed to cadmium sulfate, cadmium sulfide, and $CdCl_2$. Shiraishi et al. (1972) observed a marked increase in the frequency of chromatid breaks, translocation, and dicentric chromosomes in human leukocytes cultured for 4-8 h in a medium containing cadmium sulfide at 62 µg/L. Rohr and Bauchinger (1976) studied fibroblast cell cultures from Chinese hamsters that had been exposed to cadmium sulfate at concentrations ranging from 1 µg/L to l0 mg/L. At concentrations exceeding 100 µg/L, the mitotic index was significantly reduced, and at concentrations exceeding 0.5 mg/L, chromosome damage was seen. Saplakoglu and Iscan (1998) reported that when

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sister chromatid exchanges (SCE) were analyzed in PHA-stimulated peripheral lymphocyte cultures exposed to varying concentrations of $CdCl_2$ (10^{-7} to 10^{-3} M) at two different stages of the cell cycle, G_0 and early S phase, a statistically significant increase in SCE was observed only when cultures were exposed at S phase and not during G_0. The authors suggested that the stage of the cell cycle in which the measurements are made may be a source of contradictory results published in the literature on the effect of $CdCl_2$ on the induction of SCE in human lymphocyte cultures.

Cadmium induced DNA single-strand breaks and DNA-protein crosslinks in V79 Chinese hamster cells (Ochi and Ohsawa 1983). Amacher and Paillet (1980) and Oberly et al. (1982) observed an enhanced number of mutations at the thymidine kinase locus in mouse lymphoma L51784/TK+/− cells. A concentration-dependent increase in the frequency of 6-thioguanine-resistant mutations at the HPRT locus in V79 cells was observed by Ochi and Ohsawa (1983).

In spite of the fact that ip injections of $CdCl_2$ at 0.42-6.75 mg/kg into mice have been shown to induce genotoxic damage (CAs, SCE, micronuclei, and sperm-head abnormalities) in somatic and germ cells (Mukherjee et al. 1988), data from humans are inconclusive.

Cadmium concentrations as $CdCl_2$ ranging from 0.3 to 5.6 µg Cd/L (Paton and Allison 1972) or cadmium at 0.6 and 6 mg/L (Deknudt and Deminatti 1978) added to human lymphocytes and fibroblasts cultured for periods ranging from 24 to 72 h, did not produce any CAs. On the other hand, when human lymphocytes in culture were exposed to cadmium at 1.1 mg/L for 4 h (Andersen and Ronne 1983), reduced chromosome length, an indication of translocation, was observed.

Bruce and Heddle (1979) found that when hybrid mice (C57BL/6 × C3H/He) were injected ip with doses ranging up to 20 mg/kg, no changes were noted in the micronucleus test or in the sperm-head abnormality assay. On the contrary, Mukherjee et al. (1988) reported that when $CdCl_2$ was injected ip into albino Swiss, 8- to 10-wk-old male mice at doses of 0, 0.42, 0.84, 1.68, 3.37, or 6.75 mg/kg, significant increases occurred in the frequency of SCE in bone marrow assayed 24 h after injection in all treatment groups above 0.42 mg/kg. The incidence of micronucleated cells per 500 polychromatic erythrocytes was found to be increased only at the highest dose. The authors also used the same doses to determine the effect of cadmium on the induction of sperm-head abnormalities, but the mice were injected with the doses daily for 5 consecutive d. After 35 d, 500 sperms isolated from the cauda epididymis were evaluated for abnormal sperm morphology, and significant increases in abnormal

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sperm heads were observed in all treatment groups. Fahmy and Aly (2000) evaluated the in vivo and in vitro genotoxic effects of $CdCl_2$ in the chromosomes of mice. After 24 h, a single ip injection of $CdCl_2$ at 1.9, 5.7, or 7.6 mg/kg, induced a significant and dose-dependent increase in the percentage of polychromatic erythrocytes with micronuclei, and the last two dosed groups showed increased frequency (less than twofold that of controls) of SCE in the bone marrow. The changes at 48 h postinjection were less pronounced than those at 24 h. In germ cell (primary spermatocytes) samples taken 12 d after the single injection, CAs were observed at a dose of 5.7 mg/kg. There was also a pronounced reduction in the number of spermatocytes at all doses of $CdCl_2$ (0.9, 1.9, and 5.7 mg/kg).

Cadmium and Cancer

Several epidemiologic studies have attempted to establish a link between occupational exposure to cadmium and the incidence of lung, prostate, and bladder cancer among workers in a variety of industries in various countries (Kjellström et al. 1979; IARC 1993; Sorahan and Lancashire 1994, 1997; Waalkes 2000; Sorahan and Esmen 2004). This has been well summarized in a recent review (Verougstraete et al. 2003). The results are conflicting or difficult to interpret because subjects were co-exposed to other heavy metals such as nickel, arsenic, and lead (for example, battery plant workers were exposed to both nickel and cadmium, and workers in the cadmium-recovery industry were co-exposed to arsenic). The National Institute for Occupational Safety and Health (NIOSH) came to the following conclusions based on their evaluation of existing data several years ago and has not updated this information:

> In a study of 292 cadmium production workers who had a minimum of 2 years of employment between 1940 and 1969, a statistically significant excess of deaths from all malignancies and from lung cancer was observed in the entire cohort. In addition, a statistically significant excess of deaths from prostate cancer was detected among workers who lived for at least 20 years after the date of first working in a cadmium production facility. Some of these workers had been hired before 1926, when arsenic (a known human lung carcinogen) was produced in the plant. NIOSH considered the body of toxicologic and epidemiologic evidence for carcinogenicity to be

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> inconclusive and recommended against basing a standard on potential human carcinogenicity.

However, the criteria document stated, "This recommendation should be reconsidered if additional data on these points that warrant such reconsideration are developed" (Current Intelligent Bulletin 42, September 27, 1984).

However, the International Agency for Research on Cancer (IARC) had concluded that sufficient evidence existed to classify cadmium and cadmium compounds as human carcinogens. IARC (1993) had identified seven cohort studies of multinational origin, and they were all occupational and case-control studies. According to IARC (1993), in several cohort studies of workers exposed to various cadmium compounds, the risk of death from lung cancer is increased. IARC did not find any excess prostatic cancer. Although excess prostate cancer was found initially in a U.S. cadmium-recovery plant, the relative risk was found to be nonsignificant later on. On evaluation of animal carcinogenicity data, IARC (1993) concluded that although cadmium and cadmium compounds have been tested by oral administration in several studies in mice and rats, most of the studies were inadequate for an evaluation of carcinogenicity. There have not been any updates or follow-ups released by IARC since 1993. IARC also evaluated several animal studies that tested carcinogenic action of cadmium and cadmium compounds via single or multiple injections (sc, intramuscular, or ip) and noted that in addition to local sarcomas in rats and mice, a variety of tumors such as malignant tumors of the peritoneal cavity, testicular tumors, and tumors of the prostate were reported in these studies. IARC concluded that there is sufficient evidence based on these studies that cadmium and cadmium compounds are carcinogenic to animals.

In a follow-up study of cancer incidence and mortality in Swedish factory workers exposed to cadmium and nickel, Jarup et al. (1998) concluded that there was no exposure relation between cumulative cadmium exposure and risk of lung cancer. The increase in incidence of prostate cancers was not statistically significant and could not be confirmed by some cohort studies. A population-based case-control study was carried out in Montreal, Canada, using 484 pathologically confirmed cases of bladder cancer. The odds ratios estimated by the logistic regression analyses indicated that only a weak association existed between bladder cancer and exposure to cadmium compounds from various occupations (Siemiatycki et al. 1994). The National Toxicology Program (NTP), in its 11th report on carcinogens (NTP 2005), stated that cadmium and

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cadmium compounds are human carcinogens. This conclusion was based on "sufficient evidence of carcinogenicity in humans, including epidemiological and mechanistic information that indicate a causal relationship between exposure to cadmium and cadmium compounds and human cancer" (NTP 2005).

Very recently, Verougstraete et al. (2003) compiled all cancer data published since the IARC evaluation of 1993 and selected from them, under strict criteria for systematic analysis, all studies of cohorts exposed to cadmium and cadmium compounds that assessed the association between cadmium exposure and lung and/or prostate cancer. In total, the authors analyzed 17 plant cohort studies. The major finding was that increased mortality from lung cancer is of only borderline significance. The concentration versus response did not follow an expected trend. Only three studies had updates on prostate cancer. The authors concluded that, considering the results of the most recent updates, for lung cancer, the relative risk is lower than suggested earlier in the absence of nickel and arsenic and the association between cadmium exposure and prostate cancer was not confirmed. Furthermore, there is no increased risk of cancer for populations environmentally exposed to cadmium (Verougstraete et al. 2003).

These conclusions from data collected from occupational exposures of cadmium are not significantly relevant to cancer risk from exposure by oral ingestion. One of the reasons is that absorption of cadmium from the lung is much greater than that from the GI tract, and it is unrealistic to expect such high concentrations of cadmium absorption from spaceflight potable water. Similarly, cancer data from animals receiving cadmium by iv, sc, or intramuscular injection (Waalkes 2003) are not very useful for assessing risk by the oral route, in addition to the fact that the doses are high. No definitive human data exist to indicate that long-duration exposure to low levels of cadmium by the oral route will result in an increased incidence of malignant tumors. A few available epidemiologic studies of cancer rates among humans exposed to cadmium orally, although relevant to our analysis, are limited in their usefulness because the exposures were not estimated.

Shigematsu (1984) conducted a retrospective mortality study for three areas of Japan, categorized on the basis of cadmium content of the polluted rice that was ingested. No significant differences were found in mortality or prostate cancer. No significant increase in cancer rates was found among residents of a cadmium-polluted village in England (Inskip et al. 1982) or in prostate, kidney, or urinary tract cancer among residents of a cadmium-polluted area of Belgium (Lauwerys and De Wals 1981).

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However, in a Canadian study, prostate cancer incidence seemed to parallel increased cadmium concentrations in water, soil, or grain crops in Alberta, Canada. Incidence of prostate cancer per 100,000 individuals for all urban communities was 35.6, 10.6 in the low-cadmium community, and 53.2 in the high-cadmium community (Bako et al. 1982). Nakagawa et al. (1987, 1993) noted that mortality from malignant neoplasms in the inhabitants of cadmium-polluted areas of Japan with increased urinary excretion of retinal-binding protein was no different from that of inhabitants of unpolluted areas. Overall, there is little evidence of an association between oral exposure to cadmium and increased cancer rates in humans.

Several animal studies have been conducted to assess the carcinogenic risk from exposure to cadmium by oral route. Schroeder et al. (1965) exposed mice to cadmium acetate in drinking water (5 mg/L) for their whole lifetimes and did not find an excessive number of tumors of any type in males or females. When $CdSO_4$ was administered to rats via stomach tube once weekly for 2 y (cadmium at 0.09, 0.18, and 0.35 mg/kg/wk), there was no evidence of increased incidence of prostate tumors or any increase in lung or liver tumors compared to controls. Similar results were observed in mice (n = 50) that received weekly gavage doses of cadmium at 0.44, 0.88, or 1.75 mg/kg/wk for 18 mo (Levy et al. 1973, 1975; Levy and Clark 1975).

Kanisawa and Schroeder (1969) reported seven malignant tumors in 47 cadmium-exposed rats (5 mg/L in drinking water) compared with two malignant tumors in 34 controls. The authors concluded that because the number of animals was low, the statistical power for the increased incidence of tumors was poor. Loeser (1980) did not find any change in the incidence of benign or malignant neoplasia in groups of 50 male and 50 female Wistar rats fed $CdCl_2$ in their diet at 1, 3, 10, or 50 ppm for 2 y. A few additional animal studies designed to evaluate noncancer effects of chronic-duration oral cadmium exposure have indicated no dose-related increases in tumors (Fingerle et al. 1982; Watanabe et al. 1986) in rats or mice.

Waalkes and associates evaluated oral carcinogenic potency of cadmium in two separate studies. In one, cadmium was administered via the diet to male Wistar rats, and in the other, cadmium was given via drinking water to Noble rats. In the cadmium-diet study, Waalkes and Rehm (1992) evaluated the effect of chronic dietary zinc deficiency on the carcinogenic potential of dietary cadmium in groups of male Wistar (WF/NCr) rats fed diets adequate (60 ppm) or marginally deficient (7 ppm) in zinc and containing cadmium at various concentrations (0, 25,

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50, 100, or 200 ppm). Lesions were assessed over the next 77 wk. The incidence of prostatic proliferative lesions and atrophy, the incidence of large granular leukocytic leukemia, and the incidence of interstitial cell tumors of the testes were evaluated. Only data from zinc-adequate groups will be mentioned here. In treated groups fed cadmium at 50 ppm, the incidence of prostatic proliferative lesions, both hyperplasias and adenomas, was higher than in controls (1.8%) and treated groups fed cadmium at 50 ppm (20%). However, in 100 and 200 ppm dose groups, the incidence was only 13% and 11.5%, respectively, showing a lack of clear dose response. This is above the concentration used in the rat study by Loeser (1980). Cadmium treatment also resulted in an increased incidence of large granular lymphocytic leukemia (maximum 4.8-fold over control). Only in rats receiving cadmium at 200 ppm were testicular interstitial tumors significantly increased (treated 6/27, controls 1/28). Up to and including a 100 ppm concentration, the incidences were comparable to controls. On the basis of these results for prostate tissue, testicular tumors, and leukemia, Vainio et al. (1994), at the IARC, emphasized that cadmium cannot be considered to be a noncarcinogen by the oral route. In a comment article, Collins et al. (1996) reconsidered all the data from the Waalkes and Rehm study and the comments from Vaino et al. (1994) and came to the conclusion that the cancer potency of low concentrations of ingested cadmium would be low relative to that of inhaled cadmium.

Waalkes (1999) studied the effects of oral doses of cadmium (in drinking water) in Noble (NBL/Cr) rats to characterize proliferative lesions of the prostate and the kidneys. This strain of rats is known to be susceptible to the induction of prostate tumors by chemicals (Noble 1982). Cadmium as $CdCl_2$ was given ad libitum throughout the study in the drinking water at 0, 25, 50, 100, and 200 ppm to groups of male rats, and the rats were observed for up to 102 wk. The authors reported that cadmium did not affect water consumption. Taking body weight into consideration, the doses of cadmium can be calculated as 0, 0.37, 0.75, 1.5, and 3.5 mg/kg/d. The proliferative lesions seen in prostate were intraepithelial with multiple foci (Category A2, as the author calls it) with a prevalence in the dorsolateral lobe. At higher doses, the incidence of prostatic proliferative lesions was reduced to control levels. The loss of prostatic response at the higher doses was likely because of diminished testicular function (testicular atrophy) secondary to cadmium treatment.

In the testes, the total number of proliferative lesions and the severity of interstitial cell hyperplasia was significantly higher than in controls only at cadmium at 200 ppm. Renal tumors occurred only in groups treated with cadmium at 100 and 200 ppm, and only three of these oc-

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curred. The incidence of pheochromocytomas of the adrenal was also increased by cadmium but only at the 50 ppm dose, and there were no significant trends based on cadmium dose. These results indicate that oral exposure to cadmium can induce proliferative lesions in the prostate and kidney of the Noble rat. The rat strain used (Noble) in the study is well known to be a sensitive strain for developing prostate tumors. Hence, use of data from this study will not be suitable for NASA.

Nishiyama et al. (2003) have shown that in C3H/HeN mice (who have a high frequency of spontaneous hepatocarcinogenesis) and A/J mice (who have a high frequency of spontaneous hepatitis), dietary supplementation with cadmium as $CdCl_2$ at 50 ppm (daily dose of approximately 0.1 mg per animal) for 54 wk inhibits spontaneous carcinogenesis in C3H/HeN and spontaneous hepatitis in A/J mice. Control diets contributed only 0.02 µg/d per animal. According to the authors, this may be because of cadmium-induced increases in hepatic MT and hepatic zinc concentration noted in this study. Cadmium-induced nephrosis was not observed in this study.

Reproductive Toxicity

No human epidemiologic studies have documented any reproductive toxicity elicited exclusively by exposure to cadmium salts via oral ingestion. However, several animal studies in rats and mice have addressed the adverse effects of cadmium on the reproductive system, and a vast number of them involves studies where cadmium was administered by sc or ip injection; for example, Laskey et al. (1984) reported that weight of testes, seminal vesicles, and epididymides were reduced by at least 40% in male rats injected at doses of 1.8 or 3.66 mg/kg and observed 14 d later. Such data are difficult to extrapolate to oral ingestion.

Kotsonis and Klaassen (1978) did not find any adverse effect on testicular function, as assessed by the number of pregnancies and number of fetuses per pregnant rat when untreated female rats were mated with male Sprague-Dawley rats that received cadmium at 0, 10, 30, or 100 mg/L via drinking water for 24 wk. In rats exposed to cadmium at 0.001, 0.01, or 0.1 mg/L in drinking water for 30 d, Dixon et al. (1976) did not find any effect on several parameters assessed for adverse reproductive effects such as changes in weight of testes, prostate, and seminal vesicles, or on fertility (fertility indices measured by number of resorptions or number of viable fetuses). Male Long-Evans hooded rats, 100 d old, were exposed to cadmium in distilled water at 0, 17.2, 34.4, or 68.8 mg/L

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for 70-80 d, and male reproductive system parameters (histology and a number of spermatogenesis parameters) and measures of fertility and pregnancy outcome were examined. Electron microscopic observation of the testes of the high-dose group indicated no difference in spermatogenesis, Leydig cell morphology, or testicular vessels between controls and the high-dose group. There were no differences in the weight of cauda or testes or in sperm morphology or sperm count (Zenick et al. 1982). Pregnant rats were given $CdCl_2$ as a gavage at 2, 4, 8, 12, 20, and 40 mg/kg/d from day 7 to 16 of gestation (Baranski et al. 1982). On day 21, tissues were removed and weighed, and the number of corpora lutea was counted. The uterus was opened and the numbers of live and dead fetuses and early and late resorptions were recorded. Live fetuses were examined for crown-rump length and external malformations. The highest dose resulted in significant maternal toxicity (reduction in body weight, abnormal histopathology of liver and adrenals), placental injury, and increased fetal cadmium. A significant reduction of live fetuses and a significant increase of resorptions per litter were seen only in the 40 mg/kg dose group. Fetal development was retarded in groups dosed at 2-20 mg/kg. The skeletal morphology indicated delayed ossification, which was not dose dependent, and the authors concluded that it is more attributed to retarded development. No teratogenic effects were observed (Baranski et al. 1982). In a later experiment, Baranski (1987) exposed pregnant female rats to cadmium at 60 and 180 ppm (9 and 29 mg/kg/d, respectively) in their drinking water from day 1 to 20 of gestation. The average numbers of total implantations, corpora lutea, live and dead fetuses, resorptions, and postimplantation losses were not different from untreated control pregnant rats. External examination of fetuses did not reveal any gross malformations in the rats administered cadmium in drinking water at 60 and 180 ppm. There was, however, fetal growth retardation. There was a reduction of hematocrit in the fetal blood and not in the maternal blood in the 60 ppm group. The changes in fetal hematocrit did not follow a dose-response effect. A significant dose-dependent decrease in water consumption, which could cause hemoconcentration, might be the reason for not seeing a reduction of hematocrit and hemoglobin. No changes in resorptions or live fetuses/litter were seen. Fetal body weight and length were decreased in both groups, although litter size was not affected (Baranski 1987).

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Developmental Effects

The limited data on developmental effects in humans from exposure to cadmium indicate several confounding factors such as lead and nickel, and no data are available from exposures through oral ingestion (ATSDR 1999). There have been several reports in the literature of rodent studies aimed at assessing the adverse developmental effect of cadmium exposure via several routes (inhalation, subcutaneous injections, iv, oral ingestion via gavage, feed, and water), and the results are equivocal: reduced fetal weight, birth weight, skeletal malformations, and behavioral changes in the pups. Malformations or skeletal effects reported include fused lower limbs, absence of one or more limbs, and delayed ossification of the sternum and ribs (Baranski 1985); dysplasia of facial bones and rear limbs, edema, exenteration, cryptorchism, and palatoschisis (Machemer and Lorke 1981); and sharp angulation of the distal third of the tail (Schroeder and Mitchener 1971). Adverse effects of cadmium exposure prior to and during gestation on the neurobehavioral development in offspring have also been reported. $CdCl_2$ was administered by gavage to female rats 5 d/wk for 5 wk and then during mating and gestation periods at doses of 0.04, 0.4, and 4.0 mg/kg/d. The exploratory locomotor activity of 2-mo-old males and females born to rats given 0.4 and 4 mg/kg/d was significantly reduced. The progeny of cadmium-treated females showed decreased performance in the rota-rod test. In general, the degree of behavioral impairment was dose-related (Baranski et al. 1983).

Cadmium and Effects on Bone

Numerous epidemiologic and experimental studies have been conducted on the effects of cadmium exposure on bone, particularly reduction in bone mineral density. Cadmium can cause osteoporosis (low bone mass and increase in bone fragility) and also osteomalacia (generalized bone pain, tenderness, decreased mineralization). Epidemiologic results from China, Europe, and Japan have been very consistent. For example, the high prevalence of bone damage in cadmium-exposed patients with *itai-itai* disease and the high cadmium concentrations in their skeletons are well recognized (Blainey et al. 1980; Friberg et al. 1985; Nogawa et al. 1987; Noda and Kitagawa 1990; Kido et al. 1991a, b; Tsuritani et al. 1996; Staessen et al. 1999; Alfven et al. 2000; Nordberg et al. 2002). In a

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Chinese epidemiology study, Nordberg et al. (2002) reported finding decreased bone mineral density in postmenopausal women with heightened U-Cd and blood cadmium (B-Cd) concentrations and in men with heightened cadmium concentrations resulting from environmental cadmium pollution.

Several experimental rodent studies on this effect of cadmium are available in the literature, some that used confounding conditions such as ovariectomy or some nutritional deficiency such as zinc and calcium. The reduction in bone mineral density has been attributed to the effect of cadmium on bone resorption in female mice (Bhattacharyya et al. 1988a, b). When bone G1 protein was used as an indicator of bone damage, its concentrations correlated positively with U-Cd, B-Cd and β-2m, and microdensitometry indicators for bone damage (Kido et al. 1991a, b). Rats administered cadmium for 60 wk by oral gavage had less bone mineral density in the femur and greater excretion of bone-specific markers in the urine than before they were exposed to cadmium (Ohta et al. 2000).

Although the effect of cadmium on bone may be direct (Ogoshi et al. 1989) or secondary to renal damage, one of the mechanisms suggested is the disturbance of vitamin D metabolism in the kidney by cadmium. Accumulated cadmium in the kidney reduces the generation of active vitamin D (1, 25,(OH)2D), the lack of which delays calcium uptake by the GI track and calcium reabsorption from the kidney proximal tubules. Under normal circumstances, 98% of the calcium is reabsorbed at the kidney proximal tubules, and tubular damage by cadmium leads to calciuria. Nogawa et al. (1987) found decreased serum 1-α-25-dihydroxy vitamin D concentrations in the serum of inhabitants environmentally exposed to cadmium; this indicated that vitamin D metabolism may be a key factor (also see Nogawa et al. 1990; Aoshima and Kasuya 1991; Chalkley et al. 1998). It has also been stated that serum ALP activity and the urinary excretion of calcium had a significantly positive correlation with U-Cd in both men and women (Staessen and Lauwerys 1993).

Epidemiologic studies have evaluated the possibility that renal dysfunction and osteoporosis are associated (Nogawa et al. 1987; Jin et al. 2004). As stated above, cadmium accumulated in the proximal renal tubules inhibits the generation of active vitamin D in renal tubular cells, which in turn inhibits the active reabsorption of calcium in the distal convoluted tubule (Nogawa et al. 1987; Kjellström 1992; Tsuritani et al. 1992; Tsuritani et al. 1996). Thus, the excretion of calcium would increase, which can lead to resorption of bone calcium to maintain circulating calcium. For example, on the basis of observations from 5 patients with *itai-itai* disease, 36 cadmium-exposed residents with renal tubular

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damage, and 17 nonexposed individuals, Nogawa et al. (1987) showed that cadmium-induced bone effects were mainly attributable to a disturbance in vitamin D and parathyroid hormone metabolism that was caused by the cadmium-induced kidney damage. This cadmium-bone-mineral interaction and kidney damage has been reviewed by Berglund et al. (2000).

Jin et al. (2004) examined the relationship between cadmium nephropathy and its effect on bone in a population aged >35 y living in a cadmium-polluted area near a smelter in China. They concluded that the prevalence of renal dysfunction was significantly higher in persons who had osteoporosis than in those who did not. Furthermore, they found a significant correlation between the severity of tubular damage and osteoporosis: those without tubular damage did not have osteoporosis, whereas those with tubular damage did. However, glomerular dysfunction played a smaller role: osteoporosis was not related to the severity of glomerular damage (Jin et al. 2004).

In a cross-sectional study in Belgium of people exposed to cadmium (n = 1,700), cadmium dose was significantly associated with tubular renal dysfunction and urinary excretion of calcium (Staessen et al. 1999). The authors also suggested that cadmium exposures can promote skeletal demineralization, which can lead to bone fractures. Alfven et al. (2000) evaluated whether long-term low-level cadmium exposure, through environment or occupation, increased the risk of osteoporosis (decreased forearm bone mineral density) in a total of 1,753 subjects (520 men and 544 women) aged 16-80 y who resided in a community in southern Sweden where nickel-cadmium batteries and heat exchangers for cars were manufactured. The authors concluded that a dose-response relationship existed between cadmium dose (U-Cd concentrations used as dose estimates and protein-HC used as an index of tubular damage) and osteoporosis. Individuals, both men and women, with higher tubular proteinuria had a lower bone mineral density. The environment was substantially polluted with both lead and cadmium from both nickel-cadmium-battery plants and also lead-battery plants. Similar results were reported in another recent study by Hayashi et al. (2003) that evaluated urinary excretion rates of calcium and phosphorus among the inhabitants (n = 3,164) of the cadmium-polluted KaKehashi River basin of Japan. The rate of excretion of these minerals by inhabitants of the polluted area was significantly greater than that of people in a control area.

Wang et al. (2003) studied the effect of cadmium exposure on bone mineral density using a study population of 302 males and 488 females over age 35 who resided near a cadmium smelter in China. The average

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cadmium concentration in rice produced in the residents' own fields was 3.7 ± 1.8 mg/kg, which is 18-fold higher than the hygienic standard. They reported that for subjects of both sexes over age 60, the decline in bone mineral density in subjects from a heavily cadmium-polluted area was greater than that in subjects from control areas. A dose-effect relationship between cadmium dose and decline in bone mineral density was also evident.

Very recently, Alfven et al. (2004) reported results of analyzing the relationship between low-level cadmium exposure and forearm fractures in 479 men and 542 women who were either occupationally or environmentally exposed to cadmium. Fracture hazard ratio increased as a function of cadmium exposure. The exposure was estimated from U-Cd concentrations. The authors estimated the fracture hazard ratio increased by 18% per unit of U-Cd (nanomoles [nmole]/mmole creatinine) (Alfven et al. 2004).

In a rat study, Brzoska et al. (2001) reported that 12 wk of exposure to cadmium at 50 mg/L in drinking water resulted in a decrease in ash weight, concentration and amount of calcium, and the percentage of nonorganic-components content of tibial bone. Also very recently, Brzoska et al. (2004) used a rat model to study the mineral status, mechanical properties, and incidence of deformities and fractures of the lumbar spine (L1-L5 vertebrates) in young female Wistar rats exposed to cadmium at 1, 5, 50, or 100 mg/L in drinking water for 12 mo and determined the above-mentioned parameters at 3, 6, 9, and 12 mo. It was reported that a dose- and time-dependent decrease was observed in bone mineral content (BMC) and density as well as ash weight. At 50 and 100 mg/L dose concentrations, deformities and fractures of the lumbar vertebral body were severely affected.

Cadmium Exposure and Renal Stones

Increased prevalence of kidney stones in workers occupationally exposed to cadmium has been reported in several studies (see Friberg et al. 1986). Jarup and Elinder (1993) conducted a study on a group of 902 male workers who were employed for at least 1 y in a Swedish cadmium battery factory between 1931 and 1982. They sent a questionnaire about the occurrence of kidney stones to 601 living workers and 267 relatives of deceased workers. Seventy-three workers reported that renal calculi appeared after their employment. Cumulative exposure for each employee was computed from the cadmium concentrations monitored in the

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air and their years of employment. Smoking habits were also taken into account. The data from living workers were also correlated with U-Cd and urinary β-2m data that were collected. The rate of incidence of kidney stones was computed for three cumulative exposure categories: less than 250, 250-5,000, and greater than 5,000 µg per cubic centimenter (cm^3); cumulative exposure to 250 $\mu g/m^3$ was considered an internal control. All biologic data indicated that the higher the internal cadmium dose, the greater the degree of tubular damage; and the higher the degree of tubular damage, the higher the rate of incidence of kidney stones. For example, 13 of 33 workers (about 39%) who had a β-2m concentration greater than 34 µg/mmole of creatinine had kidney stones (Jarup and Elinder 1993).

Rationale for Spaceflight Safety Factor for Bone Effects

Microgravity induces adverse changes in the musculoskeletal system. Because of skeletal unloading, bone tissue is lost during space missions, and this has been the subject of numerous in-flight and ground-based microgravity (weightlessness) simulation studies (see LeBlanc 1998; Smith et al. 1999; Smith and Heer 2002). Urinary excretion rates of several biochemical markers, especially some bone-specific ones such as deoxypyridinoline, provide evidence for bone loss (Smith et al. 1998). For example, crewmembers (cosmonauts) who spent 4-12 mo on the Russian Mir space station lost areal bone mineral density at an average monthly rate of 0.3% from the total skeleton (97% of which was from the pelvis and legs) (LeBlanc et al. 2000; also see review by Smith and Heer 2002). Regional bone mineral density measurements made using dual-energy x-ray absorptiometry showed a loss of 1.06% per mo from the spine and 1.15-1.56% per mo from the hip (LeBlanc et al. 2000). Very recently, Lang et al. (2004) used volumetric quantitative computed tomography (vQCT) and dual-energy x-ray absorptiometry to measure cortical and trabecular bone loss in 14 astronauts (13 men and 1 woman) who spent 4-6 mo on the ISS. Bone mineral density -loss rates of 0.9% per mo at the spine and 1.4-1.5% at the hip were noted. The authors also measured losses from individual areas of the hip (Lang et al. 2004).

As described earlier, cadmium exposure has been reported in several investigations to affect bone mineral density. To protect against cadmium potentiating the spaceflight-induced bone changes, an estimated spaceflight safety factor of 3 is needed in cases where an AC is derived using bone effects as adverse end points.

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Spaceflight and Renal-Stone Risk

Negative calcium balance through increased excretion in feces and urine was observed during Skylab (28, 59, and 84 d) and Mir missions. Increased calcium in urine because of bone shedding has been proposed. Factors that increase the risk of renal stone formation are increased urinary calcium concentration; supersaturation of calcium oxalate (with subsequent formation of calcium oxalate crystals in renal epithelial cells) and brushite (calcium phosphate) uric acid saturation and decreased urine volume, magnesium, and citrate (Whitson et al. 2001b). It was suggested that urinary calcium may be a parameter critical for the increased risk of renal stone formation during and after spaceflight (Whitson et al. 1993; 1997; 1999; 2001a,b), because the majority of renal stones formed are composed of calcium phosphate and calcium oxalate. Hence, a safety factor of 3 should be applied to adverse end points that relate to bone damage by cadmium.

Why Spaceflight Safety Factor for Nephrotoxicity Is Not Needed

In several areas in this document, results have been described from human epidemiologic studies that showed that a relationship seems to exist between tubular damage and osteoporosis or bone damage; the most well-known example is the subjects suffering from *itai-itai* disease in Japan. One of the mechanisms proposed was that the accumulation of cadmium in the kidney directly inhibits the activation of vitamin D; this may lead to bone resorption, as described above. Hence, one would be tempted to apply a spaceflight safety factor for nephrotoxicity because it will lead to bone resorption, which is a well-known problem for astronauts. However, many of the human epidemiology studies involve long-term exposures to high concentrations of environmental cadmium. Although links were established, quantitative estimates could not be gathered. Ohta et al. (2000) conducted a study designed to simultaneously measure bone resorption parameters and nephrotoxic parameters to clearly show dose and duration of exposure and the relationship between renal dysfunction and bone disorder in male rats after long-term oral administration of cadmium by gavage. The study is described in detail elsewhere in this document. The important conclusion from the study is that a decrease in bone mineral density can be observed both before and after the manifestation of renal dysfunction or damage, but the amount of decrease depends on the dose and duration of exposure to cadmium. In

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this study, as indicated by bone mineral density and the urinary excretion of pyridinoline and deoxypyridinoline, the bone damage occurred only at doses of 20, 30, and 60 mg/kg, as early as 3 wk after exposure. However, when kidney tubular damage was seen as early as 10 wk in the 5 mg/kg dose group, no change in bone mineral density or in the excretion of bone markers in the urine was seen, even at 60 wk. It is unrealistic to expect high cadmium concentrations in ISS water, and, at the cadmium concentrations that will be established as ACs for 1,000 d (or more), changes in the kidney, if any, will not be a driver for bone resorption. Hence, no additional safety factor would need to be applied to an AC determined using nephrotoxicity as the adverse end point.

Gender Differences and Cadmium Metabolism

The cadmium toxicology literature includes numerous studies containing data that indicate gender differences in cadmium toxicity. It is far beyond the scope of this document to discuss these individually. Only a summary of such observations can be included. In the cross-sectional study in Belgium, for the same degree of environmental exposure, the cadmium body burden was higher in women than men. Buchet et al. (1990) reported that women had higher blood and urinary concentrations of cadmium. Higher fractional absorption of cadmium in adults who have low iron stores have been reported (Flanagan et al. 1978; Shaikh and Smith 1980). Women have been found to have a higher incidence of low body stores of iron (nonoccupational, nonsmoker exposure subset of NHANES III data plotted by Choudhury et al. [2001]). A Thai study by Satarug et al. (2004) reported that the prevalence of low iron stores (serum ferritin < 20 μg/L) were 16% in nonsmoking women (n = 99) and only 2% in men; those women with low iron stores showed a 3.4-fold greater cadmium burden than women with normal iron stores (Satarug et al. 2004). A Swedish farm study with 48 women and 57 men indicated that when men and women who had never smoked were compared, women had 1.8-fold higher B-Cd and 1.4 times higher U-Cd, even though on a per-body-weight basis, women consumed less cadmium (Olsson et al. 2002). Various rodent studies have shown a higher body burden in females than in males with similar concentrations of cadmium exposure (Buhler et al. 1981; Bhattacharyya et al. 1982; Foulkes 1986). For example, when rats were provided water or food containing cadmium as $^{109}CdCl_2$ in the range of 1-1,000 ng/g and tissue cadmium concentrations determined at 1, 2, 4, 8, and 12 wk, it was found that female

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rats accumulated cadmium at a higher rate and retained a greater percentage of the ingested CD than male rats (Buhler et al. 1981). The cadmium Dietary Exposure Model (CDEM) predicts that to obtain a kidney burden of cadmium at 10 mg, the drinking water concentrations of cadmium for males should be 50 µg/L and for females 20 µg /L. This indicates that women have a higher rate of systemic uptake (Choudhury et al. 2001). It is well known that almost all *itai-itai* disease patients in the cadmium-polluted areas of Japan are women. Sex-related differences in vitamin D metabolism because of environmental cadmium exposure have been proposed as a reason for the vulnerability of women to bone damage (Tsuritani et al. 1992). In the Tsuritani et al. study, the authors reported decreased serum 1, 25-dihydroxy vitamin D, and an increase in parathyroid hormone that was more pronounced in women than in men who had similar degrees of renal dysfunction. This difference may be related to differences in calcium metabolism. It is well known that a significant dose-response relationship exists between the prevalence of hypercalciuria and the excretion of urinary cadmium, and a significantly increased prevalence of calciuria was found when excretion of urinary cadmium exceeded 2 µg/g creatinine (Wu et al. 2001).

A list of studies on the toxicity of cadmium from oral ingestion described here is summarized in Table 5-3.

RATIONALE

The following paragraphs provide a rationale for proposing guideline values for cadmium in drinking water for 1 d, 10 d, 100 d, and 1,000 d for NASA's spacecraft water (see Table 5-4). The values listed were based on ACs for each duration according to *Methods for Developing Spacecraft Water Exposure Guidelines* (NRC 2000).

A review of studies of cadmium exposure via ingestion indicates that there are several toxicity end points such as GI effects/GI mucosal injury, hepatic necrosis, nephrotoxicity, skeletal-muscular system toxicity (osteotoxicity), reproductive toxicity, neurotoxicity, immunotoxicity, and hematotoxicity. Some of the effects can be considered local effects that develop rapidly, and/or systemic effects that are documented after absorption and accumulation in target tissues.

Nordberg et al. (1973) and Buchet et al. (1980) were selected for the derivation of the 1-day and 1000-day ACs, respectively, because both studies are based on human data and derive the lowest concentrations at which adverse effects are observed.

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TABLE 5-3 Toxicity Summary

Chemical Form and Dose	Expected Duration	Species	Adverse Effects	Reference
$CdCl_2$; cadmium at 5 mg/L	Consumed a contaminated beverage (acute)	Human child	Nausea, vomiting, abdominal distress, and diarrhea; LOAEL = 0.43 mg/L	Nordberg et al. 1973; CEC 1978; Elinder 1986
Cadmium iodide; cadmium at 25 mg/kg/d	Oral ingestion, single dose (acute)	Human	Fatal dose; died in 7 d; necropsy revealed damage to the heart, liver, kidney, and GI tract	Wisniewska-Knypt et al. 1971
$CdCl_2$; cadmium at 1.9 g/kg	Oral ingestion, single dose (acute)	Human	Death in 30 h; hemorrhagic necrosis of the stomach and GI tract; pulmonary edema; focal hepatic necrosis; pancreatic hemorrhage; normal kidney	Buckler et al. 1986
$CdCl_2$; cadmium at 29 mg/kg/d	Gavage, single bolus	Rat	50% died in 8 d	Kostial et al. 1978
$CdCl_2$; 25, 51, 107, and 225 mg/kg	Gavage, single bolus	Sprague-Dawley rats, male and female	Males: 3/10 died at 107, 225 mg/kg; no abnormal gross pathology; no changes in hematology, serum chemistry, or urine analysis; serum ALP decreased in both sexes; absolute and relative lung weights increased	Borzelleca et al. 1989
$CdCl_2$; cadmium at 75 mg/kg/d	Gavage, single bolus	Sprague-Dawley rat	Necrosis of liver cells and focal degeneration	Shimizu and Morita 1990
$CdCl_2$; cadmium at 15.7, 30.4, 59.6, and 88.8 mg/kg/d	Gavage, single bolus	MBA mouse	Gastritis and enteritis; necrosis of GI tract; fatty infiltration of liver cells; hepatic necrosis; tubular necrosis; hyaline casts	Andersen et al. 1988

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TABLE 5-3 Continued

Chemical Form and Dose	Expected Duration	Species	Adverse Effects	Reference
$CdCl_2$; 0, 25, 50, 100, and 150 mg/kg/d	Gavage (once); data collected at 2 and 14 d	Sprague-Dawley rats, male	Lower hexobarbital oxidase activity; decreased motor activity; testicular necrosis; decreased spermatogenesis; decreased urine flow and increased protein excretion at 3 d, followed by a recovery; focal tubular necrosis	Kotsonis and Klaassen 1977
$CdCl_2$; 50 mg/kg once or 5 mg/kg once/wk for 10 wk	Gavage; evaluated at 12, 18, and 30 mo	Wistar rat, male	Food and water consumption and general appearance and behavior were comparable to controls; no testicular lesions	Bomhard et al. 1987
$CdCl_2$; single dose of cadmium at 100 or 200 mg/kg	Gavage; terminated at 6 mo	Wistar rats, male	Several deaths within 3 d; severe testicular necrosis and fibrosis; hemorrhages in testes and epididymides	Bomhard et al. 1987
$CdCl_2$; cadmium at 112 mg/kg/d	Gavage (once)	ICR mouse	Epithelial necrosis of the stomach; hepatocellular coagulate necrosis	Basinger et al. 1988
$CdCl_2$; 25, 51,107 and 225 mg/kg/d (or cadmium at 12.25, 25, 52, and 110 mg/kg/d)	Gavage; 1/d for 10 d	Sprague-Dawley rats, male and female	All died at high dose; 30-40% death in next two lower doses; mild hepatic focal necrosis at the high dose; abnormal kidney histopathology in both male and female; varied as a function of dose	Borzelleca et al. 1989

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$CdCl_2$; calculated mean doses of cadmium at 1.1, 7.8, and 11.1 mg/kg/d	Drinking water, 10 d	Sprague-Dawley rats, male and female	No deaths; in male and female high-dose rats, increase in BUN, decreases in serum ALP and serum protein; no dose dependent changes in hematology; increased protein in urine at middose; decreased organ weights and ratios in males and not in females; reduced water consumption	Borzelleca et al. 1989
$CdCl_2$ at 1.8; cadmium at 6.13,18.4, and 61.3 mg/kg/d (0, 3, 10, or 100 ppm)	Feed, 10 d	Rat, pregnant	100 ppm in the feed did not affect embryogenesis	Machemer and Lorke 1981
$CdCl_2$; cadmium at 1.8, 6.13, 18.4, or 61.3 mg/kg/d (0, 3, 10, and 100 ppm)	Gavage, 1/d for 10 d	Rat, pregnant	25% decrease in body weight; intestinal necrosis, hemorrhage, and ulcers; decreased fertility; maternal toxicity; teratogenic effect (by gavages)	Machemer and Lorke 1981
$CdCl_2$; cadmium at 12 mg/kg/d	Drinking water, 12 d	Wistar rat	Anemia	Sakata et al. 1988
$CdCl_2$; cadmium at 1.1, 5, 20, and 40 ppm/kg	Feed, 12, 18, and 22 mo	Rat, female	No renal or hepatic lesions; incidence and severity of spontaneous nephropathy was not different from controls	Shibutani et al. 2000

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TABLE 5-3 Continued

Chemical Form and Dose	Expected Duration	Species	Adverse Effects	Reference
$CdCl_2$; 0, 8, 40, 200, and 600 ppm/kg/d	Feed; data collected at 2, 4, 8 mo	Sprague-Dawley rat, female	Hepatotoxicity (liver-cell necrosis) at 2 mo in ≥200 ppm group; renal toxicity at 4 mo and anemia and decreased hematopoiesis at 8 mo; reduction of cancellous bone in the femur; renal tubular degeneration in ≥200 ppm group from 2 mo; no renal lesions at 8 mo in 40 ppm group	Mitsumori et al. 1988
$CdCl_2$; cadmium at 30 mg/kg/d	Drinking water, 2-10 mo	Sprague-Dawley rat	Urinary excretion of β-2m	Bernard et al. 1988
$CdCl_2$; cadmium at 0.04, 0.4, and 4.0 mg/kg/d	Gavage, 5 d/wk for 5 wk	Rat, female	In offspring, decreased locomotor activity and behavioral impairment	Baranski et al. 1983
$CdCl_2$; cadmium at 0.1, 1.0, and 10.0 mg/kg	Gavage, 6 wk and 3 wk during mating for a total of 9 wk	Sprague-Dawley rats, male and female	Number of total implantations and live fetuses decreased significantly at 10 mg/kg group as well as the number of pregnant females; increased number of resorbed fetuses and growth retardation of fetuses; dominant lethal tests by crossing cadmium-treated males and untreated females suggested cadmium did not affect male sterility, the females were affected most	Sutou et al. 1980b

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Cadmium sulfate; cadmium at 20 mg/kg	Gavage, gestation day 6 to 14 (during embryogenesis)	Rats, pregnant	Reproductive performance and teratogenic effects studied: no reproductive effects (change in the number of implantation sites, resorption, live fetuses/litter, fetal weight, number of corpora lutea); changes in teratogenic parameters: induced external malformations, skeletal anomalies, and visceral anomalies	Salvatori et al. 2004
$CdCl_2$; cadmium at 25, 50, and 100 ppm (or 4, 8 and 16 mg/kg/d	Drinking water, 90 d	SPF Wistar rats, female	No change in hematocrit or hemoglobin; decreased serum iron and serum ALP at 25 ppm and higher; 30% increase in protein at 50 ppm and higher	Prigge 1978
Cd^{+2} (salt not specified); 20, 40, and 80 ppm (cadmium at 2.9, 5.8, and 11.6 mg/kg/d)	Drinking water, 14 wk	Rat, young	Increased kidney and testes weights; increased femur weight with increased water content and decreased minerals; symptomatic osteoporosis; hematotoxicity including depressed erythrocytes and hematocrit	Pleasants et al. 1992, 1993
$CdCl_2$; 250-500 mg/kg (cadmium as $CdCl_2$ at 0, 0.0031, 0.0062, 0.0125, 0.025, and 0.05%)	Feed, 100 d	Rat, male albino	Growth retardation; even 0.0031% of cadmium produced severe anemia as early as 2 mo; morphologic changes such as focal necrosis were seen; kidneys of two highest doses showed swelling and granulation of epithelium of convoluted tubules; marked atrophy and inflammation of the pancreas were noted	Wilson 1941
$CdCl_2$; cadmium at 12 mg/kg/d	Drinking water, 12, 26, 50, or 100 d	Wistar rat, male	Iron-deficient anemia	Sakata et al. 1988

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TABLE 5-3 Continued

Chemical Form and Dose	Expected Duration	Species	Adverse Effects	Reference
$CdCl_2$; cadmium at 50 ppm (3.6 mg/kg/d)	Drinking water, 120 d	Wistar rat	Reductions in hematocrit and hemoglobin; marked degeneration and necrosis of the glomeruli and hypertrophy of the kidneys; thinning of bone cortex; increased serum urea and ALP	Itokawa et al. 1974
$CdCl_2$; 10, 30, and 100 ppm (0, 1.15, 2.92, and 8.51 mg/kg/d)	Drinking water, 24 wk; data collected throughout the duration	Sprague-Dawley rats, male	Decreased water consumption in 30 and 100 ppm group at week 1; decreased motor activity at 3 wk; increased protein excretion from week 6 in 30 and 100 ppm group; focal tubular necrosis by week 24	Kotsonis and Klaassen 1978
$CdCl_2$; cadmium at 2.5 mg/kg	Drinking water, 6 mo	CD mouse	Reproductive failure	Schroeder and Mitchener 1971
$CdCl_2$; 0, 8, 40, 200, and 600 ppm	Feed, 2, 4, and 8 mo; 600 ppm group killed at 4 mo	Sprague-Dawley rat, female	In the 600 ppm group, at 4 mo, anemia and decreased hematopoiesis in the bone marrow and reduction of cancellous bone in their femurs seen in addition to periportal liver-cell necrosis; in 200 ppm and high-dosed grouped, renal toxicity (degeneration of proximal tubular epithelia and hepatotoxicity such as vacuolar degeneration with apoptotic cells) were seen as easily as 2 mo	Mitsumori et al. 1998
$CdCl_2$; 57 mg/kg/d	Drinking water, 12 mo	Mice	Hematotoxic; decreased erythroid cells in bone marrow	Hays and Margaretten 1985
$CdCl_2$; 13 mg/kg	Drinking water, 18 mo	Rats	Proteinuria	Bernard et al. 1992

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$CdCl_2$; 3.5 and 17.5 mg/kg	Feed, 72 wk	Rats	Increase in LDH and GST starting at 13 wk (hepatotoxic)	Bomhard et al. 1984
$CdCl_2$; cadmium at 0.1, 0.5, 2.5, 5, 10, and 50 ppm	Drinking water, for up to 1 y; 50 ppm only for 90 d.	Sprague-Dawley rats	High-dose rats developed anemia in 2 wk; continued to 3 mo; 50% reduction in water consumption in 2 wk; 10 ppm a NOAEL for various effects for 90 d	Decker et al. 1958
$CdCl_2$; cadmium at 0, 25, 50,100, and 200 ppm	Feed, 77 wk	Wistar WF/NCr rats	At 50 ppm, prostatic proliferative lesions, both hyperplasia and adenomas, were increased without a clear dose response; cadmium also increased leukemia and testicular tumors in 50 and 100 ppm but not in 200 ppm group; benign interstitial tumors of the testes increased at 200 ppm group	Waalkes and Rehm 1992
$CdCl_2$; 4 mg/kg/d	Feed, 90 wk	Monkey	Clinical signs of anemia; pale feces	Masaoka et al. 1994
$CdCl_2$; 0.12, 0.4, and 4.0 mg/kg	Feed, 9 y	Rhesus monkey	Decreased food consumption, body weight, and growth rate; anemia, proteinuria, and glucosuria,	Masaoka et al. 1994

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TABLE 5-3 Continued

Chemical Form and Dose	Expected Duration	Species	Adverse Effects	Reference
$CdCl_2$; cadmium at 10 mg/L	Drinking water, three-generation study	Mice	Toxic effects on the first generation (infantile death and runts); F2 generation same as F1 and also congenital abnormalities; F3: 3/5 pairs failed to breed (reproductive toxicity)	Schroeder and Mitchener 1971
$CdCl_2$; cadmium at 0.3, 3, 30, and 90 mg/kg	Feed, 10 mo	Wistar rats, male	At 4 mo, only LDH excretion was found to be increased in the 90 mg group; however, at 8 and 10 mo, not only was there an increase in LDH, other renal enzymes such as NAG and ALP were also found to be increased, which indicates the nephrotoxic effects of cadmium progressed (or became more pronounced) as a function of time; at 10 mo, both 30 and 90 mg/kg dose groups indicated abnormal histopathology; however, there were no increase in urinary enzyme excretions even at 10 mo for doses up to and including 30 mg/kg	Groten et al. 1994
$CdCl_2$; cadmium at 0, 25, 50, 100, and 200 ppm	Drinking water, 102 wk	Noble (NBL/Cr) rat, male	Prostatic lesions; testicular lesions, hyperplasia, pheochromocytomas of the adrenal; renal tumors; no significant trend in some	Waalkes et al. 1999
$CdCl_2$; cadmium at 2, 4, 8, 12, 20, and 40 mg/kg/d	Gavages, gestation day 7-16	Rats, pregnant	Reduction of live fetuses in the 40 mg/kg dose group; increased number of resorptions per litter; fetal development was retarded in groups dosed at 2-20 mg/kg; no teratogenic effects	Baranski et al. 1982

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$CdCl_2$; cadmium at 60 and 180 ppm	Drinking water, gestation day 1-20	Rats, pregnant	No change in total implantations, corpora lutea, live and dead fetuses, resorptions and postimplantation losses; no gross malformations; there was fetal growth retardation and decreased fetal body weight and length, but litter size was not affected	Baranski 1987
$CdCl_2$; 200 ppm	Drinking water, 11 mo	Sprague-Dawley rats, female	At 8 mo, proteinuria was seen; kidney cortex level off at a value of 250 µg/g wet weight; increased urinary excretion of gamma-globulins; aminoaciduria; slight tubular dysfunction	Bernard et al. 1981
Cadmium-polluted rice (cadmium at 0. 02, 0.04, 0.12, and 1.01 ppm) or $CdCl_2$ (cadmium at 5.08, 19.8, and 40.0 ppm)	Feed, 1, 4, and 8 mo	Sprague-Dawley rats, female (n = 14)	No cadmium-related toxic changes; cadmium in the liver and kidneys at any time point at 4 and 8 mo increased dose dependently	Hiratsuka et al. 1999
$CdCl_2$; 0, 10, 50, and 250 ppm	Feed, 72 wk (18 mo)	Wistar rats, male and female	No kidney-related adverse effects up to and including 50 ppm groups; increased urinary excretion of cytosolic phosphohexose isomerase, LDH, and GST in 250 ppm group; histopathology at 72 wk revealed chronic and acute degenerative changes in the kidney	Bomhard et al. 1999

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TABLE 5-3 Toxicity Summary

Chemical Form and Dose	Expected Duration	Species	Adverse Effects	Reference
$CdCl_2$; cadmium at 0, 0.1, 1.0, and 10.0 mg/kg.	Gavages, 9 wk	Sprague-Dawley rats, male and female	Decreased food and water consumption; 10 mg/kg was a NOAEL for hematologic effects; a 10% increases in serum creatinine in 1 and 10 mg/kg male rats; no hepatotoxicity even at the high dose in male rats (NOAEL for hepatotoxicity = 10 mg/kg/d)	Sutou et al. 1980a
$CdCl_2$; 5 and 10 ppm; also used 10, 20, 40, 80, and 160 ppm for adults and older rats	Drinking water, 4 wk	Rats, young, adult, and old	Mechanical strength of bone decreased only in young rats and not in older rats	Ogoshi et al. 1989
$CdCl_2$; cadmium at 2, 5, 10, 20, 30, and 60 mg/kg	Gavage, 6 d/wk for 60 wk	Wistar rats, female	Renal dysfunction and osteotoxicity; proximal tubular degeneration; also decreased bone mineral density even at 2 mg/kg; in 30 and 60 mg dose groups, effects seen as early as 5 wk—the effects were time and dose dependent; renal and bone effects occurred at different times	Ohta et al. 2000
$CdCl_2$; 16 ppm (1.18 mg/kg/d as Cd)	Drinking water, 4, 16, 40, and 60 wk	Wistar rats	Pattern of nephropathy studied; a widespread vesiculation of proximal tubular cells with mitochondrial and lysosomal alterations; increased α-glucosidase indicated severe anatomical tubular damage; no damage to brush border even after 64 wk. Tubular damage seen as early as 16 wk.	Gatta et al. 1989

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$CdCl_2$; cadmium at 0, 3, 10, 30, and 100 ppm	Feed	Rhesus monkeys	The 100 ppm group had glucose in the urine after 48 wk, elevated urinary proteins at 98 wk, and markedly increased urine volume at week 102; no abnormalities in renal functions were noted in the 3 or 10 ppm groups; no aggravated renal dysfunction or renal failure during the 9-y study	Masaoka et al. 1994
$CdCl_2$; 0.5 or 100 ppm	Feed; Cadmium supplemented in zinc deficient (0 ppm) and adequate in zinc (30 ppm); up to 5 mo	Sprague-Dawley rats, (3-wk-old) males	In the proximal convoluted tubules of the kidneys, degenerative changes, mitochondrial swelling, and coagulative necrosis and cytoplasmic vacuolation seen in rats fed the zinc-deficient diet containing cadmium at 100 ppm; also, diminished bone growth and cortical thinning of the femur without osteomalacia seen in this group	Tanaka et al. 1995

Abbreviations: ALP, alkaline phosphate; β-2m, β-2-microglobulin; BUN, blood urea nitrogen; GI, gastrointestinal; GST, glutathione-s-transferase; LDH, lactate dehydrogenase; LOAEL, lowest-observed-adverse-effect level; NAG, β-*N*-acetylglucosaminidase; NOAEL, no-observed-adverse-effect level.

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TABLE 5-4 Spacecraft Water Exposure Guidelines for Soluble Cadmium (Salts)

Duration	SWEG (mg/L)	Toxicity End Point	Reference
1 d	1.6	Emetic effect	Nordberg et al. 1973
10 d	0.7	Effect on water intake	Kotsonis and Klaassen 1978
100 d	0.6	Osteotoxicity	Ogoshi et al. 1989, 1992
1,000 d[a]	0.022	Nephrotoxicity	Buchet et al. 1990

[a]This level will provide protection from continuous exposure to cadmium beyond 1,000 d.

In general, we have applied a spaceflight factor of 3 for certain effects that are known to be exacerbated by microgravity such as adverse changes of hematocrit or hemoglobin, effects on bone indicative of bone resorption, and dehydration or reduction in water consumption. Low fluid consumption will lead to low urine volume, which will promote renal stone formation (Whitson et al. 2001b). Usually, an intraspecies uncertainly factor (UF) is not used because astronauts come from a homogenous, healthy population and there is no evidence of a group of healthy persons having excess susceptibility to cadmium. Our search of the literature indicates there are no known hypersusceptibility factors related to cadmium except anemia or diabetes, which have no relevance to the astronaut population.

Approaches by Other Organizations

EPA (1985) determined an oral reference dose (RfD) for inorganic cadmium based on renal toxicity. They determined that the highest renal cortical concentration of cadmium that is not associated with significant proteinuria is 200 µg/g wet human renal cortex, a critical concentration for renal dysfunction. Using a toxicokinetic model, Friberg et al. (1974) estimated that a daily intake of cadmium at 0.352 mg/d (or 0.005 mg/kg) for 50 y would result in a renal cortex concentration of 200 µg/g wet tissue. This model assumed absorption of 4.5% of the daily oral dose and an excretion rate of 0.01% of the cadmium body burden. Thus, based on an estimated NOAEL of cadmium at 0.005 mg/kg/d in drinking water, and after applying a UF of 10, an RfD of 0.0005 mg/kg/d (water) was calculated; an equivalent RfD for cadmium from food is 0.001 mg/kg/d,

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assuming that the absorption from food is only half of that from water (EPA 1985; IRIS 1994).

Although no minimal risk levels (MRLs) for acute and intermediate durations of exposure to cadmium were derived by ATSDR, the chronic MRL derived was based on the report by Nogawa et al. (1989) of a dose response for renal effects of cadmium exposure of residents to cadmium-polluted rice in Japan. Urinary β-2m was used as the index of renal damage, and an abnormal β-2m was defined as a concentration greater than 1,000 µg/L, or 1,000 µg/g of creatinine, in the morning urine. From the total lifetime intake of 2,000 mg from dietary sources, or 110 µg/g/d, and using a body weight of 53 kg for Japanese adults, the acceptable intake was calculated to be 0.0021 mg/kg/d (110 µg/g/d ÷ 53 kg = 2.1 µg/kg). After applying a safety factor of 10 for human variability, ATSDR arrived at a value of 0.0002 mg/kg/d as a chronic MRL (see Table 5-5).

ATSDR also obtained permissible concentrations by using physiologically based pharmacokinetic modeling (PBPK) and benchmark dose (BMD) calculation methods carried out by the K.S. Crump Group (Clewell et al. 1997) using the Nogawa et al. (1989) data. A $BMDL_{10}$ was derived, which is defined as the 95% lower bound on the estimates of the doses predicted to correspond to 10% extra risk; that is, $BMDL_{10}$ is the lower bound on the dose BMD_{10}. Polynomial and Weibull models were used to model the data from males and females separately. The doses based on a benchmark dose approach gave a cumulative exposure of 28.2-33.4 mg/kg for males (for Weibull and polynomial model, respectively) and 19.2-23.1 mg/kg for females (for Weibull and polynomial model, respectively). Cumulative exposure was calculated for 70 y of exposure. Daily dose was calculated based on a value for 70 y of exposures and 365 d/y (total number of d = 25,550).

The resulting MRLs of 0.000075-0.00013 mg/kg/d for males and females, respectively, are 1.5-3 times lower than the current MRL by ATSDR (see Table 5-6). Because NASA would not apply an intersubject variability factor, the resulting values will be 10 times higher, and the 1,000-d AC determined is not far from values when adjusted (see section on 1,000-d AC for ingestion).

Furthermore, Clewell et al. (1997) also derived an MRL from the PBPK model based on a modified Oberdorster (1990) model that used data from Butchet et al. (1990). In this PBPK modeling approach, it was concluded that a daily oral intake of 0.84 µg/kg/d would correspond to a urinary cadmium excretion of 2.7 µg/d, assuming an excretion half-life of 20 y. After a factor of 3 was applied to reduce the LOAEL, a chronic MRL of 0.3 µg/kg/d was derived (0.84 µg/kg/d ÷ 3).

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TABLE 5-5 Current Regulatory and Guideline Levels from Other Organizations

Standards	Exposure Limit	Reference
EPA		
MCLG	0.005 mg/L	
MCL	0.005 mg/L	
1-d HA (child)	0.04 mg/L	EPA 2004
10-d HA (child)	0.04 mg/L	EPA 2004
RfD	0.0005 mg/kg/d (water)[a]	IRIS 1994
RfD[a]	0.001 mg/kg/d (food)[a]	IRIS 1994
Life-term HA	.005 mg/L	EPA 2004
Cancer grouping	Group B1[b]	IRIS 2006
Cancer grouping	Group 1[c] carcinogen	IARC 1993
ATSDR		
Acute MRL (1-14 d)	None derived	
Intermediate MRL (15-365 d)	None derived	
Chronic MRL (>365 d)	0.0002 mg/kg/d	
BMD method	0.000075-0.00013 mg/kg/d[d]	Clewell et al. 1997
PBPK approach	0.0003 mg/kg/d	Clewell et al. 1997

[a]A factor of 2 is used to account for increased absorption from water.
[b]Group B1: probable human carcinogen.
[c]Group 1: carcinogenic to humans.
[d]The values are from two types of models used.
Abbreviations: BMD, benchmark dose; HA, health advisory; IARC, International Agency for Research on Cancer; MCL, maximum contaminant level; MCLG, maximum contaminant level goal; PBPK, physiologically based pharmacokinetic; RfD, reference dose.

TABLE 5-6 Derivation of MRL Using Benchmark Dose Modeling

Gender	Model	Cumulative Exposure (mg/kg)	Daily Exposure (mg/kg/d)	With Human Variability Factor of 10 ([mg/kg/d] = [MRL])	Extrapolated Concentration in Water (μg/L)[a]
Males	Weibull	28.2	0.0011	0.00011	2.75
Males	Polynomial	33.4	0.00131	0.000131	3.275
Females	Weibull	19.2	0.00075	0.000075	1.875
Females	Polynomial	23.1	0.0009	0.00009	2.25

[a]Multiplying the MRLs with 70 kg nominal human body weight and divide by 2.8 L/d, the NASA nominal water volume, and converting mg to μg.
Source: Data from Clewell et al. 1997.

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To compare the relative bioavailability of cadmium from water and food, Ruoff et al. (1994) compiled and analyzed the published studies in which rats were exposed to $CdCl_2$ in standard chow or drinking water. Relative bioavailability was assessed from estimates of the rate of accumulation of cadmium in kidney cortex or liver. The data were subjected to a linear regression analysis, with dose as the independent variable and tissue accumulation rate as the dependent variable, to determine whether bioavailability of cadmium was significantly different for different routes of administration. They concluded that in rats receiving food and drinking water ad libitum, the bioavailability of cadmium in drinking water was not significantly different ($P > 0.05$) from the bioavailability of cadmium in food when doses were less than 4 mg/kg/d. The authors also emphasized the fact that changes in food or water consumption should be taken into consideration in the exposure estimates. Studies of the effect of total diet composition on the bioavailability of cadmium may be more relevant than are studies of the effect of the exposure medium. Therefore, they recommend that distinct RfDs for cadmium in food and drinking water should not be based on the assumption that the bioavailability of cadmium in drinking water is greater than that of cadmium in food. EPA has used a factor of 2 when deriving an RfD for cadmium from water (IRIS 1994). One important consideration is that, in chronic-exposure protocols in which animals access food and water ad libitum, the compound being tested will mix with the components of the diet in the GI tract irrespective of whether it comes from water or diet. Therefore, for chronic durations, it may not be necessary to distinguish between the two routes.

There is no acceptable daily intake (ADI) for cadmium, but the World Health Organization (WHO) proposed a provisional tolerable weekly intake (PTWI), the dietary exposure level that can be ingested weekly over a lifetime without appreciable health risk (WHO 1993, 2001). From the human epidemiologic data, the 33rd Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1988 proposed that in the renal cortex, the critical concentration of cadmium that produces renal tubular dysfunction in 10% of the population is about 200 µg/g kidney. The JECFA committee also pointed out that a regular dietary intake of cadmium at 175 µg/man/d (2.9 µg/kg/d) would cause the concentration of cadmium in the renal cortex to reach a concentration of 200 µg/g in 50 y. Assuming absorption of 5% and a daily excretion rate of 0.005% of body burden, they recommended that the total intake should not exceed 1 µg/kg/d continuously for 50 y or 7 µg/kg/wk (PTWI). At the 55th

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JECPA meeting in 2000 and at the 61st meeting in 2003, the committee did not find a need to revise these values (WHO 2001).

Derivation of AC for Various Durations of Exposures Through the Oral Route

Numerous studies in the literature have reported toxicity of orally administered cadmium in several animal experiments, but the medium of administration has been quite diverse. Cadmium had been administered by gavage, added to the diet, or added to the drinking water, so that a meaningful comparison between studies are confounded. Few studies have used multiple concentrations of cadmium that could provide dose-response data, and some have used only one concentration. The data that provided a dose-response effect have been preferentially considered in deriving ACs. Human exposure data for the oral route of exposure to cadmium were for only chronic-duration exposures, and most of the data are from people who were exposed to cadmium through their diet.

1-d AC for Ingestion

For acute toxicity studies, the only data that are available are the gavage studies. Drinking water regimen for acute toxicity studies is not customary. The following studies were considered for determining the 1-d AC.

Nordberg et al. (1973) reported that children who drank a soft drink contaminated with cadmium vomited. The concentration of cadmium was estimated to be 16 mg/L. Using 16 mg/L as the LOAEL and applying a factor of 10 for LOAEL to NOAEL, the 1-d AC can be calculated as follows:

$$16 \text{ mg/L} \div 10 = 1.6 \text{ mg/L},$$

where
16 mg/L = LOAEL; and
10 = LOAEL to NOAEL extrapolation factor.

Thus, the 1-d AC = 1.6 mg/L.

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This concentration was derived based on the concentration of cadmium that determines the emetic effect and was not based on the dose. This is well below the emetic threshold range for cadmium, which has been estimated to be in the range of 3 to 90 mg. It is equivalent to a concentration exceeding 15 mg/L for soluble cadmium salt solutions (CEC 1978), assuming a fluid volume of 200 mL per drink and using the lowest value of the emetic threshold (3 mg) as the no-effect threshold.

A 1-d AC can also be derived from the study by Kotsonis and Klaassen (1977), who measured urine flow and hematologic parameters for 14 d after a single oral administration of various doses of radioactive $CdCl_2$ (cadmium at 0, 25, 50, 100, and 150 mg/kg) to male Sprague-Dawley rats and observed that urine flow was significantly decreased during the first 2 d in all the treated groups. However, hematocrit and blood hemoglobin concentrations measured at 2 and 14 d remained unchanged by exposure to cadmium. Daily motor activity for the initial 2-3 d was lower in the 50, 100, and 150 mg/kg groups (Kotsonis and Klaassen 1977). Considering the importance of motor activity for spaceflight, the 1-d AC was derived from a NOAEL of 25 mg/kg for this end point.

Thus, a 1-d AC for motor activity can be calculated as follows:

$$(25 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 62.5 \text{ mg/L},$$

where
25 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption.

A significant reduction in urine flow noted during the first 2 d after dosing may be because of reduced water intake. Reduced body weight noted may be because of reduced food intake. A NOAEL could not be identified for this effect. A LOAEL of cadmium at 25 mg/kg was identified. A 1-d AC can be calculated using reduced urine flow with a LOAEL of 25 mg/kg as follows:

$$(25 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 10 \times 2.8 \text{ L/d}) = 6.25 \text{ mg/L (rounded to 6 mg/L)},$$

where
25 mg/kg/d = LOAEL;
70 kg = nominal body weight;

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10 = LOAEL to NOAEL extrapolation factor;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption/d.

Acute toxicity of $CdCl_2$ was studied after single gavage doses of 0, 0.6, 3.9, 7.9, 15.7, 30.3, 59.6, and 88.8 mg/kg (Andersen et al. 1988) in 7- to 8-wk-old CBA/Bom mice. On day 10, tissue damage to stomach and duodenum was observed at 30.3 mg/kg/d. At doses higher than 30.3 mg/kg, gastric necrosis was observed. The data could not be used for a 1-d AC because histopathology was not done on mice treated at doses below 30.3 mg/kg. This dose produced a serious adverse effect, making it difficult to identify a definitive LOAEL and a NOAEL although several doses had been used.

10-d AC for Ingestion

Male Sprague-Dawley rats were exposed to cadmium at concentrations of 10, 30, and 100 ppm in drinking water for 24 wk (Kotsonis and Klaassen 1978). Several measurements were made at 3, 6, 12, and 24 wk. Food and water intake, urine flow and protein excretion, and motor activity were measured weekly. The hourly nocturnal and daily motor activities decreased with time for the 30 and 100 ppm groups. Also in these groups, nephrotoxicity indicated by increased protein concentration (mg/100 mL of urine) was seen only after week 6 of treatment. The daily water consumption was significantly lower after week 1 for the 30 ppm and 100 ppm dose groups. The motor activity decrements were seen as early as 3 wk and at as low a dose as 30 ppm. This drinking water study thus indicates that 10 ppm is the NOAEL for these effects (motor activity and reduction in water consumption). Based on water consumption and the daily mean cadmium intake per day, the dose rates were calculated as 1.15, 2.92, and 8.51 mg/kg/d for 10, 30, and 100 ppm groups, respectively.

As the effect on motor activity was seen from week 3, the data were used for the 10-d AC without any time factor. A decrease in water consumption was seen as early as 1 wk at the dose of 2.92 mg/kg. This can be used to calculate a 10-d AC after applying a time factor from 7 to 10 d.

Thus, a 10-d AC based on reduced motor activity can be calculated as follows:

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$$(1.15 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) =$$
$$3 \text{ mg/L (rounded from 2.88 mg/L)},$$

and a 10-d AC based on reduced water consumption can be calculated as follows:

$$(1.15 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times 3 \times [10/7]) =$$
$$0.7 \text{ mg/L (rounded from 0.67 mg/L)},$$

where
1.15 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption/d;
3 = spaceflight factor for decreased water consumption; and
10/7 = factor to extrapolate time from 7 to 10 d.

Borzelleca et al. (1989) conducted a 10-d short-term exposure study in which male and female rats were administered $CdCl_2$ by gavage or via drinking water for 10 consecutive d at various concentrations. Because data from the drinking water study are more appropriate and applicable for determining AC for drinking water, only the data from the drinking water protocol were used for deriving the AC for 10 d.

Male and female Sprague-Dawley-derived Wistar rats (10 each per dose) were exposed for 10 consecutive days to $CdCl_2$, which was added to the drinking water at concentrations to give theoretical doses of 2.5, 25, and 51 mg/kg/d. Exposure dose calculations based on actual water consumption resulted in actual doses of $CdCl_2$ at 1.8, 12.8, and 18.2 mg/kg/d for males and 1.8, 13.3, and 22.6 mg/kg/d for females (equivalent of cadmium at 1.1, 7.8, and 11.1 mg/kg/d). Except for some decreases in body weights and organ weights, no compound-related histopathologic effects were noted at the end of the study. Among several clinical chemistry parameters measured, only decreases (about 45%) in ALP and serum protein were seen in both male and female rats. However, the clinical relevance of these decreases in serum is not clear. An increase in serum BUN was seen at the highest dose in male rats. Qualitative urine analysis for protein (using reagent strips) indicated that a dose of cadmium at 7.8 mg/kg/d could be identified as a LOAEL and a dose of 1.1 mg/kg/d as a NOAEL for increased protein excretion in urine. The observation of increased BUN, a marker for renal dysfunction,

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in the 11.1 mg/kg/d group supports use of these data for nephrotoxic effect.

Thus, a 10-d AC for nephrotoxicity can thus be calculated as follows:

$$(1.1 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 3 \text{ mg/L (rounded from 2.75 mg/L)},$$

where
1.1 mg/kg/d = NOAEL;
70 = nominal body weight;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption.

Ogoshi et al. (1989) studied the effect of cadmium added to drinking water on the mechanical strength of bones of young, adult, and old rats after administering the dose for 4 wk. Young rats were exposed at 5 and 10 ppm, and adult rats and older rats to cadmium at 10, 20, 40, 80, and 160 ppm. A decrease in bone strength was seen only in young rats; when exposed to cadmium at 160 ppm, the adult and older rats did not show any effect at the end of 4 wk of treatment. The authors reported that the amount of cadmium accumulation in the bone correlated well with the sensitivity of the rats to effects on bone. Using a NOAEL of 160 ppm (estimated dose rate of 22 mg/kg/d) for adult and old rats for 4 wk, a 10-d AC for effects on bone can be derived as follows:

$$(22 \text{ mg/kg/d} \times 70 \text{ mg}) \div (10 \times 2.8 \text{ L/d} \times 3) = 18 \text{ mg/L (rounded)},$$

where
22 mg/kg/d = NOAEL for 4 wk;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
3 = spaceflight factor for bone effects.
(No time factor is needed; 4-wk data can be applied directly for 10 d.)

100-d AC for Ingestion

Itokawa et al. (1974) reported that 112 d (16 wk) after exposure to cadmium, significant reductions in the concentrations of erythrocytes

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(about 30%), hematocrit (about 30%), and hemoglobin (about 25%) were seen in male Wistar rats exposed to cadmium as $CdCl_2$ in drinking water (cadmium at 50 mg/L or 3.6 mg/kg/d). Although this is a drinking water study, no NOAEL was identified and there was no dose-response data. Other studies with dose-response data were preferred over this study. Data from this study were not considered for AC derivation.

Prigge (1978) exposed 12 female SPF Wistar rats (between 170 and 190 g body weight) to cadmium in drinking water at 25, 50, and 100 ppm (estimated dose rates of cadmium at 4, 8, or 16 mg/kg/d) for 90 d. Hemoglobin and hematocrit concentrations were unaltered by cadmium administration. Coincident with the higher B-Cd concentrations, proteinuria (about a 30% increase in urinary protein excretion) was observed. The 4 mg group exhibited reduced serum iron (about 30%). There was a dose-dependent increase in urinary protein excretion, which was statistically significant at doses of 8 mg/kg/d and higher. The significant reduction in serum iron may have been because of the adverse effect of cadmium on iron absorption. However, the proteinuria noted in this study is a more critical effect. Even though there was a 20% increase in protein excretion in the urine in the 4 mg/kg group, it was not statistically significant and thus was considered a NOAEL for this effect. A time extrapolation factor of 90-100 d will be used.

Thus, a 100-d AC based on renal effects can be derived from this study as follows:

$$(4 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times [100 \text{ d}/90 \text{ d}]) = 9 \text{ mg/L (rounded)},$$

where
4 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
100 d/90 d = time extrapolation factor.

The other study considered for 100-d-AC derivation is the 24-wk drinking water study. Kotsonis and Klaassen (1978) exposed rats to cadmium at concentrations of 10, 30, and 100 ppm (or cadmium at 1.15, 2.92, and 8.51 mg/kg/d, based on the amount calculated by the author) in drinking water for 24 wk. While hematocrit and hemoglobin concentrations, blood glucose concentration, liver aniline hydroxylase activity, cytochrome P-450, and hexobarbital oxidase activity were not signifi-

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cantly different from those of control animals throughout the study, the concentration of protein in the urine of the 30 and 100 ppm rats (2.92 and 8.51 mg/kg/d) was unchanged until 6 wk but was significantly higher from week 9 (after 6 wk). Slight and focal tubular necrosis was observed in these treated groups by week 24. A LOAEL of 2.92 mg/kg/d and a NOAEL of 1.15 mg/kg/d for proteinuria and tubular necrosis were identified. Renal tubular atrophy was slight and focal. Bone calcification measured (bone ash) remained unchanged. In the same study, the authors also identified cadmium at 1.15 mg/kg/d as a NOAEL for decreased motor activity (rat locomotion), which was used to assess CNS function. Beginning with week 9, motor activity of cadmium-treated animals was consistently less than those of controls. Because the rats exposed to cadmium at 1.15 mg/kg/d were unaffected, at least up to 24 wk, no time factor was applied.

An AC for decreased motor activity effects can be derived as follows:

$$(1.15 \text{ mg/kg/d} \times 70) \div (10 \times 2.8 \text{ L/d}) = 2.9 \text{ mg/L (rounded)},$$

where
1.15 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption.

A 100-d AC based on proteinuria (nephrotoxicity) can be calculated as follows:

$$(1.15 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 2.9 \text{ mg/L (rounded)},$$

where
1.15 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption.

Two studies by Pleasants et al. (1992, 1993) were evaluated for calculating a 100-d AC. In the 1992 study, male Long Evans rats were exposed to cadmium at 20 ppm and 40 ppm (2.9 and 5.8 mg/kg/d) in drinking water for 14 wk. Significant increases in the weights (g/100 g body weight) of both kidney (20%) and testes (28%) were observed. The min-

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eral content (ash, mg/100 g body weight) of the femurs decreased as a result of exposure to both doses of cadmium. This could be an indication of bone resorption, which has been reported in several cadmium-exposure studies (Kjellström 1986b; Berglund et al. 2000; Ohta et al. 2000). Although no changes in hematocrit or peripheral red blood cell counts were noted, rats exposed to cadmium at 40 ppm for 14 wk showed evidence of erythrocyte hypochromia with intracellular non-heme iron inclusions (Pleasants et al. 1992).

In the follow-up study (Pleasants et al. 1993), young Long-Evans rats (male weanling rats, 40-50 g initial body weight) were first exposed to cadmium at 40 ppm for 1 wk and then later on were exposed at 80 ppm (11.6 mg/kg/d) in their drinking water for a total of 14 wk. Cadmium-exposed rats showed significantly depressed hematocrit and erythrocyte counts. Combining the results from both studies, cadmium at 2.9 mg/kg/d seems to be a NOAEL for critical effects on the hematologic and skeletal systems. Since 14 wk is close to 100 d, no time extrapolation factor was used.

A 100-d AC for hematotoxicity and for effects on bone can be calculated as follows:

$$(2.9 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times 3) = 2.5 \text{ mg/L (rounded)},$$

where
2.9 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
3 = spaceflight factor for hematologic effects and for bone resorption.

The effects on bone are also supported by studies by Ogoshi et al. (1989, 1992). Young (3-wk-old) rats exposed to cadmium in drinking water at 5 and 10 ppm and old rats (18 mo old) that received 40 ppm showed significantly decreased compression strength (13-20%) of the femur at the distal end. The young rats were exposed for 20 wk (140 d), and older rats for 7 mo (215 d). In this study, a NOAEL of 5 ppm (0.7 mg/kg/d) was identified. A 100-d AC to protect bone effects using this value can be calculated as follows:

$$(0.7 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times 3) = 0.6 \text{ mg/L (rounded)},$$

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where
0.7 mg/kg/d = NOAEL for 20 wk;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
3 = spaceflight factor for hematologic effects and bone resorption.

Groten et al. (1994) studied renal toxicity after long-term administration of oral $CdCl_2$ for 10 mo in male Wistar rats fed diets containing $CdCl_2$ at 0, 0.3, 3, 30, and 90 mg/kg of diet. The doses were estimated to be 0, 0.027, 0.27, 2.7, and 8.1 mg/kg/d. The first sign of renal injury—increased excretion of urinary LDH activity—was seen at 120 d in rats fed cadmium at 8.1 mg/kg (90 mg/kg diet group). More pronounced proteinuria was seen after 8 and 10 mo, as indicated by increased excretion of urinary LDH, ALP (a brush-border enzyme), and NAG. No effects were seen in the 2.7 mg/kg group at 120 d. However, kidneys from the 2.7 mg/kg dose group showed an increased number of basophilic tubules at 10 mo, indicating slight nephrotoxicity. No abnormalities were detected in liver in any of the groups, nor were there any changes in the hepatotoxicity marker enzymes plasma aspartate amino transferase and alanine amino transferase. Therefore, for the sake of being conservative, the next lower dose of 0.27 mg/kg/d was identified as a NOAEL for 10 mo. This could be used for 100 d without any time extrapolation factor.

Thus, a 100-d AC can be calculated for nephrotoxicity as follows:

$$(0.27 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 0.675 \text{ mg/L or } 0.7 \text{ mg/L (rounded)},$$

where
0.27 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption.

Wilson et al. (1941) reported that rats fed cadmium in the diet at a dose rate of 2.79 mg/kg/d for 100 d suffered several serious effects such as muscle atrophy, focal kidney necrosis, tubular swelling and casts, atrophy of the pancreas, and anemia. At a higher dose, cadmium at 5.5 mg/kg/d, very significant body weight reductions were seen. In this study, 2.79 mg/kg/d was identified as a LOAEL; a NOAEL was not identified. Because this study was done several years ago and the results of

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more recent studies are available, and because a NOAEL was not identified, the data were not used for AC derivation.

1,000-d AC for Ingestion

Bomhard et al. (1999) evaluated the time course of chronic renal toxicity by cadmium in male and female Wistar rats (n = 12/group) that were fed cadmium as $CdCl_2$ in their diet at 0, 10, 50, and 250 ppm (cadmium at 0, 0.66, 3.33, or 16.7 mg/kg/d) for 72 wk. They were evaluated by measuring several urinary enzyme activities that represent different cellular compartments of the nephron, after 1, 4, 8, 13, 18, 26, 32, 45, 57, and 68 wk. The brush-border enzymes GGT, ALP, and leucine arylamidase, and lysosomal enzymes arylsulfatase A, β-galactosidase, and NAG were among those that were evaluated. At the end of the study period, the kidneys were examined histopathologically. Groups up to and including the 50 ppm group did not show any significant alterations (NOAEL). All nephrotoxic-marker enzymes clearly revealed renal damage at 250 ppm. Histopathology after 72 wk revealed not only chronic but also acute degenerative changes in the kidneys of the 250 ppm group of male and female rats, including some irregularities of the brush border lining. Thus, a NOAEL of 50 ppm or 3.33 mg/kg was identified for nephrotoxic effects.

A 1,000-d AC for nephrotoxic effects can be calculated as follows:

$$(3.33 \text{ mg/kg/d} \times 70\text{kg}) \div (10 \times 2.8 \text{ L/d} \times [1,000 \text{ d}/504 \text{ d}]) = 4.0 \text{ mg/L (rounded)},$$

where
3.33 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor;
1,000 d/504 d = time extrapolation factor from 72 wk to 1,000 d.

Another set of data that were evaluated for the 1,000-d AC for renal-effects calculation is from the Bernard et al. (1981) study. Although the authors had used only one dose, they had monitored the changes from 2 to 10 mo. In this study, 2-mo-old female Sprague-Dawley rats exposed to cadmium at 200 ppm (30 mg/kg/d) in their drinking water for 11 mo had proteinuria from month 8 of treatment. A slight tubular dysfunction was also evident from aminoaciduria. Because a NOAEL cannot be iden-

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tified, the dose of 30 mg/kg/d is considered a LOAEL. This was confirmed by the authors in another study later (Bernard et al. 1988) in which female Sprague-Dawley rats exposed to cadmium in drinking water at a concentration of 200 ppm (30 mg/kg) for 2-10 mo showed an increase in albuminuria. At 10 mo, the rats developed slight tubular damage as evidenced by increased urinary excretion of β-2m and NAG (Bernard et al. 1988). Because the initial adverse renal effect was seen at 8 mo, the 1,000-d AC will be calculated from the results of the first study as follows:

$$(30 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 10 \times 2.8 \times [1{,}000 \text{ d}/240 \text{ d}]) = 1.8 \text{ mg/L},$$

where
30 mg/kg/d = LOAEL;
70 kg = nominal body weight;
10 = LOAEL to NOAEL;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
1,000 d/240 d = time extrapolation factor.

The third and fourth studies that were considered for 1,000-d AC derivation were that of Mitsumori et al. (1998) and Shibutani et al. (2000).

In the Mitsumori et al. (1998) study, female Sprague-Dawley rats were fed a diet containing $CdCl_2$ at concentrations of 0, 8, 40, 200, and 600 ppm (0, 0.33, 1.6, 8, and 24 mg/kg/d) for 2, 4, and 8 mo from 5 wk of age. Hepatotoxicity was observed after 2 mo in the groups treated with ≥200 ppm (8 mg/kg/d). By 4 mo, the rats in the 600 ppm group had developed periportal liver cell necrosis. Renal toxicity characterized by degeneration of proximal tubular epithelia with vacuolar degeneration was apparent in the groups treated with ≥200 ppm (8 mg/kg/d) from 2 mo, becoming more prominent in the high-dose rats at 4 mo. No renal lesions were observed in the 40 ppm (1.6 mg/kg/d) group even after 8 mo. Similarly, the bile duct hyperplasia seen at 8 mo in the 200 ppm group was not seen in the group treated with 40 ppm. Therefore, 40 ppm (1.6 mg/kg/d) is also identified as a NOAEL for hepatotoxicity for 8 mo.

However, in a later study, Shibutani et al. (2000) reported that low-dose oral administration of cadmium in the diet even up to 22 mo (660 d) (at 1.1, 5, 20, or 40 ppm/kg of diet [0, 0.04, 0.2, 0.8, or 1.6 mg/kg/d]), produced only spontaneous nephropathy and no renal lesions as seen at

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short-term high-dose exposures (Shibutani et al. 2000). The results demonstrated that, in contrast to high-dose cadmium administration, treatment with 40 ppm or less for 22 mo (660 d) did not influence tubular regeneration as a component of nonspecific chronic nephropathy, suggesting that long-term administration of low levels of cadmium orally does not injure renal tubules in female rats.

Thus, taking both studies together, 40 ppm is a NOAEL for both 240 d and 660 d. As the latter one is closer to 1,000 d, it was used for deriving the 1,000-d AC.

Thus, a 1,000-d AC for nephrotoxic and hepatotoxic effects can be calculated as follows:

$$(1.6 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times [1{,}000 \text{ d}/660 \text{ d}]) = 2.63 \text{ mg/kg/d or } 3 \text{ mg/L (rounded)},$$

where
1.6 mg/kg/d = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
1,000 d/660 d = time extrapolation factor from 22 mo to 1,000 d.

Hiratsuka et al. (1999) studied rats given minimum amounts of cadmium-polluted rice or $CdCl_2$ for 8 mo. The doses used for $CdCl_2$ were cadmium at 0, 0.32, 1.28, or 2.56 mg/kg (as calculated by the author) in the diet. Biochemical and histopathologic measurements were made at 1, 4, and 8 mo. Urinary enzymes such as -glutamyl transpeptidase, LDH, and *N*-acetyl-β-d-glucosaminidase were measured as markers of adverse renal effects. Histopathology was performed on liver, kidneys, lungs, sternum, and femur. The authors concluded that at any time or at any dose, there were no signs of renal tubular damage, either chemically or histopathologically. So on the basis of these results, a NOAEL of cadmium at 2.56 mg/kg for rats can be identified.

Thus, a 1,000-d AC for nephrotoxicity can be calculated as follows:

$$(2.56 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times [1{,}000 \text{ d}/240 \text{ d}]) = 1.5 \text{ mg/L},$$

where
2.56 mg/kg/d = NOAEL;
70 kg = nominal body weight;

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10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
1,000 d/240 d = time extrapolation factor.

Another animal study that was considered was that of Gatta et al. (1989), in which changes in certain urinary enzymes specific to various anatomic areas of the kidney were evaluated. Forty Wistar rats were exposed to $CdCl_2$ at 16 ppm in drinking water for 4, 16, 40, and 60 wk (estimated dose of 0.75 mg/kg/d). No abnormal histology was seen by light or electron microscopy after 4 or 16 wk. A widespread vesiculation of proximal tubular cells with mitochondrial and lysosomal alterations was found at 40 wk and was more evident at 60 wk. The brush border never showed any damage (normal excretion pattern of GGT, an enzyme situated in this structure). Urinary α-glucosidase was increased only at 60 wk and showed the most severe anatomic damage to the proximal tubule. Urinary lysozyme, an index of tubular function, was increased at 40 and 60 wk. Having only one exposure dose, an insufficient number of controls, and a lack of paired controls for the group exposed for 60 wk made it difficult to assess spontaneous changes in the kidney of controls at 60 wk, and in general, because of poor design, this study could not be considered for AC derivation.

An epidemiologic study was conducted by Nogawa et al. (1989) on the dose-response relationship of cadmium ingestion to cadmium-induced health effects, especially to renal damage of the residents of the Kakehashi River basin in Ishikawa Prefecture in Japan, whose intake of cadmium came from food (polluted rice). β-2m was used as an index of adverse health (effect on renal cortex), and the average concentration of cadmium in the locally produced rice was used as an indicator of exposure assessment. The subjects were classified according to the average cadmium concentration in the rice in their village and the number of years of residency in that polluted area. Subjects were male and female—878 males and 972 females. Subjects from a nonpolluted neighboring area were used as controls (294 total, 133 males and 161 females). Subjects over 50 y of age were included in the study. Levels of urinary excretion that exceeded 1,000 µg/L or 1,000 µg/g creatinine were considered abnormal. The prevalence of β-2m in the males of the unpolluted area was 5.3%, and its prevalence in females was 3.1%. The subjects were divided into 12 subgroups on the basis of their length of residence. The prevalence of β-2m in each group was then calculated. A regression equation relating total cadmium intake and prevalence of β-2m was derived separately for males and females. There was a highly significant

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correlation in each of the groups between total exposure concentration and urinary β-2m. The authors concluded that a total lifetime intake of cadmium that would be comparable to that of controls (background renal excretion of β-2m) would be 2,000 mg. The authors calculated this as 110 μg/d by dividing 2,000 mg by 50 y of exposure, assuming accumulation in the renal cortex.

If NASA were to use this value of 110 μg/d, a 1,000-d AC would be derived as follows:

$$(110\ \mu g/d - 10\ \mu g/d) \div 2.8\ L/d = 0.036\ mg/L \text{ or } 36\ \mu g/L \text{ (rounded)},$$

where
110 μg/d = total acceptable cadmium intake;
10 μg/d = average daily dietary contribution; and
2.8 L/d = nominal water consumption.

The original study had a large population that included sensitive individuals (such as persons with diabetes, aged subjects, and women in menopause); therefore, no additional factors are needed.

A large-scale epidemiology study was conducted by Buchet et al. (1990) from 1985 to 1989 to assess whether environmental exposure to cadmium is associated with renal dysfunction. A total of 1,699 subjects aged 20-80 y were studied as a random sample of four areas of Belgium that had varying degrees of cadmium pollution. After standardization for several possible confounding factors, five variables (urinary excretion of retinol-binding protein, *N*-acetyl-β-glucosaminidase, β-2m, amino acids, and calcium) were significantly associated with the urinary excretion of cadmium (as a marker of cadmium body burden), suggesting the presence of tubular dysfunction. Cadmium excretion was correlated with changes in measures of proximal tubular function. The authors used multiple regressions and logistic models to study the relationship between the frequency of abnormal values of the renal parameters and the derived exposure estimates based on excretion of cadmium in the urine. There was a 10% probability that values of these variables would be abnormal when cadmium excretion exceeded 2-4 μg/24 h (range for all the nephrotoxic markers). Excretion reached this threshold in 10% of nonsmokers. There was also evidence that diabetic patients may be more susceptible to the toxic effect of cadmium on the renal proximal tubule.

The authors used a cut-off level of renal cadmium excretion of 2 μg/24 h urine sample, below which the occurrence of renal damage remains low. Buchet et al. (1990) calculated that this cadmium concentra-

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tion in the urine corresponded to 50 ppm in the renal cortex (wet weight of renal cortex) or 50 µg/g of the renal cortex, an amount of cadmium in the renal cortex that will be obtained in 50 y of daily intake of 1 µg/kg. Estimated average daily intake of cadmium in the diet by adults in the United States has been stated to be in the range of 10-20 µg/d (Pennington et al. 1986; FDA 1993).

This value of 1 µg/kg was considered for the 1,000-d AC with a background daily dietary intake of 10 µg/d (the lower end of the range of 10-20 µg/d) or 0.14 µg/kg/d.

So, daily acceptable intake is (1 µg/kg/d − 0.14 µg/kg/d) = 0.86 µg/kg/d in water.

This is equivalent to

$$(0.86\ \mu g/kg/d \times 70\ kg) \div (2.8\ L/d) = 21.5\ \mu g/L \text{ or } 22\ \mu g/L \text{ (rounded).}$$

The Buchet et al. (1990) study did not exclude unhealthy populations and considered both genders and various ages. Therefore, no other safety factor is needed for deriving the 1,000-d AC.

Thus, the 1,000-d AC for cadmium in water is 22 µg/L or 0.022 mg/L.

Another study that was evaluated for the 1,000-d AC was that of Waalkes et al. (1999) in which male Noble (NBL/Cr) rats were exposed to cadmium (in drinking water) and proliferative lesions of the prostate and the kidneys were characterized. Cadmium as $CdCl_2$ was given ad libitum in the drinking water at 0, 25, 50, 100, and 200 ppm, and rats were observed for up to 102 wk. NASA evaluated the data on renal-tumor incidence reported in the study to attempt to derive a 1,000-d AC. These data are summarized in the table below (Table 5-7).

The authors had stated that a strong trend was seen between cadmium dose and incidence of renal tumors, but they had added that a statistical significance can be seen only when these tumors of differing histogenesis are combined. Hence, the link between cadmium and renal tumors noted in the study is tenuous. NASA also discussed these data with Dr. Waalkes, the primary author of the paper, to obtain his opinion about the significance and robustness of these findings. According to the author, the incidence of renal tumors even in the 200-ppm group (3 tumors/29), should not be considered significantly different from the incidence in controls (0 tumors/29). Therefore, NASA decided that the use of the data from this study to provide a NOAEL and assess cancer risk associated with oral cadmium exposure was not warranted.

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TABLE 5-7 Effect of Cadmium Exposure via Drinking Water on the Incidence of Renal Tumors in Noble Rats

Cadmium in Water (ppm)	Group Size	Incidence of Renal Tumors[a]
0	29	0 (0%)
25	29	0 (0%)
50	29	0 (0%)
100	30	2 (7%)
200	29	3 (10%)

[a]Tumors include two mesenchymal tumors in the 100 ppm group, and one lipoma, one mesenchymal tumor, and one transitional cell carcinoma in 200 ppm group.
Source: Data from Waalkes et al. 1999.

ACs derived for 1, 10, 100, and 1,000 d, the factors used for deriving the ACs, the critical effects, and the studies used have been summarized in Table 5-8. A SWEG for each duration is also listed by taking into considerations the AC values derived for that particular duration. These final SWEG values, the critical effect, and the key study have been summarized in the Rationale section (Table 5-4).

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TABLE 5-8 Summary of Acceptable Concentrations (ACs) for Cadmium in Drinking Water

			Uncertainty Factors				Acceptable Concentration (mg/L)				
Toxicity End Point	NOAEL/LOAEL (mg/kg/d)	Species	To NOAEL	Species Factor	Time Factor	Space-flight Factor	1 d	10 d	100 d	1,000 d	Reference
Induction of vomiting; emetic stimulus	LOAEL = 16	Human	10	1	1	1	1.6	—[a]	—	—	Nordberg et al. 1973
Motor activity	NOAEL = 25	Rat	1	10	1		62.5	—	—	—	Kotsonis and Klaassen 1977
Reduced urine flow	LOAEL = 25	Rat	10	10	1	1	6	—	—	—	Kotsonis and Klaassen 1977
Neurotoxicity: motor activity	NOAEL = 1.15	Rat	1	10	1	1	—	3	—	—	Kotsonis and Klaassen 1977
Reduced water consumption	NOAEL = 1.15	Rat	1	10	1	3	—	0.7	—	—	Kotsonis and Klaassen 1977
Nephrotoxicity	NOAEL = 1.1	Rat	1	10	1	1	—	3	—	—	Borzelleca et al. 1989
Bone strength	NOAEL = 4	Rat	1	10	100/90	—	—	—	9	—	Prigge et al. 1978
CNS effect: decreased motor activity	NOAEL = 1.15	Rat	1	10	1	1	—	—	2.9	—	Kotsonis and Klaassen 1977
Nephrotoxicity	NOAEL = 1.15	Rat	1	10	1	—	—	—	2.9	—	Kotsonis and Klaassen 1977

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Hematologic and bone effects	NOAEL = 2.90	Rat	1	10	1	3	—	—	2.5	—	Pleasants et al. 1992, 1993
Bone strength	NOAEL = 0.70	Rat	1	10	1	3	—	—	0.6	—	Ogoshi et al. 1989
Nephrotoxicity	NOAEL = 0.27	Rat	1	10	1	1	—	—	0.7	—	Groten et al. 1994
Nephrotoxicity	NOAEL = 0.66	Rat	1	10	1,000/504	1	—	—	—	4	Bomhard et al. 1999
Nephrotoxicity	LOAEL = 30	Rat	10	10	1,000/240	1	—	—	—	1.8	Bernard et al. 1988
Nephrotoxicity and hepatoxicity	NOAEL = 1.60	Rat	1	10	1,000/660	1	—	—	—	3	Shibutani et al. 2000
Nephrotoxicity	NOAEL = 2.56	Rat	1	10	1,000/240	1	—	—	—	1.5	Hiratsuka et al. 1999
Nephrotoxicity	NOAEL, see text	Human epidemiology	1	1	1	1	—	—	—	0.04	Nogawa et al. 1989
Nephrotoxicity	NOAEL, see text	Human epidemiology	1	1	1	1	—	—	—	0.022[b]	Buchet et al. 1990
SWEG							1.6	0.7	0.6	0.022	

[a] —, Not derived.
[b] At this concentration of cadmium, there would be protection with continued exposure beyond 1,000 d to a nominal lifetime.

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6

Caprolactam

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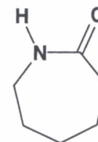
PHYSICAL AND CHEMICAL PROPERTIES

Use and Occurrence

Caprolactam is a cyclic amide, derived from epsilon-aminocaproic acid, from which nylon 6 is polymerized (see Table 6-1). Caprolactam is a monomer primarily used in the manufacture of the synthetic polymer nylon 6, fibers and resins, synthetic leather, and as a polyurethane cross linker. Nylon 6 (polycaprolactam) is used in the production of tire cords, carpeting, plastics, and food-packaging materials. Caprolactam has been

TABLE 6-1 Physical and Chemical Properties[a]

Formula	$C_6H_{11}NO$
Chemical name	Caprolactam
Synonyms	Hexahydro-2-H-azepin-2-one, 6-Aminocaproiclactam, epsilon caprolactam, 2-Oxohexamethyleneimine, 6-Hexanelactam
CAS registry no.	105-60-2 
Molecular weight	113.2
Vapor pressure	6.0 mm Hg @ 120 ºC (800 Pa at 120 ºC [ACGIH 1991]) 0.0021 mm Hg at 25 ºC
Saturated vapor concentration	13 mg/m^3
Boiling point	180 ºC at 50 mm Hg
Solubility	Very soluble in water, benzene, diethylether, and ethanol
Conversion factor	mg/m^3 = 4.6 × ppm

[a]Data from HSDB 2006 and Merck Index 1989.

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approved by the U.S. Food and Drug Administration (FDA) for use in food contact films. Occupational exposure to caprolactam occurs primarily from the manufacture of nylon 6 fibers and resins. Highly soluble in water, caprolactam leaches from clothing made from polyamide fibers when the clothing is soaked in simulated perspiration (Statsek and Ivanova 1978). It has been found in groundwater, surface waters, and finished water (IARC 1986; EPA 1988). Water produced in the Shuttle from fuel cells is iodinated, collected in large containers called contingency water containers (CWCs), and transferred to the International Space Station (ISS) for use by the crew. These CWC bags are used to store drinking water containing silver used as a biocide, and for collection and storage of humidity condensate that will be processed to potable water. The bags are lined with a material called Combitherm. When the Crew and Thermal Systems Division of the National Aeronautics and Space Administration (NASA) was performing material compatibility testing to see if CWC-stored water undergoes quality degradation, it was found that the total organic carbon (TOC) concentration increased. It was determined that caprolactam was the only contributor to this increased TOC. It was also found that the Combitherm material leaches caprolactam during storage, and irrespective of the biocide used (iodine or silver), the leaching continued. A concentration of 16 milligrams per liter (mg/L) of caprolactam was found at the end of 24 weeks (wk). Thus, NASA was prompted to evaluate caprolactam for potential health hazards at this concentration and to recommend a spacecraft water exposure guideline (SWEG).

PHARMACOKINETICS AND METABOLISM

Absorption, Disposition, and Elimination

No human data are available on the absorption of caprolactam from an oral dose. However, from an animal study of disposition pharmacokinetics described below (Unger et al. 1981), it appears that caprolactam is almost completely absorbed from the gastrointestinal (GI) tract, because within 24 hours (h), more than 80% of the administered dose (^{14}C-caprolactam) was recovered in urine, feces, and expired air.

Two sets of studies were carried out by Unger et al. (1981) on the disposition kinetics of caprolactam when dosed via the oral route. In the first set of experiments, a single oral bolus of ^{14}C-caprolactam in water

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was given to male Fischer rats at a concentration of 0.18 mg per kilogram (kg). Groups of animals were killed after 0.5, 1, 2, 3, 4, 6, 15, or 24 h following dosing; urine was also collected. In addition, exhaled ^{14}C (as carbon dioxide [CO_2]) was quantified. Twenty-four hours after a single oral bolus dose of ^{14}C-caprolactam at 0.18 mg/kg, about 77% of radioactivity was excreted in the urine, 3.5% in the feces, and 1.5% in the expired air. Elimination of radioactivity was most rapid in the urine during the initial 6 h following the dose. Analysis of urine indicated that 24 h after dosing, only 2.3% of the radioactivity was in the form of the parent compound. There were two major unidentified metabolites, one comprising 79% and the other 17.7%. After dosing, the peak concentrations of radioactivity in the tissues (nanogram [ng] equivalents of caprolactam per gram of tissue) were similar to that found in the blood (for example, it was about 128 ng/g in blood, 151 ng/g in liver, and about 140 ng/g in spleen), except for stomach (1,907 ± 286 ng/g, including contents), kidney (247 ± 19 ng/g), and bladder (1,240 ± 223 ng/g)—the tissues associated with ingestion and excretion (Unger and Friedman 1980; Unger et al. 1981).

In the second experiment, rats were orally treated with caprolactam at 1.5 g/kg for 7 days (d) and 24 h after the last dose; radioactive caprolactam was administered at the same dose. Animals were killed 6 h after receiving the radioactive dose, and their blood, tissues, urine, feces, and expired air were collected. Another set of animals was administered caprolactam containing ^{14}C at 1.5 g/kg, and 24-h urine samples were collected and analyzed for metabolites.

When a single dose of ^{14}C-caprolactam at 1.5 g/kg was administered and studied after 6 h, the pattern of distribution was the same as that observed at the low dose except that 40% of the radioactivity was still in the stomach,, whereas in the previous experiment, only 6% of the radioactivity was in the stomach after 6 h. Additionally, at 6 h, 14% was excreted in the urine in the high-dose group, and 39% was excreted in the urine in the low-dose group. The authors did not specify how much was in the stomach tissue or in the contents.

In the 7-d study, when radioactive caprolactam was given after 7 d of pretreatment, the tissue distribution was similar to that from a single bolus dose. However, at 6 h, there was a fivefold increase in the excretion of radioactive CO_2 in the expired air (about 0.25% of the administered dose). The results of this study indicated that caprolactam is very

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rapidly and almost completely absorbed at low doses and also rapidly eliminated. From the distribution of radioactivity in the GI tract, it appears that caprolactam is predominantly and directly absorbed from the stomach rather than from the intestine. The fact that liver does not show a very high percentage of the radioactivity may suggest that caprolactam either undergoes extensive biotransformation or is rapidly eliminated from the liver and finally excreted in the urine. In the high-dose group, an appreciable quantity of parent compound was excreted in the urine,, which suggests that biotransformation in liver may not be required for the elimination of caprolactam in urine. This also suggests that the metabolic pathway in liver probably becomes saturated at high doses.

Kirk et al. (1987) reported that rats fed a diet containing 3% caprolactam for 2 or 3 wk excreted approximately 16% of the caprolactam ingested as the 4-hydroxy metabolite and a small amount as the nonhydroxylated acid, 6-aminohexanoic acid. The 4-hydroxy metabolite rearranges spontaneously in acidic aqueous medium to an equilibrium mixture in which 6-amino-gamma-caprolactone is the major component and 6-amino-4-hydroxyhexanoic acid is a minor component.

The distribution of ^{14}C-caprolactam was also studied by whole-body autoradiography in male, female, and pregnant mice on day 14.5 of gestation which were given an average of ^{14}C-caprolactam at 6.6 mg/kg by oral intubation in water (Waddell et al. 1984). Pregnant mice were frozen 20 minutes (min) and 1, 3, 9, and 24 h after oral administration of the compound. From the pattern of intensity of radioactivity as measured by autoradiography, one can understand the distribution and elimination. The nonpregnant mouse was frozen 3 h after oral dosing, and two male mice were frozen 20 min and 9 h after intravenous (iv) administration. Autoradiography data indicated that radioactivity was rapidly absorbed from the stomach and distributed throughout the entire animal, including the fetuses. There was efficient elimination by the kidney and liver, as evidenced by the shift in density in autoradiograph from the stomach to these organs. Material secreted by the liver into bile and intestinal contents appeared not to be reabsorbed via the enterohepatic circulation. The kinetics of distribution and elimination appeared to be the same in male, female, and pregnant animals. The distribution into and removal from the fetuses was typical of molecules that diffuse freely across the placenta. There was no retention of radioactivity in any fetal tissue, and there was no localized concentration of caprolactam in any specific tissue.

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TOXICITY SUMMARY

Human Exposure

There are several Russian reports indicating that industrial exposures of factory workers to caprolactam resulted in various serious adverse effects on neurologic (headaches to seizures), gynecologic, obstetric, GI (nausea and vomiting), cardiovascular, dermatologic, and hepatic systems. Because these factory workers were also exposed to several organic solvents and other compounds, it is very difficult to associate these effects with caprolactam alone. Because of difficulties involved in getting full translations of these Russian articles for evaluation, these studies have not been included in this document. Caprolactam is suspected to cause contact dermatitis (allergic skin reaction) in an occupational environmental setting.

NASA Taste Tests of Caprolactam-Containing Water

The Crew and Thermal Systems Division of NASA conducted a fluid compatibility study on water stored in CWCs, the bladders of which were made of a material called Combitherm-140. Earlier results had indicated that a chemical material was leaching out of the bags, increasing the TOC in water and was traced entirely to the increased concentrations of caprolactam, one of the ingredients of the water bags. The water was of the exact composition as that of water available to crew on the ISS, water with silver biocides and minerals. The water contained silver, fluoride, calcium, and magnesium at concentrations of 0.5, 0.55, 29.4, and 4.91 mg/L, respectively. Taste tests were done at 12, 48, and 64 wk after storing ISS-quality water in CWCs using 10-12 panelists who participated in the sensory panel. The caprolactam concentrations were 4.7, 11.5, and 12.6 mg/L, respectively. The sensory panel consisted of odor, flavor, and overall acceptability. The scores were compared with the concentrations of caprolactam in the water. Water from the Johnson Space Center (JSC) drinking water and Ozarka commercial bottled water served as controls for this blinded study. Based on the results, it was concluded that at the maximum concentration of 13 mg/L of caprolactam, the water was declared acceptable for drinking. It must be stressed that the subjects tasted the water only one time and not every day over duration.

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Animal Exposure

Target organs for acute and chronic toxicities from inhalation exposures were suspected to be eye, skin, the respiratory system, neurologic system, and the GI tract (nausea and vomiting). Most of the reports on toxicity are from Russian studies using inhalation exposures on animals. In animals injected with caprolactam via iv or intraperitoneally (ip), observed effects have included reproductive/teratogenic effects, cardiovascular system effects such as hypertension and hypotension depending on the dose, anemia, and degeneration of the renal tubular epithelium (see Gross 1984).

Acute Exposures (1 d)

The National Toxicology Program (NTP) conducted a carcinogenesis bioassay of caprolactam in F344 rats and B6C3F1 mice in 1982. In the survival determination studies, rats and mice were given single gavage doses of caprolactam at 681, 1,000, 1,470, 2,150, and 3,160 mg/kg for male and female rats and 1,000, 1,470, 2,150, 3,160 and 4,640 mg/kg for male and female mice. The doses were delivered in corn oil. Deaths occurred in rats receiving more than 1,470 mg/kg (males) and 1,000 mg/kg (females). Estimated LD_{50} (the dose causing death in 50% of test subjects) values are shown in Table 6-2.

In a comparative oral toxicity study in different species of animals, a single dose of caprolactam at 1,000 mg/kg resulted in 70% lethality in mice, 60% in rabbits, 30% in guinea pigs, and 30% in rats. In all animal species, death resulted from violent epileptic convulsions, salivation, bleeding from the nostrils, respiratory arrest, tremors, and low body temperature (Savelova 1960, cited in Gross 1984).

Six hours after oral administration of a single bolus of caprolactam at 1.5 g/kg to male F344 rats, there were marked inductions of tyrosine amino transferase (TAT, L-tyrosine:2-oxoglutarate aminotransferase) and tryptophan oxygenase (TPO) (Friedman and Salerno 1980). The two enzymes are involved in the first steps of tyrosine and tryptophan catabolism, respectively. Induced activities were seen at 3 h after dosing with a maximum effect occurring at 6 h. Even 24 h postdosing, the induction of enzymes was significantly higher than in untreated controls. A dose-response study of the induction of these two enzymes after single

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TABLE 6-2 LD_{50} Values For Rats And Mice Administered Caprolactam by Oral Route[a]

Species	LD_{50} (g/kg)	Reference
Mouse (strain and sex not known)	1.20 (LD_{100})	Hohenesee 1951
Mouse (B6C3F1 male)	2.07	NTP 1982
Mouse (B6C3F1 female)	2.49	NTP 1982
Rat (strain and sex not known)	1.6	Hohenesee 1951
Rat (F344 male)	1.65	NTP 1982
Rat (F344 female)	1.21	NTP 1982

[a]For comparison, the LD_{50} dose for the mouse by ip was 0.58 g/kg and subcutaneously was 0.75 g/kg (Hohenesee 1951).

oral doses of 300, 600, 900, 1,200 and 1,500 mg/kg was conducted. Both enzymes, when measured 5 h after dosing, increased linearly with the dose up to the maximum dose tested, but TAT was induced only at doses higher than 300 mg/kg and increased by 100% at 600 mg/kg. In the case of TPO, a 100% increase was observed even at 300 mg/kg, and the activity continued to increase with doses up to 1,500 mg/kg, the maximum dose used in the studies.

In a biochemical study, 90-d-old adult female Sprague-Dawley rats (CD strain) were treated by gavage with two doses of caprolactam at 425 mg/kg (Kitchin and Brown 1989) to give a total dose in 24 h of 850 mg—one dose within 21 h of sacrifice and one 4 h before sacrifice (n = 13). Caprolactam was administered as a saline solution. Serum ornithine decarboxylase, alanine amino transferase (ALT, also called serum glutamic pyruvic transaminase, SGPT), hepatic glutathione content, hepatic cytochrome P-450 concentrations, and DNA damage (as measured by the alkaline elution of DNA) were measured. There was a statistically significant increase in the activity of SGPT (marker enzyme for abnormal liver function). Single- and double-stranded breaks in liver DNA were also measured in the study and were not different from controls. No other parameters were found to be altered.

Short-Term Exposures (2-10 d)

In another study (Friedman and Salerno 1980), caprolactam was fed to rats for 7 d as 1% and 5% of their diet. The animals were pair fed, and

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the authors estimated the doses as 1.1 g/kg/d and 1.8 g/kg/d, respectively. Liver protein synthesis was studied using incorporation of ^{3}H-leucine. Although food restriction (pair feeding) alone increased this activity by 60%, the inclusion of caprolactam blocked this increase in protein synthesis. Also, the authors reported that food-conversion efficiency (gain in weight per gram of diet consumed), was reduced by 1.8 g/kg/d. While induction of TAT was also observed in this 7-d study compared to pair-fed controls, TAT activity was lower than corresponding pair-fed controls (although, the difference was not statistically significant) (Friedman and Salerno 1980). This induction did not appear to require RNA synthesis, because RNA synthesis inhibitors did not prevent the observed induction of TAT and TPO. It must be pointed out that with a single oral bolus dose, the increased activities of TPO and TAT were several-fold higher than were observed in the week-long pair-feeding study in which the caprolactam was administered via the diet. Thus, the data will be used for the 10-d acceptable concentration (AC), which is based on the effect of caprolactam on increased amino acid catabolism, which is also reflected on the effect of protein synthesis.

Subchronic Exposures (10-100 d)

In a three-generation reproduction study by Serota et al. (1988), F344 albino rats (10 males and 20 females) were fed diets containing caprolactam at 0, 50, 250, or 500 mg/kg/d for 10 wk. Body weights of the parental generations and their offspring (in the 250 and 500 mg/kg/d groups) were significantly reduced. In this study, the authors noted that on microscopic evaluation of the kidney sections and gross lesions, there was also a slight increase in the severity of spontaneous nephropathy accompanied by the presence of granular casts in some animals of the 500-mg/kg/d group. According to the authors, this was related to the administration of caprolactam. A lowest-observed-adverse-effect level (LOAEL) of 500 mg/kg/d and a no-observed-adverse-effect-level (NOAEL) of 250 mg/kg/d were identified.

Powers et al. (1984) administered caprolactam in the feed to F344, Sprague-Dawley, and Wistar rats for 90 d at 0, 0.01, 0.05, 0.1, and 0.5% (or 0, 5, 25, 50, and 250 mg/kg/d). The authors measured glomerular filtration rates (by ^{3}H-inulin clearance) and other renal parameters (urine volume, sodium, potassium, chloride, osmolality, protein, creatinine, glucose [a proximal tubular damage marker], and alkaline phosphatase [a

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marker for kidney brush border membrane damage]). Blood samples were analyzed for blood urea nitrogen (BUN), protein, creatinine, hematocrit, sodium, potassium, and chloride. There were no significant differences in the urine parameters in any of the three strains of rats. However, a dose-related increase in BUN was observed in male F344 and Sprague-Dawley rats in the 0.1% (not statistically significant) and 0.5% caprolactam groups. If the compound is a suspect nephrotoxin, BUN is used as a marker for glomerular filtration rate (GFR); BUN rises when GFR slows. A LOAEL and a NOAEL for the increased BUN are identified as 250 mg and 50 mg/kg/d, respectively. Although there was an increase of about 15% in the ratio of kidney weight to body weight in 0.1%- and 0.5%-dose groups of male rats, this effect was not seen in female rats. Renal histopathology data showed that although eosinophilic hyaline droplets were present in the tubules of all groups including controls, they were found at a higher concentration in higher-dosage groups. In the 0.5% group, there was also an increase in the number of tubules with basophilic and hyperplasic epithelial cells. The authors suggested that a slight nephrosis might be present because of 0.5% caprolactam. Furthermore, in the 0.5% group, the frequency and degree of chronic inflammation and interstitial lymphoid cells were the highest. This occurred without any change in chronic inflammation or nephropathy. These effects were seen only in male rats of all strains, and any effect seen in females was not consistent with the dose.

Rabbits given caprolactam at 500 mg/kg/d for 6 months (mo) exhibited cellular changes in the gastric and intestinal mucosa along with lower hemoglobin values. The details of how the caprolactam was administered are not available (Savelova 1960, cited by Gross 1984).

NTP (1982) conducted a 14-d repeated-dose study in which groups of five F344 male and female rats and groups of five B6C3F1 male and female mice were fed a diet containing caprolactam at 0, 5,000, 10,000, 15,000, 20,000, or 30,000 ppm (estimated dose range of 0-4,500 mg/kg/d for rats and mice). There was no mortality in any of the species or sexes of these animals. However, NTP reported pale, mottled kidneys in all treated groups of dosed male rats in incidences of 60-100%. No histopathology was carried out to identify any specific lesions that coincide with the mottled kidneys. No such changes were seen in mice. In both the acute and 14-d studies, NTP did not look at any serum or urine clinical chemistry. Several studies have reported decreased weight gain and thus reduced body weight in animals administered caprolactam by various

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routes. When caprolactam was given in drinking water to rats (670 mg/kg/d, duration not known), weight loss was observed, and it was determined that it was due in part to decreased water intake (Goldblatt et al. 1954). Because of the lack of detailed data, this study cannot be used for AC derivations.

NTP also conducted a 13-wk subchronic feeding study (NTP 1982) in which groups of male and female F244 rats were fed diets containing caprolactam at 0, 625, 1,250, 2,500, 5,000, or 7,500 ppm (estimated dose range of 0-1,125 mg/kg for male and female rats). Male and female mice were fed diets containing caprolactam at 0, 5,000, 10,000, 15,000, 20,000, or 30,000 ppm (estimated dose range of 0-6,000 mg/kg). There were weight gain depressions and a reduction in food intake in all caprolactam-treated groups of rats. In the highest-dose group of rats, food consumption was decreased by 23% in males and by 19% in females. No compound-related adverse histopathology was noted. In mice, there were two deaths at the high dose, and as in rats, depressions in weight gain and reduced food consumption were noted.

In the early 1970s, the Central Institute of Nutrition and Food Research conducted dose-range finding and subchronic toxicity studies on caprolactam in the feed using two strains of males and females of Wistar-derived (CIVO strain) and Sprague-Dawley rats at two different concentration ranges. In the 28-d study, with caprolactam constituting 5% of the diet (about 3.8 g/kg), renal damage consisting of hyaline droplet degeneration in the epithelium of the proximal convoluted tubules was reported in both sexes of CIVO Wistar-derived rats, with minimal changes (only in males) at 1% (850 mg/kg). In the 90-d study, females of both strains of rats seemed to be less sensitive to these effects. The above mentioned effects were seen at doses greater than 0.05% in Sprague-Dawley male rats and greater than 0.3% in the Wistar-derived male rats (Wijnands and Fern 1969; de Knecht-van Eekelen and van der Meulen 1970, cited in Gross 1984). Increased kidney weights were seen only in male rats of both strains. No proteinuria could be demonstrated. The nephrotoxicity of caprolactam could be supported by the degeneration in the epithelium of the convoluted tubules of rats receiving caprolactam by ip at 50 mg/kg or 100 mg/kg for 6 mo (see Gross 1984). Because these data were presented only as an abstract and details of the data were not available for review, this data cannot be used for AC derivation. However, this study indicated that caprolactam might be a nephrotoxin.

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Chronic Exposures (>100 d)

NTP (1982) conducted a chronic feed study in which male and female F344 rats and male and female B6C3F1 mice were given caprolactam in their diet at 0, 3,750, or 7,500 ppm (estimated doses for rats of 0, 560, and 1,120 mg/kg/d) or 0, 7,500, and 15,000 ppm (estimated doses for mice of 0, 1,500, or 3,000 mg/kg/d) for a period of 103 wk. Evaluations were made of grossly visible lesions and histopathology of all organs of animals surviving at the end of 105 wk (103 wk plus 2 wk on a normal diet). Necropsies were performed. Only periodic data on body weight and food consumption were collected, and no serum or urine clinical chemistry measurements were done. From the dose-related decrement in mean body weight gain and feed consumption, it appears that the LOAEL is 1,120 mg/kg/d for the rat, and 3,000 mg/kg/d for mice (NTP 1982).

The various types of neoplasms occurring in dosed animals did not appear to be related to caprolactam ingestion. The degenerative and inflammatory lesions of the type and frequency seen in dosed rats are usually observed as a function of age in Fischer rats.

Caprolactam and Allergic Contact Dermatitis

Epsilon-aminocaproic acid, from which caprolactam is derived, has been known to cause allergic contact dermatitis (Shono 1989). Tanaka et al. (1993) presented a case report from Japan in which a 43-year (y)-old woman with a history of wearing nylon body stockings developed scaly erythema on her trunk. The lesions were resolved after she stopped wearing the body stocking. She also developed the same lesions around her waist from wearing nylon panty hose. When a patch test was done using the monomer, epsilon-aminocaproic acid 3% and 5% in petrolatum, positive reactions were obtained for both concentrations. Other cases of dermatitis have been reported in Russian factory workers involved in nylon synthesis (Kelman 1986). In one case history, a 62-y-old man who had worked in a textile factory (in the nylon drawing factory) for 29 y, presented with an 18-mo history of itchy erythematous scaly patches of eczema involving the neck, chest, and extensor limbs, although the back and flexor limbs were also involved to a lesser degree (Aguirre et al. 1995). This individual had positive reaction in the open test and patch test when caprolactam 5% aqueous patches were used. Fifteen healthy

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controls tested negative to caprolactam 5% aq. When the subject left work for 2 mo, his lesions completely resolved. These results indicate that there are only isolated case reports of allergic contact dermatitis in spite of the very wide use of nylon products. No surveys are available.

Genotoxicity

Under the International Program on Chemical Safety (IPCS), caprolactam was tested extensively in short-term mutagenicity tests, and the results were published in *Progress in Mutation Research, Vol. 5* (IPCS 1985). In addition, a special issue of *Mutation Research* (Ashby and Shelby 1989a) was devoted to studies on in vitro and in vivo genotoxicity of caprolactam (Ashby and Shelby 1989a, b). A summary of the genetic toxicology of caprolactam was published by Brady et al. (1989). A summary of caprolactam genotoxicity studies that had been present in the International Agency for Research on Cancer (IARC) monographs, Vol. 71 (1999) has been modified and included as Table 6-3.

Negative results were found in several in vitro and in vivo short-term genetic tests of caprolactam-treated bacterial, yeast, or fungal systems with or without exogenous metabolic activation as measured by gene mutations, gene conversions, or aneuploidity. In *Drosophila melanogaster*, caprolactam induced somatic cell mutations in four studies and a marginal increase in sex-linked recessive mutations in one study. No gene mutations were seen in mammalian cells exposed in vitro to caprolactam. In human lymphocyte cultures in vitro, caprolactam failed to induce sister chromatid exchanges, but it increased chromosomal aberrations.

Caprolactam treatment in vivo did not increase DNA single-stranded breaks in hepatocytes, did not induce sister chromatid exchanges, micronuclei, or chromosomal aberrations in mouse bone marrow, and did not induce morphologic abnormalities in mouse sperm. Salamone (1989) orally treated mice with various doses of caprolactam in corn oil (0, 222, 333, 500, 750, and 1,125 mg/kg) for 5 d. Sperm samples were taken 35 d after the last treatment. Abnormal sperms per 1,000 sperm were counted. There was no difference between the percentage of abnormal sperms in controls and treated groups (Henderson and Grigliatti 1992). In another study, unscheduled DNA synthesis (UDS) in F344 rat spermatocytes exposed to caprolactam was studied (Working 1989). F344 rats were gavaged with a single bolus dose of caprolactam

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in water at 750 mg/kg, spermatogenic cells were isolated at 12, 24, or 48 h after treatment, and induction of UDS was measured by quantitative autoradiography. Caprolactam did not induce UDS (see Table 6-3). In one study, when cultured human lymphocytes from one male donor and one female donor were treated at 7.5 mg/mL, slightly increased frequencies of chromosomal aberrations were seen in the cells from the male donor but not the female (Kristiansen and Scott 1989). However, in another study, Sheldon (1989b) tested caprolactam in the in vitro human lymphocyte cytogenetic assay in the presence and absence of S9 mix at dose concentrations up to 5.5 mg/mL using lymphocytes obtained from a male and a female donor. Statistically significant increases in chromosomal damage were observed in cells from both donors. Sheldon (1989b) concluded that caprolactam induces chromosomal damage in human lymphocytes in vitro at high-dose concentrations. Thus, the results are inconsistent.

Although in a few systems mutagenic responses have been documented, they are weak and occur only at very high concentrations. The results, taken as a whole, indicate that there is not strong evidence to conclude that caprolactam is genotoxic (IARC 1999).

Carcinogenicity

No epidemiologic data relevant to the carcinogenicity of caprolactam were available.

IARC has designated caprolactam as a Group 4. This is based on the studies described below. IARC states that a Group 4 “agent (mixture, exposure circumstance) is probably not carcinogenic to humans.”

A carcinogenesis bioassay of caprolactam in F344 rats and B6C3F1 mice (feed study) conducted by Litton Bionetics Inc. for NTP (Toxicology Report 214) reported that diets containing caprolactam at 3,750-7,500 parts per million (ppm) given to groups of 50 male or female F344 rats (estimated doses of 560 and 1,120 mg/kg, respectively) and 7,500 or 15,000 ppm given to groups of 50 male and female mice for 103 wk (with proper controls) did not show any carcinogenic activity (NTP 1982). Only the mean body weights of dosed rats and mice decreased compared to those of control groups in a dose-related fashion. The incidences of caprolactam-treated animals with specific-site tumors did not differ significantly from those seen in controls. For example, pituitary

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TABLE 6-3 Summary of Genotoxicity Studies on Caprolactam

Test System	End Point	Without Metabolic Activation	With Metabolic Activation	Concentrations (μg/mL unless otherwise noted) LED or HID[a]
Salmonella typhimurium TM677	Forward mutation	Negative	Negative	500
S. typhimurium TA100	Reverse mutation	Negative	Negative	500, 2,500, 5,000, 25,000
S. typhimurium TA102	Reverse mutation	Negative	Negative	5,00, 2,500
S. typhimurium TA1535	Reverse mutation	Negative	Negative	2,500, 5,000, 25,000
S. typhimurium TA1537	Reverse mutation	Negative	Negative	2,500-25,000
S. typhimurium TA1538	Reverse mutation	Negative	Negative	2,500-25,000
S. typhimurium TA98	Reverse mutation	Negative	Negative	500-25,000
S. typhimurium TA97	Reverse mutation	Negative	Negative	500-5,000
S. typhimurium D7	Gene conversion	Negative	Negative	5,000
S. typhimurium JD1	Gene conversion	Negative	Negative	2,000
Yeast				
Saccharomyces cerevisiae PV-2, PV-3	Gene conversion	Negative	Negative	1,000
S. cerevisiae D7-144	Gene conversion	Positive	Positive	400
S. cerevisiae D7	Gene conversion	Negative	Negative	2,000
S. cerevisiae D7	Homozygosis	Negative	Negative	5,000
S. cerevisiae PV-4a and 4b	Homozygosis	Negative	Negative	1,000
S. cerevisiae D6, D61M	Homozygosis	Negative	Negative	5,000, 15,000
S. cerevisiae D5	Forward mutation	Negative	Not tested	2,000
S. cerevisiae	Reverse mutation	Negative	Negative	1,000, 5,000
S. cerevisiae xv185-14C	Reverse mutation	Positive	Positive	100

(Continued)

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TABLE 6-3 Continued

Test System	End Point	Without Metabolic Activation	With Metabolic Activation	Concentrations (μg/mL unless otherwise noted) LED or HID[a]
S. cerevisiae D6, D61M	Aneuploidy	Negative	Negative	5,000
S. cerevisiae D6, D61M	Aneuploidy	Positive	Not tested	7,500
S. cerevisiae RS112 Yeast DEL assay	Recombination	Negative	Negative	0 -40,000
Fruit fly				
Drosophila melanogaster	Genetic crossing over/recombination	Negative	NA	565, 5,000 ppm in feed
D. melanogaster	Somatic mutation	Positive	NA	425, 565, 1,000 ppm in feed
D. melanogaster	Sex-linked recessive lethal mutation	Positive	NA	1,700 ppm in feed
D. melanogaster	Sex-linked recessive lethal mutation	Negative	NA	15,000 ppm injection
CHO cells, rat hepatocytes	DNA single- stranded breaks, unscheduled DNA synthesis	Negative	Negative	113-11,300
Chinese hamster V79 lung cells (in vitro)	Gene mutations (*hprt* locus)	Negative	Negative	1,000-3,000
Mouse lymphoma L5178Y cells (in vitro)	Gene mutations (*tk* locus)	Negative	Negative	200-15,000
Balb/c-3T3 cells	Gene mutation (Ouabain resistance)	Negative	Negative	15,000
CHO cells and Chinese hamster lung V79 cells, rat liver cell line	Sister chromatid exchange	Negative	Negative	1,130-17,000

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CHO cells	Micronuclei in vitro	Negative	Negative	113
Chinese hamster lung cells (V79) and CHO cells	Chromosomal aberrations	Negative	Negative	2,000-17,000
Mouse 3T3 cells, 10T1/2 cells	Cell transformation	Positive	Positive	12,500-4,570
Syrian hamster embryo cells, RLV Fischer rat cells, SHE/simian adenovirus SA7 (SA7) viral enhancement	Cell transformation	Negative	Not tested	50-5,000
Human lymphocytes in vitro	Gene mutation	Negative	Negative	8,000
Human lymphocytes in vitro	Sister chromatid exchange	Negative	Negative	1,000
Human lymphocytes in vitro	Chromosomal aberrations	Positive, negative, and questionable	Various	270 (+ ve), 4,250 (+ ve), 7,500 (?)
Rat hepatocytes (in vivo), spermatocytes	In vivo: DNA single-stranded breaks and UDS	Negative	NA	425-750 mg/kg po, 500 mg/kg ip
Pregnant mice	Mouse spot test (color spots in the offspring)	Positive	NA	500 mg/kg ip injected 9 d postconception
Pregnant mice	Mouse spot test	Positive	NA	400 mg/kg ip
Mouse bone marrow in vivo	Micronucleus test	Negative	NA	500, 700 mg/kg ip, 700 mg/kg po
Chinese hamster lung cells V79 in vitro	Cell-to-cell communication	Negative	Not tested	400, 2,250

[a]LED, if the results are positive, the highest value represents the "least effective dose"; HID, if the results are negative, the highest value represents the "highest ineffective dose" (see IARC 1999). NA = Not applicable

Abbreviations: CHO, Chinese hamster ovary cells; ip, intraperitoneally; ppm, parts per million; UDS, unscheduled DNA synthesis.

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carcinomas in male rats showed a positive trend with dose: 0/46 in controls, 0/49 at low dose, and 3/43 at high dose. In female rats, the trend was not dose specific: 2/49 in control, 1/49 at medium dose, and 1/47 in high dose. Although increased proportions of interstitial-cell tumors of the testis were seen in the highest-dose groups, this occurred at concentrations of historical controls. This response was not considered to be associated with the administration of caprolactam.

The tumor-promoting potential of caprolactam by oral feeding was also evaluated in two multistage tumor promotion studies in rats. The studies indicated that caprolactam is not a tumor promoter. Fukushima et al. (1991) conducted a complex multistage initiation-promotion study on a group of male F344/DuCrj rats. Rats received ip injections of *N*-nitrosodiethylamine followed by four ip injections of *N*-methyl-*N*-nitrosourea followed by 0.1% *N*-bis (2-hydroxy-propyl) nitrosamine in their drinking water for 2 wk. The rats were then given caprolactam at 10,000 mg/kg diet for 16 wk. In addition, five rats received vehicle without carcinogens during the first step of the treatment period and were then given 10,000 mg/kg diet for 16 wk. Histologic examination of most organs and any gross lesions and quantification of the placental form of glutathione S-transferase positive (GST-P) foci of the liver were performed after 20 wk of caprolactam treatment. Caprolactam showed no modifying effect on carcinogenesis in any organ after a 16-wk treatment.

In another study by Hasegawa and Ito (1992), male F344 rats were administered a single ip injection of *N*-diethyl nitrosamine in 9% weight per volume (w/v) of saline at 200 mg/kg. After a 2-wk recovery period, rats were given either 10,000 mg/kg diet or basal diet for 6 wk. A two-thirds partial hepatectomy was done at week 3, and animals were killed at week 8. GST-P foci of the liver were quantified. There were no significant differences in either the numbers or areas of GST-P foci between the caprolactam-treated group and the caprolactam-untreated group, indicating that there was no modifying effect by caprolactam.

Recently, caprolactam was tested in a transgenic p53-deficient mouse bioassay (p53 +/− heterozygous model) because this mouse has been found to be highly susceptible to the induction of tumors when challenged with certain carcinogens. Wild-type strain C57BLp53 (+/−) and C57BL/6 transgenic p53-deficient mice were exposed to caprolactam at 15,000 ppm in their diet for up to 26 wk. The cell-replicating fraction (RF) in the liver was determined, and no consistent and pertinent changes were present in the treated mice. Caprolactam did not produce neoplasms of any type in p53 (+/−) mice (Iatropoulos et al. 2001). The data cannot

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be considered conclusive, as diethylnitrosamine, a classical liver carcinogen, did not show any tumors in this mouse strain.

Reproductive Toxicity

Russian animal studies have reported that caprolactam administration affected both male and female reproductive performance in rats. Effects included shortening of the estrus cycle, a reduction in the number of follicles as well as an increase in corpora lutea, and damage to gonadal function. Because all papers are in Russian, with no English abstracts, we were unable to critically evaluate them and provide descriptions; therefore they could not be included in this document.

Serota et al. (1988) conducted a three-generation reproductive toxicity study in F344 albino rats on the effects of caprolactam in the diet at 0, 1,000, 5,000 and 10,000 ppm (estimated doses of 0, 50, 250, and 500 mg/kg). In this study, rats were given caprolactam in the diet, and in each generation, the rats were mated 10 wk after a dietary exposure to caprolactam. No treatment-related deaths, clinical signs, changes in reproductive performance, number of pups, or gross pathology findings was observed in the parental animals. However, decreases in body weight were observed in the P2 and P3 generations of rats, which showed that in utero and postnatal exposures of offspring resulted in body weight change at doses that did not cause substantial body weight reductions in adult P1 generations. It should further be noted that although caprolactam might be maternally toxic, fetotoxic, and developmentally toxic, it was not teratogenic. The offspring data revealed no treatment-related effect with respect to gross appearance, gross pathology, survival, number of pups, or percentage of male pups.

Unrelated to reproductive toxicity, histopathology findings indicated that in the high-dose P1 males, a slight increase in the severity of spontaneous nephropathies occasionally accompanied by granular casts was observed.

Developmental Toxicity

In a three-generation reproduction study discussed earlier (Serota et al. 1988), the body weights (on days 1, 7, and 21 of lactation) of offspring of rats administered caprolactam at 1,000, 5,000, 10,000 ppm in their diet for 10 wk revealed significantly lower mean values in the high-

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dose male and female animals of all filial generations. The mean body weights of both sexes in the mid-dose group were generally lower than those of the controls. These effects seem to be related to the ingestion of caprolactam.

In a developmental study designed for examining the teratogenic effects of caprolactam ingestion, Gad et al. (1987) reported that in rats gavaged orally with caprolactam during days 6-11 of gestation at doses of 100, 500, or 1,000 mg/kg/d, the incidence of resorption was nearly 10-fold higher in rats administered 1,000 mg/kg and mean implantation efficiency at the this dose was about 65% compared to 82% in controls. In this study, clinical signs were observed and noted during days 0, 6, 11, 15, and 20 of gestation. On day 20 of gestation, all surviving females were killed to examine the uterus, ovaries, and fetuses.

Gad et al. (1987) evaluated the developmental toxicity potential of caprolactam in rats and rabbits after an oral dose of various concentrations of caprolactam. In the first study, mature 8-wk-old female F344 rats (20/dose group) were intubated with caprolactam in distilled water at 0, 100, 500, or 1,000 mg/kg/d on gestation days 6-15, the period of organogenesis in rats. In the 1,000 mg/kg group, the maternal mortality was greater than 50%. On days 6-11, the mean body weight changes (and food consumption) in the two highest-dose groups were less than those of controls and the lowest-dose group. The mean incidence of resorption in the highest-dose group was nearly 10-fold higher than in the controls and all other dose groups. Skeletal variants (including incomplete ossification of the skull or vertebral column and the presence of extra ribs) were markedly increased among offspring from animals exposed to the highest dose (Gad et al. 1987). No other dose-related malformations or anomalies were noted among the offspring of any exposure group. For this rat oral gavage study in which doses of 0, 100, 500, and 1,000 mg/kg/d on gestation days 6-15 were used, 1,000 and 500 mg/kg/d appear to be the LOAEL and NOAEL, respectively, for fetal resorption (Gad et al. 1987).

In the rabbit study, pregnant New Zealand white rabbits were intubated with caprolactam intragastrically with water at 50, 150, or 250 mg/kg/d on gestation days 6-28. Four out of 25 rabbits dosed with 250 mg/kg had convulsions immediately. On day 29 of gestation, dead fetuses, empty implantation sites, and resorptions were recorded. Mortality was observed in the highest-dose group, and maternal body weight gain was significantly depressed. Significantly lower mean fetal weights were seen in the 150 and 250 mg/kg groups. An increased incidence of thirteenth ribs among fetuses whose mothers had been exposed to the high-

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est concentration of caprolactam was also reported (Gad et al. 1987). A NOAEL of 50 mg/kg is identified in this species. Pregnant astronauts are barred from spaceflight, and consideration of developmental effects in this section is included only for completeness of analyses (NRC 2000).

Neurologic Effects

A large number of sedative and convulsing agents are lactam derivatives, and thus there has been considerable interest in whether caprolactam can induce such activity. When administered parenterally, caprolactam caused convulsions and death. It has been shown that at high doses, caprolactam has convulsant properties (Elison et al. 1971), and the convulsion-inducing caprolactams may be acting as gamma-aminobutyric acid antagonists (Kerr et al. 1976; Skerritt et al. 1985; Kerr et al. 1986). Some of the LD_{50} studies have reported that death was caused by convulsions. Caprolactam itself is a weak agent compared to the alkylated derivatives of this lactam. Lack of effect of caprolactam when injected intraventricularly is probably because of its poor penetration of the blood brain barrier. The metabolite of caprolactam, the epsilon-aminocaproic acid, is weaker than caprolactam as a convulsant. In a Russian study, it was reported that rats administered caprolactam at a concentration of 15 mg/kg/d (probably in feed) for 2 mo developed loss of reflexes and other nonspecific effects probably related to neurologic systems (Savelova 1960, cited in Gross 1984). Because of a lack of available details on these experiments, data on neurologic end points could not be used for AC derivation.

A list of studies on the toxicity from oral intake of caprolactam is summarized in Table 6-4.

Current Regulatory and Guideline Levels from Other Organizations

The Office of the Safe Drinking Water of the U.S. Environmental Protection Agency (EPA) has not proposed a maximum contaminant level (MCL), a secondary maximum contaminant level (SMCL), nor a maximum contaminant level goal (MCLG). The Agency for Toxic Substances and Disease Registry (ATSDR) has not developed a toxicologic profile for caprolactam, and thus no minimal risk levels (MRLs) are known for oral exposure through drinking water or diet. In 1977, the Safe Drinking Water Committee of the National Research Council (NAS

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1977) concluded that in view of the paucity of data on the long-term oral toxicity of caprolactam, estimates of the toxicity of low-dose oral exposures cannot be made with any confidence. However, EPA has derived an RfD (oral reference dose) for caprolactam (EPA 1988) (see Table 6-5) using the reductions in body weights of offspring in the three-generation reproduction study with rats (Serota et al. 1988).

Rationale

The following discussion provides a rationale for proposing SWEG values for caprolactam in drinking water for 1 d, 10 d, 100 d, and 1,000 d (see Table 6-6). The values listed were based on ACs for each duration according to *Methods for Developing Spacecraft Water Exposure Guidelines* (NRC 2000). An intraspecies factor is not usually used, because astronauts come from a homogenous healthy population and there is no evidence of a group of healthy persons having excess susceptibility to caprolactam. However, if there are known variations in the metabolism of the compound or susceptibility to the compound regardless of health status, then an individual factor could be applied. Although developmental data are included in the document under the toxicity section, it is only for comprehensiveness, and the data will not be used in setting SWEGs, because pregnant astronauts are barred from spaceflight.

A search of the literature indicates there are few case reports and no survey reports of known allergic reactions to caprolactam in an occupational setting. There are no reports of such allergic reactions from ingestion. The long-term oral studies on animals have not evaluated this end point. A review of the caprolactam ingestion studies indicates that there are very few toxicity end points. Most of the reported studies indicate reduced food intake and decreased body weight gain at high doses. It is not clear if the reduced food intake noted in many studies included here is because of taste aversion (palatability problem) or because of neurologic effects.

Approaches Used by Other Organizations to Derive Their Values

EPA derived the RfD for caprolactam from the results of the three-generation reproduction study by Serota et al. (1988). This study has been described in detail under “Reproductive Toxicity.” In this study, male and female F344 rats were fed diets containing caprolactam at 0,

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TABLE 6-4 Toxicity Summary

Dosing Mode	Dose and Duration	Species	Effects	Reference
Gavage	1.2 g/kg, once	Mouse	Death; LD_{100}	Hokenesee 1951
Gavage (in corn oil)	0, 681, 1,000, 1,470, 2,150, or 3,160 mg/kg	Rat, male and female	Death; LD_{50} of 1.65 g/kg	NTP 1982
Gavage (in corn oil)	0, 1,000, 1,470, 2,150, 3,160, or 4,640 mg/kg	Mouse, male and female	Death; LD_{50} of 2.07-2.49 g/kg	NTP 1982
Gavage	1.6 g/kg, once	Rat	Death; LD_{50} of 1.6 g/kg	Hohenesee 1951
Gavage	1,000 mg/kg, once	Rat, mouse, guinea pig, rabbits	Lethality 30% in rats, 70% in mice, 30% in guinea pigs, and 60% in rabbits	See Gross 1984
Oral bolus	1.5 g/kg, once	Rat, male	Induction of TAT and TPO, max at 6 h	Friedman and Salerno 1980
Oral bolus	0, 300, 600, 900, 1,200, or 1,500 mg/kg	Rat, male	Induction of TAT and TPO, only above 300 mg/kg	Friedman and Salerno 1980
Gavage	425 mg/kg, two doses in 1 d	Rat, female	Increased SGPT	Kitchin and Brown 1989
Diet	1% and 5% (1.0 g/kg and 1.8 g/kg measured), pair fed for 7 d	Rat, male	Inhibition of protein synthesis; significant decrease in food consumption; body weight reductions; induction of TAT and TPO	Friedman and Salerno 1980
Diet	0, 250, 500, 750, 1,000, or 1,500 mg/kg, 14 d	Rat, male and female	No deaths; pale, mottled kidneys in all dosed groups of male rats; incidences of 60-100%	NTP 1982
Diet	0, 250, 500, 750, 1,000, or 1,500 mg/kg, 14 d	Mouse, male and female	No deaths; no compound-related effects	NTP 1982

(Continued)

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TABLE 6-4 Continued

Dosing Mode	Dose and Duration	Species	Effects	Reference
Gavage	0, 100, 500, or 1,000 mg/kg/d, gestation days 6-15	Rat, female	Developmental toxicity study: fetal resorptions at high dose but none at 500 mg/kg; no skeletal anomalies	Gad et al. 1987
Gavage	0, 50, 150, or 250 mg/kg/d, gestation days 6-28	Rabbit, female	Developmental toxicity study: maternal deaths at 250 mg/kg; increased incidence of 13th ribs; fetotoxicity as lower fetal weights at 150 and 250 mg/kg group.	Gad et al. 1987
Diet	0, 625, 1,250, 2,500, 5,000, or 7,500 ppm (for dose rates see text), 90 d	Rat, male and female	No compound-related deaths; reduced body weight gains; about 20% decrease in food consumption	NTP 1982
Diet	0, 5,000, 10,000, 15,000, or 20,000, 30,000 ppm (for dose rates see text), 90 d	Mouse, male and female	Deaths in the two high-dose groups; very severe reductions in body weight (25-63%); no compound-related histopathologic effects seen; food consumption not reported	NTP 1982
Diet	500 mg/kg, 90 d	Rat (two strains)	Degenerative changes in the convoluted tubular epithelium (hyaline droplets)	Gross 1984
Diet	0, 100, 500, or 1,000 mg/kg, three-generation reproductive study, 3- to 10-wk treatment phases	Rat, male and female, young	Reduced food consumption at high doses in all three generations; reduced pup weights	Serota et al. 1988

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Diet	0, 3,750, or 7,500 ppm (estimated doses included in the document), 2 y	Rat, male and female	Food consumption reduced 20-30%; no significant increases in various neoplasms; increase in trend (positive with respect to dose) of male interstitial testicular tumors, also in pituitary carcinomas; NTP declared this not significant	NTP 1982
Diet	0, 7,500, or 15,000 ppm (estimated doses included in the document), 2 y	Mouse, male and female	No increase in severity or frequency of degenerative changes in the lesions noted in dosed versus controls; alveolar/bronchiolar adenomas or lymphomas or leukemia of hematopoietic systems showed a negative trend with respect to dose	NTP 1982

Abbreviations: LD_{50}, the dose lethal to 50% of test subjects; LD_{100}, the dose lethal to 100% of test subjects; NTP, National Toxicology Program; SGPT, serum glutamic-pyruvic transaminase, also known as serum alanine aminotransferase; TAT, tyrosine aminotransferase; TPO, tryptophan oxygenase.

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TABLE 6-5 Current Regulated/Recommended Limit Values

Limit Values	Action	Data Source
RfD	0.5 mg/kg/d	IRIS 1998
Cancer grouping	Group D[a]	IRIS 1998
IARC Cancer grouping	Group 4[b]	IARC 1986

[a]Group D = Not classifiable as to human carcinogenicity.
[b]Group 4 = No evidence of carcinogenicity. IARC's decision is chemical specific and does not consider routes of exposure.
Note: EPA did not develop a MCL, SMCL, MCLG, or any drinking water health advisories (HA) for 1 d, short-term, or long-term exposure. Similarly, ATSDR also did not derive any MRL for acute, intermediate, or long-term durations.

TABLE 6-6 Spacecraft Water Exposure Guidelines for Caprolactam[a]

Duration	SWEG (mg/L)	Toxicity End Point	Reference
1 d	200	Hepatotoxicity	Kitchin and Brown 1989
10 d	100	Renal effects (adopted 100-d SWEG)[b]	Powers et al. 1984
100 d	100	Increased BUN and abnormal renal histopathology	Powers et al. 1984
1,000 d	100	Based on low toxicity, used 100-d AC	Adopted 100-d AC; no suitable data available

[a]These values will not be protective of individuals who may be allergic to nylon who may show hypersensitivity to caprolactam ingestion. No data is available to determine whether any safety factor would be needed.
[b]The rationale for adopting the 100-d SWEG as 10-d SWEG has been discussed at the end of the 100-d AC derivation section of this chapter.

1,000, 5,000, or 10,000 ppm (0, 50, 250, or 500 mg/kg/d as calculated by EPA) for three generations. Mean body weight and food consumption were reduced in both parental generations of the 250- and 500-mg/kg/d groups. Body weight of offspring was reduced at these dietary concentrations. Histopathology indicated a slight increase in the severity of nephropathy in males in the high-dose group of the first parental generation. No adverse effects were noted at 1,000 ppm (50 mg/kg/d); therefore, 1,000 ppm was chosen as the NOAEL to serve as the basis for the RfD. Thus, EPA did not use any specific end point for obtaining this NOAEL. A modifying factor of 100 for uncertainty was used that included 10 for the species factor and 10 for uncertainty in the threshold for sensitive humans.

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Thus, an RfD of 0.5 mg/kg/d or 35 mg/d for a 70 kg human was derived. If we were to extend the calculations for use by NASA, an AC for more than 1,000 d would be as follows:

$$(\text{RfD} \times 70 \text{ kg}) \div 2.8 \text{ L/d} = 12.5 \text{ mg/L}.$$

It should be noted that EPA did not use any time factor even though the three-generation exposure in this study consisted of a total of 30 wk, or 210 d, and not a lifetime for rats or mice.

1-d AC

The data on sensory threshold values measured for caprolactam cannot be used. The olfactory threshold was found to be 0.3 mg per cubic meter (m^3). Caprolactam is not expected to vaporize out of water because of its low vapor pressure at room temperature. Therefore, an odor threshold value is not calculated. NASA taste tests indicated that at least up to a concentration of 13 mg/L, water containing caprolactam will not be objectionable to drink. It may be overly conservative to use this value as a 1-d AC because such data for higher concentrations are not available.

TAT and TPO are two enzymes that are involved in the first steps of tyrosine and tryptophan metabolism. After oral administration of a bolus of caprolactam at 1.5 g/kg to male F344 rats, there were marked inductions of TAT and TPO activities at 3 h with maximum inductions at 6 h (Friedman and Salerno 1980). Although these data pertain to the induction of two important amino acid metabolizing enzymes by caprolactam, whether it can be considered an adverse effect in this acute exposure is questionable. Thus, the data were not used for deriving a 1-d AC.

A second study that was considered for the 1-d AC was that of Kitchin and Brown (1989). Oral ingestion of caprolactam at 425 mg/kg (twice) by gavage resulted in a significant increase in the activity of ALT (30%) over controls. Clinically, an increase in this enzyme indicates hepatocyte necrosis or damage. While 850 mg/kg appears to be a LOAEL, a NOAEL was not identified, because there were no dose-response data. However, with the changes in the liver protein metabolism enzymes, TAT and TPO, reported by Friedman and Salerno (1980), in conjunction with these changes reported by Kitchin and Brown (1989), it can be concluded that caprolactam resulted in adverse effects on liver. Because the investigators administered one dose at 21 h and one more dose 4 h before sacrifice, the effect may have been from the combined dose of 850 mg/kg. Therefore, this dose will be used in deriving an AC.

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The 1-d AC was calculated based on hepatotoxicity as follows:

$$(850 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 10 \times 2.8 \text{ L/d}) = 200 \text{ mg/L}$$
(rounded from 212.5 mg/L),

where
850 mg/kg = LOAEL;
70 kg = nominal body weight;
10 = LOAEL to NOAEL;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption.

10-d AC for Ingestion

Data from Friedman and Salerno (1980), in which rats fed caprolactam in their diet for 7 d at doses of 1.1 and 1.8 g/kg (calculated by the authors), showed inhibition of liver protein synthesis and also induction of liver TPO measured at the end of the treatment period. Diet restriction increased protein synthesis by at least 63%, but a dose of 1.8 g/kg blocked this increase. Also, TPO activity was found to be increased at the 1.1 g/kg dose. Therefore, 1.1 g/kg is used as the LOAEL for the induction of this enzyme. Although we considered that the change in activity of the enzyme may be an adaptive response to a single dose, the change in conjunction with an inhibition of protein synthesis allows us to use these data to derive a 10-day AC after applying a time extrapolation factor.

10-d AC for adverse effect on amino acid metabolism is calculated as follows:

$$(1{,}100 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 10 \times 2.8 \text{ L/d} \times [10 \text{ d}/7 \text{ d}]) =$$
200 mg/L (rounded from 193 mg/L),

where
1,100 mg /kg = LOAEL;
70 kg = nominal body weight;
10 = LOAEL to NOAEL;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
10 d/7 d = time extrapolation factor.

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100-d AC for Ingestion

Powers et al. (1984) studied three strains of male rats fed diets containing caprolactam at doses of 0, 0.01, 0.05, 0.1, 0.5, and 2.5% (or 0, 1, 10, 50, and 250 mg/kg/d) for 90 d. A dose-related increase in BUN was observed in male F344 and Sprague-Dawley rats at 0.1% and 0.5% dose groups but was statistically significant only in the 0.5% dose group. If the compound is a suspected nephrotoxin, BUN is used as a marker for GFR; BUN rises when GFR slows. A LOAEL and NOAEL for increased BUN are identified as 250 and 50 mg/kg/d, respectively. Although there was an increase of about 15% in the kidney weight to body weight ratio in the 0.1% and 0.5% dose groups of male rats, this effect was not seen in female rats. The increase in the ratio is because of an absolute increase in kidney weight, probably indicating hypertrophy of the kidney. Renal histopathology data revealed that, although eosinophilic hyaline droplets were present in the tubules of all groups including controls, this was found at a higher concentration in higher-dosage groups. Although the numbers were minimal, the number of rats affected increased with the dose of caprolactam. These effects were seen only in male rats of all strains, and any effect seen in females was not consistent with the dose.

Thus, a 100-d AC for renal toxicity can be derived as follows:

$$(50 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times [100 \text{ d}/90 \text{ d}]) = 100 \text{ mg/L (rounded from 112 mg/L)},$$

where
50 mg/kg/d = NOAEL for BUN;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
100 d/90 d = time extrapolation factor.

Another study that was considered for calculating a 100-d AC was Serota et al. (1988), a three-generation reproductive study in which rats were fed diets containing caprolactam at 0, 50, 250, or 500 mg/kg/d. In this study, the authors noted that there was a slight increase in the severity of nephropathy on histologic examination of the male rats of the 500 mg/kg/d group. The duration of each generation was 10 wk (70 d). Body weights of the parental generations and their offspring (of the 250 and 500 mg/kg/d dose groups) were significantly reduced. The reported slight increase in severity in spontaneous nephropathy in exposed groups, ac-

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companied by the presence of granular casts in some rats, was used as a toxicologic end point. A NOAEL of 250 mg/kg/d for severity of nephropathy was identified. The developmental effects observed in the study are not the basis for the 100-d AC.

Thus, a 100-day AC for nephropathy can be derived as follows:

$$(250\ \text{mg/kg} \times 70\ \text{kg}) \div (10 \times 2.8\ \text{L/d} \times [100\ \text{d}/70\ \text{d}]) = 438\ \text{mg/L (rounded)},$$

where
250 mg/kg = NOAEL;
70 kg = nominal body weight;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
100 d/70 d = time extrapolation factor.

NASA's Position and Rationale for 10-d and 100-d ACs

The SWEG for the 100-d duration will be accepted as the SWEG for 10-d duration, instead of the calculated value of 200 mg/L as 10-d AC. The reasons are outlined below.

The 1-d AC was based on hepatotoxic effects derived from a LOAEL of 850 mg/kg. A factor of 10 was used for LOAEL to NOAEL. The 10-d AC was based on adverse effects on amino acid catabolism and protein synthesis (Friedman and Salermo 1980). This data was used to calculate a 10-d AC of caprolactam at 200 mg/L. In this study, hepatotoxic indices were not measured. It is quite possible that 10 d of continuous ingestion of water containing caprolactam at 200 mg/L will lead to hepatoxicity, because it approaches the LOAEL for hepatotoxicity observed in 1 d and will not provide any margin of safety. The rationale for using 100 mg/L as the 10-d AC and for adopting the 100-d AC obtained using the Powers et al. (1984) study is as follows: NTP (1982) conducted a carcinogenesis bioassay of caprolactam in the feed for 103 wk in F344 rats and B6C3F1 mice. The study also included a 1-d gavage and a 14-d feed protocol. In these protocols, the focus was on food and water consumption, body weight gain, and survival rates in addition to general clinical observations and necropsy. NTP reported that at the end of 2 wk, there were no deaths; however, pale, mottled kidneys occurred in all groups of dosed rats in incidences of 60-100%. NTP did not discuss the significance of this observation. The calculated dose rates for male and female rats for the lowest-dose group that showed the pale, mottled kid-

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neys are about 860 mg/kg/d, and 750 mg/kg/d respectively. Although the biologic significance of mottled kidney is not clear, it appears that the kidney is somewhat affected. This is somewhat in concordant with the observations from the 90-d study of Powers et al. (1984) that showed that the kidney is a target organ. NTP did not conduct any clinical chemistry on blood or urine. Therefore, it was decided to adopt the 100-d AC derived from the Powers et al. (1984) study for 10 d, because it would offer enough margin of safety for kidney effects and for hepatotoxicity. In addition, the 28-d and 90-d rat subchronic caprolactam-feed study conducted by the Central Institute of Nutrition in the Netherlands reported changes in the kidney resulting from caprolactam feeding. However, as details of the study were unavailable as a full report, the data could not be critically evaluated for NASA in the determination of 10-d or 100-d AC.

1,000-d AC for Ingestion

The 2-y carcinogenesis bioassay study sponsored by NTP (1982) is the only study that can be considered for 1,000-d AC derivation. In this study, rats and mice were exposed to caprolactam in their diet for 2 y (male and female F344 rats to 0, 3,750, or 7,500 ppm, and male and female B6C3F1 mice to 0, 7,500, or 15,000 ppm). These mean concentrations for males and females corresponded to estimated doses of 0, 560, and 1,120 mg/kg/d for rats and 0, 1,500, and 3,000 mg/kg/d for mice. The feed consumption in the highest-dose groups was only 70-80% of that of controls, and thus, body weights were lower. The NRC committee had recommended in the past that body weight changes should not be used to set ACs when it is known that changes occurred because of reduced food consumption. According to the NTP report (NTP 1982), the frequency of the large number of degenerative, proliferative, and inflammatory lesions encountered in caprolactam-treated rats was not different from that in control rats. However, there were some observations of toxicologic concern. One is that in rats, testicular interstitial cell tumors were observed in increasing proportions as a function of dose, even though they occur historically at 80%. The Cochran-Armitage linear trend analysis was statistically significant. Also, carcinomas of the pituitary were observed in increased proportions in high-dose male rats. The Cochran-Armitage linear trend analysis was statistically significant in the positive direction. However, the committee recommended that NTP data could not be used to set an AC based on non-neoplastic lesions, because

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NTP concluded that it did not find any evidence of neoplastic or non-neoplastic lesions related to dietary caprolactam.

The 100-d AC was adopted as the 1,000-d AC in the absence of sufficient data for long- term ingestion. The use of the 100-d AC without any time factor for 1,000 d was justified by the fact that caprolactam does not accumulate in the body and is excreted efficiently. Furthermore, if one were to use the lowest dose (560 mg/kg/d) used in the NTP 2-y carcinogenicity study for rats, which did not produce any compound-related long-term adverse tissue pathology, and calculate an AC using only the species extrapolation factor, a concentration of 1,400 mg/L would be obtained as the AC. Thus, the use of 100 mg/L as the 1,000-d AC is justified and conservative. See Table 6-7 for a summary of all ACs and final SWEGs for all durations.

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TABLE 6-7 Acceptable Concentrations (ACs) for Caprolactam in Water

Toxicity End Point	LOAEL or NOAEL (mg/kg/d)	Species	Modifying Factors: To NOAEL	Species	Exposure Time	Space-flight	Acceptable Concentrations (mg/L): 1 d	10 d	100 d	1,000 d	Reference
Hepatotoxicity	LOAEL = 850	Rat	10	10	1	1	200				Kitchin and Brown (1989)
Increased amino acid catabolism	LOAEL = 1,100	Rat	10	10	10 d/7 d	1		200			Friedman and Salerno (1980)
Renal toxicity: increased BUN; renal histopathology	NOAEL = 50	Rat	1	10	100 d/ 90 d	1		100 (adopted 100-d AC)	100		Powers et al. (1984)
Increased severity of nephropathy	NOAEL = 250	Rat	1	10	100 d/ 70 d	1			438		Serota et al. (1988)
No chronic toxicity data										No suitable data available	
SWEG[a]							200	100	100[b]	100[c]	

[a]Tabulated values may not protect against water that has a taste to it. The levels are protective against adverse health effects.
[b]Based on evaluation of summary of ACs, the 100-d AC of 100 mg/L will be used for 100 and 1,000 d.
[c]No suitable data available for deriving 1000-d AC.
Note: The rationale for adopting the 100-d SWEG as 10-d SWEG has been discussed at the end of the 100-d AC derivation section in this document. Also, these values will not be protective of individuals who may be allergic to nylon who may show hypersensitivity to caprolactam ingestion. No data is available to determine or justify whether any safety factor would be needed.

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7

Formaldehyde

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OCCURRENCE AND USE

Formaldehyde (HCHO) is an organic substance that is widely used and can occur as a result of both natural and anthropogenic processes (see Table 7-1). At room temperature, formaldehyde exists as a colorless gas with a distinct and pungent odor. Formaldehyde has been widely used since the early 1900s in commercial and industrial applications. Much of its use centers around the manufacturing of plastics and resins (such as urea-formaldehyde resins) or applications in the production of chemical intermediates. Other diverse uses include application as a preservative for biologic samples, an ingredient in shampoos and household cleaning agents, use as an agricultural fumigant, and use in various products as an antimicrobial agent. In addition to these numerous commercial

TABLE 7-1 Physical and Chemical Properties

Formula	HCHO
Synonym	methanal, formic aldehyde, methyl aldehyde
CAS registry no.	50-00-0
Molecular weight	30.0
Boiling point	−19.5°C
Melting point	−92 °C
Water solubility	55 g/100 mL
Vapor pressure	10 mm Hg at −88°C
Vapor density	1.08
Specific gravity	0.81 at 20°C
Log K_{ow}	0.35

H
|
C = O
|
H

Source: Data from ATDSR 1999.

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applications, formaldehyde is a common combustion byproduct and can be measured at low concentrations in almost all ambient air samples. Although it is often thought of as strictly an environmental pollutant, formaldehyde is a normal metabolic product formed endogenously from the breakdown of serine (Restani and Galli 1991) and serves as an intermediary cellular metabolite in the biosynthesis of purines, thymidine, and several amino acids (ATSDR 1999). Formaldehyde is also a normal byproduct from the metabolism of many *N*-methyl substituted drugs and other xenobiotics (Dahl and Hadley 1983). Endogenous concentrations of formaldehyde in blood are estimated at 2.6 micrograms per gram (µg/g) for humans (Heck et al. 1985). Dietary exposure is also relevant because formaldehyde is found naturally (and even as a food additive) in some animal products, fruits, vegetables, cheeses, seafood, and other commodities (Restani and Galli 1991).

Formaldehyde is a common contaminant that may be discharged to the spacecraft environment from both direct and indirect sources. Its occurrence in water is also closely tied to releases to air, thus providing a good example of the interdependency of these media onboard the space shuttle and International Space Station (ISS). Formaldehyde is occasionally detected, although at relatively low concentrations (1-3 µg per liter [L]), in drinking water provided to the ISS from ground-based sources (Schultz 2004). However, much higher concentrations of formaldehyde (up to 9,000 µg/L) have been measured in the humidity condensate onboard the ISS (Schultz 2004).

The relatively high concentrations of formaldehyde in this condensate are largely attributable to the number of sources that may release formaldehyde, as well as to its chemical and physical properties. Formaldehyde is one of the most common indoor air pollutants. Formaldehyde can be off-gassed from textiles, foam insulation, resins, epoxies, and a myriad of other substances commonly encountered in the indoor environment (both ground based and in orbit). Furthermore, formaldehyde can be formed through secondary reactions of other indoor air pollutants (for example, methane, pinene), especially in the presence of higher temperatures and/or chemical oxidizers. Studies by the National Aeronautics and Space Administration (NASA) have frequently observed formaldehyde releases from Delrin and other commonly used industrial materials (James 2004). Once present in the air, the high water solubility of formaldehyde relative to its vapor pressure (a relationship expressed by its Henry’s law constant) results in a significant removal of formaldehyde from the air by condensing moisture. Given this interdependency, exposure to formaldehyde through drinking water ingestion is a relevant exposure pathway on-orbit, despite ground-based experiences that suggest

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that this pathway might be insignificant in comparison to inhalation exposures.

TOXICOKINETICS

For the purposes of this document, the toxicokinetics of ingested formaldehyde will be highlighted. As with any chemical, a thorough understanding of its absorption, metabolism, distribution, and elimination is crucial to gaining a perspective on its toxicity and to the setting of appropriate spacecraft water exposure guidelines (SWEGs).

Absorption

Formaldehyde is readily absorbed into gastrointestinal (GI) mucosal cells following oral exposures (IARC 1995). However, it is difficult to distinguish the fraction of formaldehyde that is ultimately absorbed across the GI tract (ATSDR 1999). As evidenced by rapid increases in formic acid concentrations in the blood (within minutes per observations of Eells et al. 1981), absorbed formaldehyde is either metabolized to formate in the GI tract (which is then quickly absorbed), or formaldehyde is quickly absorbed and metabolized to formate in the blood (Galli et al. 1983; Burkhart et al. 1990). No studies were found that were able to distinguish the oral absorption kinetics of formaldehyde apart from the kinetics of its metabolism to formic acid.

Metabolism

Although metabolism rates are thought to vary somewhat across species, the basic sequence of metabolism is the same in all mammalian systems (Pandey et al. 2000). Figure 7-1 describes the basic metabolic process by which formaldehyde is converted to formate/formic acid and ultimately to carbon dioxide and water. The conversion of formaldehyde to formic acid is mainly catalyzed by the formaldehyde dehydrogenase (FDH)/alcohol dehydrogenase 3 complex (with glutathione [GSH] and nicotinamide adenine dinucleotide [NAD+] acting as cofactors), because of its specific affinity for formaldehyde, although other enzyme systems also have the capability to oxidize aldehydes (Teng et al. 2001). FDH (which is also defined as alcohol dehydrogenase [ADH3] according to

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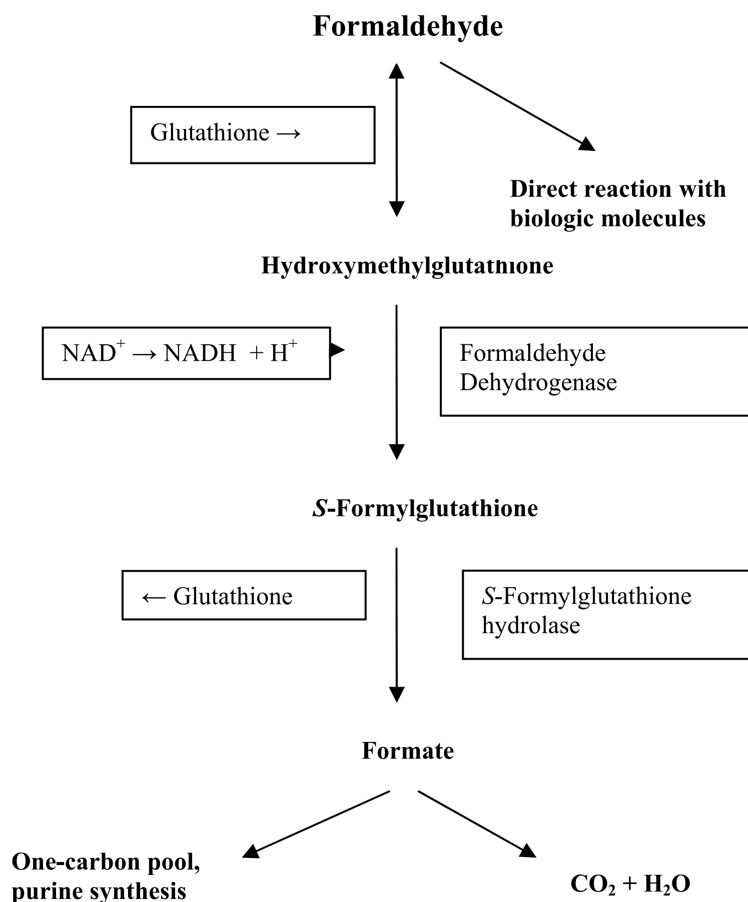

FIGURE 7-1 Metabolism and biologic fate of formaldehyde. Source: Modified figure printed with permission; copyright 1995, World Health Organization.

current nomenclature) is present in almost all tissues but is especially prevalent in the liver and erythrocytes and is very efficient in acting upon endogenous or exogenous formaldehyde. Researchers have observed polymorphisms in the ADH3 gene that encodes FDH/ADH3 among several population groups, and this variability suggests that the capacity to metabolize formaldehyde will likely exhibit some interindividual variation (Hedberg et al. 2001). Upon metabolism from formaldehyde, formic acid can then be metabolized to carbon dioxide and water, although much more slowly than the FDH/ADH3-dependent metabolism of formaldehyde (55 minute [min] plasma half-life as compared to 1 min with

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formaldehyde) (Stratemann et al. 1968). This metabolic step relies upon tetrahydrofolate-dependent enzymatic action (Pandey et al. 2000). Alternately, formic acid can be excreted as a salt in the urine or can enter the one-carbon metabolic pool (Restani and Galli 1991) where it can be incorporated as a methyl group into nucleic acids and proteins (Thrasher and Kilburn 2001).

Distribution and Elimination

In rodents, Buss et al. (1964) observed that about 40% of radiolabeled formaldehyde that was administered orally (7 milligrams per kilogram [mg/kg] of body weight dose) was eliminated as carbon dioxide within 12 hours (h), while another estimated 10% was eliminated in urine and 1% in feces. Incorporation into macromolecules was postulated by the authors to account for much of the remaining radiolabeled carbon. Formaldehyde is not generally expected to be absorbed into the bloodstream and carried as an unmetabolized molecule to other organ systems. Thus, distribution and excretion are not thought to be significant considerations with formaldehyde exposure and toxicity. Instead, its rapid metabolism and reactivity suggest that it will either be metabolized or it will exert its toxic effects locally at the point of exposure. It is postulated that the toxicity of formaldehyde is evidenced when exposure is of sufficient magnitude that this detoxification mechanism for formaldehyde is saturated and the reactive formaldehyde molecule is allowed to exert its effects on local tissues and macromolecules (for example, proteins and DNA) (Heck and Casanova 1990).

TOXICITY SUMMARY

Given its commercial importance and potential for human exposure, formaldehyde has been the subject of a relatively large amount of toxicologic research. Much of this work has been focused on investigations of inhalation exposures to formaldehyde, and the literature on its oral toxicity is not quite as robust. However, there are a number of studies (both human and animal) focusing on a variety of toxicologic end points and exposure durations that provide a basis for setting SWEGs for formaldehyde. The following discussion is not meant to represent a comprehensive review of all available data on the toxicity of formaldehyde. Instead, the focus is on those studies that appear to be the most applica-

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ble to SWEG development (that is, exposure to formaldehyde through drinking water).

The discussion of the oral toxicity of formaldehyde is organized into four different categories based on the duration of the exposures: acute (1-5 days [d]), short-term (6-30 d), subchronic (30-180 d), and chronic (180 d to lifetime). Data for these individual exposure durations generally correspond to timeframes relevant to the development of 1-, 10-, 100-, and 1,000-d SWEGs, respectively. Complete descriptions of studies cited in the discussion below are provided in Table 7-2. It is important to note that some of the data discussed below came from studies involving gavage exposures to formaldehyde. These results should be viewed with some caution because gavage exposures may have a greater potential to overwhelm formaldehyde metabolic capabilities, resulting in greater distribution and toxicity of formaldehyde than might be experienced with episodic drinking water exposures.

Acute Toxicity (1-5 d)

Several human cases of accidental, homicidal, or suicidal ingestion of formaldehyde have been reported in the literature (Eells et al. 1981; Kochhar et al. 1986; Burkhart et al. 1990). Eells et al. (1981) reported on the death of a 41-year [y]-old woman following the intentional ingestion of formaldehyde (620 mg/kg). The woman was cyanotic and severely hypotensive when admitted to the emergency room and died within 28 h of admission. Observed toxicologic effects prior to death included renal failure, abdominal pains, and symptoms of metabolic acidosis. Similar effects were reported by Burkhart et al. (1990) after the ingestion of formaldehyde at 520 mg/kg in a suicide attempt. The victim complained of difficulty breathing and severe abdominal pains before experiencing a significant drop in blood pressure and slipping into a coma. Upon autopsy, the stomach of the victim was reported to be hard, white, and leathery. In a nonfatal case, Kochhar et al. (1986) described a 26-y-old woman who accidentally ingested formaldehyde at 230 mg/kg. The patient experienced ulceration and sloughing of the soft palate and posterior pharyngeal wall and epiglottis, as well as ulceration of the stomach and upper GI tract. Mild tachycardia was also observed following the ingestion of formaldehyde. In summary, acute oral human exposures to formaldehyde have resulted in adverse respiratory, cardiac, GI, metabolic, renal, and neurologic effects.

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TABLE 7-2 Toxicity Summary

Dose/Route	Exposure Duration	Species	Effects	Reference
Acute Exposures				
520 mg/kg/dm, oral	One event	Human, male (n = 1)	Death; decreased blood pressure; GI irritation; cardiac arrest; acidosis	Burkhart et al. 1990
620 mg/kg/d, oral	One event	Human, female (n = 1)	Death; decreased blood pressure; GI irritation; acidosis; loss of consciousness	Eells et al. 1981
230 mg/kg/d, oral	One event	Human, female (n = 1)	Ulceration of esophagus mucosa; GI irritation; tachycardia	Kochhar et al. 1986
Short-Term Exposures				
185 mg/kg/d (LOAEL), gavage	Gestation days 6-15	CD-1 mice, female	Increased mortality (22 of 34 mice); no observed teratogenic effects	Marks et al. 1980
40 ppm (NOAEL), formaldehyde vapor	Gestation days 6-20, 6 h/d	Sprague-Dawley rats, female (n = 25)	No reproductive or developmental effects observed at concentrations of 0, 5, 10, 20, and 40 ppm; maternal toxicity in the 40-ppm group was observed	Saillenfait et al. 1989
10 ppm (NOAEL), formaldehyde vapor	Gestation days 6-15, 6 h/d	Sprague-Dawley rats, female (n = 25)	No reproductive or developmental effects observed with exposures of 0, 2, 5, or 10 ppm	Martin 1990
10,000 mg/L (as HMT) (NOAEL), drinking water	2 wk (exposures to dams continued through gestation and lactation)	Wistar rats, male and female	No malformations noted in 124 pups born to females exposed to 0 or 1% hexamethylenetetramine (HMT)	Della Port et al. 1970
80 mg/kg/d (LOAEL), gavage	4 wk	Wistar rats, male	Reduced weight gain	Vargova et al. 1993

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20 mg/kg/d (LOAEL), gavage	4 wk	Wistar rats, male	Dose-dependent reduced antibody response	Vargova et al. 1993
125 mg/kg/d (LOAEL), 25 mg/kg/d (NOAEL), drinking water	4 wk	Wistar rats, male and female	Significant hyperkeratosis of the stomach; focal gastritis; bodyweight reductions; decreases in blood protein and albumin concentrations; increased kidney weight	Til et al. 1988
30 mg/L (0.003 %) (LOAEL), auxiliary spray test	2 wk	Humans	No ACD other than very mild dermatitis observed (2/13 subjects) in formaldehyde-sensitized individuals at this concentration	Jordan et al. 1979
300 mg/kg/d (LOAEL), drinking water	24 mo, but effects observed after several wk	Wistar rats, male and female	Mortality as early as 9 d, reduced weight gain and food/water intake	Tobe et al. 1989
82 mg/kg/d (LOAEL), drinking water	24 mo, but effects observed after 1 wk	Wistar rats, male	Decreased bodyweight and liquid consumption	Til et al. 1989
Subchronic Exposures				
150 mg/kg/d (rats) (LOAEL), drinking water. 100 mg/kg/d (dogs) (LOAEL), 50 mg/kg/d (NOAEL), oral in feed	90 d	Sprague-Dawley rats, male and female; beagles, male and female	Reduced weight gain; reduction in water/feed consumption	Johannsen et al. 1986
9.4 mg/kg/d (NOAEL), oral in feed	52 d, gestation days 4-56	Beagles, female	No effect on pregnancy rate, weight gain, length of gestation, or malformation	Hurni and Ohder 1973

(Continued)

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TABLE 7-2 Continued

Dose/Route	Exposure Duration	Species	Effects	Reference
Chronic Exposures				
258 mg/kg/d (LOAEL), drinking water	32 wk	Wistar rats, male	Reduced weight gain; gastric ulceration; papillomas; regenerative mucosa	Takahashi et al. 1986
82 mg/kg/d (LOAEL), 15 mg/kg/d (NOAEL), drinking water	24 mo	Wistar rats, male	Reduced body weight; thickened limiting ridge; gastric ulceration; mucosal thickening	Til et al. 1989
300 mg/kg/d (severe effects), 50 mg/kg/d (LOAEL), 10 mg/kg/d, (NOAEL), drinking water	24 mo	Wistar rats, male and female	Significant mortality; non-neoplastic gastric lesions; hyperplasia of fundic mucosa; reduced weight gain; lower food and water intake	Tobe et al. 1989
(1) 188 mg/kg/d (2) 125 mg/kg/d (3) 63 mg/kg/d (4) 13 mg/kg/d (5) 6 mg/kg/d (6) 1 mg/kg/d Drinking water	104 wk	Sprague-Dawley rats, male and female (n = 50 each)	Leukemia incidence (male and female) by dose group (1) 22,14% (2) 12,14% (3) 16,8% (4) 10,8% (5) 10,8% (6) 2,4% 4,3% (control) 10,6% (methanol)	Soffritti et al. 1989

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(1) 188 mg/kg/d (2) 125 mg/kg/d (3) 63 mg/kg/d (4) 13 mg/kg/d (5) 6 mg/kg/d (6) 1 mg/kg/d Drinking water	104 wk	Sprague-Dawley rats, male and female	GI tract malignant neoplasias (male and female) (1) 6,0% (2) 2,0% (3) 0,0% (4) 0,0% (5) 0,4% (6) 4,0% 0% (control) 0% (methanol)	Soffritti et al. 1989
313 mg/kg/d Drinking water	104 wk	Sprague-Dawley breeders (n = 36) and their offspring (n = 73)	Malignant GI neoplasias (male and female): 2.8% in breeders 15.1% in offspring 0% in controls Leukemias (male and female) 11% in breeders 5.5% in offspring 2.5% in control 5.5% methanol	Soffritti et al. 1989

(Continued)

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TABLE 7-2 Continued

Dose/Route	Exposure Duration	Species	Effects	Reference
(1) 188 mg/kg/d (2) 125 mg/kg/d (3) 63 mg/kg/d (4) 13 mg/kg/d (5) 6 mg/kg/d (6) 1 mg/kg/d Drinking water	104 wk	Sprague-Dawley rats, male and female (n = 50 each)	Leukemias (M,F) (1) 22,18% (2) 18,24% (3) 6,20% (4) 16,12% (5) 8,12% (6) 4,6% 14,6% (control) 11,10% (methanol)	Soffritti et al. 2002
(1) 188 mg/kg/d (incidences primarily limited to highest dose group), drinking water	104 wk	Sprague-Dawley rats, male and female (n = 50 each)	Glandular stomach: 12% adenomatous polyp (male only), 0% in control Intestine: 10% malignant tumors (male only), 0% in control	Soffritti et al. 2002
(1) 188 mg/kg/d (2) 125 mg/kg/d (3) 63 mg/kg/d (4) 13 mg/kg/d (5) 6 mg/kg/d (6) 1 mg/kg/d Drinking water	104 wk	Sprague-Dawley rats, male (n = 50)	Testes: interstitial-cell adenoma (1) 18% (2) 24% (3) 20% (4) 12% (5) 12% (6) 6% 10% (control) 6% (methanol)	Soffritti et al. 2002

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(1) 188 mg/kg/d (2) 125 mg/kg/d (3) 63 mg/kg/d (4) 13 mg/kg/d (5) 6 mg/kg/d (6) 1 mg/kg/d Drinking water	104 wk	Sprague-Dawley rats, female (n = 50)	Mammary gland adenocarcinomas (1) 22% (2) 18% (3) 6% (4) 16% (5) 8% (6) 4% 11% (control) 14% (methanol)	Soffritti et al. 2002

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One consistent symptom following acute human ingestion to formaldehyde is GI irritation and abdominal pains, with varying degrees of severity. It is thought that the irritation produced by formaldehyde may be related to the precipitation of proteins or the formation of protonated hydroxymethyl amino acid derivatives that occurs in conjunction with high concentrations of formaldehyde exposure (Loomis 1979). Another common observation following acute exposures is that metabolic acidosis occurs as a result of the metabolism of formaldehyde and the subsequent build up of formic acid (Eells et al. 1981).

Short-Term Toxicity (6-30 d)

Marks et al. (1980) found that formaldehyde (in a solution with 12-15% methanol) administered by gavage on days 6-15 of gestation at doses of 185 mg/kg/d resulted in the death of 22 of 34 treated pregnant mice within 18 d. No mortality was observed at a dose of 75 mg/kg/d. However, the authors noted that the methanol fraction may have contributed to the observed mortality. Tobe et al. (1989) observed mortality within the first 9 d of drinking water administration of formaldehyde at 300 mg/kg/d in Wistar rats, but not at 10 or 50 mg/kg/d. Other drinking water (Johannsen et al. 1986; Takahashi et al. 1986) and gavage (Vargova et al. 1993) studies failed to observe mortality in rats exposed to formaldehyde orally at doses as high as 258 mg/kg/d.

Tobe et al. (1989) reported significantly reduced bodyweight gain and water and food intake in Wistar rats evaluated weekly after being exposed to formaldehyde at 300 mg/kg/d (5,000 mg/L) in drinking water. Values for similar end points evaluated for the 10 and 50 mg/kg/d groups (200 and 1,000 mg/L, respectively) were not statistically different than the control group values. Johannsen et al. (1986) reported that rats administered formaldehyde at 150 and 225 mg/kg/d by gavage in a 2-week (wk) pilot study displayed mean body weight reduction proportional to dose, along with reduced food and water consumption. Vargova et al. (1993) found significantly reduced mean body weights relative to controls for rats exposed to 80 mg/kg/d, but not for the 20 and 40 mg/kg/d exposure groups. Body weight was also not reduced in rats exposed for 4 wk to formaldehyde in drinking water at doses as high as 125 mg/kg/d in a study conducted by Til et al. (1988).

Til et al. (1988) did report other effects following subacute oral exposures to formaldehyde. The authors exposed Wistar rats to formaldehyde at 0, 5, 25, and 125 mg/kg/d in drinking water for 4 wk. In the

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highest-dose group, the authors observed decreased protein and albumin concentrations in blood plasma and slight increases in the weight of the kidneys relative to controls (female only). However, the most marked observation was significant hyperkeratosis and thickening of the limiting ridge in the forestomach and focal gastritis in the glandular stomach, all observed in the 125 mg/kg/d group. The authors did not observe these effects in the 25 mg/kg/d group and suggested that dose as a no-observed-adverse-effect level (NOAEL) for formaldehyde. This distinct portal-of-entry irritation is consistent with results seen in both shorter- and longer-duration studies of formaldehyde across a variety of species including humans (ATSDR 1999).

Vargova et al. (1993) investigated the potential for formaldehyde to affect the immune system during short-term subacute exposures. Rats were administered formaldehyde by gavage at doses of 0, 20, 40, and 80 mg/kg/d for 28 d. Hematologic and clinical chemistry data revealed some significant differences relative to controls (for example, increased hematocrit, hemoglobin, and erythrocytes, and decreased monocyte and lymphocyte percentages), primarily in the highest-dose group. Increased absolute and relative lymph node weights were observed in the 40- and 80-mg/kg/d groups. Perhaps the most notable finding was dose-dependent reductions in antibody responses (immunoglobulin G [IgG] and M [IgM]) in a hemagglutination assay across all dose groups. Results from several other immunologic assays that were utilized in this study failed to show significant adverse effects.

Subchronic Toxicity (30-180 d)

A small number of studies addressing subchronic oral exposures to formaldehyde were available in the scientific literature. Johannsen et al. (1986) administered formaldehyde to rats and dogs for 90 d via their drinking water and diet, respectively. Sprague-Dawley rats were dosed with 0, 50, 100, and 150 mg/kg/d, and dogs were exposed to formaldehyde through their feed at doses of 0, 50, 75, and 100 mg/kg/d. Compared to controls, treated rats exhibited a dose-dependent decrease in the volume of water consumed (up to 31% in the males from the 150 mg/kg/d group). Significant reductions in body weight gain were also observed for rats of both sexes at 150 mg/kg/d and in male rats at 100 mg/kg/d. For the dogs, reduced food consumption compared to controls was statistically significant above 50 mg/kg/d, although reduced weight gain was only observed at the 100 mg/kg/d dose. For both rats and dogs,

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hematologic and clinical chemistry analysis did not demonstrate any significant exposure-related changes from nonexposed controls. Additionally, no differences in the weights of critical organs including kidney, liver, heart, spleen, and thyroid were observed. Another important observation was that gross and histopathologic evaluations did not reveal inflammation of the GI mucosa or lesions in either species, even in the highest-exposure groups. These negative findings were consistent with observations of Vargova et al. (1993), but were in contrast to the Til et al. (1988) findings, which observed signs of GI irritation at similar doses.

Chronic Toxicity (180 d to lifetime)

Systemic Effects

Several chronic-duration studies in which the systemic oral toxicity of formaldehyde was assessed are available in the scientific literature. Some of these studies included end points with shorter-term observations by the authors (for example, increased mortality, reduced weight gain, lower water consumption) that have already been presented and discussed in the acute, short-term, or subchronic sections of this document.

Tobe et al. (1989) conducted a 2-y study in which formaldehyde at 0, 10, 50, and 300 mg/kg/d was administered to male and female Wistar rats in drinking water (0, 200, 1,000, and 5,000 mg/L). The general condition of the rats in the highest-dose group was poor, with reduced weight gain and reduced water and food intake noted by the authors. No significant differences were noted with the lower-dose groups. Mortality, which was observed as early as 9 d into the study, was 100% by the end of the study for both males and females in the highest-dose groups.

A significant observation in the Tobe et al. (1989) study was marked ulceration and tissue changes within the forestomach and glandular stomach of rats of either sex following chronic formaldehyde exposure at the 300 mg/kg/d and 50 mg/kg/d doses. The highest-dose group, necropsied after 12 months (mo) of exposure, showed the highest frequency of effects with all of the rats exhibiting squamous cell hyperplasia (with or without hyperkeratosis) and 10/12 exhibiting basal cell hyperplasia of the forestomach. Effects were also observed in the glandular stomach at this dose, with erosion and ulceration and glandular hyperplasia along the limiting ridge. No effects were observed in the lower-dose groups at the 12-mo necropsy. At the 18-mo and 2-y necropsies, forestomach hyperkeratosis (2/14 rats) was reported for the 50-mg/kg group,

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but no effects on the glandular stomach were observed. No lesions of the forestomach or glandular stomach were observed in the 10 mg/kg/d exposure group, and this concentration was viewed by the authors as a NOAEL for formaldehyde with respect to these effects.

Til et al. (1989) conducted a similar 2-y study in which Wistar rats (70 male and 70 female in each dose group) were dosed with 0, 1.2, 15, and 82 mg/kg/d (males) and 0, 1.8, 21, and 109 mg/kg/d (females) via drinking water . Unlike the aforementioned Tobe et al. (1989) study, the general condition of the animals was not significantly affected, even for the highest-dose groups. No differences in mortality were noted relative to controls. However, the high-dose group did exhibit statistically significant decreases in water and food consumption and reduced mean body weights. Absolute decreases in kidney, testes, heart, and liver weights were noted, although they were attributed to the overall reductions in weight gain for the rats.

As with the Tobe et al. (1989) study, one of the notable findings of the Til et al. (1989) study was the observed GI effects following drinking water exposures to formaldehyde. Necropsies performed at weeks 53, 79, and 105 noted pronounced inflammatory changes in the forestomach and glandular stomach of rats in the high-dose group. These effects were noted in both male and female rats. Within the forestomach, histopathologic examination revealed papillary epithelial hyperplasia along with hyperkeratosis along the limiting ridge. In many rats in this dose group, this inflammation was advanced enough to be considered ulceration. In the glandular stomach, almost all rats exhibited chronic atrophic gastritis of varying severity. The affected mucosa was often reduced in width and had extensive lesions and ulceration. Rats in the 15 mg/kg/d (male) and 21 mg/kg/d groups (female) did not exhibit statistically significant gastric effects relative to controls, and these formaldehyde concentrations were considered to be NOAELs for these effects.

Carcinogenic Potential

Human Epidemiology Summary

Because of its commercial importance and because of widespread occupational exposures to formaldehyde, there is a wealth of human epidemiologic studies (over 40 case-control or cohort studies and several meta-analyses) that have assessed chronic inhalation exposures to formaldehyde and the potential for these exposures to result in the devel-

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opment of cancer (see ATSDR 1999 and WHO 2002 for a comprehensive review of available studies). No human epidemiologic studies specific to oral cancers associated with formaldehyde exposures were found.

Given the reactivity and water solubility of formaldehyde and the available evidence from laboratory animal inhalation exposures, many epidemiologic studies have focused on upper respiratory tract cancer incidences. With respect to sino-nasal and nasopharyngeal cancers, some recent studies and at least two meta-analyses reported an exposure-response relationship (Blair et al. 1990; Partanen 1993; Hauptmann et al. 2004), although other studies and reviews failed to find such a relationship (Collins et al. 1997; Marsh et al. 2002; Coggon et al. 2003; Pinkerton et al. 2004), and the evidence is considered by some to be equivocal (ATSDR 1999; WHO 2002). Hauptmann et al. (2004) reported an association between increasing risk of nasopharyngeal cancers and highest peak and cumulative exposures (but not for average exposures or duration of exposure). The significant trend was based on a small number (nine) of cancer cases, two of which were from the control group. In contrast, Collins et al. (1997) conducted a meta-analysis of sino-nasal and nasopharyngeal cancers reported in 47 occupational epidemiologic studies involving formaldehyde inhalation. They found that although a few of the studies reported increased incidences of these cancers, the findings from the majority of the studies were negative. After correcting for underreporting of negative study results, a relative risk estimate of 1.8 (95% confidence interval [CI] = 1.4-2.3) for case-control studies and 0.3 (95% CI = 0.1-0.9) for cohort studies was found. The authors concluded that these observations do not support the contention that there is a significant association between formaldehyde inhalation and sino-nasal or nasopharyngeal cancers. Similarly, Coggon et al. (2003) conducted a follow-up evaluation of a cohort of over 14,000 chemical workers who were exposed to formaldehyde by inhalation. The authors did not find an association between formaldehyde exposures and nasopharyngeal cancers and concluded that "overall, the epidemiologic evidence now available indicates that if formaldehyde does cause nasopharyngeal cancers, then the increased risk is small." However, the International Agency for Research on Cancer (IARC 2004) recently upgraded its classification of formaldehyde from Group 2A to Group 1 (sufficient evidence from experimental animals and humans to conclude it is carcinogenic to humans), based on their opinion that there is sufficient epidemiologic evidence that formaldehyde causes nasopharyngeal cancers in humans. The National Toxicology Program (NTP) classifies formaldehyde as "reasonably anticipated to be a human carcinogen" (NTP 2005).

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Although scanty, there is some epidemiologic evidence that formaldehyde could result in lymphohematopoietic cancers and lung cancers. In regard to lung cancer, Coggon et al. (2003) reported increased incidences for their cohort (standardized mortality rate [SMR] of 1.28 for the high-exposure group), but there was no association with duration of exposure, and the authors cautioned that these results need to be further investigated (for example, closer evaluation of confounding factors). Collins et al. (1997) found no increased lung cancer incidences for industrial workers in the available cohorts or in the case-control studies and concluded that the available epidemiologic evidence did not support an association between formaldehyde exposure and lung cancers. Similarly, Hauptmann et al. (2004) conducted a follow-up study of a cohort of over 25,000 industrial workers from 10 U.S. plants and did not find a positive association between formaldehyde exposure and lung cancer risks.

With respect to lymphohematopoietic cancers in this same cohort, Hauptmann et al. (2003) reported relative risks for leukemia of 1.15 (95% CI = 0.4-3.2) and 2.49 (95% CI = 1-6) when average formaldehyde exposures were 0.5-0.9 parts per million (ppm) and > 1 ppm, respectively (larger relative risks were reported when grouping by peak formaldehyde exposure concentrations). However, the leukemia relative risks were not positively associated with cumulative formaldehyde exposure or duration of exposure, and all myeloid tumor types (for example, acute and chronic) were lumped together despite their different etiologies. The authors stated that these results should be viewed with caution because the overall body of evidence for an association between formaldehyde and leukemia is mixed. Pinkerton et al. (2004) also reported an increased risk of myeloid leukemia in association with duration of formaldehyde exposure in their review of a cohort of over 11,000 U.S. garment workers. In contrast, Coggon et al. (2003) did not find increased mortality from leukemia in their cohort, even in evaluating the subset of workers with highest formaldehyde exposure. The need for caution in interpreting non-respiratory tract cancers with formaldehyde is echoed by the World Health Organization (WHO 2002) who states, “Available evidence for these tumors at sites other than the respiratory tract does not, therefore, fulfill traditional criteria of causality (for example, consistency, biologic plausibility) for associations observed in epidemiological studies.” IARC (2004) concluded that there was “strong, but not sufficient evidence for a causal association between leukemia and occupational exposure to formaldehyde,” citing existing uncertainties and inconsistencies among different cohorts.

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Discussion of Animal Data

There is a wealth of animal data on formaldehyde carcinogenesis associated with inhalation exposure, and these findings are important to consider in evaluating cancer risks from oral exposures, especially with respect to the assumed mode of action and the types of tissues most likely to be affected. Inhalation of formaldehyde has been shown to induce nasal tumors (squamous cell carcinomas) in rats in several animal bioassays (Swenberg et al. 1980; Albert et al. 1982; Kerns et al. 1983; Monticello et al. 1996). Largely based on this inhalation evidence, formaldehyde is classified by the U.S. Environmental Protection Agency (EPA) as a probable (B1) human carcinogen (IRIS 2004). As mentioned above, IARC recently elevated its rating of formaldehyde and now lists it as a Group 1 carcinogen (IARC 2004). The carcinogenic potential of formaldehyde is supported by its demonstrated cytotoxicity and genotoxicity (Yager et al. 1986; Casanova et al. 1989; Ballarin et al. 1992), the reactivity of the molecule, and its ability to be incorporated into other critical macromolecules (Heck and Casanova 1990). There has been a significant focus in the scientific literature on the mechanism of nasal tumor induction with formaldehyde (CIIT 1999; WHO 2002; Conolly et al. 2003) and the relevancy of rodent tumor data to human health risk assessment (particularly in extrapolating from high to low levels of exposure). Nasal tumors are believed to be largely dependent on cytotoxicity and cell proliferation at the portal-of-entry (that is, nasal epithelium), although mutagenicity mediated through DNA-protein cross-links (DPX) may also be a factor (Schlosser et al. 2003). Inhalation studies have generally observed that exposure concentrations of formaldehyde that were high enough to produce nasal tumors were also high enough to produce nasal lesions and necrosis of the epithelium and that exposure concentrations that were not sufficient to result in these necrotic effects also did not cause nasal tumors (Heck and Casanova 1990; Morgan 1997). Tumors at sites distant from the portal of entry have generally not been demonstrated in inhalation studies with formaldehyde (ATSDR 1999).

Fewer studies have attempted to evaluate the carcinogenicity of formaldehyde following oral ingestion. However, five animal studies are available that provide data pertinent to the setting of guidelines for acceptable concentrations (ACs) of formaldehyde in drinking water.

As described previously, both Tobe et al. (1989) and Til et al. (1989) conducted 2-y exposure studies in which Wistar rats were administered formaldehyde through drinking water. Both of these studies observed inflammation and necrosis in the forestomachs and glandular

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stomachs of the rats. However, in both studies, evaluation of multiple organ systems did not find any increased tumor incidence in the formaldehyde-treatment groups relative to controls.

Takahashi et al. (1986) assessed the potential for oral exposures of formaldehyde (single dose group at 258 mg/kg/d) to promote gastric tumors in rats. Forestomach papillomas were observed in 8 of 10 formaldehyde-treated rats, which was statistically significant relative to controls. Til et al. (1989) have questioned whether these benign papillomas, which were not found to be statistically significant during their own study, might actually be characterized as papillary epithelial hyperplasia. It is unclear, however, whether this difference, species variations, or other factors might explain apparent inconsistencies between these animal studies.

There are two studies that reported positive associations between cancer and the ingestion of formaldehyde by rats (Soffritti et al. 1989, 2002). The earlier study involved two separate experiments. The first involved the administration of 0, 1, 6, 13, 63, 125, and 188 mg/kg/d to male and female Sprague-Dawley rats through their drinking water (0, 10, 50, 500, 1,000, 1,500, and 2,500 mg/L, respectively) for a total of 104 wk. In addition to the normal controls, another control group of rats were exposed to drinking water containing 15 mg/L of methanol. In the second experiment from the same Soffritti et al. (1989) study, two groups of male and female breeders were exposed to formaldehyde in drinking water at doses of 0 and 313 mg/kg/d from 25 wk of age for 104 wk. Offspring from these breeders were then exposed to the same concentrations of formaldehyde for 104 wk.

The findings from the Soffritti et al. (1989) study were very different from other similarly designed long-term studies of oral exposure to formaldehyde with respect to the assessment of its carcinogenic potential. Increased incidences of GI tract tumors (both benign and malignant) were observed in the highest-does group (188 mg/kg/d) for male and female rats exposed from 6 wk of age and in both female breeders and offspring when dosed with 313 mg/kg/d. Although the observed incidences were low, the authors noted that no similar tumors were reported in the control groups and that these cancers are very rare for untreated rats in their breeding colony. There were some inconsistencies in GI tumor incidence between breeders and offspring (for example, female breeders dosed with 313 mg/kg/d had no malignant GI tumors, whereas 21% of female offspring were positive at this exposure concentration) observed in the second experiment from this study.

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Cancers at other sites were also reported in the Soffritti et al. (1989) study. An increased incidence of leukemias (mostly lymphoblastic leukemias and lymphosarcomas) was observed in both male and female rats exposed to formaldehyde in the first experiment of the study. This effect was most observable at the highest exposure concentration, where leukemias were found in 22% of the male rats and 14% of the female rats compared to only 3-4% of controls. Leukemia incidence for both male and female rats and their offspring were approximately twice as high as controls in the second experiment (Soffritti et al. 1989). Soffritti et al. (2002) followed up with a similar 2-y study of oral formaldehyde exposure to Sprague-Dawley rats through drinking water (0, 10, 50, 500, 1,000, 1,500, and 2,500 mg/L). Estimated dose concentrations in this study were 0, methanol control, and formaldehyde at 1, 6, 13, 63, 125, and 188 mg/kg/d (ATSDR 1999). Fifty male and 50 female rats per group were dosed from 6 wk of age. This study found similar results to the earlier Soffritti et al. study (1989), with some malignant intestinal cancers (adenocarcinomas and leiomyosarcomas) largely limited to the 188 mg/kg/d group (3/50 in male rats). This study also noted adenomatous polyps (6/50 for male rats) and adenocarcinomas (2/50 for female rats) in the glandular stomach in the 188 mg/kg/d group (not reported in the earlier study).

Cancers within hemolymphoreticular tissue (lymphomas or leukemias) were observed in 46% of the male rats and 20% of the female rats at the highest-dose concentration, compared with only 7-8% of the rats in the control group. Other tumors of significance were interstitial cell adenomas within the testis (significant relative to controls at the 63, 125, and 188 mg/kg/d groups) and mammary gland adenocarcinomas (significant relative to controls at the top two doses and in the 13 mg/kg/d group).

Organoleptic Considerations

Although not strictly a health consideration, organoleptic characteristics (for example, taste, odor) can affect the palatability of drinking water and can indirectly contribute to or exacerbate crew dehydration if noticeable enough to discourage crew water consumption. Formaldehyde has a characteristic odor that is frequently described as strong, pungent, and irritating (ACGIH 1994). In water, this odor threshold is approximately 50 mg/L (ATSDR 1999; HSDB 1999). As cited in these same sources, formaldehyde in drinking water can also impart an undesirable taste at this concentration.

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Genotoxicity

Formaldehyde is a very reactive molecule that can readily interact with proteins, DNA, RNA, and other critical macromolecules (formaldehyde has an electrophilic carbonyl group that has an affinity for nucleophilic sites on these molecules) (Feron et al. 1991). The genotoxicity of formaldehyde has been observed (although not strictly by the oral-ingestion route) through a number of in vivo (Lam et al. 1985; Chebotarev et al. 1986; Yager et al. 1986; Casanova et al. 1991; Ballarin et al. 1992) and in vitro (Glass et al. 1986; Schmid et al. 1986; Grafstrom et al. 1993; Merk and Speit 1998) studies. In vivo and in vitro studies in which genotoxicity was not observed are also available (DeFlora 1981; Dallas et al. 1992; Vasudeva and Anand 1996). These and other studies have been the focus of several thorough genotoxicity reviews for formaldehyde (Ma and Harris 1988; Heck and Casanova 1990; IARC 1995; ATSDR 1999; WHO 2002). General genotoxicity observations are summarized briefly in the following sections.

In Vitro Assays

The genotoxicity of formaldehyde has been observed in many, but not all, bacterial and mammalian in vitro assays (IARC1995; WHO 2002). Positive mutagenicity with *Salmonella typhimurium* and *Escherichia coli* has been reported for formaldehyde (Donovan et al. 1983; Connor et al. 1985; Glass et al. 1986) in both the presence and absence of metabolic activation. With cultured human cell lines (for example, bronchial fibroblast, lymphocytes, and tracheal epithelial cells) genotoxic effects ranging from chromosomal aberrations, sister chromatid exchange, and DNA damage have been observed (Kreiger and Garry 1983; Schmid et al. 1986; Dresp and Bauchinger 1988). Similar results have been reported in cell lines from other mammalian test species (Miller and Costa 1989; Grafstrom et al. 1993).

In Vivo Observations

Although no genotoxicity studies involving oral exposures to formaldehyde are available, there are a number of in vivo inhalation exposure studies (in both humans and rodents) that suggest formaldehyde is genotoxic. Other negative studies are also available (Dallas 1992;

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Vasudeva and Anand 1996). With humans, positive genotoxic effects include lymphocyte chromosomal aberrations (Chebotarev et al. 1986) and sister chromatid exchange (Yager et al. 1986), along with increases in micronuclei formation in cells of the nasal passage. As discussed previously, genotoxicity at sites distant from the portal of entry is unlikely. This conclusion is generally supported by available genotoxicity studies (Klingerman et al. 1984; Dallas 1992; ATSDR 1999; WHO 2002).

There has been a particular research focus placed on the significance of DNA-protein crosslinks. These complexes between DNA bases and proteins bound by crosslinks are formed in response to formaldehyde exposure (Swenberg et al. 1980). It is postulated that these crosslinks could result in mutations and/or chromosomal aberrations if not repaired prior to cell replication (Morgan 1997; Klaassen 2001) or that repair and DNA regeneration could promote cell proliferation (Heck and Casanova 1990). This potential is heightened by the observation that these crosslinks are relatively stable and can be formed following formaldehyde exposures that are not cytotoxic (Merk and Speit 1998). However, there is still some debate in the literature as to whether genotoxic expression through DNA-protein crosslinks is the exact mechanism of formaldehyde carcinogenesis.

Reproductive and Developmental Toxicity

The reactivity of formaldehyde and the ability of the body to metabolize it significantly limit the potential for formaldehyde exposure to result in adverse reproductive or developmental effects (Collins et al. 2001). Several publications have reviewed the human epidemiologic data on reproductive and developmental effects of formaldehyde (for example, rates of spontaneous abortion, birth defects, worker infertility, etc.) and concluded that these types of effects are unlikely with occupational exposures to formaldehyde (ATSDR 1999; Collins et al. 2001; WHO 2002).

There are a number of published animal studies that have assessed the reproductive and/or developmental toxicity of formaldehyde through a variety of exposure routes. Collins et al. (2001) conducted a thorough review of these animal studies and concluded that they generally did not report positive reproductive or developmental effects in association with formaldehyde exposure. In briefly describing those studies here, a focus was placed on summarizing those exposures most relevant to SWEG development. Hurni and Ohder (1973) evaluated the reproductive toxicity

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of formaldehyde in female beagles exposed to formaldehyde at 9.4 mg/kg/d in their food on days 4-56 after mating. When compared to controls, no effects on pregnancy rates, length of gestation, litter size, or other reproductive end points were noted. Also, no internal or skeletal teratogenic anomalies were observed in the pups born to exposed females. In another study, Marks et al. (1980) exposed pregnant mice to formaldehyde orally (gavage) on days 6-15 of gestation. Formaldehyde doses were as high as 185 mg/kg/d. Despite the gavage exposures and significant observed mortality (23/34) to the dams in the highest-dose group, necropsy revealed no statistically significant teratogenic effects in the fetuses when compared to controls. Similarly, Della Porta et al. (1970) observed no malformations in 124 pups from female rats exposed to formaldehyde through drinking water at 0 or 1% hexamethylenetetramine over 2 wk of exposure.

One inhalation study that did observe reproductive effects with formaldehyde was a Russian study (Guseva 1972) in which groups of male rats were exposed to formaldehyde by inhalation for 4 h/d, 5 d/wk for 6 mo. The formaldehyde concentrations were 0, 0.1, and 0.2 ppm. After mating with unexposed females, reproductive effects were assessed. The males in the highest-exposure group exhibited a significant decrease is testicular DNA, although there were no observed effects on the fetus (abnormalities in fetal weight, litter size, incidence of birth defects, etc.). There are also several Russian studies that have suggested that there is a relationship between formaldehyde exposure and various developmental effects in rats (Gofmekler 1968; Gofmekler et al. 1968; Pushkina et al. 1968). In these studies, rats were exposed to formaldehyde 24 h/d by inhalation for 10-15 d, at concentrations of 0, 0.01, and 0.83 ppm. These effects included increases in fetal body weight, a reduced number of fetuses per litter, reduced nucleic acid levels in fetuses, and fetal histopathologic changes. Specific study details have been found to be lacking or inconsistent, and these results have not been duplicated in other studies (Collins et al. 2001). They are also inconsistent with the findings of Saillenfait et al. (1989) who evaluated Sprague-Dawley rats exposed to up to 40 ppm on gestations days 6-20 and observed no changes in a number of reproductive variables (for example, number of resorptions, implantation), and they are inconsistent with the work of Martin (1990) who evaluated a wide variety of reproductive and developmental end points and observed no effects in Sprague-Dawley rats exposed to formaldehyde at concentrations as high as 10 ppm (6 h/d on gestation days 6-15).

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Allergic Contact Dermatitis

Sufficient exposure to formaldehyde can elicit allergic contact dermatitis (ACD), and patch test results suggest that approximately 1-4% of individuals may have ACD from exposure (Marks et al. 1995). These reactions may be exhibited as simple drying and reddening of the skin to perifolicular dermatitis and edema (Loomis 1979). There are a tremendous number of studies that have demonstrated that closed-patch testing can result in ACD following formaldehyde exposure (see ATSDR 1999). Many of these studies are designed to evaluate relatively high concentrations of formaldehyde (that is, 1-2%), concentrations that have been shown to result in irritation in the general population (Trattner et al. 1998; WHO 2002). These studies do not provide useful information on water concentrations likely to produce a response in individuals with ACD.

One study (Jordan et al. 1979) provided particularly useful information on the potential for low-level exposure to formaldehyde in water to elicit ACD. In this human study, nine individuals with ACD consented to continuous closed-patch testing with formaldehyde at concentrations of 1, 30, 60, and 100 ppm (0.003-0.01%). The testing was continued for a total of 168 h, with midstudy evaluation at 72 and 120 h. The same testing regime was followed with four non-ACD individuals, and these subjects failed to exhibit any ACD response, even to the highest concentration tested. For the individuals with ACD, a concentration-dependent response to formaldehyde was observed, with 6/9 subjects responding at 100 ppm, 5/9 responding at 60 ppm, and 4/9 responding at 30 ppm by the end of the study. The authors noted that the reaction times and responses seen with the 100 ppm exposure group clearly indicated that 100 ppm was sufficient to produce ACD. However, with the 30 ppm exposure group, the intensity of the response lessened with time and there was some question as to the significance of the patch test findings, especially given that closed-patch testing is extremely rigorous. To address this issue, the authors also performed repeated axillary spray tests on 13 subjects with ACD. Solutions of formaldehyde at 30 ppm were sprayed onto the axilla twice a day for 2 wk. This type of experimental design is likely to be more applicable than the closed-patch testing to on-orbit exposures to formaldehyde in water. With the exception of two individuals, no response was observed in this testing. The two individuals who might be characterized as responders only displayed very mild perifolicular dermatitis after 2 wk of testing. The subjects reacting to formaldehyde at 30 ppm in the closed-patch testing did not respond to this same concentra-

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tion of formaldehyde in the axillary spray testing. The authors concluded that contact with formaldehyde below 30 ppm can be tolerated without notable response (even by individuals with ACD) for extended-exposure durations (Jordan et al. 1979).

Spaceflight Effects

As discussed previously, human oral exposure to formaldehyde has been shown to cause adverse cardiac effects ranging from sinus tachycardia, reduced blood pressure, and even cardiac arrest. However, these effects have only been observed at acute doses (200-600 mg/kg/d) during accidental or suicidal poisoning incidences described previously. These are not expected to be critical effects of formaldehyde that are relevant to exposures which could foreseeably occur in the spacecraft environment. With respect to hematologic effects, only one of the reviewed acute poisoning cases observed any effect (intravascular coagulopathy), and numerous reviewed animal studies of various durations generally did not report any statistically significant hematologic effects.

LIMITS SET BY OTHER ORGANIZATIONS

In order to provide context for the development of SWEGs for formaldehyde, an attempt was made to summarize health-based exposure limits for formaldehyde in drinking water that have been adopted or published by various organizations external to NASA (Table 7-3). These organizations include federal agencies such as EPA and the Agency for Toxic Substances and Disease Registry (ATSDR), as well as state environmental agencies and other organizations that may have their own drinking water quality standards. Certain exposure assumptions (for example, drinking water intake rates), assumed receptors (for example, child, adult), and policy decisions (for example, target cancer risk levels) are inherent to some of the drinking water standards provided in Table 7-3, in accordance with their intended application. Because these exposure assumptions differ, any differences in allowable concentrations among the various groups may be attributable to factors other than just the toxicity factor that is utilized. In cases where a toxicity value (for example, EPA's reference dose [RfD]) was available, standard drinking water risk assessment assumptions for the general population (70 kg body weight, 2 L/d ingestion) were used to derive a water equivalent concentration.

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TABLE 7-3 Standards and Guidelines for Formaldehyde Set by Other Organizations

Organization	Standard	Value	Water Equivalent (mg/L)	Reference
EPA	Oral RfD	Chronic duration (0.2 mg/kg/d)	7	IRIS 2004
EPA	HAL	Oral RfD (0.2 mg/kg/d)	10, 1 d 5, 10 d 7, DWEL 1, lifetime	EPA 2002
ATSDR	MRL	Intermediate duration (0.3 mg/kg/d)	10	ATSDR 1999
		Chronic duration (0.2 mg/kg/d)	7	
State of California	Action level	EPA oral RfD	0.1	CDHS 2003
Florida	Water guideline	—	6	FSTRAC 1999
Maine	Water guideline	—	0.03	FSTRAC 1999
Minnesota	Water guideline	—	1	FSTRAC 1999
New Jersey	Water guideline	—	0.1	FSTRAC 1999
Texas	Water guideline	EPA oral RfD	4.9	TCEQ 2003
Wisconsin	Water guideline	—	1	FSTRAC 1999

[a]EPA health advisories are provided for different exposure durations. The 1- and 10-d values are based on assumed exposures by a child. DWELs are based on adult exposures, assuming all exposures are through drinking water. Lifetime water values are health-based goals that include formaldehyde contributions from other routes.
Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; DWEL, drinking water equivalent levels; EPA, U.S. Environmental Protection Agency; HAL, health advisory level; mg/L, milligrams per liter; MRL, minimal risk level; Rfd, reference dose.

EPA References

No maximum contaminant level (MCL) has been established by EPA for formaldehyde in drinking water. However, nonenforceable

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health-effects guideline values have been calculated for formaldehyde by the EPA Office of Water. These guidelines are based on the oral RfD and are calculated for different exposure durations (1 d, 10 d, lifetime). Although the guidelines are one of the few sets of available guidelines that include consideration for shorter-duration exposures, they are not completely applicable to the SWEG process, because they are not specifically based on short-term toxicity studies and are intended to address sensitive childhood exposures.

The EPA has established an oral RfD for formaldehyde for use in evaluating its chronic noncancer health risks. An RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of adverse effects over a lifetime (IRIS 2004). The oral RfD for formaldehyde is 0.2 mg/kg/d and was last revised on EPA's Integrated Risk Information System (IRIS) in 1990. The basis for the RfD is the Til et al. (1989) 2-y study that observed adverse noncancer effects following oral exposure to formaldehyde in drinking water. Administered formaldehyde doses were 0, 1.2, 15, and 82 mg/kg/d for males and 0, 1.8, 21, and 109 mg/kg/d for females. At the highest dose level in both sexes, significant changes in the histopathology of the forestomach and glandular stomach were noted. These effects were evidenced as papillary epithelial hyperplasia accompanied by erosions/ulceration in the forestomach and chronic gastritis in the glandular stomach. These effects were not observed in the lower-exposure groups, and a NOAEL of 15 mg/kg/d was used as the basis for the RfD. A total uncertainty factor (UF) of 100 was applied to account for interspecies (10) and intraspecies differences (10).

ATSDR MRLs

In its 1999 Toxicologic Profile for Formaldehyde, ATSDR established minimal risk levels (MRLs) for formaldehyde. An oral MRL is a daily exposure dose for the general population (including sensitive receptors) that is expected to be without appreciable risk of adverse noncancer health effects. ATSDR has provided intermediate duration (15-364 d) and chronic duration (365 d to lifetime) MRLs for formaldehyde. An acute-duration MRL was not derived because of an overall lack of suitable dose-response data for these types of exposures to formaldehyde (ATSDR 1999).

The intermediate-duration MRL is based on the 4-wk Wistar rat

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study conducted by Til et al. (1988). Male and female rats were administered formaldehyde at doses of 0, 5, 25, and 125 mg/kg/d in drinking water. In the highest-dose group, exposed rats exhibited decreased food and water intake, along with slight changes is blood chemistry. However, the most marked effects were seen in the GI tract, with significant thickening of the limiting ridge in the forestomach along with hyperkeratosis and mucosal lesions. Focal atrophic gastritis was also observed in the glandular stomach. ATSDR utilized the NOAEL from this study (25 mg/kg/d) and applied a UF of 10 for extrapolation from animals to humans and another factor of 10 for human variability (final intermediate duration MRL of 0.3 mg/kg/d).

Consistent with the EPA oral RfD, the chronic-duration ATSDR MRL for formaldehyde is based on the results from Til et al. (1989). This 2-y drinking water study, which observed significant histopathologic changes in the rat forestomach and glandular stomach, has been described previously in this document. In deriving the MRL, ATSDR used the NOAEL of 82 mg/kg/d and applied a combined UF of 100 (10 for human variability and 10 for extrapolation from animals to humans). This resulted in a final chronic-duration MRL of 0.2 mg/kg/d.

Applying drinking water risk assessment assumptions for the general population (70 kg body weight, 2 L/d ingestion), these MRLs for intermediate- and chronic-duration exposure to formaldehyde would correspond to drinking water equivalent concentrations of 10 mg/L and 7 mg/L, respectively.

State Approaches

Most states do not derive their own toxicity factors. Instead, they typically have a hierarchy of recognized sources of toxicity information (for example, EPA RfD, ATSDR MRL) that are utilized. These toxicity factors are applied in risk calculations that also incorporate exposure assumptions and policy decisions (for example, evaluating a child versus an adult receptor) in the derivation of drinking water standards, action levels, or health-based guidelines. As can be seen from those examples provided in Table 7-3, state drinking water guidelines for formaldehyde can vary significantly (0.03-6 mg/L). Some of this variation can be explained solely by the addition of relative source contribution terms (often a factor of 5), which lower the allowable concentration of formaldehyde in water to account for non-drinking water exposures. California, which has an action level of 0.1 mg/L for formaldehyde in drinking water, used

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the Til et al. (1989) NOAEL but applied an additional UF (as compared to EPA or ATSDR) of 10 to account for inadequacies in the toxicity database. The action level is also reflective of a relative source contribution of 20% in drinking water (that is, lowering the action level by a factor of 5). California raised this action level in 2000, having previously used an action level for formaldehyde of 0.03 mg/L. These observations may explain the low drinking water guidelines listed for New Jersey (0.1 mg/L) and Maine (0.03 mg/L), because it is not uncommon for other states to replicate California's approaches in developing their own guidelines. It should be noted that all state and federal agencies appeared to use the systemic (noncancer) toxicity of formaldehyde as the basis for decision making.

RATIONALE FOR SWEGS

In the next section of this SWEG document, the justification for the establishment of formaldehyde SWEGs for 1-, 10-, 100-, and 1,000-d durations (as shown in Table 7-4) is provided. ACs for each end point are summarized in Table 7-5. To a significant degree, critical toxicity studies that formed the basis for many of the state and federal drinking water guidelines discussed will also play a role in SWEG development. However, because the target population is not the same for SWEGs and state and federal drinking water standards, it should not be surprising that the guidelines will differ as well.

Several studies involving gavage exposures to formaldehyde were not considered in SWEG development. Although data from gavage studies can often be utilized for toxicologic purposes, with formaldehyde it is especially critical that the route of administration match up with conditions that are most relevant to drinking water exposures (that is, sustained, relatively low-level exposures). Gavage exposures may increase

TABLE 7-4 Spacecraft Water Exposure Guidelines for Formaldehyde

Duration	Concentration (mg/L)	Target Toxicity
1 d	20	Gastric irritation; allergic contact dermatitis
10 d	20	Gastric irritation; allergic contact dermatitis
100 d	12	Gastric irritation; allergic contact dermatitis
1,000 d	12	Gastric irritation; allergic contact dermatitis

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TABLE 7-5 Acceptable Concentrations (ACs)

End Point, Exposure Data, Reference	Species	Uncertainty Factor					ACs (mg/L)			
		NOAEL	Exposure Time	Species	Space-flight	Inter-Individual	1 d	10 d	100 d	1,000 d
Gastric Irritation										
25 mg/kg/d (NOAEL) 4-wk drinking water study (Til et al. 1988)	Wistar rats	1	1	10	1	3	20	20	—[a]	—
15 mg/kg/d (NOAEL) 2-y drinking water study (Til et al. 1989)	Wistar rats	1	1	10	1	3	—	—	12	12
Allergic Contact Dermatitis										
30 mg/L (LOAEL)[b] 2-wk axillary spray test (Jordan et al. 1979)	Human	1	1	1	1	1	30	30	30	30
Organoleptic Concerns										
50 mg/L taste and odor threshold (HSDB 1999)	Human	1	1	1	1	1	—	50	50	50
SWEG							20	20	12	12

[a]—, not applicable

[b]In this study, a very mild response was noted in 2 of 13 susceptible individuals. The authors concluded that concentrations below 30 mg/L should not cause notable ACD effects, even for susceptible individuals. A much higher LOAEL/NOAEL would be more appropriate for the 96-99% of the population that does not have ACD.

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the potential for formaldehyde to remain unmetabolized and circulate through the bloodstream in a manner that would not occur following administration of equivalent daily doses in drinking water.

Irritation

Studies from both humans and laboratory animals have shown that irritation and other adverse GI effects can occur with sufficient oral exposure to formaldehyde. Although no designed studies were found that dealt specifically with very short-term exposures (1-2 d) to formaldehyde, observations from longer-duration studies suggest that this is a credible adverse effect to be considered in AC development.

The AC for this irritation is based on the NOAEL (25 mg/kg/d) for the Til et al. (1988) 4-wk administration of formaldehyde to rats in drinking water. At the next highest dose level (125 mg/kg/d), significant thickening of the limiting ridge and hyperkeratosis in the forestomach, along with gastritis in the glandular stomach were observed. A UF of 10 was applied to account for necessary species extrapolation, and a standard drinking water rate (2.8 L/d) and body weight (70 kg) for astronauts was assumed in calculating the AC. It is not possible to ascertain exactly how long an exposure was necessary to result in the observed gastric effects. However, this and other studies (Til et al. 1989; Tobe et al. 1989), have reported reduced food and drinking water intake (proceeding observed GI effects) in formaldehyde-exposed rats as early as the first week of exposure. Although a single day of exposure may not be sufficient to produce the marked GI changes observed in these animal studies, the establishment of an AC for this end point is supported by the potential for milder gastric irritation.

With inhalation exposures, some variability in human response to the irritant effects of formaldehyde has been shown. Individual sensitivity may be partially related to observed genetic polymorphisms which influence the activity of FDH/ADH3, the key enzyme involved in the rapid metabolism and detoxification of formaldehyde (Hedberg 2001). Given that a range of irritant responses is likely with drinking water exposures to formaldehyde, and considering that this type of sensitivity is not evaluated in astronaut health screening, an additional UF of 3 was applied in calculating the AC to account for variability in individual response.

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1- and 10-d ACs = (25 mg/kg/d [NOAEL] × 70 kg) ÷ (2.8L/d × 10 [species] × 3 [interindividua]) = 20 mg/L (rounded).

In regard to the 100- and 1,000-d ACs, Til et al. (1989) conducted a 2-y drinking water study where formaldehyde was administered to male and female rats at various doses. At the highest doses (82 mg/kg/d in males, 109 mg/kg/d in females), significant and marked GI effects were observed relative to controls. Effects were observed during the 53-, 79-, and 105-wk autopsy evaluations. These effects consisted of ulceration and hyperkeratosis in the forestomach (especially along the limiting ridge) and focal gastritis and significant mucosal inflammation in the glandular stomach. A NOAEL of 15 mg/kg/d was noted for these effects. These results were consistent with the findings of Til et al. (1988), who conducted a similar study over a 4-wk exposure period. A UF of 10 was applied to account for species extrapolation, along with an additional factor of 3 to address variations in individual response to formaldehyde. A standard drinking water rate (2.8 L/d) and body weight (70 kg) for astronauts was assumed in calculating the AC.

The rationale for applying the results of a chronic study to the 100-d SWEG is that there is sufficient evidence that the gastric effects observed in the chronic study could occur over a shorter timeframe. Til et al. (1988) observed the same gastric changes in their 4-wk study, at very similar doses (125 mg/kg/d as compared to 82 and 109 mg/kg/d in the chronic study). Also, Til et al. (1989) observed significantly reduced weight gain, water intake, and food intake throughout much of their study (especially with the male rats), which is suggestive that there were treatment-related effects relevant to the 100-d exposure timeframe.

100- and 1,000-d ACs = (15 mg/kg/d [NOAEL] × 70kg) ÷ (2.8 L/d × 10 [species] × 3 [interindividual]) = 12 mg/L (rounded).

Allergic Contact Dermatitis

Jordan et al. (1979) evaluated low-level exposure to formaldehyde with respect to the potential for elicitation of ACD. Among other tests, the authors performed repeated axillary spray tests on 13 human volunteers with ACD. Solutions of 30 ppm (0.003%) formaldehyde were sprayed onto the axilla twice a day for 2 wk. This type of experimental design is likely to be more applicable than closed patch testing to on-orbit exposures to formaldehyde in water. With the exception of two in-

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dividuals, no response was observed in this testing. The two individuals who might be characterized as responders only displayed very mild perifolicular dermatitis after 2 wk of testing. The authors concluded that formaldehyde concentrations below 30 ppm can be tolerated without notable response (even by individuals with ACD) for extended exposure durations (Jordan et al. 1979).

In developing ACs for ACD, the 30 mg/L threshold was taken as the starting concentration that will be protective of responsive individuals. Adjustment to estimate a NOAEL was not accomplished. With ACD, it can be difficult, if not impossible, to establish an AC that will protect all individuals (Loomis 1979). For example, one highly responsive individual was reported to elicit an ACD response to formaldehyde at concentrations in water as low as 0.2 ppm (Horsfall 1934). However, contact with water containing less than 30 mg/L of formaldehyde is unlikely to result in any notable ACD response, even for individuals who already have ACD (WHO 2002). Since the Jordan et al. results are based on a human study of individuals with ACD, no adjustment was necessary for species extrapolation or to ensure protection of a susceptible population. Also, accounting for the small number of volunteers is not considered necessary given the populations studied and the mildness of the potential response to this low concentration of formaldehyde.

There is no evidence in the literature to suggest that low-level exposures to formaldehyde in water will cause ACD in crew members who do not already have ACD. Also, given the ubiquitous ground-based use of formaldehyde (for example, use in consumer products, laboratories), it is likely that crew members would have experienced more-significant exposures to formaldehyde prior to flight. With respect to the 100-d and 1,000-d ACs for ACD, it is probable that any ACD response would have been experienced within the timeframe (2-wk study) observed by Jordan et al., and no adjustment was made in setting the 100- or 1,000-d ACs. A significantly higher AC for this end point would be acceptable for the vast majority (96-99%) of the population (Marks et al. 1995) that does not already have contact dermatitis from exposure.

All timeframe ACs = 30 mg/L.

Carcinogenicity

Interpretation of the Soffritti et al.'s (1989, 2002) cancer findings for formaldehyde in terms of their applicability to SWEG derivation is complex. First, two chronic duration animal studies (Til et al. 1989; Tobe

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et al. 1989) did not report increased tumor incidence in the GI tract or other evaluated organ systems. As discussed previously, Soffritti et al. (1989, 2002) reported both GI tumors and cancers at other sites distant from the portal of entry. With respect to observed GI tumors, it is biologically plausible that sufficient formaldehyde exposure could result in cancers at the portal of entry (through incorporation of formaldehyde into critical macromolecules and tissue damage and cell proliferation at the point of contact). However, given the rapid metabolism of formaldehyde, it is likely that a threshold would exist below which formaldehyde ingestion would not pose a credible cancer risk (CIIT 1999). Additionally, both Soffritti et al. studies showed a relatively low incidence of treatment-related GI tumors, and these tumors were generally limited to the high-exposure groups (188 and 313 mg/kg/d). Consistent with the understood mechanism of action of formaldehyde in the development of tumors in association with inhalation exposures, oral portal-of-entry tumors would likely be dependent on cytotoxicity, hyperplasia, and regenerative cell proliferation (CIIT 1999; WHO 2002). Even when considering the possibility of a mutagenic mechanism of action (expressed through DNA-protein crosslink formation), cancer risks associated with low levels of formaldehyde exposure would be much lower than those based on extrapolations from doses sufficient to evidence cytotoxicity and cell proliferation (Schlosser et al. 2003). Overall, these studies suggest that formaldehyde exposures that do not result in GI irritation or damage are also unlikely to represent a concern for GI cancers. ACs were not established for GI cancers based on this assessment. This approach is consistent with the WHO (2002) conclusion that the "lack of evidence for the potential carcinogenicity of ingested formaldehyde precludes an analysis of exposure–response for this effect."

With respect to the Soffritti et al. observations of tumors at sites distant from the portal of entry (that is, leukemias, mammary gland adenocarcinomas, testicular adenomas), there is uncertainty as to their applicability to SWEG development. The Soffritti et al. findings contradict other drinking water studies (Til et al. 1989; Tobe et al. 1989) in which these types of tumors were not observed, and are inconsistent with the lack of increased concentrations of formaldehyde in the blood (Heck et al. 1985) and the general absence of tumors at sites other than the portal of entry with inhalation exposures to formaldehyde. The findings from the Soffritti et al. (1989) study were reviewed by WHO (2002) and IRIS (2004) but were not utilized in development of oral-cancer risk estimates by these organizations. Similarly, ACs for these cancer end points were not developed in this document.

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8

Formate

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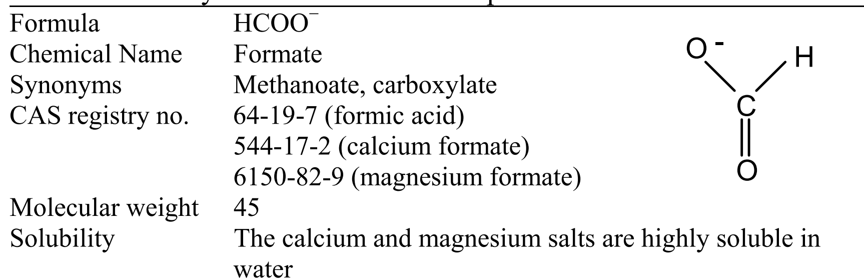
TABLE 8-1 Physical and Chemical Properties



Formula	$HCOO^-$
Chemical Name	Formate
Synonyms	Methanoate, carboxylate
CAS registry no.	64-19-7 (formic acid) 544-17-2 (calcium formate) 6150-82-9 (magnesium formate)
Molecular weight	45
Solubility	The calcium and magnesium salts are highly soluble in water

Formate is the anion of formic acid, the simplest organic acid.

Source: Data from HSDB 2006.

OCCURRENCE AND USE

Formic acid is found in the venom of ants, from which it got its name (Latin *formica*), as well as in the venoms of other insects and a variety of plants. Formate ions ($COOH^-$) are generated in mammalian tissues during normal metabolism of glyoxylate. Formate ions are also generated as an intermediate during mammalian metabolism of methanol and formaldehyde to carbon dioxide (CO_2). In industry, formic acid is used as a food preservative, in a variety of chemical syntheses, in tanning leather, as a decalcifying agent, and in the preservation of silage (Liesivuori 1986; Dorland 1988; Thompson 1992). Endogenous concentrations of formate in human blood are in the range of 0.1-0.2 micromoles per milliliter (µmole/mL) (4.5-9.0 micrograms per milliliter [µg/mL]) (Medinsky and Dorman 1995). See Table 8-1 for the physical–chemical properties.

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Formate is added to potable water on the International Space Station (ISS) in the process of adding minerals to pure water to make it more palatable. Highly pure water is supplemented with calcium and magnesium as the formate and acetate salts by adding 100 mL of a stock containing formate at 37.8 g/L to 44 L of pure (fuel-cell generated) water to yield about 86 mg/L (0.52 millimoles per liter [mmol/L]). Normally, the room-temperature water-dispensing port on the ISS is supplied with this U.S. formate-containing water, sometimes diluted with non-formate-containing water from other sources, while heated water (without formate) comes from Russian-supplied sources (either regenerated or natural water from Russia). If all the drinking water were formate-containing U.S. water, an astronaut would ingest 241 mg per day (d), at a consumption rate of 2.8 L/d of water.

TOXICOKINETICS AND METABOLISM

The distribution, metabolism, and elimination of formate have been studied in humans, nonhuman primates, rodents, and pigs.

Absorption

Gastrointestinal (GI) absorption of formate is almost total, as seen in cases of accidental and intentional self poisoning with formic acid solutions (Naik et al. 1980). No data were found on the rate of absorption of formate from the GI tract.

Distribution

Absorbed formate is distributed in the body water compartment, which in humans, is about 0.5 L per kilogram (kg) (Liesivuori and Savolainen 1991). No data were found on tissue-specific differences in the distribution of formate.

Metabolism

The major route of formate metabolism in the rat, monkey, and presumably in humans is oxidation to CO_2 via the folate biochemical path-

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way. Formate enters the pathway by combining with tetrahydrofolate. In a tetrahydrofolate-dependent reaction, formic acid is slowly metabolized to CO_2 in primates but is metabolized about twice as rapidly in nonprimates (McMartin et al. 1977).

Formic acid is produced during metabolism of methanol in humans and animals, and the delayed toxicity of ingested methanol in humans appears to be due to the toxicity of formate. The susceptibility of various species to methanol toxicity is inversely related to the rate of tetrahydrofolate-dependent oxidation of formate to CO_2 (Black et al. 1985). Two mechanisms may be operative in explaining low formate oxidation in species susceptible to methanol toxicity: low hepatic tetrahydrofolate concentrations and reduced hepatic 10-formyltetrahydrofolate dehydrogenase activity (Johlin et al. 1987). The activity of 10-formyltetrahydrofolate dehydrogenase, the enzyme that catalyzes the final step of formate oxidation to CO_2, has been reported to be markedly (four- to fivefold) reduced in both monkey and human liver compared to rodent liver, consistent with lower formate oxidation rates in primates. Both of these mechanisms should apply to ingested formate (for example, in spacecraft drinking water) as well as formate generated by the metabolism of methanol.

Excretion

The majority of ingested formate is excreted as its metabolite, CO_2. Renal elimination of formate appears to be minimal (Kerns et al. 2002), being 5% in humans (Lund 1948). In a later study, when sodium formate was given orally to humans, 2.1% of the dose was detected in urine collected over 24 hours (h) (Malorny 1969).

In monkeys, whose sensitivity to the ocular toxicity of methanol is similar to that of humans, the half-life of formic acid in the blood is about 31-51 minutes (min) for intravenous (iv) doses of 50-470 mg/kg (Clay et al. 1975). At low doses (≤100 mg/kg), iv-infused formic acid is cleared from monkeys with a half-life of 31 min (Liesivuori and Savolainen 1991). The half-life increases with increasing formate dose, indicating a nonlinearity in formate disposition. Data from animal studies (Clay et al. 1975; McMartin et al. 1977) suggest that this represents an unusual dose-dependent metabolic clearance that is the result of depletion of the co-substrate (tetrahydrofolate) for the 10-formyl tetrahydrofolate dehydrogenase reaction. Under this kinetic condition, the elimination of formate will appear first-order with increasing doses, but the apparent elimination rate constant will decline with increasing doses

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(Galinsky and Levy 1981). This behavior is only an approximation of more-complex formate elimination kinetics that will occur with a dynamic state of depletion and repletion of the co-substrate as formate is introduced into the body and then eliminated. However, at every dose studied, formate elimination in monkeys appears to behave as a pseudo-first-order process over a period of time when approximately 75-87% of the dose had been eliminated (Clay et al. 1975). In humans who had ingested toxic doses of methanol and had been treated with fomepizole and folate supplementation, the endogenous elimination half-life of formate was reported to be 3.4 h (Kerns et al. 2002). Interpretation of formate half-lives calculated using data from methanol intoxications, however, is complicated by the simultaneous production of new formate because of continuing methanol metabolism. The observed formate half-life could be formation-rate limited and not elimination-rate limited. The true formate half-life could be less than or equal to that observed after methanol ingestion. Thus, the 3.4-h half-life value may not be relevant when the exposure to formate is not from the ingestion of methanol.

The formate concentrations in the blood of a methanol-intoxicated 21-year (y)-old patient remained relatively constant until blood pH was normalized by bicarbonate treatment, after which formate concentrations declined, suggesting that formate elimination may be pH dependent (Jacobsen et al. 1988).

TOXICITY SUMMARY

Formic acid is highly irritating and is caustic to the skin.

The main toxic effects associated with the ingestion of methanol (a metabolic precursor of formate) are metabolic acidosis and blindness from optic nerve injury. The visual toxicity has been shown to be independent of pH (Martin-Amat et al. 1978). The mechanism of cytotoxicity is believed to involve histotoxic hypoxia (the reduced ability of cells or tissue to accept and use oxygen from the blood, such as in cyanide poisoning) in aerobic cells caused by the impeding of oxygen metabolism (Liesivuori 1986). Optic nerve cells have relatively few mitochondria and thus have a high sensitivity to histotoxic hypoxia (Jacobsen and McMartin 1986). Formate acts as a reversible inhibitor of the mitochondrial cytochrome c oxidase complex (Nicholls 1975, 1976; Erecinska and Wilson 1980) with an apparent inhibition constant (*Ki*) between 1 and 30 millimolar (mM) (45-1,350 µg/mL) at pH 7.4, 30˚C (Nicholls 1975, 1976), depending on the reduction state of the system. Inhibition of aero-

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bic respiration is known to stimulate anaerobic glycolysis, resulting in increased lactate production and intra- and extracellular lactic acidosis (Seisjo 1992). Metabolic and lactic acidosis are both hallmark features of severe human methanol intoxication (Erlanson et al. 1965; Koivusalo 1970; Jacobsen and McMartin 1986). The acidosis causes a decreased production of adenosine triphosphate (ATP) (Liesivuori and Savolainen 1991).

Interpretation of toxicity data from experiments involving exposures to methanol is complicated by interspecies as well as nutritional status-dependent differences in the rate of metabolism of methanol. Although reports from Finland (Liesivuori et al. 1987; Liesivuori and Savolainen 1991) and the United States (Martin-Amat et al. 1977, 1978; McMartin et al. 1977, 1980; Jacobsen et al. 1988; Johlin et al. 1989; Tephly 1991; Eells et al. 1996) implicate formate as the toxic agent in methanol poisoning, dissenting investigators from Japan (Hayasaka et al. 2001) assert that formaldehyde is the toxic agent in methanol-induced eye toxicity.

Acute and Short-Term Exposures (≤10 d)

Moore et al. (1994) reported that iv folinic acid and furosemide and urinary alkalinization resulted in the survival after attempted suicide of a woman who ingested 110 g of formic acid. They state that ingestion of over 60 g of formic acid is potentially fatal. A successful suicide reported by Westphal et al. (2001) was attributed to massive acidosis, hemolysis, bleeding complications, and hepatic and renal failure after ingestion of an estimated 44-88 mL of a decalcifying agent containing 60% volume per volume (v/v) formic acid. Westphal's review of the literature indicated that formic acid concentrations in the blood of healthy humans range from 3.2-56 µg/mL and that ingestion of less than 30 g of formic acid was never fatal. Westphal et al.'s reported value of formate at 56 µg/mL blood in unexposed humans appears to be a typographical error, however, based on examination of his cited sources for this value. The highest value found in any of the articles cited by Westphal et al. was 5.6 ± 4.5 µg/mL (Baumann and Angerer 1979).

No reports were found in the literature of ocular toxicity in humans who had ingested formic acid or formate, whereas ocular toxicity is commonly seen in cases of human exposure to methanol. McMartin et al. (1980) reported severe metabolic acidosis and optic disc edema in two patients whose blood formate concentrations were 11.1 and 26.0 mil-

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liequivalents (mEq) per milliliter when measured after hospitalization for methanol intoxication. The patient with the higher blood formate concentration died despite aggressive treatment (bicarbonate, iv 10% ethanol, and hemodialysis) of both patients.

Histotoxicity (manifested as extracellular calcium casts) has been reported in several tissues examined 1 h after the fifth daily dosing in male New Zealand rabbits (3,070 ± 220 g) given daily iv 1 mL doses of formate at 100 mg/kg (Liesivuori et al. 1987). If we assume a body water volume to body mass ratio of about 700 mL water per kilogram, the rabbits had about 2,200 mL body water, and the formate concentration immediately after the injection would have been 307 mg/2,200 mL = 140 µg/mL body water. Measured blood formate concentrations were 0.7 µmole/g (29 µg/mL) at 1 h after the fifth daily dose, 0.5 µmole/g (21 µg/mL) at 2 h, and 0.2 µmole/g (8.4 µg/mL) at 20 h. Thus, it appears that 80% of the administered dose of formate had disappeared from the blood by 1 h postdosing, 5% more during the second hour postdosing, and 6% during the following 18 h. This is consistent with a half-life of formate in rabbits of about 26 min. The reduction of blood formate concentrations could be caused by either rapid sequestration of formate into other tissues or by rapid metabolism of the formate. No mention was made in this report of any ocular toxicity in the rabbits.

In humans, permanent visual damage has been associated with prolonged exposures (>24 h) to blood formate concentrations > 7 mM (315 µg/mL) produced during methanol intoxications (Hayreh et al. 1977, 1980; Jacobsen and McMartin 1986). The formate concentration in these patients (315 µg/mL) was higher than the initial concentration calculated for the rabbits (140 µg/mL) in Leisivuori's study. Also, the fact that formate was being constantly produced by metabolism of ingested methanol means that elevated blood concentrations of formate were maintained for a prolonged period in methanol-intoxicated humans as opposed to rapidly declining to near background levels in the formate-treated rabbits, presumably from metabolism. Clinical observations in methanol-intoxicated humans have shown that in its initial stages, developing ocular toxicity can be reversed, even >24 h after methanol ingestion, by treatments such as bicarbonate, fomepizole or ethanol, iv folinic acid, or dialysis, which reduce blood formate concentrations and metabolic acidosis (Barceloux et al. 2002). Such reversibility is consistent with a mechanism of formate toxicity involving gradual optic nerve histotoxicity due to prolonged metabolic hypoxia and with the reversibility of formate inhibition of cytochrome c oxidase (Nicholls 1975).

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Martin-Amat et al. (1978) determined that the ocular toxicity (edema of the optic disc and loss of pupillary response) of methanol could be reproduced in male rhesus monkeys by iv infusion of sodium formate at 0.5 M, even when bicarbonate was administered to prevent acidosis. Infusion at a rate of about 3.1 mEq/kg/h (formate at 140 mg/kg/h) after a loading dose of 1.25 mmole/kg (formate at 56 mg/kg) was calculated so as to maintain 10-30 mEq/L (450-1,350 μg/mL) formate in the blood over a period of 25-39 h. Note that because a quasi-steady state was achieved, the infusion rate is also an estimate of the rate of removal of formate from the blood by metabolism and excretion. In most of the treated animals, no pupillary response to light was observed at between 24 and 48 h after the onset of formate infusion, but in one of the four monkeys tested, normal pupillary reflexes and only moderate optic disc edema were observed at 25 h after initiation of treatment. In this monkey, the maximum blood concentration of formate achieved was 540 μg/mL at 25 h postinitiation of treatment, compared to 900-1,530 μg/mL for the other three monkeys, as measured at later times (39-50 h post-initiation of treatment). The onset of ocular toxicity generally occurred more rapidly in the formate-treated animals than in monkeys that had received methanol in previous studies.

A more sensitive test for ocular toxicity (measurement of the reductions in the a and b waves of electroretinograms, [ERGs]) was used by Eells et al. (1996) to demonstrate that intraperitoneal (ip) injections of methanol in a regimen that maintained blood formate concentrations at 4-6 mM (180-270 μg/mL) for 60 h showed evidence of causing retinal dysfunction in the absence of retinal histopathology in nitrous oxide (N_2O)-treated rats. The retinal dysfunction was not correlated with any clinical signs. The rats in this experiment were made to more closely resemble humans and nonhuman primates in their methanol sensitivity by using subanesthetic concentrations of N_2O to selectively inhibit formate oxidation by inactivating the enzyme methionine synthetase, thereby reducing the production of tetrahydrofolate (Eells et al. 1996). The 4-6 mM (180-270 μg/mL) concentrations of formate in blood were maintained in this experiment by ip injections of methanol at 2 g/kg in 12 h intervals. Significant reductions in ERG a-wave and b-wave amplitude were not observed until 60 h after methanol administration, although blood formate concentrations reached the plateau level of about 180 μg/mL by 12 h, the earliest time of measurement. Another group of rats in this experiment was treated with higher doses of methanol so that blood formate concentrations increased almost linearly from a baseline of 0.8 mM (36 μg/mL) to 7 mM (315 μg/mL) at 12 h to 15 mM (675 μg/mL) at 60 h after methanol administration. ERGs of these high-dose rats showed reduc-

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tions in the b wave as early as 24 h after methanol administration and profound attenuation or complete elimination of the b wave by 48-60 h after methanol administration. Significant but less pronounced reductions in the a wave were seen in the high-dose animals. Histopathologic changes including edema in the outer nuclear layer and vacuolization in the photoreceptors and the bases of retinal pigment epithelium cells were seen in the high-dose but not the low-dose rats at 48 h after methanol administration.

The ocular toxicities of 1% methanol, 0.1% or 1% formaldehyde, or 1% formate were compared in rabbits after a single intravitreal injection of 100 µL (Hayasaka et al. 2001). The eyes were examined ophthalmoscopically at 1 d, 2 d, 1 week (wk), 2 wk, and 1 month (mo) after treatment. Mild inflammation was seen at 1 d and 2 d for all chemicals, but by 1 wk, the eyes treated with methanol and formate appeared nearly normal while those treated with 0.1% formaldehyde showed retinal vessel dilation and those treated with 1% formaldehyde showed mild posterior subcapsular cataract, retinal vessel dilation, and retinal hemorrhages. At 1 mo, animals who received 0.1% or 1% formaldehyde showed mild subcapsular cataract, vessel dilation, and juxtapapillary retinal hemorrhages. Histopathologic study of the eyes showed nearly normal retinas in animals that received vehicle, methanol, or formate, but disorganized ganglion cell layer and outer nuclear layer in eyes that received 0.1% formaldehyde, and markedly disorganized retina in eyes that received 1% formaldehyde. Similar differences were seen in the optic nerves, with formaldehyde-treated eyes showing vacuolizations. The calculated intravitreal concentrations of the treatment chemicals were methanol at 700 µg/mL, formate at 700 µg/mL, and formaldehyde at 70 µg/mL and 700 µg/mL. The report did not consider the potential for dilution of intravitreal chemicals into the systemic circulation. Equally importantly, because these experiments involved a single dose of formate and methanol, the resulting reversible intracellular hypoxia may not have been prolonged enough to produce lasting injury, whereas the high reactivity of formaldehyde would be expected to cause immediate local injury.

From the above observations, it appears that the production of ocular toxicity by formate requires that the formate blood concentration remain elevated (≥180 µg/mL) for at least 24 h. There are not sufficient data, however, to predict with confidence the effects of repeated low doses of formate such that blood concentrations oscillate between toxic and nontoxic concentrations.

The amount of formate needed to achieve a blood formate concentration (180 µg/mL) in humans that has been reported (Eells et al. 1996)

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to cause electrophysiologic toxicity (but no histopathology) in retinas of N_2O-treated rats for exposures of up to 60 h would require ingestion of a bolus of formate at about 9.1 g. This value was calculated (180 mg/L × 70 kg × 0.72 L/kg = 9,070 mg) assuming that formate is distributed throughout the water that makes up 72% (Lentner 1981) of a 70 kg body, assuming 100% uptake of ingested formate and no metabolism or excretion so that peak concentrations could be achieved and maintained.

Subchronic and Chronic Exposures (≥10 d)

No reports were found describing exposures to formate with durations of 10 d or more.

Genotoxicity

In tests by the National Toxicology Program (NTP), formic acid was found not to be mutagenic to *Salmonella typhimurium*, with or without metabolic activation (Thompson 1992). No genotoxicity studies on mammalian cells were found.

Reproductive Toxicity

No reports were found describing the reproductive toxicity of formate.

Developmental and Fetal Toxicity

No reports were found describing the developmental or fetal toxicity of formate.

Summary

The published literature on the toxic effects of formate in humans and animals is summarized in Table 8-2. Probably because it is not a common contaminant and because it is a normal product of metabolism, no organizations have set drinking water exposure limits for formate.

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TABLE 8-2 Toxicity Summary

Dose and Route	Exposure Duration	Species and Strain	Effects	Reference
Pre-exposure				
0.1-0.2 µmole/mL (4.5-9.0 µg/mL)	Endogenous in blood	Human	Endogenous formate concentration in human blood	Medinsky and Dorman 1995
Acute and Short Term Exposures (≤10 d)				
Effects on vision:				
0.5 M sodium formate buffer (Na:H=10:1) 140 mg/kg/h iv infusion	24-48 h	*Maccaca mulatta* monkey, male; n = 3/4	No pupillary reflexes in response to light	Martin-Amat et al. 1978
0.5 M sodium formate buffer (Na:H=10:1) 140 mg/kg/h iv infusion	39-50 h	*Maccaca mulatta* monkey, male; n = 4/4	Optic disc edema with normal vascular bed and intracellular edema with intraaxonal swelling	Martin-Amat et al. 1978
180-270 µg/mL blood pre-exposed to 1:1 N_2O/O_2	48 h	Long-Evans rats, male	NOAEL for retinal dysfunction (ERG changes) with no clinical signs	Eells et al. 1996
180-270 µg/mL blood pre-exposed to 1:1 N_2O/O_2	60 h	Long-Evans rats, male	LOAEL for retinal dysfunction (ERG changes), but no metabolic acidosis or retinal histopathology	Eells et al. 1996
315-675 µg/mL blood pre-exposed to 1:1 N_2O/O_2	48 h	Long-Evans rats, male	LOAEL for retinal dysfunction (ERG changes), metabolic acidosis, and retinal histopathology	Eells et al. 1996

(Continued)

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TABLE 8-2 Continued

Dose and Route	Exposure Duration	Species and Strain	Effects	Reference
Histotoxic hypoxia:				
100 mg/kg/d; iv for 5 d	1/d × 5 d	New Zealand rabbit, male	Histotoxicity including calcium aggregates in the kidneys, liver, heart, and brain, and an increased number of myelin figures in cardiac cells	Liesivuori et al. 1987
Lethality:				
348 µg/mL in blood at admission	Unknown	Human	Shock, metabolic acidosis, hemolysis.	Verstraete et al. 1989
1.2 mg/mL in blood at 8 h posthospitalization	Unknown	Human	Coma; severe metabolic acidosis; optic disc edema	McMartin et al. 1980

Abbreviations: ERG, electroretinogram; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level.

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RATIONALE

Table 8-3 lists formate SWEGs for 1-, 10-, 100-, and 1,000-d durations, that were calculated as follows. Acceptable concentrations (ACs) were determined following the guidelines of the National Research Council (NRC 2000). ACs were calculated assuming consumption of 2.8 L of water per day. This includes an average of 800 mL/d of water used to prepare and reconstitute food in addition to 2 L/d for drinking. A value of 70 kg was used as the mass of an average astronaut. A number of conservative assumptions were made in calculating the spacecraft water exposure guidelines (SWEG) values for formate based on the retinal toxicity data reported by Eells et al. (1996). First of all, the experiments reported by Eells et al. involved an artificial-exposure scenario (ip methanol and inhaled N_2O) designed as a mechanistic model rather than as a means of extrapolating toxic exposures. Also, although the weight of evidence in both humans and animals, as presented above in the toxicity sections, strongly indicates that retinal toxicity requires 24-60 h of continuous blood formate concentrations > 315 µg/mL, SWEG values were calculated to avoid even brief, transient blood formate concentrations ≥180 µg/mL. A 1-h blood half-life, twice that reported for monkeys (whose sensitivity to formate is similar to humans), iv infused at ≤100 mg/kg, was assumed in calculating SWEG values. It is assumed that an iv-infused bolus of formate at 100 mg/kg would be equivalent to a concentration of 100 mg/kg × 70 kg ÷ 670 mL = 10,448 µg/mL in 670 mL of drinking water. The only reported value found for the half-life of formate in humans was 3.4 h, as seen in methanol-intoxicated individuals (Kerns et al. 2002). This clinical half-life is almost certainly greater than the true half-life of formate, because of continuing production of large amounts of new formate from methanol in the intoxicated individuals.

Spaceflight Effects

None of the reported adverse effects of formate exposures are known to be affected by spaceflight.

1-d SWEG

Because one of the sources of ISS water is supplemented with multiple formate salts that result in a formate concentration of 86 mg/L

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TABLE 8-3 Spacecraft Water Exposure Guidelines for Formate

Duration	mg/L	Target Toxicity
1 d	10,000	Ocular effects (abnormal ERG)
10 d	2,500	Ocular effects (abnormal ERG)
100 d	2,500	Ocular effects (abnormal ERG)
1,000 d	2,500	Ocular effects (abnormal ERG)

(86 µg/mL) in that source of drinking water, daily consumption of formate from ISS water is ≤ 2.8 L/d × 86 mg/L ≈ ≤ 240 mg/d. No data were found on the rate of absorption of formate from the gut; therefore, the most conservative assumption is instantaneous 100% absorption and distribution into a body water volume of 0.72 L/kg × 70 kg = 50.4 L. The weight of evidence suggests that humans can metabolize formate at about the same rate (half-life = 0.5 h) as monkeys, because they have comparable hepatic concentrations of tetrahydrofolate (Tephly 1991), but the only published half-life for formate in humans is 3.5 h as reported for methanol-intoxicated humans treated with folinic acid (Kerns et al. 2002). Use of such a prolonged half-life in calculations of acceptable (that is, low) formate concentrations, however, would be excessively conservative. To allow for interindividual differences in metabolic rates and temporal fluctuations in hepatic tetrahydrofolate concentrations, it should be sufficient to assume a maximum half-life of 1 h for individuals exposed to formate (not derived as a product of methanol metabolism). We can mathematically model the kinetics of blood formate concentrations by making the following assumptions:

- The background blood formate concentrations in humans are ≤10 µg/mL. This value represents the mean +2 standard deviations for the highest value reported by Baumann and Angerer (1979) for unexposed healthy humans and agrees well with the higher of the endogenous blood formate concentrations (4.5-9.0 µg/mL) reported by Medinski and Dorman (1995).
- The half-life of formate in the blood of humans is ≤1 h unless the blood formate is a product of metabolism of ingested methanol.
- The 2.8 L/d average water consumption is divided into 5 × 400 mL beverages (3 with meals + 1 midafternoon + 1 before bedtime) and 3 × 270 mL for food hydration.

For a one-compartment model, the concentration of formate in the

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blood resulting from ingestion of water containing formate can be calculated using the following equation:

μg formate after ingestion per mL blood = μg formate before ingestion per mL blood + (μg formate per mL in water × mL of water ingested)/mL blood.

The amount of formate in the blood at *t* h after ingestion, assuming a 1-h half-life, can be calculated using the following equation:

$$\mu\text{g formate }(t) = \mu\text{g formate (initial)} \times e^{(-0.69315\,t)}$$

Figure 8-1 illustrates the predicted kinetics of formate concentrations in the blood over a >5-d period in crewmembers ingesting 2.8 L/d of U.S.-provided ISS water (containing formate at 86 μg/mL) fractionated over a

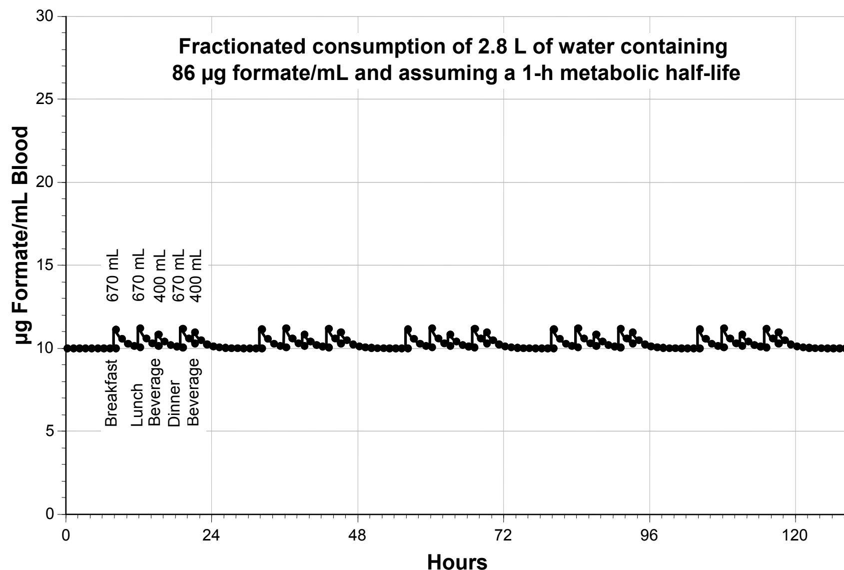

FIGURE 8-1 Calculated blood formate kinetics for a 70 kg person ingesting water containing formate at 86 μg/mL. The daily pattern of ingestion was assumed to be 670 mL at 8 h (for example, 8:00 a.m.), 670 mL at 12 h, 400 mL at 15 h, 670 mL at 19 h, and 400 mL at 21 h. The metabolic half-life was assumed to be 1 h.

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conservatively low number of portions (that is, high volume of water per portion). Consumption of up to 670 mL of this water increases the peak blood concentrations by ~1 µg/mL, but the concentrations return to baseline between ingested portions. Baseline concentrations of blood formate have been reported by various authors to range from 4.9 to 10.3 µg/mL (Bouchard et al. 2001); thus, a transient increase of ~1 µg/mL would be highly unlikely to cause any adverse effects.

To estimate an AC of formate in drinking water for a 1-d exposure duration, one can select a concentration that is below the lowest concentration reported to cause minimal effects. As noted earlier, visual-system damage in humans has been associated with prolonged exposures (>24 h) to blood formate concentrations > 315 µg/mL. Eells et al. (1996) reported that blood formate concentrations of 180-279 µg/mL in rats (treated with N_2O to make their response to formate similar to that of humans and repeatedly ip injected with methanol) were a NOAEL for retinal dysfunction, histopathology, and metabolic acidosis for exposures of 48 h and a LOAEL for retinal dysfunction, still without histopathology or metabolic acidosis, for exposures of 60 h. ERGs of high-dose (315 µg/mL at 12 h up to 675 µg/mL at 60 h) rats showed reductions in the b wave as early as 24 h after methanol administration and profound attenuation or complete elimination of the b wave by 48-60 h after methanol administration. Thus, the sensitivity of rats in Eells et al.'s 1996 study to formate-induced ocular toxicity is similar to that of humans, and concentrations of formate in drinking water that do not increase blood formate concentrations above 180 µg/mL should be acceptable for humans. Figure 8-2 shows that drinking water containing formate at 10,000 µg/mL (10,000 ppm or 10 g/L) meets this goal because peak blood formate concentrations are about 150 µg/mL. After ingestion of each portion of water, the blood formate concentration decreases rapidly, returning to baseline within 20 h after the first ingestion.

The weight of evidence strongly indicates that the ocular toxicity of formate is caused by prolonged cellular hypoxia and that mild ocular symptoms (for example, blurred vision) can be reversed if the blood concentration of formate is promptly reduced. Thus, a 1-d exposure to formate concentrations that transiently approach (but do not reach) concentrations that are minimally toxic to the retina when maintained for 60 h would not be expected to cause any ocular toxicity.

As illustrated in Figure 8-2, continued exposure to formate at 10,000 µg/mL drinking water for several days results in a repetition each day of the same pattern of blood formate concentrations seen on the first day and of formate concentrations returning to baseline values before the

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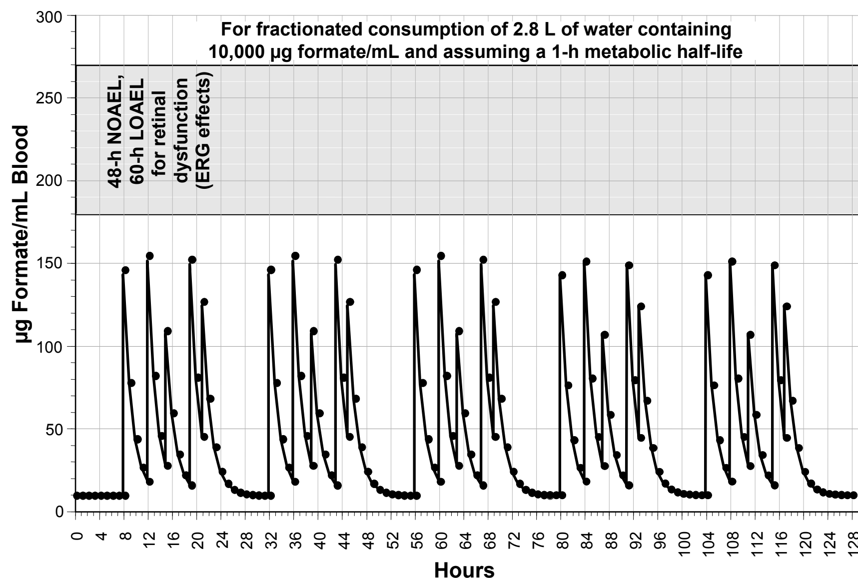

FIGURE 8-2 Calculated blood formate kinetics for a 70-kg person ingesting water containing formate at 10,000 µg/mL. The pattern of ingestion was assumed to be 670 mL at 8 h (for example, 8:00 a.m.), 670 mL at 12 h, 400 mL at 15 h, 670 mL at 19 h, and 400 mL at 21 h. The metabolic half-life was assumed to be 1 h.

first exposure of each 24-h period. At 10,000 µg/mL in the water, the maximum blood formate concentration achieved is about 150 µg/mL, which is below the lower borderline of the concentration range reported by Eells et al. (1996) to be a LOAEL for ocular toxicity for a continuous 60-h exposure.

10-d, 100-d, and 1,000-d SWEGs

Experimental data on which one could base the calculation of a safe concentration of formate in drinking water for exposure durations of ≥ 10 d are unavailable, so one must extrapolate from the available data on shorter-term exposures based on reasonable assumptions. Although the kinetics of the ocular toxicity of formate is, to some extent, dependent on both the concentration of formate in the blood and the duration of exposure, the weight of evidence indicates that there is an effective threshold concentration of formate below which ocular toxicity will not occur re-

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gardless of the duration of exposure. This can also be inferred from the fact that formate is present at concentrations up to about 10 µg/mL in normal blood as a product of metabolism.

One approach to establishing longer-term SWEGs is to determine a concentration of formate that would cause only minimal inhibition of the mitochondrial cytochrome c oxidase whose inactivity leads to the retinal edema responsible for clinical ocular toxicity. Based on the lower limit of the *Ki* range (45-1,350 µg/mL) for formate determined by Nicholls (1976), an intracellular formate concentration as low as 45 µg/mL can reversibly produce about 50% inhibition of the enzymatic activity of the cytochrome c oxidase complex. The formate concentration in drinking water that would produce a blood formate concentration of 45 µg/mL can be calculated, assuming a drinking water volume of 670 mL and a body water volume of 50.4 L as follows:

µg/mL water = (45 µg/mL − 10 µg/mL endogenous) × 50,400 mL ÷ 670 mL = 2,633 µg/mL, rounded to 2,500 µg/mL.

Peak concentrations of 45 µg/mL blood, such as would be produced by the ingestion of water containing formate at 2,500 µg/mL, would be transient, however (see Figure 8-3). Thus, the level of inhibition of cytochrome c oxidase would peak at about 50% and decline to near baseline before the next exposure (drink). Such a pattern of brief, partial inhibition of cytochrome c oxidase is not expected to result in any clinical or even histologic adverse effects. Therefore, the ACs and SWEGs for all exposure durations longer than 1 d can be set at 2,500 µg/mL in drinking water (see Table 8-4 for a summary of ACs and SWEGs):

10-d SWEG = 2,500 µg/mL
100-d SWEG = 2,500 µg/mL
1,000-d SWEG = 2,500 µg/mL

RECOMMENDATIONS

To refine the data on which the longer-term SWEGs are based, studies should be performed to determine the minimum level of inhibition of cytochrome c oxidase that leads to detectable ocular toxicity and the concentration of formate that will produce that level of inhibition.

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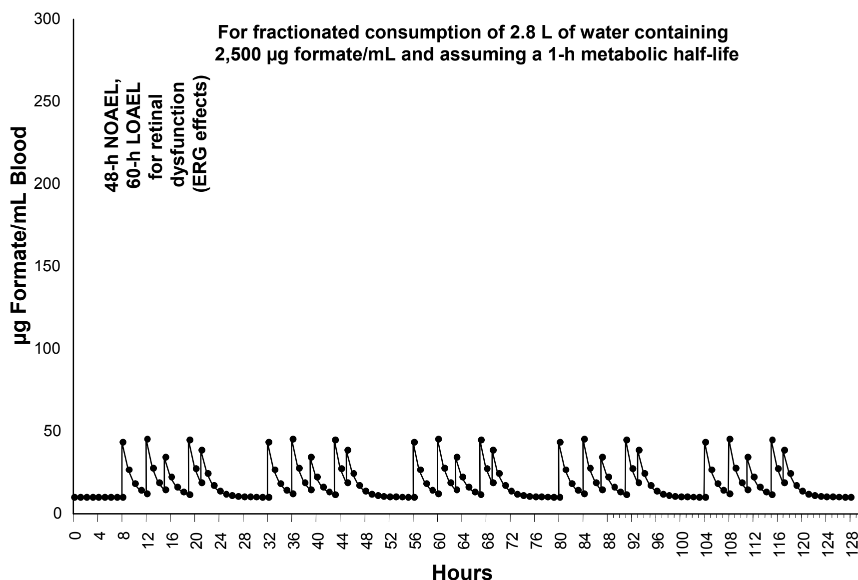

FIGURE 8-3 Calculated blood formate kinetics for a 70-kg person ingesting water containing formate at 2,500 μg/mL. The pattern of ingestion was assumed to be 670 mL at 8 h (for example, 8:00 a.m.), 670 mL at 12 h, 400 mL at 15 h, 670 mL at 19 h, and 400 mL at 21 h. The metabolic half-life was assumed to be 1 h.

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TABLE 8-4 Acceptable Concentrations (ACs)

			Uncertainty Factors					Acceptable Concentrations (mg/L)			
End Point	Exposure Data	Species and Reference	Inter-individual	To NOAEL	Inter-species	Exposure Time	Space-flight	1 d	10 d	100 d	1,000 d
48-h NOAEL for ERG effects	Injections (ip) of methanol to produce blood concentrations of 180-270 μg/mL	Rat, male (Eells 1996)	1	1	1	1	1	10,000	—	—	—
60-h LOAEL for ERG effects	Injections (ip) of methanol to produce blood concentrations of 180-270 μg/mL	Rat, male (Eells 1996)	1	1	1	1	1	—	2,500	2,500	2,500
SWEG								10,000	2,500	2,500	2,500

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9

Manganese (Inorganic Salts)

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OCCURRENCE AND USE

Manganese (Mn) is a component of several minerals in the earth's crust (rock, soil) and is an abundant element. It is found in most food and in drinking water and is distributed to the environment through dust and industrial emissions during the production of iron alloys and during coal burning. Manganese as methylcyclopentadienyl manganese tricarbonyl (MMT) was used as an additive to gasoline to improve octane rating, to replace tetraethyl lead as an antiknock agent. It has been used in Canada since 1977 (Crump 2000), but it was banned from use as a gasoline additive in the United States because of concerns about neurologic effects from the inhalation of particulate emissions of combustion products of MMT, namely manganese phosphate and manganese sulfate ($MnSO_4$) (see Davis 1999). Manganese exists in several different oxidation states (see Table 9-1). The most common ones are the +2 and +4; the former is the one most commonly found in biologic systems including humans. Manganese chloride ($MnCl_2$), $MnSO_4$, and manganese acetate are the most soluble forms. Manganese oxide, although frequently encountered in the work place, is very insoluble.

Manganese as a metal is primarily used in steel manufacturing. It is also used as a micronutrient in fertilizers and in animal feeds. The sulfate salts are used extensively as nutritional supplements for humans and animals and in dyes and varnishes.

Manganese is an essential element needed for the normal physiologic function of all animal species, including humans. Deficiencies of manganese produce abnormalities in brain function, skeleton and cartilage formation, reproduction, and glucose tolerance and are associated with osteoporosis (see Freeland-Graves 1994). A variety of enzymes

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TABLE 9-1 Physical and Chemical Properties of Some Manganese Compounds[a]

Chemical	Manganese	Manganese Chloride	Manganese Sulfate	Manganese Acetate	Manganese Dioxide
Formula	Mn	$MnCl_2$	$MnSO_4$	Mn $(CH3COO)_2$	MnO_2
Molecular weight	54.94	125.84	151	173	86.94
Percent manganese	100%	43.66%	36.38%	31.75%	63.19%
Solubility in water	Decomposes slowly	723 g/L at 25°C	529 g/L at 5°C	970 g/L at 25°C	Insoluble
General form	—	$MnCl_2 \cdot 4H_2O$	$MnSO_4 \cdot H_2O$	$MnAc \cdot 4H_2O$	
Molecular weight		197.91	169	245	
Percent manganese		27.76%	32.50%	22.40%	

[a]Other common manganese-containing inorganic compounds, such as potassium permanganate ($KMnO_4$), and other compounds, such as the tetraoxides, carbonate, and nitrates of manganese, are not included here.
Sources: Data from Aldrich Company 2006; Bingham et al. 2001; Merck 1989.

have been reported to interact with Mn^{+2} or depend on Mn^{+2} for either catalysis or regulatory properties. Thus, it plays an important role in energy metabolism, bone mineralization, protein and energy metabolism, and the metabolic regulation of several enzymes. Manganese has been reported to activate transferases, decarboxylases, and hydrolases (Wedler 1994). It is also an integral part of mitochondrial superoxide dismutase (thereby playing a role in the protection of free superoxide radical species), carboxylase, and liver arginase (NRC 1989).

Food is the major source of manganese, and different foods vary widely in manganese concentration. Nuts and grains contain a high concentration (18-46 parts per million [ppm]) (IOM 2001). For example, Greger (1999) reported that the average intake from Western and vegetarian diets was in the range of 0.7-10.9 mg/d. Milk products contain about 4 ppm. Other investigators have reported various values. The estimated dietary intakes of several nutritional elements for specific age groups have been reported in the U.S. Food and Drug Administration's (FDA's) *Total Diet Study* and updated at various times (1974-1982, 1982-1984, 1982-1986, 1982-1989, 1982-1991, and 1991-1997). Ac-

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cording to the dietary intake data the from FDA's *Total Diet Study, 1991-1997* (see IOM 2001, Appendix E), the mean daily intakes of manganese for men of age groups 19-30, 31-50, 51-70, and 71+ have been reported to be 3.07, 3.27, 3.07, and 2.82 mg/d, respectively. The corresponding intakes for women of the same age groups are 2.34, 2.43, 2.42, and 2.43 mg/d, respectively. As for the usual amounts of manganese taken as supplements by men and women, according to the National Health and Nutrition Examination Survey (NHANES III, 1988-1994), they are 2.49 mg/d (median or 50th percentile) for men and 2.37 mg/d (median) for women, and the 95th percentile is 5.07 mg/d for both men and women (see IOM 2001, Appendix C-20).

Dissolved manganese was detected in surface water in 51% of 1,577 samples, with a mean of 59 micrograms per liter (μg/L) (range of 0.3-3,230 μg/L). A later survey from 286 locations indicated the mean concentration was 24 μg/L and the range was from 11 μg/L (25th percentile) to more than 51 μg/L (75th percentile) (Smith et al. 1987). A 1962 survey of a public drinking water supply reported a concentration of 100 μg/L of manganese (Durfor and Becker 1964). Other reports estimated the values to be very low, between 4 and 32 μg/L (NRC 1980). The U.S. Environmental Protection Agency's (EPA's) secondary maximum contaminant limit (SMCL) of 50 μg/mL was exceeded a few times in both the recycled water and in the humidity condensate samples collected from space missions. At least once, a maximum of 150 μg/L was found in the reprocessed water. When a particular component is frequently found in water samples, even if it is at concentrations under the National Aeronautics and Space Administration (NASA) interim levels, a spacecraft water exposure guideline (SWEG) is determined. The main reasons for this are a concern that the component could break through the water processing system and the fact that no real-time monitoring instruments are on board the International Space Station (ISS).

PHARMACOKINETICS AND METABOLISM

General

There are no systematic human studies on the pharmacokinetics of manganese after its ingestion. There are several reports of elimination rates of manganese in human subjects occupationally exposed to manganese via inhalation. In all these studies, the elimination was measured based on the injection of tracer doses of radioactive manganese and elimination based on the injection of tracer doses of radioactive manga-

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nese and elimination followed via whole-body disappearance of radioactivity over time. One must consider that the metabolic handling of manganese absorbed from the diet is different from that introduced through intravenous (iv) injection (Davidsson et al.1989a) in spite of the fact that in plasma ^{54}Mn is carried by transferrin, regardless of the route, iv or oral, of administration (Davidsson et al.1989c).

In a study of five men and six women administered oral loads of elemental manganese at 40 mg per kilogram (kg) of body weight after fasting, the mean T-max (time to reach the peak plasma level) ranged from 1 to 3 hours (h). Then the concentrations gradually decreased to initial levels over the next 4 h. The time to reach the C-max (maximum blood concentration) varied with subjects (Freeland-Graves and Lin 1991). These authors did not calculate any other pharmacokinetic parameters.

In a recent study, the toxicokinetics of manganese were investigated in male and female rats following a single iv or oral dose of $MnCl_2$ (6 mg/kg) (Zheng et al. 2000). For the oral dosing, rats were fasted for 12 h before dosing. Upon iv administration of $MnCl_2$, manganese rapidly disappeared from blood, with a terminal elimination half-life ($t_{½}$) of 1.83 h and plasma clearance (CL) of 0.43 L/h/kg. After oral administration of $MnCl_2$, manganese was rapidly absorbed from the gastrointestinal (GI) tract and entered the systemic circulation (T-max = 0.25 h; C-max = 0.3 µg/mL). The absolute oral bioavailability was about 13% (calculated from the area under the curves from iv and oral dosing). The elimination half-life for the iv dose was 1.83 h compared to 4.6 h for the oral bolus. Plasma concentrations returned to predose levels 12 h after dosing.

Absorption

Orally ingested manganese is absorbed from the GI tract, but not very efficiently, and the review of literature indicates that only about 3-5% is absorbed in humans and animals. Under normal conditions, the absorption of Mn^{+2} is low because of poor solubility of the cationic Mn^{+2} such as in $MnCl_2$ and $MnSO_4$, in the alkaline pH of the intestine. Mn^{+2} absorption in the GI tract is controlled by homeostatic mechanisms; the absorption rate depends on the amount ingested and the plasma levels of Mn^{+2}. The manganese ion is transported across gut walls by both active transport (based on in vitro studies using the everted intestinal sacs) and by diffusion (Cikrt and Vostal 1969, as cited in WHO 1981), with the diffusion process taking place in iron-overloaded states. However, Garcia-Aranda et al. (1983) using an in vivo perfusion system and perfusing

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segments of either jejunum or ileum with isotonic solutions containing $MnSO_4$ at 0.0125-0.1 millimoles (mM) concluded that absorption of manganese in rat intestines takes place through a high-affinity, low-capacity, active transport mechanism and suggested that the diffusion-mediated transport plays only a limited role. Manganese absorption (fractional absorption) from diet has been found to vary according to the amount of manganese in the diet. For example, using a rat model and using ^{54}Mn retention method, Davis et al. (1992a, b) found that the absorption of manganese from a manganese-deficient diet was at least twofold higher than from a manganese-adequate diet or a diet containing higher concentrations of manganese measured after 7 weeks (wk) of feeding.

Lee and Johnson (1988) reported that in rats fed diets containing manganese at between 1.3 and 82.4 mg/kg for 7 or 14 d and administered a tracer dose of ^{54}Mn by gavage, increasing dietary manganese reduced manganese absorption and enhanced ^{54}Mn excretion. Absorption of ^{54}Mn by fasted, gavaged rats was four times higher than in unfasted gavaged rats (Lee and Johnson 1988).

Mena et al. (1969) found that only about 3% of the administered dose of $MnCl_2$ was absorbed by human subjects, and the difference between the lowest and highest value among the 11 normal subjects was fivefold, as measured by the retention of ^{54}Mn and whole-body counting daily for 2 wk. Similar levels of absorption from oral ingestion have been reported by Davidsson et al. (1988, 1989a). It has to be noted that the estimation of absorption using nutrition-balance studies is confounded by the fact that the GI tract is not only the site of absorption but also the principal site of elimination and where the exsorption of endogenous manganese takes place. Estimated absorption measured using retention of ^{54}Mn in 14 men for 10 d was 5.9% ± 4.8%; however, the range was 0.8-16%. The interindividual variation was large (Davidson et al. 1989b). Using a rat model, Davis et al. (1993) reported that young, growing rats fed manganese at 45 µg/g in their diet absorbed 8.2% of their manganese intake and then lost 37% of this absorbed manganese through gut endogenous losses.

The literature indicates the amount of manganese absorbed depends not only on the total amount of it present in food but also on several dietary ingredients that influence the absorption of manganese (Bales et al. 1987; Lee and Johnson 1989; Davidsson et al. 1991). For example, phytate, tannins, oxalates, and fiber inhibit manganese uptake (fractional absorption) from the GI tract. Phytate (myo-inositol hexaphosphate, IP6) is the major storage form of phosphorus in plants, and cereal foods contain large amounts of IP6. IP6 possesses a high potential for chelating

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minerals, such as iron (as Fe^{+2}), zinc (as Zn^{+2}), magnesium (as Mg^{+2}), calcium (as Ca^{+2}), and Mn^{+2}, and, thus, IP6 has a negative influence on the bioavailability of elements from food. Davidsson et al. (1991) studied in humans the effect of adding phytate, phosphate, and ascorbic acid to infant formula and studied manganese absorption using radionuclide techniques. They found no significant differences in manganese absorption, although the addition of calcium to human milk decreased manganese absorption. This seems to contradict the report by Bales et al. (1987) who observed a decrease, apparently caused by the fiber and phytate content of food. In another study by Schwartz et al. (1986) in which adult human males ingested a high-fiber diet containing manganese at 12-17.7 mg/d via wheat bread and bran muffins, there was no net retention, or a negative balance or only a mild positive balance of manganese was observed. Johnson et al. (1991) found that in men and women, absorption of manganese from lettuce was higher than spinach and less so from sunflower seeds compared to that from wheat. Davidsson et al. (1991) reported humans absorbed a higher percentage of manganese from human milk than from cow's milk or soy formula.

Johnson et al. (1991) stated that in humans, manganese absorption tended to be greater from $MnCl_2$ in demineralized water (which ranged from 7.74% to 10.24%) than from foods (vegetables, wheat, and nuts). However, the biologic half-life of manganese from either source is the same. EPA has recommended using an additional factor of at least 3 if an assessment made from the ingestion of manganese from food is extrapolated to drinking water (EPA 1996). One might think, based on the characteristics of other divalent cationic metals, that manganese absorption from water would be much greater than from food. However, no definitive experiments have documented this. Davidsson et al. (1988, 1989a) reported that in humans, the absorption of manganese from these two sources is comparable. If a difference exists, it is masked by vast interindividual variations in (human) absorption of manganese (determined using ^{54}Mn retention with intrinsic and extrinsic labeling of the meal). Mena (1974) reported an absorption of 70% from young rats compared to only 1-2% in adult rats. Similarly, Lonnerdal et al. (1987) reported that in neonatal rats, manganese is absorbed at a very high level (as high as 80%) until 14 d, where it drops to 30% by day 18 and to 3-4% when the animal reaches maturity. The proposed reason that this phenomenon is because of the lack of development of the excretory pathway in the neonates (Miller et al. 1975) has been debated by Ballatori et al. (1987), who in their studies on dose dependency of biliary excretion of intraperitonealy (ip) injected manganese in adult and 14-d-old rats, concluded

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that at doses higher than tracer doses used in previous studies neonatal rats can excrete as much as adult rats.

Manganese Absorption and the Influence of Other Minerals

Several minerals have been shown to interact with the absorption of manganese, including iron, calcium, zinc, magnesium, cobalt, and iodine. Interaction of iron with manganese has been the subject of numerous human and animal studies (see Finley and Davis 1999), which have shown metabolic interactions between these metals. Available evidence indicates body stores of iron and manganese inhibit each other's absorption (Kies 1994), perhaps because of a competition via a common transport protein such as the divalent metal transporter. Diet-induced iron deficiency can increase the GI absorption of manganese, and supplementing the diet with iron suppresses this enhanced manganese absorption (Davis et al. 1992b; Chua and Morgan 1996). Chua and Morgan also observed that the supplementation of manganese via drinking water (manganese acetate at 2 g/L) increased ^{59}Fe uptake from plasma in the brain, liver, and kidneys, which was not seen when iron was loaded in the diet (Chua and Morgan 1996). But a critical observation is that both iron depletion and loading in the diet increased the brain concentrations of manganese. In humans, supplementation of iron decreases not only the absorption of manganese from the diet (Lonnerdal et al. 1987) but also its retention in the body (Kies 1987). Pertinent to the objective of this document, excess manganese will inhibit the intestinal absorption of iron and could lead to iron deficiency and to anemia. However, Davis and Greger (1992) did not find any change in the iron status of 47 women supplemented with manganese at 15 mg/d for 124 d. Recent work by Zheng et al. (1999) on iron homeostasis in rats after chronic exposure to $MnCl_2$ indicates that exposure to manganese alters iron homeostasis, possibly by expediting the influx of iron from the systemic circulation to the cerebral compartment of the brain. It is interesting to note that iron overload in the brain has been thought to be responsible for Parkinson's disease (PD), causing iron-mediated oxidative stress in the brain and consequent degeneration of neurons. Finley (1999) and Finley et al. (1994) investigated possible gender differences in the influence of iron status (using serum ferritin concentration as the marker), especially on manganese absorption and half-life of manganese (using ^{54}Mn) in men and women. They found that higher ferritin concentrations reduced manganese absorption in young women. Although men absorbed much less manganese than women, manganese had a longer half-life in men (Finley

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et al. 1994). These findings may be related to the differences in serum ferritin between men and women. In an another study (Finley 1999), it was reported that dietary-manganese concentrations did not affect manganese status, but the absorption of manganese from a low-manganese diet by women with high ferritin concentrations was very low compared to the absorption by women with low ferritin concentrations. In this study, 11-15 women received a diet containing manganese at 0.7 mg/d or 9.5 mg/d for 60 d. Retention of ^{54}Mn, measured after 60 d, indicated that a greater percent of the test dose was retained by women on the low-manganese diet than by those consuming the high-manganese diet. Thus, for manganese absorption and biologic half-life, there was a significant interaction between ferritin status and dietary manganese. The results indicated that dietary manganese intake was not associated with any clinically significant changes, especially at the high dose (9.5 mg/d). It is worth noting that in a population-based study of manganese conducted in southwest Quebec, Baldwin et al. (1999) showed that blood manganese (Mn-B) was negatively correlated with age and serum iron in women, whereas serum iron was negatively correlated with age and not Mn-B in men.

Erikson et al. (2002), from their studies on rats fed an iron-deficient diet and groups fed iron-deficient and manganese-supplemented diets (iron-deficient + manganese), concluded that both iron-deficient and iron-deficient + manganese diets significantly increased the concentration of manganese across the brain regions compared to control groups. Based on the concentrations of glutamate, gamma-aminobutyric acid (GABA), and taurin, the authors concluded that iron-deficiency is a significant risk for the central nervous system (CNS) because of increased manganese accumulation and also that the observed changes in the neurochemicals can be attributed to manganese accumulation (Erikson et al. 2002). Recently, Erikson et al. (2004) showed that in 21-d-old male Sprague-Dawley rats given an iron-deficient diet supplemented with manganese (100 mg/kg diet), there was not only an increased overall brain manganese concentration, but this increase was seen in the globus pallidus and substantia nigra in both the groups of rats on an iron-deficient diet and those on an iron-sufficient diet supplemented with manganese compared with controls. In the caudate putamen, the increase was seen only with the manganese-supplemented iron-deficient group (Erikson et al. 2004). An increase in manganese concentration in the globus pallidus, where increased accumulation causes manganism, and observed increased concentrations of divalent metal transporter (DMT-1) protein levels seen in globus pallidus in iron-deficient animals underscores the importance of the interaction of iron and manganese.

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Several animal investigations suggested that a high intake of calcium can affect manganese balance and vice versa. This has prompted several studies evaluating this interaction in humans because of concerns over increased use of calcium supplements for osteoporosis, particularly among postmenopausal women. Mixed results have been reported. Variations such as the chemical form of calcium, the concentration of calcium, and/or its ratio to manganese may be a reason. An inhibitory effect of calcium on manganese absorption in humans was found in women consuming calcium at 400 and 6.3 mg/d, and manganese negative balance was found in men who took supplements of calcium at 916 mg either as lactate or as milk (McDermott and Kies 1987). Freeland-Graves and Lin (1991) observed that the addition of calcium as calcium carbonate $CaCO_3$ or 2% milk, which provided calcium at 800 mg and manganese at 40 mg, given to adult subjects essentially blocked the plasma uptake of manganese (indicating inhibition of absorption). Similar results were obtained by Davidsson et al. (1991). However, no effect was found by Johnson et al. (1991). The differences may be accounted for by the relative amounts of manganese and calcium. In the study by Johnson et al. (1991), the human subjects were fed conventional diets containing manganese at 1 or 5.6 mg/d with calcium at 587 or 1,336 mg/d in a 2 × 2 factorial design. ^{54}Mn was used to study the absorption of manganese. Biologic half-life was unaffected by calcium concentration. Spencer et al. (1979) found little effect on the excretion or the retention of manganese in adults when 200 or 800 or 1,500 mg/d of calcium was provided as a supplement.

Transport of Manganese Absorbed from Oral Ingestion

Considerable speculation exists in the literature about the oxidation state of manganese that binds to the manganese transport protein in the plasma and is speculated to be responsible for manganese toxicity. Several carrier/transfer proteins have been proposed for manganese, including serum albumin, transferrin (Scheuhammer and Cherian 1985), transmanganin (Cotzias 1962), and beta-1-globulin (Foradori et al. 1967). The finding of significantly different turnover rates of manganese in humans (as measured by the whole-body retention of ^{54}Mn) after iv and oral administration of ^{54}Mn seems to indicate that humans have at least two different Mn^{+} binding proteins. Davidsson et al. (1989c) identified transferrin as the only major plasma carrier protein when manganese is administered orally or by iv. It has been proposed that after ingestion of Mn^{+2} and after absorption from the gut, manganese binds to alpha-2-macro-

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globulin in the plasma (Gibbons et al. 1976) and reaches the liver. While Mn^{+2} is traversing the liver, a major amount of it is secreted into the bile while a small amount is oxidized by liver ceruloplasmin to Mn^{+3}. Transferrin has a high binding affinity for Mn^{+3}. This enters the circulation as Mn^{+3}-transferrin conjugate and is transported to tissues (Aisen et al. 1969; Aschner and Aschner 1991). That transferrin is the only transport mechanism of manganese from liver to the brain through the systemic circulation has been questioned by other investigators. For example, in the transferrin knock-out mice, the uptake of injected ^{54}Mn in the brain (and other tissues) was comparable to that found in wild mice (Dickinson et al. 1996).

In the last few years, a significant amount of research has been carried out to delineate the complex diffusion-mediated and transporter-mediated processes by which manganese is taken up and transported into the brain and the dependence of these mechanisms on the route of administration. In short, the results indicate that the transport of manganese into the brain takes place in three different ways, and some of these are more relevant to inhaled manganese than to exposure by other routes. One of them is the uptake and transport of manganese via primary and secondary olfactory neurons in pike and has received significant attention by researchers (see Tjalve and Henriksson 1999). Because systemically absorbed manganese enters the brain through the blood brain barrier, olfactory transport through olfactory neurons, relevant to transport of inhaled manganese, will not be relevant here. The other proposed routes are a saturable, transferrin-independent transport across the blood brain barrier, the transferrin-dependent transferring receptor, and DMT-1, which is also an iron-transporter protein in the brain. There is a considerable amount of speculation as to which of these is most important (see Aschner and Gannon 1994; Aschner et al. 1999; Malecki et al. 1999; Aschner 2000; Crossgrove and Yokel 2004) and whether the distribution of manganese among the target brain regions are different depending on the transport system.

Distribution

Normal human and animal tissues contain manganese. Human tissues contain manganese at 0.1-1 µg/g tissue (Tipton and Cook 1963; Sumino et al. 1975), with the highest concentrations in the liver, pancreas, and kidney. Tissue manganese concentrations are controlled by the homeostatic control mechanism through absorption and elimination; thus, the liver and intestines play important roles in maintaining manga-

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nese status (see, for example, Bertinchamps et al. 1966; Papavasiliou et al. 1966; Abrams et al. 1976). The profile of tissue distribution of manganese seems to vary with the routes of administration and various salt forms of manganese (see Davis et al. 1993 and Roels et al. 1997). Therefore, descriptions of studies on other routes of administration will be limited. Maximal concentration depends on time, due to different rates of uptake by the tissues, and different rates of tissue elimination half-lives for manganese. For example, after a single dose, it takes several days for the brain to reach the maximum concentrations (see Newland et al. 1987).

Rats given a single, oral dose of manganese as $MnCl_2$ at 416 mg/kg had little tissue accumulation of manganese 14 d later (Holbrook et al. 1975). This pattern is thought to result from a homeostatic mechanism that leads to decreased absorption and/or increased excretion of manganese when the intake of manganese is high (Mena et al. 1967; Abrams et al. 1976; Ballatori et al. 1987).

A study in which the retention of a single oral dose of radiolabeled manganese was measured in adult and neonatal rats indicated that 6 d after exposure, tissue retention of the label was much greater in pups (67%) than in adults (0.18%) (Kostial et al. 1989).

A study by Lai et al. (1991) confirmed that chronic exposure to $MnCl_2$ (1 and 10 mg/mL) in drinking water increased brain manganese concentrations; rats exposed to manganese from conception to 120 d had much higher concentrations than controls. Lai et al. (1992) determined several neurochemical parameters in brain regions of rats chronically treated with $MnCl_2{\cdot}4H_2O$ at 20 mg/mL drinking water throughout development until adulthood. The highest increases of manganese accumulation in manganese-treated rats were found in the hypothalamus, (increase of 530%) and striatum (an increase of 479%), and the increase in other regions were between 152% and 250%. Chronic $MnCl_2$ exposure in drinking water (20 mg/mL) throughout development until adulthood was found to alter brain regional manganese concentrations in neonatal rats. However, the regional manganese differences were less pronounced in weanling and adult rats (Chan et al. 1992). These results indicate that manganese accumulates in the brain particularly during neonatal exposure.

Manganese content as a function of the salt form of various tissues was reported in a dietary study (Komura and Sakamoto 1991). Elevated manganese concentrations were found in the organs of male mice fed $MnCl_2$, manganese acetate, manganese carbonate ($MnCO_3$), or manganese dioxide (MnO_2) at about 200 mg/kg/day for 100 d. Concentrations of manganese in the tissues were generally higher from $MnCO_3$ and

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manganese acetate than from $MnCl_2$, but the pattern of tissue storage (μg/g tissue) was very similar, in the order of liver > kidney > bone > pancreas > brain > spleen > muscle. Manganese concentrations in the tissues were lowest from MnO_2.

Prolonged oral exposure to manganese compounds results in increased manganese concentrations in all tissues, but the magnitude of the increase diminishes over time. Rehnberg et al. (1980) reported that in animals receiving manganese as Mn_3O_4 at 3,550 ppm in their diet (compared to control animals receiving a normal diet [50 ppm]) the increases in manganese concentrations were in the order of liver > brain > kidney > testes. The concentrations observed after 60 and 224 d indicated that elimination of the excess manganese was slower from the brain than from liver and kidney. Such data are not available for soluble manganese compounds.

Several studies in the literature have described the regional brain distribution of manganese in rodents, primates, and in humans occupationally exposed to manganese. Positron Emission Tomography (PET) scans, magnetic resonance imaging (MRI) scans, and neutron activation analysis techniques had been used. The primary goal was to see whether there is preferential accumulation of manganese in regions associated with the extrapyramidal system that may explain the extrapyrimidal effects from manganese intoxication. But in most of these studies, manganese was administered either via iv or ip injection and not as an oral dose. However, some results are important. In humans, manganese is primarily found in the striatum, globus pallidus, and substantia nigra (Newland et al. 1989; Calne et al. 1994; Pal et al. 1999; and references cited therein). Newland et al. (1989), using proton nuclear magnetic resonance, studied the distribution of manganese in several separate and specific brain regions after manganese administration. Monkeys either inhaled $MnCl_2$ aerosol or were iv injected various doses. Data suggested selective affinity for manganese in globus pallidus and in the pituitary gland. Effects in caudate and putamen effects were intermediate and little effect appeared in gray and white matter, compared to pre-exposure to manganese (Newland et al. 1989). According to Newland and Weiss (1992), there was a correlation between the intensity of MRI and the behavioral effects of manganese, which corresponded to an increase in the manganese content of the globus pallidus and substantia nigra. Scheuhammer and Cherian (1981) reported the rate of uptake and regional distribution of manganese in 13 different regions of the brains of rats administered manganese as $MnCl_2$ as an ip injection for 30 d. The results indicated that manganese accumulated more rapidly in the striatum, thalamus, and midbrain compared to other brain regions. Also, a signifi-

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cant positive correlation between iron and manganese distribution was noted (Scheuhammer and Cherian 1981).

Roels et al. (1997) studied the absorption and cerebral distribution of manganese with respect to the route of administration and the chemical form of manganese. Male adult rats received either $MnCl_2{\cdot}4H_2O$ or MnO_2 once a week for 4 wk at a dose of 24.3 of mg/kg body weight by gavage or 1.22 mg/kg by ip or intratracheal instillation. Four days after the last administration, the rats were killed, and the concentration of manganese was measured in blood, liver, and cerebral tissues (cortex, cerebellum, and striatum). The concentration of liver manganese was affected by neither the route of administration nor its chemical form. This was not so when the concentrations of manganese in the brain were determined. The extent of increase in cortex manganese concentration in the $MnCl_2$-gavaged group was less than in the MnO_2-gavaged groups. Gavage resulted in a lower increase in cerebellum manganese than other routes did. However, when the results from the oral doses of the chloride and the oxide form of manganese were compared, the increase in steady-state blood manganese concentrations over those of the controls (about 1,000 nanograms [ng] per 100 mL) was about the same, even though blood concentrations reached a maximum very rapidly (Roels et al. 1997).

Excretion

Earlier studies reported that injected radioactive manganese quickly disappears from the blood and enters the mitochondria in the liver and the pancreas. Excess manganese is excreted primarily via bile (Bertinchamps et al. 1966; Papavasiliou et al. 1966; Klaassen 1976). The liver is the major excretory organ for manganese; the concentrations in bile can be more than 150-fold higher than in plasma. Fifty percent of a $MnCl_2$ dose was excreted in the feces within 1 d (Klaassen 1974, 1976), and 85% by day 23 (Dastur et al. 1971). It has been shown that hepatic dysfunction leads to manganese overload in the brain as indicated by the abnormal T1-weighted MRI (Hauser et al. 1996). Less than 1% of a dose of manganese is excreted in the urine within 5 d after an iv injection (Klaassen 1974). However, when the hepatic passage is blocked, the pancreas serves as the reserve organ of excretion (Papavasiliou et al. 1966). The fact that the liver is a major organ for the disposition of manganese has been realized in terms of manganese neurotoxicity in many clinical instances of progressive liver failure, end-stage liver disease,

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cholestasis, and chronic hepatic encephalopathy (Layrargues et al. 1998; Malecki et al. 1999).

In a recent study by Davis et al. (1993), it was reported that the intestinal wall could also contribute significantly to manganese that is excreted. It was shown in this study that young rats fed manganese at 45 mg/kg/d absorbed 8% of the manganese intake and that 37% of the amount absorbed was lost through the intestinal wall. Although manganese is excreted in urine, urinary manganese did not increase with an increasing duration of supplemental oral manganese intake (Davis and Greger 1992).

Newland et al. (1987, 1989) studied the kinetics of uptake and elimination of manganese administered to monkeys via inhalation (of tracer as aerosol) and also via subchronic subcutaneous (sc) administration using an osmotic mini pump for 50 d (a total of $MnCl_2$ at 400 mg mixed with a radioactive tracer of manganese). In this investigation, two female macaque monkeys were used for the inhalation study, and one for the sc infusion study. The gamma emission from the tracer was counted to determine uptake and elimination of manganese from the chest and head and elimination by fecal route. Radioactivity was monitored for over a year. The authors observed different rates of elimination from the brain after inhalation and after sc infusion. Inhalation exposure led to slow uptake, and the peak activity in the head was reached in about 40 d (the head uptake phase had a half-life of 10 d). Elimination from the head occurred with a $t_{½}$ of 223-267 d, and it appeared to take place in a single phase. Manganese was detected in the chest area (probably lung) even after 500 d, and the disappearance of manganese from the chest had three elimination phases. The slowest phase had a half-life of 94-187 d. The monkey that received sc doses of manganese received a cumulative dose of $MnCl_2$ at 400 mg and $^{54}MnCl_2$ at 200 microcuries (µCi) as an sc continuous infusion from ALZET osmotic pumps over 6 wk. In this monkey, the head activity increased gradually, and after the pump was removed, the concentrations in head and feces declined abruptly. The disappearance of manganese from the head was biphasic in this treatment protocol and occurred at a rate 4.5 times higher than after acute inhalation of the aerosol.

Fecal elimination from these routes also differed significantly, although the clearance followed two-phase kinetics. According to the authors, the kinetics of inhalation exposure indicated that the lungs served as a primary reservoir for replenishing head concentrations long after the cessation of exposure and also that elimination of manganese from the lungs is very slow. The authors acknowledged the fact that the differences in the rate of elimination from the brain taken up by these routes

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may have been because of the vast difference in the doses used (Newland 1999).

Cotzias et al. (1968) reported that after a single iv injection of ^{54}Mn, normal human subjects had a whole-body manganese clearance of 37.5 d and a brain clearance of about 54 d (similar to the infusion in monkeys, as described above), whereas the elimination times from individual tissues were different. These results indicate that tissue storage and tissue-specific uptake are important. Mena et al. (1969) reported that in iron-deficient anemic patients (four men and nine women), the half-life of elimination (measured by total body retention of ^{54}Mn) was only 23 d compared to a $t_{½}$ of 37 ± 7 d in normal individuals. This indicates that iron reserves affect manganese retention in the body. The biologic half-life of manganese in blood ranges from 12 h in healthy miners to 40 h in healthy volunteers iv injected with ^{54}Mn. (Mahoney and Small 1968). Based on the retention of manganese in the whole body in three subjects, it was reported by these authors that manganese elimination from the body could be described by two phases. The faster phase has an elimination half-life of 4 d, and the slower phase has a half-life of 39 d from the whole body from normal subjects (an average of 70% of the injected dose was eliminated in this phase). Sixty days after the initiation of the study (with an injection of a radioactive dose of manganese), one of the subjects began to ingest a solution containing manganese as $MnCl_2$ at 800 mg/d for the subsequent 35 d (Mahoney and Small 1968). One must note that the number of subjects was only two. According to Mena et al. (1969), in subjects with higher oral intake, manganese was eliminated at an increased rate. In miners suffering from manganese intoxication, the half-life of manganese is 34 d, whereas healthy miners had a fast turnover of only 15 d. Also, in mice, Britton and Cotzias (1966) reported a two-compartmental whole-body clearance of manganese, as reported in humans. However, the amount and the half-time of elimination from fast and slow compartments were different from humans. These studies show that with high- and low-manganese diets, these half-lives changed considerably. When the manganese intake is high, the slow elimination phase essentially disappeared (Mahoney and Small 1966).

Suzuki et al. (1974, as cited in WHO 1981) demonstrated that in mice, the whole-body clearance half-life, as measured by the administration of a radioactive tracer dose at the end of the treatment periods, varied with the dose (the preloading). For example, in mice that received $MnCl_2$ at 20, 100, and 2,000 mg/L for 26-30 d, the half-lives were 6, 3, and 1.5 d, respectively. Lee and Johnson (1988) observed that orally administered ^{54}Mn had a shorter biologic half-life than injected ^{54}Mn and tissue distribution of ^{54}Mn differed in rats given ^{54}Mn by different routes.

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Toxicity Summary

A vast amount of the existing literature on the adverse effects of exposure to manganese indicates that the CNS is the most important target of manganese toxicity. The most important event of neurotoxicity is the performance degradation, irrespective of route of exposure. Optimum crew performance is of paramount importance for NASA's mission success. Therefore, an overview of manganese-induced neurotoxicity will be presented here.

In humans, neurotoxicity from manganese is usually a consequence of chronic inhalation over a period of several years, of high concentrations of airborne manganese in the form of manganese fumes and manganese oxide dusts in occupational and industrial settings (ferromanganese factories or mining operations). Although the primary objective of this document is to derive a SWEG for manganese from data relevant to exposures through the oral route, a review of the neurophysiologic, neurofunctional, and neuropathologic effects of manganese exposure from inhalation-exposure studies has led to a valuable understanding of the progression of adverse health effects from the early signs of manganese toxicity to the onset of clinically observable conditions, such as manganism, a disease that resembles PD. For this reason, results from studies of inhalation exposures to manganese are important and thus have been described here.

However, it must be mentioned that NASA does not anticipate having a high concentration of manganese in processed water, so the neurologic symptoms and neuropathology seen in the established phase of manganese neurotoxicity in chronically exposed subjects are not likely to occur. NASA is interested in subtle neurobehavioral effects usually seen in the early phase of manganese toxicity and in understanding how neurobiologists have attempted to relate tests to performance decrements (see the Mergler et al. [1999] study discussed below).

Manganese Neurotoxicity in General

A review of the metabolism of manganese indicates that although clear differences exist in the kinetics of uptake, distribution, and excretion of manganese between inhalation exposure and the oral route, the critical adverse effect is on the CNS as stated above.

The basal ganglia are the part of the nervous system that coordinates movement and motor functions and are implicated as being affected when abnormal movements (hypo- and hyperkinesia) and neuro-

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psychiatric symptoms are present. The basal ganglia include the caudate nucleus and putamen (which are collectively called the striatum) and the globus pallidus, subthalamic nuclei, and substantia nigra. It has been reported in several investigations with human and nonhuman primate models that upon exposure to manganese, increased deposition of manganese occurs in the globus pallidus, striatum, and substantia nigra. This leads to a depletion of dopamine (DA) (and perhaps other biogenic amines) in the basal ganglia because of accelerated oxidation and consequently to the degeneration of neurons and associated nuclei of the extrapyramidal system. Damage to neurons leads to an extrapyramidal disorder called manganism, which resembles PD. Symptoms include generalized weakness, apathy, anorexia, stiffness of the legs, and muscle pain. (Mena et al. 1967; Tanaka and Lieben 1969) (see Table 9-2). One of the most important differences between PD and manganism is the lack of clinical response by patients with the latter disease to the drug levodopa (Olanow et al. 1996).

In "manganese madness" (Pal et al. 1999), the initial acute psychotic episodes lead to more serious symptoms similar to those of PD, such as mask-like faces, stooped posture, tremors, impaired speech, and rigidity of limbs, along with dystonia and severe gait disturbance (motor effects). These symptoms exhibit several years after the cessation of exposure to manganese fumes or dust. In cases of low-dose exposure, one can detect changes in neuromotor functions such as poorer subtle motor activity.

Manganese Neurotoxicity from Inhalation Exposures

A vast amount of data has accumulated regarding the toxicity of manganese to nervous system parameters and functions. Inhalation exposure to high concentrations of manganese as dust or manganese oxide fumes has been known to result in a syndrome of profound neurologic effects in humans. Scanty evidence is available that oral exposure to manganese leads to neurologic effects in humans. The results strongly indicate that neurotoxic effects are progressive and continue even in the absence of continued exposure. The results of a few population-based studies designed to assess early CNS alterations, to develop sensitive methods to identify dysfunction on a continuous scale, and to examine doses of manganese associated with neurotoxic outcomes are discussed below. In the recent past, astrocytes have been implicated as playing a very important role in the pathophysiologic mechanism in manganese

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TABLE 9-2 Phases of Manganese Neurotoxicity[a]

Phase	Neurologic Outcome and Parameters
First phase	Characterized by nonspecific symptoms: anorexia, apathy, headache, hypersomnia, spasms, weariness in legs, irritability, pain in the joints
Second phase (established phase)	Signs of basal ganglial dysfunction: speech disturbance, expressionless face, altered gait, and fine tremor
Third phase (final phase)	Muscular rigidity, staggering gait ("cock walk"), fine tremor

[a]Course and degree of manganese toxicity varies with individuals. Psychiatric symptoms ("manganese madness," compulsive and aberrant behavior, emotional lability) may also be manifested.
Source: Data from Mergler 1999.

neurotoxicity, at least for chronic-exposure scenarios. Astrocytes have a high affinity for manganese; this leads to sequestration of manganese in mitochondria followed by the disruption of several key cell functions, including oxidative phosphorylation (see Normandin et al. 2002; Normandin and Hazell 2002).

Roels et al. (1987) conducted a cross-sectional epidemiologic study among 141 male subjects exposed to inorganic manganese in a plant that produced manganese oxide and salt (mean age, 34.3 years [y]; mean duration of exposure, 7.1 y; average exposure, 1 mg/m^3). The authors of this study concluded that psychomotor tests are more sensitive than neurologic examinations and detect manganese neurotoxicity much earlier. They also tried to relate tissue manganese to the psychomotor test responses. A matched control group consisted of 104 subjects. The intensity of manganese exposure was moderate. Manganese was measured in blood and in urine. Mn-B in manganese-exposed male workers ranged from 0.10-3.59 µg per deciliter (dL), compared with 0.04-1.31 µg/dL in 104 control subjects. A significantly higher prevalence of cough in cold season, dyspnea, and episodes of acute bronchitis were found in the manganese group. Significant changes were found in simple reaction time (visual), audioverbal short-term memory capacity, and hand tremor (hand-eye coordination, hand steadiness). The concentration of manganese in urine did not correlate with either the exposure duration or the adverse effects measured in the tests. The significant observation was that the prevalence of disturbances in hand tremor was related to Mn-B. According to the authors, the response to the eye-hand coordination test suggests that a Mn-B threshold of about 1 µg/dL of whole blood exists, and that a person exposed to airborne manganese dust (total dust) at

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about 1 mg per cubic meter (m^3) (time-weighted average [TWA]) for less than 20 y may present preclinical signs of manganese neurotoxicity (Roels et al. 1987).

Roels et al. (1992) conducted another study in 92 workers in a battery plant who had been exposed to MnO_2 for an average of 5.3 y. The concentration of respirable and total manganese dust in air, as measured by personal sampling, was about 0.2 mg/m^3 and 0.95 mg/m^3, respectively. Although no differences in neurologic symptoms were found between exposed and control workers, the visual reaction time, hand-eye coordination, and hand steadiness of both group were significantly impaired. In a continuation of this study (a prospective study) aimed at assessing the reversibility of neurobehavioral effects in workers in the dry-alkaline battery plant in Belgium, Roels et al. (1999) noted that even though the amount of total manganese dust (MnO_2 particulates) decreased in the plant because of abatement programs over the years, the time courses of the hand-steadiness and visual-reaction test results from these workers showed the absence of any improvement, suggesting that hand stability (postural tremor) and simple visual reaction time were irreversibly impaired (or performance leveled off even when the manganese concentration in air was reduced). In this study, the workers had been divided into low-, medium-, and high-dose exposure groups. In a separate follow-up study of 24 people who had previous occupational exposure to manganese but who had not been exposed to manganese for at least 3 y, a significant improvement in hand-eye coordination was measured, but there was no significant change in the deficit in hand steadiness or visual reaction time (Roels et al. 1999).

Mergler (1999) reviewed the effects of low-level exposure to manganese in a general population, and in this review, the author indicated that dose-effect relations may vary with the different parameters of neurotoxic outcome. For example, Lucchini et al. (1995) reported that there was a good relationship between Mn-B (used as a reflection of cumulative manganese body burden) and certain neurotoxicity parameters, such as finger tapping, visual perception speed, short-term memory, and the ability to add. Mergler et al. (1999) assessed nervous system functions in residents exposed to manganese from a variety of environmental sources (a nonoccupationally exposed population) in southwest Quebec, Canada. The subjects were drawn from seven postal code regions near a former manganese alloy plant that was a potential source of manganese pollution. MMT from gasoline used in Canada was the other main source of environmental manganese. The subjects were 273 persons (151 women and 122 men); blood lead, iron, and mercury concentrations were measured in addition to manganese. Mn-B ranged from 2.5-15.9 µg/L (me-

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dian: 7.3 µg/L). Mn-B was significantly higher in women (7.70 µg/L) than in men (geometric mean of 6.60 µg/L). Nervous system assessments included computer- and hand-administered neurobehavioral tests, computerized neuromotor tests, sensory evaluation, and a neurologic examination. Neurologic outcomes were examined with respect to Mn-B. Results revealed that higher concentrations of Mn-B (7.5 µg/L) were significantly associated with changes in coordinated upper-limb movements and poorer learning and recall. Further analyses revealed that with increasing log Mn-B, performance on a pointing task (hand-eye coordination) was poorer and frequency dispersion of hand-arm tremor decreased, and harmonic index increased and the velocity of a pronation/supination arm movement was slower. Men older than 50 y whose Mn-B was > 7.5 µg/L showed significant disturbances in several mood symptoms. In addition, the results indicated that motor slowing associated with increased Mn-B concentrations are most likely to manifest after age 50. Gender differences were observed for portions of the neurologic examination (finger, hand, arm, and foot movements) and in learning and recall tests. The gender differences were also observed for mood and postural stability. The results suggested that men might be at greater risk than women, although effects were also observed in women. It must be pointed out that samples of drinking water from participating residences were analyzed for manganese (Mn-W). Two hundred seventy-eight manganese-W samples were obtained and the geometric mean was 4.11 µg/L (0.5-71.1 µg/L). There was no relation between Mn-B and manganese-W. Mn-B was highest in geographical areas with the highest concentrations of airborne manganese, which suggests that neurobehavioral and neurophysiologic alterations may be primarily caused by airborne manganese (Baldwin et al. 1999).

Acute, Subacute, Subchronic, and Chronic Toxicity Studies

Few reports exist of toxic effects of excess manganese in humans exposed via ingestion (water or food). A significant number of research reports on manganese in humans have focused on its bioavailability and mass balance to determine a reasonable recommended dietary allowance (RDA) for manganese and to elucidate the interaction of other minerals (especially iron and calcium) with manganese absorption. Several of these studies have also focused on infant nutrition and manganese status. Significant amounts of data have been obtained from animal experiments on the toxicity of supplemented manganese. However, some subacute, subchronic, and chronic studies have shown several interspecies and in-

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traspecies variations. This might result from a wide variation in the requirement for manganese among species. Rodents' requirements are much higher than humans'. The estimated requirement of manganese of rats is a 50-mg/kg diet (Rogers 1979). This corresponds to 2.5 mg/kg, whereas the estimated safe and adequate daily dietary intake (ESADDI) for humans is 2-5 mg/d or 0.03-0.07 mg/kg/d (NRC 1989)—two orders of magnitude lower than that for rodents. Thus, extrapolation of rodent data to humans should be approached cautiously.

ACUTE EFFECTS

1 d

Freeland-Graves and Lin (1991) conducted a study with human subjects in which six young adults (18-26 y of age; gender not provided), were administered manganese as $MnCl_2$ at 40 mg as a gelatin capsule, and plasma uptake of manganese was measured over 4 h. The peak concentration of manganese in plasma occurred between 1 and 3 h, and the maximum concentration was 2 nanomoles (nmoles) per liter or about 110 ng/L of plasma. This amount of manganese caused no discomfort to the GI systems of the subjects. In this study, no other clinical or neurologic parameters were measured. However, the blood manganese data can be compared with the data from a community study from Mergler et al. (1999), who studied neurologic parameters in subjects whose Mn-B ranged from 2.5 to 15.9 μg/L (median, 7.3 μg/L). Neurologic outcomes were examined with respect to Mn-B. The authors reported that subtle manganese-related neurologic outcomes were evident at Mn-B above 7.5 μg/L. Although in one case, total Mn-B was measured, in the other case, plasma manganese was measured. The concentration in the Freeland-Graves and Lin study was so low that it can be concluded that there would not have been any neurologic adverse effect from an acute single dose of 40 mg per subject.

A clinical case report of a patient who was on hemodialysis with a solution contaminated with manganese reported the patient had severe vomiting, abdominal pain, and increased heart rate and blood pressure. The dialysis was discontinued after 30 min. The dialysate contained $MnSO_4$ at 120 mg/L, or manganese at 40 mg/L. The patient was diagnosed with an acute pancreatitis by day 2 (Taylor and Price 1982).

Several studies indicate that the dose lethal to 50% of test subjects (LD_{50}) dose for $MnCl_2$ or $MnSO_4$, administered as a gavage, is in the range of manganese at 325 to 1,082 mg/kg/d. A summary of LD_{50} data is

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shown in Table 9-3 below. These LD_{50} values seem to be inconsistent with the data on survival rates observed in several subchronic and chronic studies in which manganese salts have been administered in the feed (see the discussion of the National Toxicology Program [NTP 1993] study below). In the NTP (1993) study, rats and mice have tolerated doses much higher than the LD_{50}. Thus, use of these LD_{50} values for calculating acute risks for 1 d must be weighed carefully.

In an LD_{50} study (Singh and Junnarkar 1991), mice that received a single gavage dose of manganese as $MnCl_2$ at 580 mg/kg body weight exhibited a decrease in spontaneous activity, alertness, muscle tone, and respiration.

Short-Term Toxicity (2-14 d)

No well-designed toxicity studies were conducted on the adverse effect of manganese ingestion, probably for ethical reasons. However, the literature is replete with human subject studies designed to evaluate manganese balance and absorption and the interaction of manganese with metals such as calcium and iron. Some of these studies included clinical observations, which can provide some insight into the adverse effects of supplemental manganese.

TABLE 9-3 Summary of LD_{50} Doses for Various Soluble Manganese Salts in Rats and Mice[a]

Species	Manganese Salt	LD_{50} (mg/kg)	Reference
Rat	Acetate	1,082	Smyth et al. 1969
Wistar rat, male	Chloride	325	Kostial et al. 1989
Wistar rat, female	Chloride	331	Kostial et al. 1989
Rat, albino, male	Chloride	804[b]	Kostial et al. 1978
Sprague-Dawley rat, male	Chloride	412	Holbrook et al. 1975
Wistar rat, male	Chloride	642	Singh and Junnarkar 1991
Mouse, albino Swiss, male	Chloride	580	Singh and Junnarkar 1991
Wistar rat, male	Sulfate	782	Singh and Junnarkar 1991
Mouse, albino Swiss,male	Sulfate	848	Singh and Junnarkar 1991

[a]All of these doses were administered one time as gavages.
[b]The number of doses not specified.

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In a 124-d supplementation study in women (placebo, n = 13; manganese-supplemented, n = 11; supplements of manganese at 15 mg/d), measurements were made on days 1, 25, 60, 89, and 124. Davis and Greger (1992) did not observe any changes for up to 124 d in hematocrit, serum iron, serum copper, serum ferritin, or serum transferrin (the last two are indexes of iron status). Manganese status was indicated by measuring lymphocyte manganese-superoxide dismutase, and an increase was seen only after 89 d of administration of the supplement.

NTP (1993) conducted 14-d, 13-wk, and 2-y toxicology and carcinogenesis feed studies of $MnSO_4 \cdot H_2O$ in F344/N rats and B6C3F1 mice. For the 14-d study, male and female rats and mice were exposed to 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm of $MnSO_4 \cdot H_2O$ in the diet. Assuming average food consumption, these correspond to dose rates of manganese at 0, 84, 165, 340, 665, and 1,265 mg/kg/d for both male and female rats. The doses varied for mice, with 0, 121, 262, 669, 1,603, and 2,500 mg/kg for male mice and 0, 240, 488, 1,068, 2,494, and 3,560 mg/kg for female mice. NTP measured hematology variables in addition to collecting body weight, food consumption, and tissue weight data 14 d after dosing.

In the NTP (1993) studies, no effect of manganese exposure on survival was observed in rats fed $MnSO_4$ at up to 50,000 ppm for 14 d. However, decreases in body weight gain were observed in male rats (57%) and in female rats (20%) fed 50,000 ppm. Decreased body weight gain was also observed in male and female mice above 1,200 ppm. NTP (1993) reported that no conclusions could be made about the body weight data because of poor randomization at initiation of the study. Male rats exposed to 50,000 ppm and all exposed female rats had diarrhea during the second week, showing GI effects. The lowest dose that caused diarrhea was 25 mg/kg. Male and female mice did not show similar effects in spite of the fact that the doses for the mice were almost 2 to 3 times those for the rats. An evaluation of hematologic parameters indicated that total leukocyte and neutrophil counts were significantly increased, particularly in the group that received $MnSO_4$ at 50,000 ppm.

A summary of results is listed below. In mice given $MnSO_4$ in the feed for 14 d (NTP 1993), no effects on hematologic parameters were noted. At necropsy, the absolute and relative liver weights of 50,000 ppm dose rats were significantly lower than those of the controls, and this seemed to be chemical related. Even at this high dose, the liver manganese concentration was only twice that of control male and female rats. In male and female mice, the liver manganese concentration was 8 to 15 times that of controls (the dose was threefold greater in mice than in rats). In both male and female rats, the number of leukocytes and seg-

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mented neutrophils were increased, especially at the highest dose (5,000 ppm, or manganese at 1,270 mg/kg/d). The increased number of leukocytes was seen at doses as low as 340 mg/kg/d in male rats, who seemed to be more sensitive to this change.

Because of the implications of such research for growing children, the literature contains reports of several investigations focused on the adverse neurologic effects of manganese exposure on neonatal rodents (Kontur and Fechter 1988; Dorman et al. 2000). Because this is not directly relevant to the purpose of this document, these studies will not be discussed here.

Short-Term Toxicity (14-100 d)

No human studies have been specifically designed to evaluate the adverse effects of ingestion of manganese. However, nutrition studies of manganese balance provide some indication of the concentrations that do not lead to any observable deleterious effects. For example, as described above, Davis and Greger (1992) did not observe any changes in hematocrit, serum ferritin, serum transferrin, serum iron, or serum copper during a 124-d supplementation study in women taking supplements of manganese at 15 mg/d. These did not change over time for up to 124 d (measurements were made on days 1, 25, 60, 89, and 124). Even the manganese status, as measured by lymphocyte manganese-superoxide dismutase, increased only at 89 d of administration of the supplement.

In a study aimed to understand the interaction of iron and manganese, especially any interaction between the absorption and retention of manganese and serum ferritin concentration, Finley (1999) administered diets that contained manganese at 0.7 or 9.5 mg/d to healthy nonpregnant women. At the end of 60 d, the authors did not find any changes in hematocrit, hemoglobin (g/L), number of erythrocytes (cells/L), white cells, or platelets in women consuming either diet. This study indicated that a total intake of 9.5 mg/d was without any effects. Neurologic indices were not measured in this study (Finley 1999).

Recently, Finley et al. (2003) evaluated the effect of two doses of manganese supplementation in healthy nonsmoking premenopausal women (n = 17) with a mean age of 35.7 ± 8 y and a mean body weight of 72.9 ± 13 kg. The aim of the study was to find out whether there are significant changes in the manganese status in the range of manganese concentrations that are present in a mixed Western diet and also to determine whether the type of dietary fat has any effect on manganese

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status. The authors concluded that manganese intake in the range of 0.8-20 mg/d for 8 wk was efficiently managed in the human system by the manganese homeostasis mechanisms, because these doses did not affect any neurologic measures and had only minor effects on psychological variables. The lower dose did not result in manganese deficiency, and the high concentrations did not lead to signs of manganese toxicity. The details are as follows. The subjects were fed nutritionally adequate diets for 8 wk, but the diet was formulated to contribute manganese at 0.8 or 20 mg/d (which was supplemented in orange juice to the basal diet). The manganese-intake protocol was done in a randomized double-blinded crossover design. In this study, manganese absorption and retention in the body were estimated from the retention of a test dose of orally administered ^{54}Mn. The authors conducted this study using two different types of fats, one enriched in saturated fatty acids (cocoa butter) and one enriched in unsaturated fatty acids (corn oil). Fat contributed 15% of the energy content of the diet. Neurologic and psychological tests were carried out to determine possible effects on psychomotor and behavioral variables. During the last week of the dietary exposure, to assess neurologic examination results and motor steadiness (tested using a steadiness tester in both dominant and nondominant hands), the subjects were examined by a board-certified clinical neurologist for the presence and severity of more than 75 neurologic signs and symptoms. These included measures used to determine manganese intoxication and measures used for PD (for details see Finley et al. 2003). Psychological assessment was done using three standardized self-report methods to evaluate broad-spectrum components related to hostility and anger. The Buss-Durke Hostility Inventory (BDHI), the State-Trait Anger Expression Inventory (STAXI), and the Interpersonal Behavior Survey (IBS) report measures were used (Finley et al. 2003). Psychological variables were converted to T-scores based on a gender- and age-appropriate normative database for evaluations. Clinical examination did not reveal any signs of neurologic impairment. No interaction was found between ingested manganese and any measure of point or line steadiness (steadiness assessment) within the same fat-type diet group. Differences in the point or line steadiness tests depended on the fat type, leading the authors to conclude that it was not the manganese in the range of concentrations used in this study, but rather the type of fat in the diet that accounted for the observed changes in neurologic and steadiness assessment.

In addition, data for several clinical parameters, such as activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, ammonia, concentrations of indicators of biliary function (bile

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acids and serum bilirubin), glucose, glucose tolerance tests, insulin, iron-binding capacity, and indicators of manganese and iron status were collected at the end of the dietary period. Manganese status was measured by lymphocyte manganese superoxide dismutase activity. Most indicators of manganese and iron status were not affected by dietary manganese. Biliary function was unaffected by diet and was normal in all subjects. Dietary manganese also did not affect the activities of antioxidant enzymes (glutathione peroxidase, catalase, and copper-zinc superoxide dismutase) in whole blood.

A case report described the symptoms of a man who mistakenly ingested low doses of potassium permanganate (manganese at about 1.8 mg/kg/d) for 4 wk and exhibited neurologic effects such as weakness and impaired mental capacity after this oral exposure (Holzgraefe et al. 1986). Although exposure was stopped after 4 wk, this subject developed a syndrome apparently similar to PD after about 9 months (mo).

Japanese Study

In a study by Kawamura et al. (1941), a group of six Japanese families (about 25 people) were exposed for approximately 2-3 mo to high concentrations of manganese in their drinking water, which came from a well. Manganese leached from an adjacent manganese battery storage area where about 300 batteries had been buried. A concentration of manganese at 14 mg/L was measured in the well water. Some of the exposed individuals exhibited muscle rigidity and tremors and mental disturbances similar to those seen in manganism. Not all members of the group were affected, but two individuals died. The actual concentration of manganese may have been 28 mg/L, as the initial well-water samples were taken 1 mo after the incident. The water also contained a high concentration of zinc. It must be pointed out that in spite of the small number of people affected, it was clear that the older individuals exhibited toxic symptoms to a greater degree than younger individuals did. The measurements were made several days after removal of the batteries. Several confounding factors, such as the potential presence of high concentrations of other metals associated with batteries, including nickel, could have caused the effects. Therefore, a LOAEL (lowest-observed-adverse-effect level) or NOAEL (no-observed-adverse-effect level) cannot be estimated from this study. The concentrations of manganese to which humans were exposed were much higher than reported in other epidemiologic studies described below.

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Animal Studies

There have been several reports on exposure through drinking water and consequent adverse effects from experiments on motor effects conducted mostly on rats. Several subchronic and chronic studies were conducted by Bonilla and coworkers on the behavioral effects and the concentrations and distribution of several biogenic amines (neurotransmitters) in rats treated with various concentrations of $MnCl_2$ in drinking water (Bonilla and Diez-Ewald 1974; Bonilla 1978, 1980, 1984; Bonilla and Prasad 1984). In the 1978 study, Bonilla observed that in male Sprague-Dawley rats (200-300 g) administered $MnCl_2$ in drinking water at 10 mg/mL for 2 mo a significant increase in GABA content of caudate nucleus (Bonilla 1978) resulted. GABA is a principal inhibitory neurotransmitter in the cerebral cortex that counterbalances neuronal excitation. The increase is neither because of an increase in GABA synthesis, because there was no change in glutamic acid decarboxylase (an enzyme responsible for GABA synthesis), or because of its increased catabolism, because the activity of GABA-transminase (the enzyme that metabolizes GABA to succinic aldehyde) was unaltered. The author speculated that the increase may be because of the inhibition of GABA outflow. Spontaneous motor activity was not measured in this study, and hence, the direct relevance of observed changes could not be determined. In a later chronic manganese-drinking-water-exposure study in rats, the author measured motor activity at different times during the interim periods (Bonilla 1984). In this study, male Sprague-Dawley rats (150-250 g body weight) were given solutions of $MnCl_2$ at concentrations of Mn^{+2} at 0.1 or 5 mg/mL in their drinking water for 8 mo. Spontaneous motor activity was measured using an optical digital animal activity monitor along with a vertical activity monitor. Various activity parameters measured indicated that at the end of the first month, the animals exhibited hyperactivity, which was seen in both groups of manganese-treated rats. However, the magnitude of the change was not a function of the dose. Furthermore, during months 2 through 5, the activities were comparable to controls. The reduced activity (hypoactivity) noted in the later months has been described under the “Exposure > 100 d” section of this chapter. The authors suspected that the initial increase in activity seen might be because of the decreased release of GABA from GABAergic neurons and the decrease in the inhibitory action on the neuronal excitation reported earlier (Bonilla 1978). The author also related that the noted hyperactivity was perhaps because of the overactivity of dopaminergic neurons, because in another study (Bonilla 1980), the author observed increased activity of tyrosine hydroxylase, an enzyme that converts tyrosine to levodopa, 3,4-

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dihydroxy-L-phenylalanine (L-DOPA) to DA, at least during the first 2 mo after manganese ingestion.

Behavioral and neurochemical changes were studied in ITRC albino rats (200-250 g) exposed daily to $MnCl_2$ in drinking water for 14 and 30 d at 1 mg/mL, an estimated dose rate of manganese at 140 mg/kg/d (Chandra 1983). Spontaneous motor activity, the conditional avoidance response test, aggressive behavior, and learning ability were assessed at 14 and 30 d. In the treated rats, hyperactivity was seen after 14 and 30 d, with greater magnitude at 30 d. A reduction in the percentage of conditional avoidance responses and an increase in fighting score were also observed after 30 d. Additionally, increased concentrations of striatal DA and norepinephrine in rats exposed to manganese may be responsible for the increased motor activity (Chandra 1983). Measurement of turnover of corpus striatal DA measured at 30 d indicated a 30% increase. Only one dose was used in this study.

Senturk and Oner (1996) administered manganese as $MnCl_2 \cdot 4H_2O$ at 357 and 714 µg/kg in water intragastrically to 2-mo-old female albino rats for 30 d. Increased manganese concentrations in brain regions and slower learning, determined by the T-maze food retrieval method, were seen. Measurements were made after 15 and 30 d of manganese treatment. In addition, significant accumulation of cholesterol in the hippocampal region was seen. Normalization of hippocampal cholesterol using a cholesterol synthesis inhibitor significantly corrected this learning impairment, without any change in the increased brain concentrations of manganese that were observed. Therefore, the authors doubted that the slower learning was caused by manganese ingestion and that manganese-induced hippocampal hypercholesterolemia was involved in the process. It must be noted that neither the learning deficit nor the cholesterol increase was prevented by the drug in rats in the high-dose group (Senturk and Oner 1996).

Increased GABA content of caudate nucleus was reported in Sprague-Dawley male rats (200-300 g body weight) administered $MnCl_2$ (10 mg/mL) in drinking water for 2 mo (Bonilla 1978). The increase did not result from an increase in GABA synthesis (as no change occurred in glutamic acid decarboxylase) nor from its increased catabolism (as the activity of GABA transminase was unaltered). The author speculated that it may have resulted from inhibition of GABA outflow. Spontaneous motor activity was not measured in this study.

In a drinking water study, male Sprague-Dawley rats were administered $MnCl_2 \cdot 4H_2O$ at a dose of 390 mg/kg/d for 60, 100, 165, or 265 d, and the concentrations of DA and its metabolite in the striatal regions of the brain (caudate nucleus and putamen) were measured (Eriksson et al.

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1987a). The rats were given manganese-containing water from 20 d of age. Rats exposed to manganese for 60 or 165 d had significantly increased concentrations of DA and its metabolites (dihydroxyphenylacetic acid [DOPAC]) in the discrete regions of the dorsal caudate-putamen. These alterations were not found in rats exposed for 100 or 265 d. The importance of the time factor has been shown in several investigations (Chandra and Shukla 1981; Eriksson et al. 1987a).

In a study by Subhash and Padmashree (1991), albino male rats were given a dose of manganese at 12 mg/kg in drinking water for 90 d, which resulted in a two- to threefold accumulation of manganese in all regions of the brain. Activities of DA β-hydroxylase and monoamine oxidase (MAO) were significantly inhibited in the striatum, hypothalamus, midbrain, and cortex ($P \leq 0.01$). MAO activity was also significantly decreased in the cerebellum and cortex. DA concentration was lower (not statistically significant) in the striatum but significantly decreased in the hippocampus and significantly increased in the midbrain. No significant changes in 5-hydroxy tryptamine (5-HT) levels were observed in any region. No neuromotor activity was measured in the study to correlate with any of these changes, and the data were not found to be useful for the derivation of the acceptable concentration (AC).

A study by Dorman et al. (2000) included a comparative evaluation of the distribution of manganese in several regions of the brain and neurotoxicity of $MnCl_2$ in neonatal (postnatal day 13, 17, and 21) and adult CD rats following subchronic high-dose oral exposure. Only data from adult rats will be described. Gavage doses of $MnCl_2{\cdot}4H_2O$ were administered to adult male CD rats at doses of $MnCl_2$ at 0, 25, and 50 mg/kg for 21 d (dose rates of manganese at 0, 7, and 14 mg/kg/d). In this study, Dorman et al. (2000) measured spontaneous motor activity using an automated photo beam activity system and passive avoidance tests to assess learning and memory. Additionally, the investigators evaluated the pulse-elicited acoustic startling reflex, and numerous functional observations, such as observations of posture, tremors, spasms, convulsions, muscle tone, and the animals' condition such as breathing pattern, ataxia, arousal, gait, and body position (see Dorman et al. 2000 for details). The authors reported that they found no statistically significant effects on motor activity that were related to manganese exposure. A significant decrease in the overall mean acoustic startle amplitude was elicited in the 25 mg/kg group but not in rats receiving manganese at 50 mg/kg, showing a lack of dose-related response.

Tissue manganese was determined in six brain regions, namely striatum, hypothalamus, hindbrain, cerebellum, hippocampus, and the rest of the brain (called "brain residue" by the authors). Increased striatal,

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cerebellar, and brain residue manganese concentrations were observed in adult rats from the 50 mg/kg group, but in the 25 mg/kg group, the increase was seen only in the "brain residue." Similarly, measurement of neurochemistry variables indicated that only minor changes occurred in neurotransmitter levels, such as a small increase in cerebellar 5-hydroxyindoleacetic acid in the low-dose group and no significant changes in striatal DA and DOPAC. There were also no changes in homovanillic acid (HVA) or serotonin (Dorman et al. 2000).

Spadoni et al. (2000) reported some selective vulnerability of pallidal neurons in the early phases of manganese intoxication. In this study, 20-d-old male Wistar rats that were provided $MnCl_2$ in drinking water at 20 mg/mL (about 550 mg/kg/d) for 3 mo accumulated manganese in liver and brain subregions. The authors did not conduct any behavioral tests in this study. Morphologic examination showed no neuronal loss or gliosis in the globus pallidus. However, when investigators attempted to isolate neurons from the globus pallidus, most neurons died, showing extreme sensitivity. The authors proposed that accumulation of Mn^{+2} in the brain mitochondria caused inhibition of mitochondrial superoxide dismutase, resulting in oxidative stress on the neurons, which led to vulnerability. The authors considered this an early phase of intoxication. Also, manganese-treated globus pallidus cells from these rats showed a peculiar response to glutamate, including irreversible cell damage. It must be noted that developing rats and not adults were used in this study. In a later study from the same laboratory, Calabresi et al. (2001) conducted biochemical, morphologic, and electrophysiologic experiments with 20-d-old male Wistar rats exposed to manganese as $MnCl_2$ in drinking water for 10 wk at a concentration of 20 mg/mL (about 550 mg/kg). The rats were tested for locomotor activity, reactivity to object novelty in an open field, and radial maze performance. Exposed rats were hyperactive (compared to controls). However, no overt signs of brain damage, such as significant neuronal loss or gliosis, were observed. The concentration of manganese (nmole/mg protein) in basal ganglia, cortex, and cerebellum was threefold higher than in the same structures in control rats, and manganese in liver was 10-fold higher. The authors also reported that in spite of the rats' hyperactive behavior, their ability to learn procedures and their spatial memory were not impaired. These results were consistent with the electrophysiologic measurements.

A variety of histologic changes in subcellular organelles (rough and smooth endoplasmic reticulum, Golgi apparatus) were observed in the livers of rats exposed to manganese as $MnCl_2$ at 12 mg/kg/d for 10 wk in drinking water (Wassermann and Wassermann 1977). According to the authors, these were only adaptive, rather than adverse effects. NTP

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(1993) conducted a 90-d subchronic study in which male and female F344/N rats and male and female B6C3F1 mice were exposed to $MnSO_4 \cdot H_2O$ in their feed (at the concentrations of $MnSO_4$ at 0-25,000 ppm for rats and 0-50,000 ppm for mice). The estimated dose rates for the 13-wk experiment groups were 0-655 mg/kg/d for rats and 0-2,300 mg/kg/d for mice. The investigators observed few differences between the responses of male and female rats. Absolute and relative liver weights of all exposed males and females in the highest-dose group were significantly lower than those of controls. In the male rats, at concentrations above 65 mg/kg/d, a decrease in lymphocytes, an increase in segmented neutrophil counts, and increases in both hematocrit and erythrocyte counts were observed. An increase in erythrocytes in female rats was seen only at the highest dose. In the females, both lymphocytes and leukocytes were decreased. No clinical or abnormal histopathology findings were significant at 13 wk.

In the NTP (1993) subchronic study described above, at the end of the 13 wk, at the highest dose of 50,000 ppm (manganese at 2,300 mg/kg), significant reductions were found in body weight of both male and female mice and in relative and absolute liver weight of males. Hematocrit, hemoglobin levels, and mean erythrocyte volumes decreased in male and female mice receiving 50,000 ppm, suggesting that the animals had a microcytic anemia (probably because of sequestration or iron deficiency). Three male high-dose mice also had epithelial hyperplasia and hyperkeratosis of the forestomach (NTP 1993). Such observations were not seen in the interim evaluation period of 15 mo in the 2-y chronic study, making it difficult to interpret these changes as being related to treatment.

Komura and Sakamoto (1991, 1992) conducted two studies aimed at examining the effects on the CNS of rather large amounts of various chemical forms of manganese (two soluble and two insoluble) added to the diet of mice—effects including behavioral alterations and concentrations of biogenic amines. One of these (Komura and Sakamoto 1991) was a short-term (100-d) study and the other (Komura and Sakamoto 1992) was a long-term (12-mo) study. Six-week-old ddY mice were exposed to manganese via a diet containing one of two water-soluble ($MnCl_2 \cdot 4H_2O$ and manganese acetate) and two water-insoluble (manganese carbonate and manganese oxide) manganese compounds at a concentration of 2 g/kg or 200 mg/kg (calculated by the authors). Spontaneous motor activity was tested once in 30 d for 30 minutes (min) using an Animex activity meter. After 100 d, red blood cell count, hematocrit, hemoglobin, and white blood cell count were determined. The only hematologic effect seen with $MnCl_2$ was a 42% decrease in white blood

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cell count. The group fed manganese acetate and $MnCO_3$ had a significantly lower red blood cell count than controls. White cell count was decreased in the group fed manganese acetate, $MnCl_2$, and MnO_2. In the NTP (1993) 13-wk $MnSO_4$ feed study in B6C3F1 mice, a decrease in white blood cell count was seen only in mice treated with more than 20 times the dose used by Komura and Sakamoto. Although one might argue that the salt ($MnSO_4$ versus four other salts) and mouse strain (B6C3F1 versus ddY mice) were different in the two studies, such a difference in response is unexpected. Even with $MnCl_2$ at 200 mg/kg, the authors did not find any change in motor activity compared to that of control mice, whereas in the manganese acetate group, it was lower at all three durations (30, 60, and 90 d). The difference between mice exposed to $MnCl_2 \cdot 4H_2O$ and manganese acetate cannot be explained. The dose used was very high and no dose-response data were reported. Because of the lack of consistent findings in hematology and low confidence in the spontaneous motor activity data, the data were not considered for AC calculation.

Chronic Toxicity (>100 d)

Human Exposure Data

Few reports exist about neurologic effects associated with human exposure to manganese from environmental sources such as drinking water. The neurotoxic effects on human populations of low-level exposure to manganese from environmental exposures have been reviewed by Mergler (1999). In this review, the authors drew attention to the progression of dysfunction leading to manganism after cessation of exposure.

In one epidemiologic study of an elderly population in Greece, Kondakis et al. (1989) evaluated an association between various concentrations of manganese and clinical symptoms akin to PD. Three areas in northwest Greece that had a range of manganese concentrations in drinking water were chosen for this study. The areas were categorized as areas A, B, and C. Manganese concentrations in natural well water were 3.6-14.6 µg/L in area A, 81.6-252.6 µg/L in area B, and 1,600-2,300 µg/L in area C. The total population of the three areas ranged from 3,200 to 4,350 people. The study included only individuals over 50 y of age, drawn from a random sample of 10% of all households. The number of subjects was 62, 49, and 77 for areas A, B, and C, respectively. The authors had stated that "all areas were similar with respect to social and dietary characteristics" and that it was improbable that dietary differ-

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ences existed, because food, fruits, and vegetables were traded among all these areas. A neurologist assigned a score value (from 0 [absent] to 3 [strong]) for each symptom for each subject based on its diagnostic value for PD. The mean combined scores for both sexes within an area were 2.7, 3.9, and 5.2 for regions A, B, and C, respectively. It must be pointed out that comparisons between sexes within an area and among areas were not very consistently significant. For example, the difference between region A and region C was statistically significant for men and not for women, even though in both genders combined, the difference in mean scores was highly significant. The data need to be interpreted cautiously. Only selected older populations were covered in this study. Although 33 symptoms were tested for presence and severity, the authors did not report which neurologic signs or symptoms increased. The investigators had assumed the symptoms of manganism are the same as those of PD. They did not measure sleep and emotional stability parameters, which were altered in miners exposed to manganese. The authors did correlate the prevalence of neurologic symptoms with the progressive concentration of manganese in the hair of the elderly subjects. Using hair manganese concentrations may have only a limited value as a measure of manganese body burden. Mn-B did not differ between areas A and C. There was no difference between genders for Mn-B or any correlation between Mn-B and hair manganese concentration. The authors did not report the general health status of this elderly population or whether subjects were taking any medications.

In a cohort study conducted in northern Germany, manganese burden from rural well water was studied cross-sectionally in two communities that had their own drinking-water wells (Vieregge et al. 1995). Study participants were randomly selected from right-handed residents over the age of 40 who had used their well as their principal source of drinking water for at least 10 y. Group A consisted of 41 subjects with a mean age of 57.5 y who were exposed to manganese at least 0.3 mg/L (range 0.3-2.16) in well water, and Group B consisted of 74 subjects with a mean age of 56.9 y who were exposed to concentrations of less than 0.05 mg/L. The authors stated that the groups were homogenous with regard to age, sex, nutritional habits, and drug intake. Structured questionnaires were used to gather data on medical and occupational history, habits, and medication use. Mean hemoglobin, ceruloplasmin, copper, and iron values, as well as results of liver function tests, were within the acceptable range in both groups. Each subject underwent a complete, standardized neurologic assessment by a neurologist who did not know the subject’s manganese exposure history. Data were collected by administering a symptom questionnaire, performing an assessment of possible PD signs

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by the Columbia University Rating Scale (CURS), and measuring the fine motor coordination of both hands using an instrument called MLS-22. MLS tests consisted of the following trials: 1) aiming, 2) steadiness, 3) line pursuit, and 4) tapping rate. No significant difference in any neurologic measure was found between groups, leading the authors to conclude that chronic exposure to drinking water containing manganese at concentrations of 0.3 mg/L will not result in detectable neurologic impairment. Several positive aspects of the quality of this study will be considered in conjunction with the study from rural Greece by Kondakis et al. (1989).

Several publications from the Ben-Gurion University of Israel discuss studies designed to evaluate the etiologic factor or factors responsible for the increased prevalence of PD in three adjacent clusters of kibbutzim (rural settlements) in southern Israel. In residents older than 40 in each of these three clusters, the prevalence of PD was 5 times greater than in residents in the remainder of the region (see Herishanu et al. 2001 and references cited therein, including Goldsmith et al. 1990). It was reported that well water and soils contained excess manganese and that Maneb (a manganese-containing fungicide) was commonly used in the area, but concentrations of these were not reported. In addition to the fact that both inorganic and organic manganese compounds were present, confounding factors such as an excess of aluminum and iron, as well as other heavy metals found in the water and soil in that region, make it difficult to use these data for quantitative risk assessment for inorganic manganese.

A few miscellaneous studies have associated environmental manganese exposures and adverse neurologic effects. Chinese children (11-13 y old) who were exposed to increased manganese concentrations (about 0.24 mg/L) in water and wheat irrigated with sewage for 3 y performed poorly in neurobehavioral tests and performed poorly at school compared to control children from a nearby village. Hair manganese concentrations reported in this study correlated negatively with performance scores (Zhang et al. 1995, based on the abstract from Medline for this Chinese paper). iron, copper, and zinc were measured in blood and hair; performance was inversely correlated only with hair manganese concentrations. Daily dietary intake of manganese was not provided. Gottschalk et al. (1991) reported significantly elevated manganese concentrations in the hair of populations of prisoners incarcerated for violent behavior compared with nonviolent subjects in the prison population. The source of exposure was not known.

Some case reports have associated adverse neurologic effects and exposure to manganese, sometimes apparently caused by the defective

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metabolism of manganese. One case history was that of a 62-y-old male who had been receiving parenteral nutrition that contained manganese (salt not known) at 2.2 mg/d for 23 mo (Ejima et al. 1992). He exhibited all of the symptoms of PD. An extrapolated oral amount of manganese was calculated as 40 mg/d. Another case was that of an 8-y-old girl who had end-stage liver disease with a block in biliary secretion and showed signs of manganism. Her intracranial T1 MRI scans indicated hyperintense globus pallidus and subthalamic nuclei. Biliary obstruction may have resulted in an increased uptake of circulating manganese by the brain (Ejima et al. 1992).

Animal Exposure Data

Considerably more data are available on the neurologic effects of manganese ingestion in animals. However, only a few studies have reported clinical signs such as weakness, ataxia, or altered gait following oral dosing. Gupta et al. (1980) conducted a study on adult male rhesus monkeys (5-6 kg) who were given $MnCl_2$ at 25 mg/kg (6.9 mg/kg/d) orally by gavage for 18 mo. The monkeys developed weakness and muscular rigidity of the lower limbs. Histologic analysis also revealed scanty neuromelanin granules and degenerated neurons in the substantia nigra with gliosis and neuronal loss. This may be related to the fact that melanins are formed by the oxidation of DA, and DA depletion may be the reason for neuromelanin depletion, as noted by several investigators.

In male albino rats exposed to $MnCl_2$ (manganese at 100 mg/kg/d) in drinking water for 360 d, catecholamines (norepinephrine [NE] and DA), HVA, MAO, and manganese were measured in the corpus striatum (Chandra and Shukla 1981) at different time intervals up to the full period of 360 d. Manganese treatment initially increased the concentrations of DA, NE, and HVA, but normal concentrations were seen from 120-240 d, and thereafter, the concentrations of these decreased significantly from 300 to 360 d. These concentrations could not be correlated with the concentration of tissue manganese in this region of the brain, which increased to a maximum at 240 d and remained at that concentration until the end of the study. In spite of the fact that the underlying cause (neuronal loss in the basal ganglia) of the psychiatric and neurologic phases of chronic manganese poisoning is known, it is difficult to determine a NOAEL for the interim duration of 100 d, because it is not clear that the early increase is an adverse effect.

Gianutsos and Murray (1982) observed that administration of manganese to mice in the form of $MnCl_2$ (4%) in the diet for 6 mo (manga-

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nese at 2,300 mg/kg/d) resulted in a decrease in concentration of DA in the striatum and olfactory tubercles. The GABA content of the striatum was higher after treatment, whereas the cerebellar GABA content did not change. Choline acetyltransferase activity remained unchanged. Changes in neurotransmitter concentrations were observed after long-term manganese administration but were not seen in mice exposed to $MnCl_2$ for 1-2 mo (Gianutsos and Murray 1982).

Similarly, in two studies by Bonilla and coworkers in which male rats were given manganese via drinking water (0.1 and 5 mg/mL) for 8 mo, an increase in motor activity during the first mo, normal activity during months 2-6, and then activity significantly lower than control after 6 months were observed (Bonilla 1984). A part of this study was described in the earlier section. Bonilla and Prasad (1984) conducted a study in male Sprague-Dawley rats on the effect of chronic intake (for 8 mo) of two concentrations ($MnCl_2$ at 0.1 and 1 mg/mL) in drinking water on the concentrations of biogenic amines and their metabolites in several regions of the brain. One of the metabolites of DA, DOPAC, was found to be significantly reduced in both striatum and hypothalamus in both of the manganese-treated groups. There was no dose-consistent change in the other metabolite, homovanillic acid. A significant decrease in noradrenaline in the pons was also observed in both treated groups. The authors did not measure locomotor activities. The changes reported in the study do not appear to be dose related (Bonilla and Prasad 1984).

Nachtman et al. (1986) examined the effects of chronic manganese exposure on locomotor activity in rats maintained on $MnCl_2{\cdot}4H_2O$ at 0 or 1 mg/mL in drinking water for 65 wk. Locomotor activity was tested in 15-min sessions several times during the study. Manganese treatment produced a significant increase in activity in weeks 5-7, control values at 8 wk, and decreases from 14 to 29 wk. Manganese-exposed animals were found to be more responsive to the effects of d-amphetamine (1.25 mg/kg) than controls. This increased responsiveness to d-amphetamine found in earlier weeks was gone at weeks 41 and 65. The results were similar to those of the study described previously.

In addition to their 100-d study described earlier, Komura and Sakamoto (1992) also conducted a 12-mo study in which male ddY mice were chronically treated with four forms of manganese ($MnCl_2{\cdot}4H_2O$, manganese acetate tetrahydrate, MnO_2, and $MnCO_3$) mixed in the diet at 2 g/kg. Biogenic amines in the brain and spontaneous motor activity were measured several times during the course of 12 mo. The manganese concentrations were higher in some parts of the brain after exposure to insoluble salts than after the soluble salts. A review of the data presented as a graph indicates that it is difficult to come to such a definite conclu-

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sion. The concentrations of hypothalamic DA correlated with the manganese concentrations, especially in the manganese acetate-exposed group. In addition, the amount of brain manganese correlated well with the extent of suppression of spontaneous motor activity measured using an Animex Activity Meter. Paradoxically, the largest manganese content was found in the cerebral cortex of mice treated with insoluble manganese compounds, $MnCO_3$ and MnO_2. In concordance with this, spontaneous motor activity was most affected in MnO_2-fed mice. In the mice exposed to soluble manganese compounds, at the end of 12 mo, there was a significant decrease in DA and an increase in HVA, indicating that metabolism of DA increased.

In the 2-y NTP (1993) study, $MnSO_4 \cdot H_2O$ was administered to rats (at doses of 0, 20, 65, and 200 mg/kg for males and 0, 23, 75, and 232 mg/kg for females) and mice (0, 52, 175, and 585 mg/kg for males and 0, 65, 228, and 731 mg/kg for females). Survival decreased in male rats fed the highest dose; however, females fed this amount of manganese were not affected. Several rats died in both the control and treated groups. But the cause of death in male rats was attributed to increased severity of nephropathy and renal failure. The survival of male and female mice that received $MnSO_4$ at 15,000 ppm (manganese at about 600-750 mg/kg/d) for 2 y was not affected. Similarly, mice tolerated doses of $MnCl_2$ as high as 2,270 mg/kg/d) in their diet for 6 mo without lethality (Gianutsos and Murray 1982). The authors did not observe any histologic changes in the lungs or cardiovascular system or any clinical signs of impaired function of these organs.

In rats fed $MnSO_4$ at as much as 15,000 ppm (up to 232 mg/kg/d) for 2 y, no histologic effects on the GI system were observed. Mice in the highest-dose groups of this study (15,000 ppm for 730 mg/kg/d) had hyperplasia, erosion, and inflammation of the forestomach, but these effects were considered minor. Significant hepatic histologic changes were observed in neither mice nor rats exposed to $MnSO_4$ in their diet for 2 y at various concentrations.

Genotoxicity

Studies of the genetic toxicology of manganese salts have been carried out using several methods, such as the *Salmonella typhimurium*/mammalian-microsome mutagenicity test (also in a preincubation-type assay), the rec assay with *Bacillus subtilis* for growth inhibition caused by DNA damage, the reversion assay with *Escherichia coli*, and many others. There appear to be some differences in results with differ-

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ent types of salt. $MnCl_2$ was negative in *S. typhimurium* strains TA98, TA102, and TA1535 and weakly positive in TA1537 without activation (Wong 1988), whereas it was weakly positive in the preincubation protocol with TA102 but not with TA100 (De Meo et al. 1991). $MnSO_4$ was negative in TA98, TA100, TA1535, TA1537, and TA97 (Mortelmans et al. 1986). $MnCl_2$ was found to be positive in one *E. coli* strain (Zakour and Glickman 1984). In the NTP (1993) bioassay, using a preincubation, $MnSO_4$ (100-10,000 µg/plate) was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, TA1535, or TA1537, with or without induced male Sprague-Dawley rat or Syrian hamster liver S9. $MnSO_4$ was tested in two laboratories.

Several manganese salts ($MnCl_2$, manganese nitrate, $MnSO_4$, manganese acetate) were positive in the *B. subtilis* rec assay (Nishioka 1975), but were negative in the same system in a different study (Kanematsu et al. 1980).

Both $MnSO_4$ and $MnCl_2$ gave mutagenic dose-response relationships on tester strain TA102 without S9 mix. The mutagenic potencies were 2.8 and 2.4 revertant/nmole for $MnSO_4$ and $MnCl_2$, respectively. $MnCl_2$ also induced DNA damage in human lymphocytes as determined by the "single cell gel assay."

While Parry (1977), using the fluctuation test in yeast, found $MnSO_4$ to be negative, Singh (1984) found $MnSO_4$ to be positive in the yeast gene conversion and reversion assay. In mammalian cell tests, $MnCl_2$ was positive for gene mutation in mouse lymphoma cells (Oberly et al. 1982), positive in the Syrian hamster ovary cells transformation assay (Casto et al. 1979), and positive in the DNA damage assay in human lymphocytes (De Meo et al. 1991). $MnCl_2$ at high concentrations (10 mM) caused single-stranded DNA breaks in both Chinese hamster ovary (CHO) cells and human fibroblast cell cultures; the CHO cells seemed to be more sensitive (Hamilton-Koch et al. 1986).

Significant increases in chromosomal aberrations in CHO cells (Galloway et al. 1987) and in sister chromatid exchanges (SCE) in mouse fibroblasts and human lymphocytes (Andersen 1983) were reported. In cytogenetic tests with CHO cells, $MnSO_4$ induced SCEs with and without S9 activation (NTP 1993).

In the in vivo genotoxicity tests, $MnSO_4$ did not induce sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster* treated with 12,500 ppm in feed or 1,000 ppm administered by injection. Similar results were obtained by Rasmuson (1985) with the somatic mutation system.

$MnSO_4$ also induced chromosomal aberrations in CHO cells in the absence or presence of S9; in the presence of S-9, no significant increase

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in chromosomal aberrations compared with the results of tests without S9 was observed. A dose response was not clear.

Joardar and Sharma (1990) reported that when male Swiss albino mice were administered various concentrations of $MnSO_4$ ($MnSO_4 \cdot H_2O$ at 10.25, 20.5, and 61 mg/100 g body weight), it was clastogenic. For the chromosomal analysis, the animals were dosed daily for up to 21 d. The chromosomal aberrations in both bone marrow and micronuclei were significantly increased. In contrast, in earlier work in albino rats treated with oral doses of $MnCl_2$, tests for induction of chromosomal aberrations in the bone marrow cells were negative. No induction of heritable translocations was observed in mice treated with $MnSO_4$ in the feed for 7 wk, nor was induction of dominant lethal mutations observed in rats gavaged with $MnSO_4$ for 1-5 d (Newell et al. 1974).

In spite of the fact that the results are mixed from both the in vivo and in vitro systems in prokaryotes, yeast, fungi, and mammalian systems, the positive results strongly indicate that excess manganese may be genotoxic.

Carcinogenicity

Manganese dusts, fumes, and soluble salts have not been recognized as possible human carcinogens by any of the regulatory and health organizations. Few animal studies have looked at the carcinogenic potency of manganese compounds. Stoner et al. (1976), using a strain A mouse lung tumor model, investigated the carcinogenic potency of $MnSO_4$ in 6- to 8-wk-old mice after a total of 22 ip injections (3/wk) of $MnSO_4$ at 132, 330, or 660 mg/kg of body weight (42.9, 107, or 214 mg/kg). The highest-dosed group had a slight increase in the number of pulmonary adenomas. Results for other groups were similar to those for the control group, which had been injected with vehicle solution. Because this strain A lung tumor model could detect only 5 of 18 known carcinogens, the validity of this result has been questioned. In an earlier study, DiPaolo (1964) injected DBA mice ip or sc with 0.1 mL of 1% $MnSO_4$ solution (manganese at 200 mg/kg per injection) twice weekly for 6 mo (62.3 mg/kg/d). After 18 mo, 67% of mice treated via sc and 41% of ip-treated mice developed lymph sarcomas (24% of animals in the control group responded similarly). The study was reported only as an abstract.

In the NTP (1993) 2-y feeding study with $MnSO_4$, when rats ingested $MnSO_4$ at 0, 1,500, 5,000, or 15,000 ppm in the diet (see

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TABLE 9-4 Incidence of Nonneoplastic Lesions of the Pancreas in Male Rats in the 2-y $MnSO_4$ Feed Study

Dose in food (ppm)	Dose (mg/kg/d)	Hyperplasia	Adenoma
0	0	0/52	0/52
1,500	20	2/50	3/50
5,000	65	2/51	4/51
15,000	200	3/51	3/51

Source: Data from NTP 1993.

Table 9-4 for estimated average doses), hyperplasia or adenomas of the pancreatic islets occurred in a few males of all the treated groups, but none were found in the controls. In addition, carcinoma of the pancreas was seen in one male rat of the highest-dose group (NTP 1993). Adenomas and carcinomas were within the NTP (1993) historical controls, even though the study controls had none.

Chronic oral exposure of mice and rats to $MnSO_4$ in the feed resulted in a marginally increased incidence of thyroidal follicular cell adenomas. From the results of this 2-y study, NTP (1993) concluded that there was no evidence of carcinogenic activity in male or female rats fed $MnSO_4$ at 1,500 or 15,000 ppm in their diet for 2 y. But there was equivocal evidence of carcinogenic activity in male and female B6C3F1 mice based on the marginally increased incidence of thyroid gland follicular cell adenoma and the significantly increased incidence of follicular cell hyperplasia.

Biologically significant changes did occur in the incidences of neoplasms and/or non-neoplastic lesions in the thyroid gland, forestomach, and liver of male and female mice.

In the thyroid glands, at the end of the 2-y study in mice, the incidence of follicular dilatation increased significantly in males exposed to $MnSO_4{\cdot}H_2O$ at 15,000 ppm and females exposed to 5,000 or 15,000 ppm (see Table 9-5). A significantly increased incidence of focal hyperplasia of follicular epithelium also occurred in males dosed with 15,000 ppm and in all manganese-fed females. Follicular cell adenomas were found in some males and females fed manganese at 15,000 ppm, but the incidence of adenomas was not considered significantly different from that of controls (NTP 1993). However, an increased incidence of follicular cell hyperplasia with adenoma leads one to conclude that there is equivocal evidence of carcinogenicity in mice.

In the forestomach in male and female mice exposed to $MnSO_4$ at 15,000 ppm, a statistically significant increased incidence of focal

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TABLE 9-5 Thyroid Lesions in Mice That Received $MnSO_4$ in the Diet for 2 Years

		Concentration of $MnSO_4$ in the Feed			
Sex	Lesions	Control	1,500 ppm	5,000 ppm	15,000 ppm
Male	Follicular dilatation	2	2	5	23[a]
	Follicular cell hyperplasia	5	2	8	27[a]
	Follicular cell adenoma	0/50	0/49	0/51	3/50
Female	Follicular dilatation	1	5	11[a]	24[a]
	Follicular cell hyperplasia	3	15[a]	27[a]	43[a]
	Follicular cell adenoma	2/50	1/50	0/49	5/51

[a]Significantly different ($p \leq 0.05$) from the control group by logistic regression test.
Note: The follicular cell adenomas were only slightly increased above the historical controls.
Source: Modified from NTP 1993.

hyperplasia of the forestomach squamous epithelium occurred, accompanied by ulceration or erosion and by inflammation with focal occurrence of infiltrating neutrophils and mononuclear leukocytes at various sites on the forestomach mucosa.

In mice at the 9-mo interim evaluation, absolute liver weights of males exposed to 15,000 ppm and females exposed to 5,000 or 15,000 ppm were significantly lower than those of controls. Because these groups also had lower mean body weights and relative liver weights were similar to those of controls, the lower absolute liver weights cannot be considered an adverse effect of the manganese dose.

Reproductive Toxicity

Few reports exist on the reproductive toxicity of manganese compounds in humans or experimental animals. All of the human data are from an occupational setting where workers were exposed to manganese dust or manganese oxide, and all pertain to long-term exposure to manganese (Baranski 1993). Manifested symptoms included loss of libido and impotence (Rodier 1955; Mena et al. 1967; Kilburn 1987) and a decrease in the number of children born to the workers exposed to manganese dust (Lauwerys et al. 1985). Gennart et al. (1992) did not find any

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difference in the reproductive rate of dry-battery plant workers exposed to 0.71 mg/m^3 of manganese dust for 6.2 y. No reports of any adverse reproductive effects in humans from the ingestion of manganese by the oral route could be located.

There are several studies that had looked at the reproductive effects of manganese after injection of the solution (ip or sc). These studies will not be discussed here. Several rodent studies have reported that ingestion of manganese in the diet or drinking water leads to adverse reproductive parameters. Laskey and coworkers conducted several experiments in mice and rats administered Mn_3O_4 in the diet (Gray and Laskey 1980; Laskey et al. 1982). This insoluble form of manganese is not relevant for determining AC for drinking water. When weanling mice were fed a diet containing Mn_3O_4 at 1,050 mg/kg/d and were sacrificed on days 58, 73, or 90, significant decreases in the growth of preputial gland, seminal vesicle, and testes were observed (Gray and Laskey 1980). In Long-Evans rats chronically exposed to dietary Mn_3O_4 (concentrations of manganese as Mn_3O_4 at 350, 1,050, and 3,500 ppm) beginning on day 1 of gestation and continuing through 224 d of age, male reproductive development was delayed by manganese treatment as indicated by testes weight, sperm count, and serum follicle-stimulating hormone and testosterone concentrations. After receiving the manganese-containing diet for 100 d, the rats were mated. When male and female rats were fed a diet containing Mn_3O_4 at 3,500 ppm/kg/d for about 100 d before breeding, male fertility was reduced; female reproductive parameters were unaffected (Laskey et al. 1982). $MnCl_2$ administered to pregnant rats in drinking water (manganese at up to 620 mg/kg/d during the gestation period) did not adversely affect litter size or the sex ratio of the pups (Pappas et al. 1997). Similarly, Kontur and Fechter (1985) did not find any effect on litter size when dams were exposed to concentrations of $MnCl_2$ as high as 1,240 mg/kg/d in drinking water. NTP (1993) did not find changes in testicular weight even at the highest concentration of $MnSO_4$ (50,000 ppm).

In a study by Joardar and Sharma (1990), gavage solutions containing $MnSO_4{\cdot}2H_2O$) at 10.25, 20.5, or 61 mg per 100 g body weight (equivalent to manganese at 33, 66, or 198 mg/kg/d) were administered to mice for 5 d. The percent of sperm with abnormal heads increased with the dose. However, studies in which a dietary regimen of $MnSO_4$ was given to rats and mice for 13 wk or 2 y revealed no gross or abnormal histopathology lesions or changes in reproductive organ weights. No other reproductive parameters were evaluated (NTP 1993).

In a study of male rats exposed to $MnSO_4$ at a concentration of 1,000 ppm (manganese at 30 mg/kg/d in drinking water) for 12 wk,

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Bataineh et al. (1998) suggested that subchronic exposure to $MnSO_4$ has adverse effects on sexual behavior, territorial aggression, and the reproductive system of the adult male rat. Although ingestion of $MnSO_4{\cdot}2H_2O$ in drinking water for 12 wk did not affect male rat fertility, the total number of resorptions increased in female rats impregnated by treated males. Only one dose was used. Confidence in these data is low because other metals tested as a part of this study, such as aluminum chloride, lead acetate, and copper chloride, produced very similar results. Such effects have not been previously reported for these metals. The concentration of $MnSO_4{\cdot}2H_2O$ used in this study (1 g/L) is rather high. Elbetieha et al. (2001), from the same laboratory, reported that when sexually mature male and female Swiss mice were exposed to $MnCl_2{\cdot}4H_2O$ for 12 wk at 1, 2, 4, and 8 g/L in drinking water, fertility was significantly reduced in males of the high-dose group. The average daily doses, calculated from the water consumption data provided by the authors, were 30, 48, 98, 196 mg/kg/d for males and 28, 52, 100, and 176 mg/kg/d for females. Ingestion of $MnCl_2{\cdot}4H_2O$ at 8,000 mg/L significantly reduced the fertility of male rats. There were no effects on the number of implantations, viable fetuses, or resorptions in untreated females impregnated by treated males. Fertility was not significantly affected in treated female mice, but the number of implantations and viable fetuses in the highest dose group ($MnCl_2$ at 8 g or 176 mg/kg/d) was reduced. A LOAEL of 196 mg/kg/d and a NOAEL of 98 mg/kg/d for adverse effects on male fertility was identified. Water consumption decreased in all dosed groups, without a clear dose-response effect. It must be noted that the dosage concentrations used were much higher than one would encounter from sources of water.

Szakmary et al. (1995) observed adverse effects of manganese on reproduction parameters of rats but not rabbits. Administering $MnCl_2$ to pregnant rabbits and rats, by gavage at doses of 0, 11, 22, and 33 mg/kg/d resulted in an increase in the postimplantation loss in the rat but not in the rabbit (as cited in ATSDR 2000).

Recently, Ponnapakkam et al. (2003) assessed organ weights and histopathology of male reproductive organs and sperm parameters in 6-wk-old male CD-1 mice (n = 12/group) that received manganese acetate at 7.5, 15, and 30 mg/kg/d as gavage doses for 43 d. The dose rates were calculated as 1.65, 3.3, and 6.6 mg/kg/d. In mice of the 15 and 30 mg/kg/d groups, cauda epididymis and testis sperm counts and sperm motility showed significant reductions. No alterations were found in male fertility (examined only in the 30 mg/kg group) based on the number of implantations, corpus lutea, number of resorption sites, and num-

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ber of live and dead fetuses, or histopathology of right testis, epididymis, seminal vesicle, or accessory glands.

Developmental Effects

There have not been many conclusive studies of the teratogenic effects of manganese exposure in humans. The incidences of neurologic disorders, birth defects, and stillbirths were elevated in a small population of people living on an island having rich manganese deposits (Kilburn 1987), but no reliable association with manganese exposure could be made.

Animal studies indicate that manganese salts can cross the placental barrier to fetuses. The FDA carried out a teratologic evaluation of $MnSO_4$ in rats, mice, hamsters, and rabbits. The compound was gavaged at different doses in each of these species (0.783-78.3 mg/kg for rats, 1.25-125 mg/kg for mice, 1.36-136 mg/kg for hamsters, and 1.12-112 mg/kg for rabbits). No significant effects were seen on maternal or fetal survival, nor were any abnormalities found in the soft or skeletal tissues of the test groups (NTIS 1973). Studies in which manganese compounds were injected ip, iv, or sc have documented fetal deaths and skeletal malformations when the compound was injected during the gestation period. These studies are not relevant to setting oral exposure guidelines for spacecraft and will not be described here.

Pappas et al. (1997) reported that when $MnCl_2$ in drinking water at 0, 2, or 10 mg/mL was provided to rat dams and their litters from conception until postnatal day 30 (PND-30), no physical abnormalities were observed in the offspring. The rats exposed to $MnCl_2$ at 10 mg/mL had increased cortical manganese concentrations and were hyperactive at PND-17.

A summary of manganese toxicity studies described in the above paragraphs is listed in Table 9-6.

LIMITS SET BY OTHER ORGANIZATIONS FOR SOLUBLE MANGANESE

Table 9-7 provides a list of the current standards and recommendations by other organizations. The EPA SMCL is 50 µg/L based on taste and discoloration. The World Health Organization's (WHO's) guideline value in drinking water for aesthetic quality is 100 µg/L.

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TABLE 9-6 Manganese Toxicity Summary

Chemical Form	Species and Gender	Mode of Dosing and Duration	Dose	Effects	LOAEL/ NOAEL (mg/kg/d)	Reference
$MnSO_4$	Human case report	Hemodialysis solution (iv)	40 mg/L; 30 min	Severe vomiting, abdominal pain, increased heart rate and blood pressure; acute pancreatitis on day 2	Serious effect; not calculated	Taylor and Price 1982
$MnCl_2$	Women	Capsule, single dose	40 mg	No adverse effect reported	NOAEL = 0.57	Freeland-Graves and Lin 1991
Manganese supplement aminoacid chelated	Women (60 kg)	Oral, 1, 25, 60, 89, and 124 d	15 mg	No adverse effects on hematocrit, serum iron, zinc and copper, or indexes of iron status; lymphocyte manganese superoxide dismutase (manganese status) did not change until day 89	NOAEL = 15 mg/d for up to 89 d; or 0.25 mg/d as supplement	Davis and Greger 1992
$MnSO_4$ supplemented in diet	Women	Oral; diet (60 d)	0.7 or 9.5 mg/d supplement	No changes in hematocrit, hemoglobin, erythrocyte count, white cells, or platelets	NOAEL = 0.15 mg/kg/d (body weight assumed to be 60 kg)	Finley 1999
Dietary intake	Women	Diet (56 d)	0.8 or 20 mg/d; 8 wk	No signs of adverse symptoms of neurologic and motor steadiness or psychological assessments attributed to Mn	NOAEL = 20 mg/d or 0.33 mg/kg/d	Finley et al. 2003

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$MnSO_4 \cdot H_2O$	Swiss albino mouse, male	Gavage, 2 doses	33, 66, and 198 mg/kg/d	Increased frequency of micronucleated polychromatic and normochromatic erythrocytes as a function of dose	LOAEL = 33	Joardar and Sharma 1990
$MnSO_4 \cdot H_2O$	Swiss albino mouse, male	Gavage, 5 doses	33, 66, and 198 mg/kg/d	Increased sperm head abnormalities as a function of dose	LOAEL = 33	Joardar and Sharma 1990
$MnSO_4$	F344/N rat, male	In feed, 14 d	0, 84, 165, 340, 665, and 1,264 mg/kg/d	Decreased neutrophils and leukocyte counts; reduced liver weight; decreased body weight	LOAEL = 1,264; NOAEL = 665	NTP 1993
$MnSO_4$	F344/N rat, female	In feed, 14 d	0, 84, 159, 340, 675, and 1,275 mg/kg/d	Decreased body weight; No hematolog , neurologic, reproductive, hepatic, or renal system effects	LOAEL=1275; NOAEL = 675	NTP 1993
$MnSO_4$	B6C3F1 mouse, male	In feed, 14 d	0,121, 262, 669, 1,603, and 3,212 mg/kg/d	No effect on body weight, hematologic , respiratory, hepatic, renal, reproductive, or cardiovascular systems	LOAEL = none; NOAEL = 3,200	NTP 1993
$MnSO_4$	B6C3F1 mouse, female	In feed, 14 d	0, 240, 488, 1,068, 2,494, and 3,560 mg/kg/d	No changes in body weight; no effect on hematologic , respiratory, hepatic, renal, reproductive. or cardiovascular system.	LOAEL = none; NOAEL = 3,500	NTP 1993

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TABLE 9-6 Continued

Chemical Form	Species and Gender	Mode of Dosing and Duration	Dose	Effects	LOAEL/ NOAEL (mg/kg/d)	Reference
$MnSO_4$	Swiss albino mouse, male	Gavage, 7, 14, 21 d	33, 66, 198	Increased chromosomal aberrations and breaks	LOAEL=33	Joardar and Sharma 1990
$MnCl_2 \cdot 4H_2O$	CD rat, male	Gavages, 21 d at 0, 25, and 50 mg/kg	7 and 14 mg/kg	No changes in clinical observations; no change in striatal DA or DOPAC; no changes in spontaneous motor activity; decreased acoustic startle reflex was noted only in low-dose group	LOAEL = 14	Dorman et al. 2000
$MnCl_2$	ITRC rats, male	Drinking water, 30 d	140 mg/kg/d	Hyperactivity, aggression, altered neurotransmitter levels, increased turnover of striatal DA	LOAEL = 140; only dose (serious effects)	Chandra et al. 1983
Manganese acetate	CD-1 mouse, male (6 wk old)	Gavages, (43 d) at 7.5, 15, and 30 mg/kg/d	1.65, 3.3, and 6.6 mg/kg/d	Significant decrease in caudal epididymal and testicular sperm counts, and sperm motility of two high-dose groups; no changes in mating behavior or fertility of male mice and no abnormal histopathology of testicular tissues (testis and epididymis) in mice treated at the highest dose; no manganese-	NOAEL = 1.65	Ponnapakkam et al. 2003

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				related changes in the fetuses delivered on gestation day 18		
$MnCl_2$	Sprague-Dawley rat, male	Drinking water, 2 mo at 10 mg/mL	400 mg/kg/d	Decreased water consumption; increased GABA in caudate nucleus; motor activity not assessed	LOAEL = 600	Bonilla 1978
$MnCl_2$	Albino rat, male	Drinking water, 90 d	12 mg/kg/d	Altered DA, serotonin, and MAO	LOAEL = 12	Subhash and Padmashree 1991
$MnCl_2$	Rat, male (20 d old)	Drinking water, 13 wk at 20 mg/L	550 mg/kg/d	No neuronal loss or gliosis in GP, but GP neurons were vulnerable to death; peculiar response to glutamate; behavioral tests not done	LOAEL = 550	Spadoni et al. 2000
$MnCl_2$	Rat, male (20 d old)	Drinking water, 10 wk at 20 mg/L	550 mg/kg/d	Rats were hyperactive in the open field tests; manganese concentrations in brain regions, morphologic assessments and electrophysiologic tests done; no overt signs of brain damage; no obvious morphologic or cytologic or cytochemic characteristics of striatum or substantia nigra	LOAEL = 550	Calabresi et al. 2001
Manganese-contaminated water	Human, male and female	Drinking water, 2-3 mo	28 mg/L estimated	Lethargy, increased muscle tonus, tremor; children not affected; microscopic changes in globus pallidus of the dead subject	LOAEL = 28 mg/L	Kawamura et al. 1941

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TABLE 9-6 Continued

Chemical Form	Species and Gender	Mode of Dosing and Duration	Dose	Effects	LOAEL/NOAEL (mg/kg/d)	Reference
$MnSO_4$ dihydrate	Sprague-Dawley rat, male	Drinking water, 12 wk	30 mg/kg/d	Suppressed sexual behavior in males and aggression; number of resorptions increased in females impregnated by exposed males	LOAEL = 30; NOAEL not known.	Bataineh et al. 1998
$MnCl_2{\cdot}4H_2O$	Swiss mice, male and female	Drinking water, 12 wk	0, 38, 76, and 152 mg/kg/d	Reduced male fertility, but no effect on implantations or fetus viability or resorptions; reduced sexual activity	LOAEL = 152; NOAEL = 76	Elbetieha et al. 2001
$MnSO_4$	F344/N rat, male	In feed, 13 wk	0, 33, 66, 113, 275, and 546 mg/kg/d	Increased neutrophil counts; decreased liver weight; no changes in respiratory, cardio, renal, endocrine, neurologic, reproductive, or immunologic systems	LOAEL = none; NOAEL = 275	NTP 1993
$MnSO_4$	F344/N rat, female	In feed, 13 wk	0, 37, 74, 183, 303, and 655 mg/kg/d	Reduced lung weight; no hematologic , respiratory, cardio, renal, hepatic, endocrine, reproductive, or immunologic effects	LOAEL = 655; NOAEL=303	NTP 1993

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$MnSO_4$	B6C3F1 mouse, male	In feed, 13 wk	0, 135, 260, 530, 1,075, and 2,300 mg/kg/d	Reduced body and liver weights; increased neutrophil; no effect on respiratory, renal, reproductive, or cardiologic systems	LOAEL = 135; NOAEL = not known for hematology /immunology	NTP 1993
$MnSO_4$	B6C3F1 mouse, female	In feed, 13 wk	0, 170, 340, 660, 1,350, and 2,800 mg/kg/d	Reduced body weight and reduced liver weight; increased neutrophil; no effect on respiratory, renal, reproductive, or cardiovascular systems	LOAEL = 170; NOAEL = not known for hematology effects	NTP 1993
$MnCl_2$, manganese acetate, $MnCO_3$, MnO_2	ddY mouse, male (6 wk old)	In feed, 100 d	200 mg/kg/d	Decreased hematocrit; erythrocytes, white blood cells; decreased spontaneous motor activity in manganese acetate group; none in $MnCl_2$ group	LOAEL = 200 (only one dose)	Komura and Sakamoto 1991
$MnCl_2$	CD-1 mouse, male	In feed, 180 d	2,300 mg/kg/d	Decreased levels of DA; increased GABA in striatum	LOAEL = 2,300 (one dose only)	Gianutsos and Murray 1982
$MnCl_2$	Sprague-Dawley, rat, male	Drinking water, 8 mo at 0.1 and 1 mg/mL	4 and 40 mg/kg/d	Decrease neurotransmitter norepinephrine in striatum and pons; significant decreases in DOPAC in striatum and hypothalamus at both doses; magnitude of decrease was not dose related	LOAEL = 4	Bonilla and Prasad 1984

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TABLE 9-6 Continued

Chemical Form	Species and Gender	Mode of Dosing and Duration	Dose	Effects	LOAEL/ NOAEL (mg/kg/d)	Reference
$MnCl_2$	Sprague-Dawley, rat, male	Drinking water, 8 mo at 0.1 and 5 mg/mL	4,200 mg/kg/d	Increased spontaneous motor activity during the first mo; decreased activity after 6 mo; extent of change same for both doses	LOAEL = 10	Bonilla 1984
$MnCl_2$, manganese-acetate, MnO_2, $MnCO_3$	ddY mouse, male	In feed, 12 mo at 2 g/kg diet	200 mg/kg/d	Reduced spontaneous motor activity; reduction in DA and increase in HVA; changes in biogenic amines	LOAEL = 200; (only one dose)	Komura and Sakamoto 1992
$MnCl_2 \cdot 4H_2O$	Sprague-Dawley, rat, male	Drinking water, 65 wk	40 mg/kg/d	Hyperactivity at week 5 to 7; control values at 8 wk; decreased at 14 to 29 wk; increased response to d-Amphetamine seen in earlier week not seen at 41and 65 wk	LOAEL = 40 (only one dose)	Nachtman et al. 1986
$MnCl_2 \cdot 4H_2O$	Rhesus monkey, male	Gavage, 18 mo	6.9 mg/kg/d	Muscular weakness; rigidity of lower limbs; histopathology confirmation	LOAEL = 6.9 (only one dose)	Gupta et al. 1980
$MnSO_4$	F344/N rat, male	In feed, 2 y	0, 20, 65, and 200 mg/kg/d	Chronic progressive nephropathy; reduced body weight; no changes in lung weight; no hematologic , GI, cardiovascular, hepatic, endocrine, reproductive, or immunologic effects	LOAEL = 200; NOAEL = 65	NTP 1993

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$MnSO_4$	F344/N rat, female	In feed, 2 y	0, 23, 75, and 232 mg/kg/d	No changes in lung weight; no hematologic , respiratory, cardiovascular, renal, hepatic, endocrine, reproductive, or immunologic effects	NOAEL = 232	NTP 1993
$MnSO_4$	B6C3F1 mouse, male	In feed, 2 y	0, 52, 175, and 585 mg/kg/d	Hyperplasia and erosion of GI tract; forestomach ulceration and inflammation; increased hematocrit, hemoglobin, and erythrocyte count; follicular hyperplasia of thyroid and dilation; no effect on body weight or respiratory, renal, reproductive, or cardiovascular systems	LOAEL =175; NOAEL = 52 for renal and body weight	NTP 1993
$MnSO_4$	B6C3F1 mouse, female	In feed, 2 y	0, 65, 228, and 731 mg/kg/d	Ulceration and inflammation of forestomach; thyroid follicular hyperplasia; 13% decrease in body weight; no significant changes in liver weight; no hematologic effects; no effect on respiratory, renal, reproductive, or cardiovascular systems	LOAEL = 65 for thyroid effect; NOAEL = not known	NTP 1993

(Continued)

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TABLE 9-6 Continued

Chemical Form	Species and Gender	Mode of Dosing and Duration	Dose	Effects	LOAEL/ NOAEL (mg/kg/d)	Reference
$MnCl_2 \cdot 4H_2O$	Wistar rat, male and female	Drinking water, 2 y	40 mg/kg/d	A developmental rat model: exposure initiated in utero; measure-ments made at 2 and 24 mo after manganese exposure; altered uptake of neurotransmitters such as DA and choline in brain regions	LOAEL = 40 (only one dose)	Lai et al. 1984
$MnCl_2 \cdot 4H_2O$	Wistar rat, male and female	Drinking water, 2 y	1, 10, 20 mg/mL	Developmental model: exposure from in utero to 2 y; brain regional distribution of manganese and other metals studied; increase in manganese accumulation caused region-specific changes in the brain iron, copper, selenium, zinc, calcium, and magnesium concentrations; subcellular fractionation indicates selective enhancement of manganese accumulates in brain mitochondria	No adverse end point studied	Lai et al. 1999
Manganese salt form not specified	Human case report	Parenteral nutrition (iv)	2.2 mg/d, 23 mo (690 d)	Developed symptoms characteristic of Parkinson's disease	Serious effect; LOAEL not estimated	Ejima et al. 1992

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Salts not specified; well water	Human, male and female; a Greek study	Drinking water, 50 y	3.6 µg/L to 2.3 mg/L	Weakness, fatigue, gait disturbances, tremors, and dystonia; increase in manganese concentrations in water correlated with higher prevalence of neurologic signs of chronic manganese poisoning based on neurologic scores (diagnostic value for Parkinson's disease)	NOAEL = 167 µg/L or 0.005 mg/kg/d	Kondakis et al. 1989
Salt form not known; well water	Human, male and female	Drinking water, 10-40 y	<0.05 mg/L or 0.3 to 2.16 mg/L	A German cohort study: no detectable neurologic impairment as determined by structured questionnaire, standardized neurologic examinations, and fine motor coordination measurements	NOAEL = 0.3-2.16 mg/L	Vieregge et al. 1995

Abbreviations: DA, dopamine; DOPAC, dihydroxyphenylacetic acid; GABA, gamma-aminobutyric acid; GI, gastrointestinal; GP, globus pallidus; HVA, homovanillic acid; LOAEL, lowest- observed-adverse-effect level; MAO, monoamine oxidase; NOAEL, no-observed-adverse-effect level.

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TABLE 9-7 Current Regulatory and Guideline Concentrations from Other Organizations

Standard	Value	Reference
EPA		
MCLG	None established	
MCL	None Established	
SMCL	0.05 mg/L (for aesthetic quality)	40 CFR 1991
1-d HA	None derived	
10-d HA	None derived	
Longer-term HA	None derived	
RfD	0.142 mg/kg/d (for food)	IRIS 1996
RfD[a]	0.050 mg/kg/d (for water)	IRIS 1996
Lifetime HA	None derived	
Cancer grouping	Group D[b]	
ATSDR (Agency for Toxic Substances and Disease Registry		
Acute MRL	None Derived	
Intermediate MRL	None Derived	
Chronic MRL	None Derived	
Other Agencies		
FDA	0.05 mg/L for bottled water	21 CFR 1993
WHO	0.1 mg/L for water aesthetic quality	WHO 1984

[a]A factor of 3 is used to account for increased absorption from water.
[b]Group D: Not classifiable as a carcinogen; inadequate or no human and animal evidence of carcinogenicity.
Abbreviations: HA, Health Advisory; MCL, maximum contaminant level; MCLG, maximum contaminant level goal; MRL, minimal risk level; RfD, reference dose, an estimate of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime; SMCL, secondary maximum contaminant level.

The Institute of Medicine (IOM) recently determined a tolerable upper intake level (UL) of 11 mg/d (IOM 2001), which was set for adults based on a NOAEL for Western diets using data from Greger (1999). UL is defined as “the highest level of daily nutrient intake that is likely to pose no risk of adverse effects in almost all individuals.” IOM (2001) also recommended an AI (acceptable intake) of 2.3 mg/d for men and 1.8 mg/d for women of 19+ y of age.

RATIONALE

The following paragraphs provide a rationale for proposing guideline limit values for manganese in spacecraft drinking water for 1 d, 10 d,

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100 d, and 1,000 d. The values listed were based on ACs for each duration according to *Methods for Developing Spacecraft Water Exposure Guidelines* (NRC 2000). Usually, an intraspecies factor is not used because astronauts, composed of men and nonpregnant women, come from a healthy population, and there is no evidence of a group of healthy persons having excess susceptibility to Mn^{+2}. However, while considering some adverse effects, consideration has to be given to the physiologic changes that occur in microgravity that could make the astronauts more susceptible to some chemicals. Our search of the literature indicates that no hypersensitivity factors are known to be related to manganese, except in neonates and those with liver disease, who are susceptible to increased toxicity from manganese ingestion. This is not an issue here. A summary of SWEGs derived for various durations is listed in Table 9-8.

A review of the manganese-ingestion studies indicates that there are two key toxicity end points: neurotoxicity and reproductive organ toxicity. The neurotoxicity literature indicates strongly that rodents may not be appropriate models for human neurotoxicity because manganese neurotoxicity often results in a behavioral syndrome and motor disturbances in humans, and the brain organization of rodents is different from that of humans. It should be pointed out that recently, Brenneman et al. (1999) concluded that the rat may be a poor model for human manganese-induced neurotoxicity because selective regional brain distribution of manganese was not observed in CD rats administered manganese as $MnCl_2$, in contrast to what has been reported to occur in humans and primates. Several studies have shown that DA levels of rodents are

TABLE 9-8 Spacecraft Water Exposure Guidelines for Soluble Manganese (Salts)

Duration	SWEG (mg/L)	Toxicity End Point	Reference
1 d	14	No adverse effects (human subject data)	Freeland-Graves and Lin 1991
10 d	5.4	No adverse effect (human subject data)	Davis and Greger 1992
100 d	1.8	No abnormal neurologic effects or clinical parameters	Davis and Greger 1992 and Finley et al. 2003
1,000 d	0.3	No neurotoxicity (human population)	Vieregge et al. 1995

Note: The 1,000-d SWEG is higher than EPA's SMCL value of 0.05 mg/L. The EPA's SMCL is based on taste and discoloration.

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affected by manganese exposure. Various indications of an initial increase in DA are followed by a normal response, which is followed by a significant decline (Cotzias et al. 1976; Gupta et al. 1980; Chandra and Shukla 1981; Barbeau 1984). Rodents have very low levels of pigment (neuromelanin) in the substantia nigra compared to those found in humans and monkeys. The argument that favors comparison between rodents and humans is that, in both cases, oral bioavailability is only 3-5%, and the clearance pathway is essentially biliary. Furthermore, rodents and humans respond to nutritional change very similarly; for example, an iron-deficient diet increases manganese absorption. Nevertheless, nonhuman animals require much more manganese than humans do. Rodents seem to tolerate much higher supplemental doses than humans, and thus, caution must be exercised when using species factors to extrapolate rodent data to humans. Rodents, like primates including humans, exhibit neurobehavioral effects attributed to manganese poisoning, but the neurologic symptoms seem to be different (McMillan 1999).

Manganese is essential for normal physiologic function because it is a cofactor for several enzymes involved in energy metabolism. In addition, it is an integral part of superoxide dismutase and pyruvate carboxylase. It seems to play a key role in the production of superoxide anions in the mitochondria of many mitochondria-rich organs, including the brain. Yet there is no RDA for manganese because it is available in numerous food sources to various degrees and no natural deficiency of manganese in humans has been encountered. In 1973, after evaluations of standard diets in the United States, England, and Holland, WHO concluded that manganese at 2-3 mg/d for adults is adequate and 8-9 mg/d is safe. The Food and Nutritional Board of the National Research Council (NRC) of the National Academy of Sciences (NAS) determined an estimated safe and adequate daily dietary intake (ESADDI), which is 2-5 mg/d for adults (NRC 1989).

NRC (1989) based this ESADDI on the metabolic study by McLeod and Robinson (1972). Four women aged 19-22 y old were fed various foods as a source of manganese for 27 d. A positive balance (of 0.32 mg) was obtained after an intake of 2.78 mg/d. Several metabolic studies conducted after this work obtained results close to this value. Also, *The Total Diet Study* (Pennington et al. 1989) conducted in the United States between 1982 and 1986 showed that the average of the intake of manganese was 2.7 mg/d for men and 2.2 mg/d for women. On the basis of the report by WHO (1973) that stated that adverse effects were seen in people consuming 8-9 mg/d, NRC suggested that the occasional intake of about 10 mg/d will not cause any adverse effects. Also,

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because of the efficiency with which humans exert homeostatic control over manganese concentrations, NRC rationalized the extra margin in the ESADDI of 10 mg/d for occasional intakes but recommended a concentration of 2-5 mg/d to provide a margin of safety on the 10 mg/d.

The ESADDI is also consistent with the U.S. Total Diet Study (Pennington and Young 1991), which summarizes the average daily dietary intakes of nutritional elements from 1982 to 1989. Furthermore, studies by Greger (1999) on differences in manganese status between individuals consuming a Western diet and a vegetarian diet indicated that there were no adverse effects in subjects consuming a diet containing manganese at 10.9 mg/d. This somewhat supports the extra allowance in the NRC's ESADDI. Freeland-Graves (1994) suggested a concentration of 3.5-7 mg/d.

EPA has determined an oral reference dose (RfD), that was not based on an observable toxicity end point. EPA based the values on the NRC (1989) recommendations and used the safe dose of 10 mg/d. Thus, an RfD for food of 0.14 mg/kg/d based on a nominal adult weight of 70 kg (10 mg/70 kg = 0.14 mg/kg/d) was derived. In determining the concentration for drinking water, EPA recommended the use of a factor of 3 to account for differences in exposure from food compared to water, assuming that absorption of manganese would be more from water than from food. Also considered is the exposure of neonates, who can absorb more manganese from formula milk. In spite of the very well known adverse neurologic effects of manganese exposure, EPA did not arrive at a regulatory enforceable value (MCL) for manganese in drinking water. Only a SMCL of 0.05 mg/L was issued for "discoloration." manganese was not on the list of drinking water priority pollutants because it is abundant in natural food and there have not been a considerable number of documented cases of high manganese concentrations in distributed water. Also, there was no convincing evidence of the prevalence of manganese-induced toxicity from drinking water in the general population. EPA derived an oral RfD only as a health advisory.

As stated earlier, the Food and Nutrition Board of the National Academy of Sciences (IOM 2001) recommended an AI of 2.3 mg/d for men 19+ y of age and 1.8 mg/d for women 19+ y of age. IOM (2001) also recommended a UL of 11 mg for both men and women 19+ y of age.

ATSDR, while recognizing that manganese is beneficial and essential at low concentrations and could be toxic to neurologic systems at high concentrations, did not derive a minimal risk level (MRL) for acute-, intermediate-, or chronic-duration exposure. Quantitative data

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were not available to derive an acute-duration oral MRL. Because no threshold levels were identifiable in the intermediate-duration experiments, no intermediate MRL was set. A chronic MRL was not derived, because the human epidemiologic studies leave a significant amount of uncertainty regarding exposure levels.

For NASA's AC calculations, it was decided not to use studies with manganese dioxide. It lacks relevance in drinking water because of its extremely poor solubility in water. The results from animal studies that used these oxides in the feed indicate that manganese from this source is systemically absorbed and stored in tissues. Secondly, it was decided not to use data from neonates because it is clear that neonates absorb manganese to a much greater extent than adults and lack development of the blood-brain barrier. Therefore, in infants, higher concentrations will be found in the brain, one of the target organs. Although several changes in neurotransmitters (an initial increase in DA followed by a longer-term decrease) in response to manganese ingestion are very similar in rodents and humans, there have been reservations about the validity of extrapolating rodent neurotoxicity data to humans. As stated earlier, rodents have very low levels of neuromelanin in the highly pigmented regions of the brain.

A survey of literature of manganese toxicity strongly indicates increased absorption of inhaled manganese compared with ingested manganese, hypersusceptibility of infants and neonates (from the lack of development of the blood-brain barrier, resulting in higher uptake of manganese by the brain) and elderly persons, and increased absorption associated with iron deficiency. These exposure scenarios will not be applicable to spaceflight crew exposure; data from them will not be useful for deriving a SWEG. Data from studies that used Mn_2O_3 or MnO_2 will not be useful, because of their low solubility in water. For example, as described earlier, Roels et al. (1997), using MnO_2 and $MnCl_2$ and orally administering these manganese compounds to adult rats, observed significant differences in manganese concentrations in blood, liver, and cerebral tissues such as cortex, cerebellum, and striatum. In contrast to $MnCl_2$ given orally, MnO_2 did not increase blood and cerebral manganese concentration to a significant extent, perhaps because of low bioavailability from the lack of intestinal absorption. Thus, pharmacokinetic considerations favor the use of data from studies that used soluble manganese salts (Roels et al. 1997). However, a major issue is deciding the appropriateness of using or extrapolating manganese toxicity data from rodents to humans. According to McMillan (1999), rats can be used as the model because a) the largest database on manganese-induced neu-

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rotoxicity (neurobehavioral effects) is available for rodents, especially the rat; b) in spite of the fact that gross neurologic syndromes are not seen (extrapyramidal syndromes) in rats after manganese administration and the fact that rats do not have pigmentation in the substantia nigra, manganese compounds produce behavioral effects and neurochemical (neurotransmitter) alterations in rats that are similar to those seen in subhuman primates and in humans. For example, the biphasic response reported in monkeys with respect to locomotor activities is similar to those reported in rats. But according to Newland (1999), the effects are not consistent and are influenced greatly by dose and duration. NASA's reservation of using the rat neurotoxic effects data comes mainly from the vast difference in the responses between rodents and primates as excellently summarized by Newland (1999) in a comparative chart depicting various changes in neurotransmitter levels and neurobehavioral effects in rodents and primates as a function of cumulative doses of manganese. This makes it difficult to derive a human equivalent dose. The monkey data can be useful because the nonhuman primates exhibit very similar effects (neurologic symptoms and disorders) seen in humans exposed to high doses of manganese. Rodent studies will still be included in the following discussions.

1-d AC for Ingestion

The human study in which one individual developed pancreatitis after being exposed for 30 min to a hemodialysis solution contaminated with manganese could not be used because it had only one subject, the dose was not known, and the effects were very serious. Also, the individual's health was already compromised, and exposure was via the iv route (Taylor and Price 1982). Other case reports cited in this document on manganese intoxication during total parenteral nutrition also can not be used, not only because of the route of administration, but also because the subjects' health status was compromised.

No acute toxicity data are available from which to derive a 1-d AC. The LD_{50} values cannot be used, because the doses are not concordant with the survival data from the 14-d, 13-wk, and 2-y NTP $MnSO_4$ feed study (1993). It is difficult to determine if the mode of administration (delivery by diet or by gavage) could explain the differences in the LD_{50} and the survival of animals exposed through the diet. Comparing the mortality data from the LD_{50} dosage studies and the long-term NTP diet

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study (1993), it seems the availability of manganese from a single bolus and from small doses may be very different.

It was decided to use the observations from human subject manganese nutrition balance studies to derive ACs for acute and short durations.

Freeland-Graves and Lin (1991) conducted a human subject study in which six young adults were administered manganese as $MnCl_2$ at 40 mg as a supplement in a gelatin capsule, and plasma uptake of manganese was measured over 4 h. The peak concentration of manganese in plasma was about 110 ng/L. This amount of manganese was without any discomfort to the GI system of the subjects. In this study, no other clinical or neurologic parameters were measured. The Mn-B data from this study was compared with data reported from a community study by Mergler et al. (1999), who determined neurologic parameters in subjects whose Mn-B ranged from 2.5-15.9 µg/L (median: 7.3 µg/L). Neurologic outcomes were examined with respect to Mn-B. The authors reported that subtle manganese-related neurologic outcomes were evident at Mn-B concentrations above 7.5 µg/L. In one case, total Mn-B was measured, whereas in the other case, plasma manganese was measured. The concentration in the Freeland-Graves and Lin study was so low that one can conclude that there would not have been any neurologic adverse effect attributed to an acute single dose of 40 mg per subject. Therefore, using 40 mg/d as the NOAEL and 2.8 L/d as the nominal water consumption, a 1-d AC can be calculated as follows:

$$(40\ mg/d) \div (2.8\ L/d) = 14\ mg/L\ (rounded).$$

10-d AC for Ingestion

Human subject studies designed to study manganese absorption and retention were evaluated to derive an AC for 10 d. In a study by Davis and Greger (1992) during a 124-d supplementation study in women (placebo, n = 13; manganese supplemented, n = 11), supplements of manganese at 15 mg/d did not affect levels of hematocrit, serum ferritin, serum transferrin (both indexes of iron status), serum iron, or serum copper. These did not change over time for up to 124 d when measurements were made on days 1, 25, 60, 89, and 124. Even in the manganese status, as measured by the lymphocyte manganese-superoxide dismutase, an increase was seen only after 89 d of administration of the supplement. Thus, an amount of 15 mg/d can be identified as a NOAEL. Even though

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the total numbers of subjects are ≤100 and NASA usually applies a "low n" factor if a human study indicates only a NOAEL and not a LOAEL (NRC 2000), we decided not to use the factor, because the results show that there is enough margin of safety between 10 and 89 d. Thus, a 10-d NOAEL will be 15 mg/d.

A 10-d AC for hematology and iron and copper status end points can be derived as follows:

$$(15 \text{ mg/d}) \div (2.8 \text{ L/d}) = 5.4 \text{ mg/L (rounded)}.$$

A second study that was considered for deriving the 10-d AC is that of Finley and co-workers (Finley 1999). In the first study designed for evaluating the interaction of iron and manganese, Finley did not find any changes at the end of 60 d in nonpregnant women consuming a diet containing manganese at either 0.7 or 9.5 mg/d. Hematocrit, hemoglobin (g/L), number of erythrocytes (cells/L), white blood cells, and platelets were measured. This study indicated that a total intake of 9.5 mg/d was without any effects, a NOAEL for 60 d. However, neurologic indices were not measured in this study.

In another study conducted recently, Finley et al. (2003) evaluated the effect of two concentrations (0.8 and 20 mg/d) of manganese supplementation on manganese status in healthy nonsmoking premenopausal women (n = 17).[1] These manganese concentrations are usually present in a mixed Western diet. They also studied the influence of type of dietary fat on manganese status. In addition, data for several clinical parameters such as activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, concentrations of ammonia, bile acids, and serum bilirubin—indicators of biliary function—glucose, glucose tolerance tests, insulin, iron-binding capacity, and indicators of manganese and iron status were collected at the end of the dietary period. Manganese status was measured by lymphocyte manganese superoxide dismutase activity. Most indicators of manganese and iron status were not affected by dietary manganese. Biliary function was unaffected by diet and was normal in all subjects. Additionally, dietary manganese did not affect the activities of antioxidant enzymes (glutathione peroxidase, cata-

[1]The 17 women made up two study populations. The first (n = 11) included women who ate all meals and participated in testing procedures in the metabolic ward. The second (n = 6) included women who lived in the metabolic ward for the duration of the study.

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lase, and copper-zinc superoxide dismutase in whole blood) (Finley et al. 2003).

Neurologic and psychological tests were carried out to determine possible effects on psychomotor and behavioral function during the last week of the dietary exposure. The authors concluded that manganese intake in the range of 0.8-20 mg/d for 8 wk was efficiently managed in the human system by the manganese homeostasis mechanisms, because these doses did not affect any neurologic measures and had only minor effects on psychological variables. Clinical examination did not reveal any signs of neurologic impairment. No interaction was found between ingested manganese and any measure of point or line steadiness (steadiness assessment) within the same-type-fat-diet group (Finley et al. 2003). Thus, the highest amount of manganese at 20 mg Mn/d can be identified as a NOAEL for 10-d.

A 10-d AC for no liver, hematologic, or neurotoxic effects can be calculated as follows:

$$(20 \text{ mg/d}) \div (2.8 \text{ L/d}) = 7 \text{ mg/L (rounded)}.$$

A Japanese study by Kawamura et al. (1941) was also considered for deriving a 10-d AC. A group of six Japanese families (about 25 people) was exposed for approximately 2-3 mo to high concentrations of manganese that leached into their drinking water from an adjacent manganese battery storage area where about 300 batteries had been buried. Manganese at a concentration of 14 mg/L was measured in the well water when the subjects became sick. Because the initial well-water samples were taken 1 mo after the incident, the concentration of manganese to which the subjects were exposed was estimated to be 28 mg/L. Some of the exposed individuals exhibited muscle rigidity and tremors and mental disturbances similar to those seen in manganism. Not all in the group were affected, but two individuals died. These data could not be used because of several confounding factors such as the presence of high concentrations of other metals associated with batteries, including nickel. Effects were seen only in older individuals.

Some rodent studies were evaluated but were not used for deriving the 10-d AC in preference to available data from human studies.

In the 14-d NTP (1993) study in which male and female rats and mice ingested $MnSO_4$ in the diet (0-1,275 mg/kg/d for rats and 0-3,560 mg/kg/d for mice), the number of leukocytes and segmented neutrophils increased in male rats and in female rats only at the highest dose. Male rats seemed to be more sensitive, and a NOAEL of 84 mg/kg/d for an

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increase in leukocytes and a NOAEL of 665 mg/kg/d for an increase in neutrophils can be identified. A gross comparison of such changes with the changes noted at 13 wk in the 13-wk study and at the 9-mo interim evaluation point of the 2-y study at similar doses indicates that the changes in hematologic parameters are transient or adaptive. In addition, the increases in leukocytes and segmented neutrophils may be too non-specific as toxicologic indicators to be considered for AC derivation. Thus, the data were not used for deriving the 10-d AC. Mice did not show any notable changes in hematology.

In this study, the authors reported that female rats of all treated groups had diarrhea during the second wk. This may have been because of some GI disturbances. The lowest dose for this effect was manganese at 84 mg/kg/d. Male rats were not as sensitive in that only the 50,000 ppm group exhibited this effect. Because a similar effect was not reported in the 13-wk study in which rats were exposed to some similar doses (0, 1,600, 3,130, 6,250, 12,500, and 25,000 ppm), using diarrhea as an end point for 10-d AC derivation was not justified.

Behavioral and neurochemical changes were studied in ITRC albino rats exposed daily to $MnCl_2$ for 14 and 30 d at 1 mg/mL in drinking water—at an estimated dose of 140 mg/kg/d (Chandra 1983). In the treated rats, hyperactivity was seen after 14 and 30 d, with greater magnitude of alteration in the latter period. Only one dose was used in this study. In preference to the human subject data, these rodent data were not used for AC derivation.

Bonilla (1984), using two different doses of manganese, reported similar results that male Sprague-Dawley rats (150-250 g body weight) given manganese as $MnCl_2$ at 0.1 or 5 mg/mL (about 10 and 500 mg/kg/d, respectively) in drinking water for 8 mo showed a significant increase in spontaneous motor activity in the first mo at both doses. Thereafter, from months 2-7, the activity returned to normal. However, during the eighth mo, the rats exhibited hypoactivity. Both hypo- and hyperactivity were not dose-dependent. The later changes in activity were consistent with the marked decrease in DA (Bonilla and Diez-Ewald 1974). The total activity also changed in a similar manner. In another study that measured motor activity, Nachtman et al. (1986) exposed rats to $MnCl_2$ at 1 mg/mL in drinking water (estimated dose of manganese at 30 mg/kg/d) for 65 wk, and locomotor activity was tested at weekly intervals from week 1 to week 13. Locomotor activity was increased during weeks 5-7 and returned to normal at 8 wk. Only one dose was used. These studies and studies reported by others indicate that the earliest effect of manganese ingestion is the hyper motor activity. All

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these studies reported early hyperactivity at low doses of Mn; however, two of the studies, those of Chandra (1983) and Nachtman et al. (1986), used only one dose. Bonilla's 1984 study showed that although two doses were used, the magnitude of the changes were not dose dependent (dose rate was not proportional to dose). Similar results for the concentration of some neurochemicals in the brain have been described earlier in this document. Therefore, NASA decided not to use these data for deriving a 10-d AC.

Another rodent study that was evaluated for 10-d AC derivation was that of Dorman et al. (2000). In this study, the authors evaluated changes in the distribution of manganese in several regions of the brain and neurotoxicity in adult CD rats after gavage doses of $MnCl_2 \cdot 4H_2O$ at 0, 25, and 50 mg/kg body weight for 21 d. In this study, Dorman et al. (2000) measured spontaneous motor activity, using an automated photo beam activity system and used passive avoidance tests to assess learning and memory. Additionally, observations of the pulse-elicited acoustic startling reflex and numerous other functions were made (see Dorman et al. 2000 for details). There were no statistically significant effects related to manganese exposure on motor activity. A significant decrease, observed in the overall mean acoustic startle amplitude elicited in the 25 mg/kg group, was not seen in rats of 50 mg/kg, thus showing a lack of dose-related response. Therefore, for motor activity, a calculated dose rate of manganese at 14 mg/kg/d seems to be a NOAEL. Similarly, there were no significant changes in striatal DA and DOPAC or in the concentrations of HVA or serotonin (Dorman et al. 2000) in any of the dosed groups compared to controls. A 10-d AC can be calculated using a NOAEL of 14 mg/kg/d for neurotoxicity and a species factor of 10 and 2.8 L/d as daily water consumption:

$$(14 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 35 \text{ mg/L}.$$

Although the authors had performed extensive evaluations and more than one dose had been used in contrast to many previous studies, the AC derived will not be used, because of uncertainties associated with deriving human equivalent concentrations.

The other study that was considered for setting a 10-d AC was that of Joardar and Sharma (1990). Male Swiss albino mice were administered $MnSO_4$ by oral bolus for 3 wk at doses of 33, 66, and 198 mg/kg. Total chromosomal aberrations (CA) and breaks per cell (BC) in bone marrow of mice (n = 5) showed significant increases at 7, 14, and 21 d. An evaluation of micronucleus (MN) formation in bone marrow erythro-

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cytes showed that MN percent was significantly elevated in both polychromatic and normochromatic erythrocytes. The animals received only two doses within an interval of 24 h, and the slides were prepared 6 h after the second dose. The committee had concerns that the authors had only presented the total chromosomal aberrations without including the specific nature of the aberrations, such as chromatid gaps, breaks, fragments, isochromatid gaps, chromatid exchanges, and double minutes. Also, there was no change as a function of duration of a particular dosing. Because of limited confidence in the data, it was not considered for calculating AC.

The changes in reproduction toxicity parameters observed in this study were also evaluated for 10-d AC derivation (Joardar and Sharma 1990). Male adult Swiss mice received doses of manganese (as $MnSO_4 \cdot H_2O$) as a gavage for 5 d, and the animals were killed 35 d after treatment to remove the caudate epididymis. The dose rates were 33, 66, and 198 mg/kg/d. The percentage of abnormal sperms increased in all treated groups as a function of dose. A NOAEL could not be identified for sperm head abnormalities.

The NTP (1993) study in which both male rats and male mice were fed diets containing manganese as $MnSO_4$ at much higher doses than used in the Joardar and Sharma study (about 1,300 mg/kg for 14 d) did not find any change in weight, gross morphology, or histology in the reproductive organs at the end of 14 d or at the end of 13 wk. NTP (1993) did not measure sperm head abnormalities; the rodents were exposed to manganese via the diet; and the mouse species studied were different from that used in the Joardar and Sharma study. The magnitude of the effects is questionable. In the Joardar study, $MnSO_4$ was given orally as a single bolus. Several articles in the literature report that inhalation exposure to high concentrations of manganese oxide produced decreased fertility, sperm counts, and testosterone in young animals. although some results cannot be directly compared, the effects reported by Joardar and Sharma were too pronounced after just five doses of $MnSO_4$ administered to adult mice. Also, the percent of abnormal sperm in the untreated control mice seems to be low for mice. The dose-response curve is also quite steep. Because of limited confidence in the data, the study could not be used for deriving 10-d AC.

100-d AC for Ingestion

There are no human studies designed directly to evaluate the toxicity of ingested manganese. However, there are data from human-subject

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manganese balance studies that can be evaluated for a 100-d AC. Numerous rodent studies have been conducted to assess manganese toxicity by the oral route (drinking water, diet, and gavages), several of which had used developing animals.

First, the 13-wk data from the NTP (1993) study, which used $MnSO_4$ at 0-25,000 ppm in the diet, were evaluated for deriving a 100-day AC. The decreased body weights and significantly lowered absolute and relative liver weights could not be used for AC derivation without an abnormal histology being reported. The increased hematocrit and erythrocytes seen in male rats at the end of 13 wk were not seen in the 9-mo interim evaluation, so the effect seems somewhat transient. Significant increases in neutrophils and decreases in lymphocytes were not seen at 9 mo. Similarly, changes seen in the female rats at 13 wk were not seen at later times. Because of the transient nature of these changes, the data were not used for AC derivation.

Inhibition of DA hydroxylase and MAO, and altered DA and serotonin concentrations in certain regions of the brain were reported by Subhash and Padmashree (1991) for albino male rats exposed to $MnCl_2$ at 12 mg/kg/d in drinking water for 90 d. A significant decrease in the hippocampus but a significant increase in midbrain DA levels indicates that the effect of manganese is region-specific in the brain. However, these neurochemical changes by themselves could not be used as specific functional deficiencies in the treated rats. The profile of changes attributed to manganese in neurotransmitters is quite complex and depends on duration and dose rate. Because of the biphasic nature of these changes and their direct relation to motor activity, this study, which lacks a dose and duration response, was not used for calculating an AC for 100 d.

The other study that was considered for the 100-d AC was the study by Wassermann and Wassermann (1977), in which rats received $MnCl_2$ at 200 ppm in drinking water for 10 wk (12 mg/kg/d). The investigators reported ultrastructural changes in liver morphology that included an increased amount of rough endoplasmic reticulum, a proliferated smooth endoplasmic reticulum, and prominent golgi apparatuses in the biliary area. These data were not considered for AC derivation because of the very low sample size (n = 3) of the treated group.

The following human data were evaluated. Kawamura and coworkers (1941) reported a study of members of five Japanese families who were exposed to high concentrations of manganese in drinking water from wells contaminated with materials from several hundred dry batteries buried nearby. Twenty-five cases of manganese poisoning were reported, with symptoms of lethargy, increased muscle tonus, tremor,

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and other symptoms. Three individuals died. The concentration of manganese in water samples was estimated retrospectively to be 28 mg/L at the time of exposure. Analysis of the severity of symptoms with respect to the age of the exposed subjects indicated that the older the individual, the more severe the symptoms were. Children were not affected. Autopsy results from one subject who died indicated high concentrations of brain manganese and microscopic changes in the globus pallidus. The length of exposure to manganese was estimated to be 2-3 mo. The neurologic symptoms of manganism or PD develop over a longer period (long latency period), so that in spite of the autopsy findings, it is questionable if the effects seen in this case are entirely because of manganese in the water. They may have been caused by other contaminants leaching from the batteries. At least one of the contaminants was zinc, which seemed not to be related to the symptoms seen. Thus, this study could not be used for 100-d AC calculations.

In order to derive a 100-d AC for manganese, the doses used in the nutrition balance and excretion studies that employed moderate durations were used. For example, Davis and Greger (1992), in their studies to identify indexes for manganese status, carried out a study in 47 nonsmoking young women (aged about 25 y and weighing about 60 kg) supplemented with manganese at 15 mg/d for 124 d. The authors did not observe any changes over time for up to 124 d when measurements were made on days 1, 25, 60, 89, and 124 of hematocrit, serum ferritin, serum transferrin (both indexes of iron status), serum iron, or serum copper. Even for the manganese status as measured by the lymphocyte manganese-superoxide dismutase, an increase was seen only after 89 d of administration of the supplement. Davis and Greger (1992) did not report any toxic symptoms or any obvious neurobehavioral changes. It must be noted that no neurologic tests were done in this study. Therefore, manganese at 15 mg/d can be considered a NOAEL for 100 d. Because observations for critical parameters were made at 124 d, no time extrapolation factor is needed. However, the number of subjects who were given manganese supplements was only 11, and thus, a factor for uncertainty may be required on the NOAEL. This factor will be equal to $10\sqrt{11}$, which is 3.02. An additional consideration is that the investigators used subjects of 60 kg body weight. So in terms of dose, the actual NOAEL will be 15 mg/d divided by 60 = 0.25 mg/kg/d, which will be used in the calculation.

Thus, the 100-d AC for no adverse effects on critical hematology variables and also on iron status can be derived using a NOAEL of

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0.25 mg/kg/d, 70 kg as the nominal body weight, 2.8 L/d as the nominal water consumption, and 3.02 as the NOAEL uncertainty factor:

$$(0.25 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times 3.02) = 2 \text{ mg/L (rounded)}.$$

In a study on the interaction of iron and manganese, Finley (1999) administered diets that contained manganese at 0.7 or 9.5 mg/d to healthy nonpregnant women. The authors did not find any changes in hematocrit, hemoglobin (g/L), number of erythrocytes (cells/L), white blood cells, and platelets at the end of 60 d in women consuming either diet. This study indicated that a total intake of 9.5 mg/d was without any effect. Neurologic indices were not measured in this study (Finley 1999). A NOAEL of 9.5 mg/d for at least 60 d can be identified. As the Davis and Greger study indicated, a higher NOAEL for a longer time would be preferred.

Recently, Finley et al. (2003) evaluated the effect of two concentrations (0.8 and 20 mg/d) of manganese supplementation in healthy non-smoking premenopausal women (n = 17) with a mean age of 35.7 ± 8 y and a mean body weight of 72.9 ± 13 kg. This study has been discussed in detail earlier in the section on a 10-d AC for ingestion. The authors concluded that manganese intake in the range of 0.8-20 mg/d for 60 d was efficiently managed by the body's homeostatic control mechanism for manganese. Neurologic and psychological tests were carried out to determine possible effects on psychomotor and behavioral effects. Measurements of the severity of more than 75 neurologic signs and symptoms included measures used for manganese intoxication and for PD (for details see Finley et al. 2003). Clinical examination did not reveal any signs of neurologic impairment. No interaction was found between ingested manganese and any measure of point or line steadiness (steadiness assessment) within the diet group ingesting the same type of fat.

In addition, data for several clinical parameters (activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase; ammonia, bile acids, and serum bilirubin concentrations (indicators of biliary function); glucose, glucose tolerance tests, insulin, iron binding capacity, and indicators of manganese and iron status) were collected at the end of the dietary period. Manganese status was measured by lymphocyte-manganese superoxide dismutase activity. Most indicators of manganese and iron status were not affected by dietary manganese. Biliary function was unaffected by diet and was normal in all subjects. Dietary manganese also did not affect the activities of antioxidant enzymes (glutathione peroxidase, catalase, and copper-zinc superoxide dismutase)

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in whole blood. This study indicated that manganese at 20 mg/d could be identified as a NOAEL for at least 60 d. The strength of this study is that extensive adverse health-related indices were measured, including the psychological parameters. However, for this subchronic study, it may be necessary to apply a "low n" factor for uncertainty to the NOAEL, because this study did not identify a LOAEL (NRC 2000). Thus, a factor for uncertainty on the NOAEL will be equal to $(10/\sqrt{6})$, which is about 4, where 6 is the number of subjects used for this particular protocol. This study was carried on for up to 60 d, and one might need a time extrapolation factor of 100 d/60 d, which is 1.67. In this case, the uncertainty factor of 4 can be used as a combined factor, because it will be protective of the time extrapolation factor of 1.67.

Thus, using a NOAEL of 20 mg/d, 2.8 L/d as the nominal drinking water volume, and a combined factor of 4 for duration and uncertainty on NOAEL, a 100-d AC can be calculated as follows:

$$(20 \text{ mg/d}) \div (2.8 \text{ L/d} \times 4) = 1.8 \text{ mg/L (rounded)}.$$

Mean age and body weight of women used in the Finley study were 35 y old and 70 kg, respectively; therefore, there is no need to use any adjustment factor for body weight.

1,000-d AC for Ingestion

A few human studies were considered for AC derivation for 1,000 d. Ejima et al. (1992) reported a case study of manganese intoxication in a 62-y-old man who received total parenteral nutrition that provided manganese at 2.2 mg/d for 23 mo (690 d). He developed symptoms characteristic of PD. This study could not be used for deriving the 1,000-d AC, because it involved only one subject, the subject's health was already compromised, and the dose was given intravenously. Extrapolation to an oral dose cannot be done with enough certainty, taking into account only the mean human absorption of manganese by the oral route.

The second study considered was the Kondakis study, which was based on epidemiologic studies in northwest Greece (Kondakis et al. 1989). Three different ranges of manganese concentration were found in the drinking water of three areas, called areas A, B, and C in order of increasing concentration of manganese (see Table 9-9).

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TABLE 9-9 Summary of Manganese Concentrations in Water from Different Areas

Area	Number of Samples	Range of Manganese Concentration (µg/L)	Mean Manganese Concentration (µg/L)
A	62	3.6-4.6	9
B	49	81.6-252.5	167
C	77	1,800-2,300	1,950

Source: Data from Kondakis et al. 1989.

The presence and intensity scores for 33 neurologic symptoms indicated that the manganese-W in area C could be considered a LOAEL and manganese-W in area B a NOAEL. The duration of exposure is assumed to be 10-50 y, because the individuals selected for this study were over 50 y old (both sexes were included). The authors communicated to EPA that the median concentration of manganese in the water of Area B was 167 µg/L. Assuming that this is a NOAEL, the AC will be 0.167 mg/L. The mean manganese concentrations in hair were 3.51, 4.49, and 10.99 µg/g dry weight of hair for areas A, B, and C, respectively. According to the authors, there was a good correlation of manganese concentrations in hair with manganese concentrations in the water of the corresponding area. The age range of subjects was narrow.

There are, however, some drawbacks to the way the data were collected, and much uncertainty existed. The dietary manganese status of the selected population is not known. The effects may have been because of consumption of water over several years, and there is no history of how the concentrations changed with time. The neurologist was not blinded, because the sequence of data collection was not randomized. The exposure time varied widely, from 10-50 y. Neurologic signs of aging may have significantly confounded the study. The data collection was done only once. Some of the neurologic symptom variables included in the data collection usually vary with the time of day and probably biased the results. The neurologic scores had a wide range, with considerable overlapping among the groups. For example, for both sexes, the ranges of values were 0-21 for area A, 0-43 for area B, and 0-29 for area C. A nonparametric statistical analysis using Mann-Whitney, Kruskal-Wallis, and Jonckheere tests was used. No data on medical history or the use of medications were collected in this study.

The third set of data considered for the 1,000-d AC is that of Vieregge et al. (1995) who conducted a neurologic assessment of two

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TABLE 9-10 Summary of Manganese Concentrations in Water in Two Areas in Vieregge et al. (1995) Study

Group Area	Number of Subjects	Sex	Mean Age ± SD (Years)	Age Range	Manganese Concentrations in Water
A	41	Male (21) Female (20)	57.5 ± 10.3	41-84	<0.05 mg/L
B	74	Male (41) Female (33)	56.9 ± 11.8	41-86	0.3 to 2.16 mg/L

Note: In the Kondakis et al. (1989) study, the mean ages ranged from 65.4 ± 6.3 to 67.6 ± 8.4 for areas A, B, and C.
Source: Data from Vieregge et al. (1995).

communities in northern Germany, in which manganese in drinking water water was 0.05 mg/L and 0.3-2.16 mg/L. A brief summary of the details are in Table 9-10.

The mean age of subjects in the Vieregge et al. (1995) study is at least 10 y younger than in the Kondakis et al. (1989) study. In this study, during the neurologic evaluation, the clinical investigator was blinded to the manganese exposure concentration. The study used not only a structured questionnaire but also a validated neurologic examination, tests using instrumentation for fine motor performance, and a CURS for signs of PD. The authors reported that there were no neurologic effects of exposure, even when the manganese concentrations in the well water were in the range of 0.3-2.1 mg/L for up to 40 y. Because it has the strength of presenting human population data that assessed a critical toxicologic end point, this study was chosen for deriving a 1,000-d AC.

Because we do not back-extrapolate from 40 y to 1,000 d, the AC represents a significant safety margin of 40 y over 1,000 d. Choosing the lowest exposure level of the high-concentration community water, that is, 0.3 mg/L (over the dietary contributions), should eliminate a risk of neurologic effects from manganese. The maximum concentrations of manganese observed in the water samples collected from the previous U.S.-Mir missions did not exceed 0.15 mg/L.

A 1,000-d AC based on a NOAEL for neurologic effects in humans is 0.3 mg/L.

Some observations from the chronic animal manganese-exposure studies on the effect of manganese ingestion were also considered. First, the 2-y NTP (1993) study was evaluated for deriving the 1,000-d AC. The dietary concentrations of $MnSO_4$ were 0, 1,500, 5,000, and 15,000 ppm. The estimated dose rates were manganese at 65, 200, and 615

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mg/kg for male rats and 70, 230, and 715 mg/kg for female rats. There was an increased severity of nephropathy and renal failure in male rats that were exposed to the highest dose (615 mg/kg/d) and died during the course of the study. NTP (1993) did not report whether the lower-dose groups had the same effects. For nephropathy and renal failure, manganese at 65 mg/kg/d can be considered a NOAEL for renal effects. The adenomas and carcinomas were within NTP (1993) historical controls for rats of this age and thus were not taken into consideration.

A 1,000-d AC for renal effects can be calculated as follows:

(65 mg/kg/d × 70 kg) ÷ (10 × 2.8 L/d) = 163 mg/L (rounded),

where
65 mg/kg/d = NOAEL;
70 kg= nominal body weight;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption.

In both male and female mice in the 2-y NTP (1993) study, significant increases in focal hyperplasia of the forestomach and ulceration and inflammation were reported in the highest-dose group (15,000 ppm, corresponding to a maximum of manganese at 731 mg/kg/d). No such changes were reported in the lower-dose groups. A NOAEL of manganese at 175 mg/kg/d, based on the lowest dose for male mice, was identified.

A 1,000-d AC for GI-system effects can be calculated as follows:

(175 mg/kg/d × 70 kg) ÷ (10 × 2.8 L/d) = 437 mg/L (rounded),

where
175 mg/kg/d = NOAEL;
70 kg = nominal human body weight;
10 = species extrapolation factor; and
2.8 L/d = nominal water consumption.

An AC was also calculated based on the adverse effect on the thyroid glands of manganese-exposed animals. In mice, at the end of the 2 y of exposure to $MnSO_4$ in the diet, the incidence of follicular dilatation increased significantly in 15,000 ppm dosed males (manganese at 585 mg/kg) and in 5,000 ppm (228 mg/kg/d as manganese) and 15,000 ppm (731 mg/kg/d manganese) females (NTP 1993). A significantly increased

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incidence of focal hyperplasia of follicular epithelium also occurred in the 15,000 ppm dosed males and in all manganese-fed females. The number of follicular cell adenomas found in some males and females fed manganese at 15,000 ppm was not considered significantly different from that of controls (NTP 1993). This is shown in Table 9-11.

Female mice seem to be sensitive to thyroid lesions from manganese exposure. Thus, the calculation of the AC for 1,000 d was based on data from female mice only. Factors of 70 kg for nominal body weight, 10 for species extrapolation, and 2.8 L/d for nominal water consumptions were used. A time factor to extrapolate to 1,000 d was not used.

A 1,000-d AC for follicular dilatation based on a $BMDL_{01}$ of manganese at 8.4 mg/kg/d is derived as follows:

$$(8.4 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 21 \text{ mg/L}.$$

A 1,000-d AC for follicular hyperplasia using a $BMDL_{01}$ of manganese at 17.5 mg/kg/d is derived as follows:

$$(17.5 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 44 \text{ mg/L (rounded)}.$$

A manganese toxicity study with nonhuman primates was also considered for the 1,000-d AC (Gupta et al. 1980). A group of four monkeys administered manganese at 6.9 mg/kg/d as an oral bolus for 18 mo

TABLE 9-11 Thyroid Lesions in Mice in 2-y NTP Study

Lesion	LOAEL (mg/kg)	NOAEL (mg/kg)	$BMDL_{01}$ (mg/kg)
Male Mice			
Follicular dilatation	585	175	12
Follicular cell hyperplasia	585	175	18.5
Female Mice			
Follicular dilatation	228	65	8.4
Follicular cell hyperplasia	65	Not known	17.5

Note: The response data were also processed using the benchmark dose (BMD) approach using the EPA BMD software. $BMDL_{01,}$ the effective dose or the benchmark dose corresponding to the 95% lower confidence limit of 1% response (benchmark response level = 1%), was used. The LOAEL and NOAEL are included for informational purposes only.
Source: Data from NTP 1993.

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developed muscular weakness and rigidity of the lower limbs. Histologic analysis showed degenerated neurons in the substantia nigra and scanty neuromelanin granules. Because this study was done in primates, who are the best available models for humans, especially for neurotoxic outcomes of manganese, these data may be quite suitable. The confirmation by histology is strength. The biggest drawback is that only one dose and only four monkeys per group were used. A survey of literature strongly indicates that neurologic effects and all characteristics of neurotoxicity typically seen in manganese-exposed workers have been documented in primate models, in spite of the fact that most of the primate model studies involved administration of the dose via iv or sc (Olanow et al. 1996; Newland 1999). Additionally, the use of primate data is well justified because Eriksson et al. (1987b), who studied the effects of manganese dioxide (long-term sc injections) on monkeys, concluded that manganese-exposed monkeys revealed a response pattern very similar to humans, as judged by a combined eurochemical, histologic, and neurophysiologic evaluation. Also, Eriksson et al. (1992) observed that manganese-induced brain lesions, as measured by PET scans and MRIs, in *Macaca fascicularis* monkeys were very similar to those reported in humans exposed to excess manganese via occupational exposures, total parenteral nutrition with excessive or contaminated with manganese, and hepatobiliary disease (see Lucchini et al. 2000).

1,000-d AC based on neurotoxicity is equated as follows:

$$(6.9\ \text{mg/kg/d} \times 70\ \text{kg}) \div (10 \times 3 \times 2.8\ \text{L/d} \times [1{,}000\ \text{d} / 540\ \text{d}]) = 3\ \text{mg/L (rounded)},$$

where
6.9 mg/kg/d = LOAEL;
10 = LOAEL to NOAEL;
70 kg = nominal body weight;
3 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
1,000 d/540 d = time extrapolation factor from 18 mo to 1,000 d.

A summary of ACs and SWEGs for various durations is listed in Table 9-12.

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TABLE 9-12 Acceptable Concentration (ACs) and SWEGs for Manganese

			Modificaton Factors				AC (mg/L)				
Toxicity End Point	Exposure	Species	To NOAEL	Species Factor	Time Factor	Spaceflight Factor	1 d	10 d	100 d	1,000 d	Primary Study
No adverse effects (human subjects)	NOAEL= 40 mg/d	Human	1	1	1	1	14	—	—	—	Freeland-Graves and Lin 1991
No adverse effect on hematologic parameters or iron or copper status	NOAEL = 15 mg/d	Human	1	1	1	1	—	5.4	—	—	Davis and Greger 1992
No adverse effect on neurologic parameters or serum clinical chemistry	NOAEL = 20 mg/d	Human	1	1	1	1	—	7.0	—	—	Finley et al.2003
No abnormal hematologic parameters or iron or copper status	NOAEL = 0.25 mg/kg/d	Human	3.02 for low *n*	1	1	1	—	—	2	—	Davis and Greger 1992
No adverse neurologic effects or serum clinical chemistry	NOAEL = 20 mg/d	Human	4 for low *n*	1	1	1	—	—	1.8	—	Finley et al. 2003
Nephropathy and renal failure	NOAEL = 65 mg/kg	Rats	1	10	1	1	—	—	—	163	NTP 1993
Forestomach hyperplasia and inflammation	NOAEL = 175 mg/kg	Rats	1	10	1	1	—	—	—	437	NTP 1993
Thyroid lesions: follicular dilatation	$BMDL_{01}$ = 8.4 mg/kg	Mice	1	10	1	1	—	—	—	21	NTP 1993

(Continued)

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TABLE 9-12 Continued

			Modificaton Factors				AC (mg/L)				
Toxicity End Point	Exposure	Species	To NOAEL	Species Factor	Time Factor	Spaceflight Factor	1 d	10 d	100 d	1,000 d	Primary Study
Thyroid lesions: follicular cell hyperplasia	$BMDL_{01}$ = 17.5 mg/kg	Mice	1	10	1	1	—	—	—	44	NTP 1993
Neurotoxicity (histopathologic lesions, muscular weakness, rigidity of lower limbs)	LOAEL = 6.9 mg/kg	Primates	10	3	1,000 d/ 540 d	1	—	—	—	3.0	Gupta et al. 1980
Neurotoxicity (absence of adverse neurotoxic indices)	NOAEL = 0.3 mg/L	Human	1	1	1	1	—	—	—	0.3	Vieregge et al. 1995
SWEG							14	5.4	1.8	0.3	

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10

Total Organic Carbon

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GOALS OF A TOTAL ORGANIC CARBON STANDARD

Because NASA probably will not have the real-time capability to quantify all individual organics that might break through the water recovery system (WRS), a spacecraft water exposure guideline (SWEG) for total organic carbon (TOC) will have to serve as a first-line screening parameter for water quality. For this screening purpose, an upper limit SWEG for TOC is proposed rather than guidelines for different exposure periods, so the approach taken to estimating the SWEG is different than for individual chemicals. That approach and the assumptions made are detailed below.

Compliance with the SWEG for total organic carbon will accomplish the primary goal of ensuring, with a high degree of confidence, that the crew is not exposed to potentially harmful chemicals as they consume processed water over a period of time up to 100 days (d). TOC is the total measured mass of carbon per unit volume in a water sample minus the carbon present from carbon dioxide and bicarbonate. Secondarily, the standard may ensure that the water is readily palatable; although, the standard will not guarantee that the water will have no taste or odor. If the crew discovers that the water is not readily palatable, they can mix in flavoring or recycle it through the water recovery system. The crew should not have their water consumption discouraged by water that is not readily palatable.

We will assume that any reactions between organic carbon compounds and other components of the processed water will be limited to concentrations that do not pose an *indirect* health concern. For example, organic carbon may act as a substrate for microbial growth, indirectly

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threatening crew health; conversely, the presence of disinfectants such as chlorine, and possibly iodine, can form toxic compounds in the presence of organic carbon (Miettinen et al. 1996). These considerations are mitigated by the fact that TOC will be measured *in situ* soon after the sample is acquired. Hence, there will be insufficient time for these secondary reactions to occur before the measurement is taken. Thus, in creating a standard, we will not attempt to compensate for microbial growth due to the presence of organic carbon, nor will we be concerned with chemical reactions that could increase the toxicity of the water.

ASSUMPTIONS

To meet the primary goal stated above, the WRS must be bounded, or known, in a number of ways. The input water, which can come from humidity condensate, urine, or makeup sources (e.g., water brought up from the ground or obtained through fuel cells), must be reasonably well characterized so that, knowing the elements of the processing system, one can predict the most likely organic components to break through to the product water.

We will assume that the WRS will be taken offline under conditions where the load may *knowingly* exceed capacity or might damage the processor's capability. For example, the WRS would not process humidity condensate immediately after a serious fire or after leakage of certain air pollutants such as ammonia from the U.S. Laboratory Module of the International Space Station (ISS).

We will assume that microbial control is accomplished with iodine or silver and that there will be no mixing of the product water with other water that may have an organic residue from a biocide. Specifically, we will assume that none of the water for analysis has originated from an ethanol tincture of iodine.

We will further assume that any other treatment of the post-process water will not involve an addition of organically contaminated water. For example, we will assume that the process of adding minerals does not involve the use of an organic counter ion such as formate. We will assume that any mixing in of ground-supplied water will not provide contamination from unusual pollutants (for example, pesticides and chloroform). Such additions to the product water totally confound the interpretation of the TOC measurement. Alternatively, quantification of the confounding compounds would enable the calculation of a TOC measurement that could be compared to the standard.

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Finally, we will assume that the measurement of TOC is performed within a short period of time after the sample collection, so that there is no question about the decay of TOC as the sample is retained. For example, microbial metabolism could alter the concentration of TOC if the sample is not kept cold or treated with a biocide. The U.S. Environmental Protection Agency (EPA) requires analysis of a water sample for TOC immediately, or within 28 d if the sample is refrigerated and acidified to a pH < 2 (Clesceri et al. 1998).

COMPOSITION OF ORGANIC CARBON IN RECOVERED WATER

Ground-Based Testing

High-fidelity, ground-based testing of a WRS can be helpful when predicting the organic components of processed water. Far more data can be obtained from these tests than from ones performed in space; the input load to the processor can be controlled, and supporting data can be taken at various points within the WRS. In addition, ground-based data can be acquired quickly before chemical concentrations such as TOC are changed by the presence of microorganisms.

One such test lasted for 128 d at the Marshall Spaceflight Center and involved input from many sources, including humidity condensate, urine, and personal hygiene (Carter 1997). During the test, the nominal concentration of TOC in processed water was between 0.2 and 0.5 milligrams per liter (mg/L); however, an excursion to 1.6 mg/L was observed on day 7 when a software anomaly allowed intermediate processor water to be mixed into the product water tank. A smaller anomaly of 0.6 mg/L was noted on day 9 of the test. The average concentration of TOC during the test was 0.30 mg/L, and the TOC component averages were as follows: 2-propanol, 0.14 mg/L; acetone, 0.12 mg/L; ethanol, 0.16 mg/L; and methanol, 0.21 mg/L, accounting for 100% of the TOC concentration. The concentrations of each of these components in the water were well below concentrations that would pose a health risk.

In another series of tests conducted at Johnson Space Center, humans were placed in a closed environment for up to 91 d, and water was recovered from all available sources, including condensate, urine, hygiene water, and wash water. The first phase used tap water, the second phase used physiochemical recovery, and the third phase used physio-

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TABLE 10-1 TOC in Recovered Water

Test	Duration (d)	Lowest TOC (mg/L)	Highest TOC (mg/L)	No. of Samples Taken	Comments
Phase I	15	0.24	0.43	2	Public water supply
Phase II	30	0.10	0.24	7	Humidity condensate and reprocessed urine
Phase IIa	60	0.14	0.53	51	Was most like ISS processor
Phase III	91	0.06	0.29	45	Biologic recovery system added

Abbreviations: ISS, International Space Station; No., number.
Source: Data from Pierre et al. 2002.

chemical and biologic recovery systems (Pierre et al. 2002). The TOC ranges were as shown in Table 10-1. The TOC was managed to prevent the crew from drinking water in which the concentration of TOC was above 0.5 mg/L, which was the standard at that time. Eleven times during Phase IIa, the recovered water was reprocessed because the TOC concentration exceeded 0.5 mg/L (Pierre et al. 2002). The most consistently found organic compounds during the recovery tests (Phases II, IIa, and III) were acetone, toluene, and formaldehyde. Typically, they were found at about 10 micrograms (µg) per liter or less in the samples. The percentage of identified components in the TOC concentration was very low. Other low-molecular-weight compounds found occasionally in processed water and their maximum concentrations were as follows: 2-propanol, 175 µg/L; methanol, 274 µg/L; bis-2 ethylhexyl phthalate, 28 µg/L; methyl sulfone, 54 µg/L; oxalate, 410 µg/L; lactate, 1,100 µg/L; urea, 300 µg/L; 4-methyl-2-pentanone, 47 µg/L; and 2-methyl-2,4-pentanediol, 34 µg/L.

Mir

During the late 1990s, the National Aeronautics and Space Administration (NASA) participated in a cooperative program with Russia in the operation of their space station Mir. As part of that program, seven samples of hot, regenerated water from the Mir WRS during missions Mir 18 and Mir 19 were tested in ground-based laboratories for TOC and or-

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ganic components. In five of the samples, the range of TOC concentrations was 1.5-5.4 mg/L (Pierre et al. 1996). The components that exceeded 0.01 mg/L in any of the samples were as follows: acetone, 3/5; chloroform, 1/5; benzothiazole, 2/5; 2-methylthiobenzothiazole, 2/5; *N*-phenyl-2-naphthylamine, 1/5; and formaldehyde, 3/5. Acetone and formaldehyde were the most prevalent of these individual components. The percentage of the identified individual components composing the TOC was very low in these samples, typically only 3-6%. The samples were not evaluated for potential changes in components from the date of sampling until analysis in the laboratory.

In the final report of the Mir Phase 1 project, the potable water pollutants were provided in order of decreasing average concentrations (Pierre et al. 1999). It is essential to note that the potable water consisted of reprocessed water supplemented with water obtained from municipal ground sources and on-orbit sources that had magnesium and calcium formate salts added, as well as an iodine tincture containing ethanol. In decreasing order, the 10 highest averages in the potable water were as follows: formate, ethylene glycol, ethanol, acetate, acetone, chloroform, methanol, di-*n*-butyl phthalate, benzothiazole, and formaldehyde. Ethylene glycol broke through the processor once at a concentration of 46 mg/L because of its overwhelming concentration in the humidity condensate. This resulted in a TOC concentration of 25 mg/L. The addition of a catalytic reactor to the processing system after this problem much reduced the ethylene glycol concentrations in potable water. Chloroform was believed to originate from ground-supplied water.

ISS

Two types of TOC measurements have been taken in water processed for consumption aboard the ISS. An on-board analyzer has been sporadically available to take measurements from freshly obtained samples, and an archival method has been used to sample product water and take ground-based measurements from those samples, which can be several months old by the time they reach the lab. Refrigerator space is not available aboard the ISS, so these samples remain at ambient temperature during the storage period.

Although no formal health or epidemiologic studies have been conducted, major adverse health effects have not been reported during mission debriefings after flights in which the TOC concentrations in ISS water were as high as 30 mg/L (Plumlee et al. 2002). The current standard

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for ISS is 20 mg/L (MORD 2000). Much of the TOC comes from the deliberate addition of formate and, to a lesser extent, residual ethanol in shuttle-derived water. If these contributions are subtracted from the total, then the "corrected" TOC concentrations have ranged from 0.1 to 1.2 mg/L through August 25, 2003, except for a sample obtained in a unique sampler (Straub et al. 2004).

ORGANIC COMPOUNDS AT RISK FOR BREAKTHROUGH

Russian WRS

A scaled-down, ground-based test conducted in Russian laboratories involved injecting actual humidity condensate recovered from shuttle flights STS-89 and STS-91 while docked to the Mir into a model of the Mir WRS (Mudgett et al. 1999). The system was operated until organic carbon compounds began to break through. The initial breakthrough came from methanol, ethanol, acetic acid, ethylene glycol, and propylene glycol. The TOC increased from 1 part per million (ppm), at a cumulative throughput of 100 L, to 25 ppm after a cumulative throughput of 590 L.

Planned U.S. WRSs

Although the U.S. WRS will recover potable water from urine, the Russian and U.S. WRSs are similar. Thus, the breakthrough products would be expected to be similar. On the basis of a personal communication (L. Carter, Marshall Space Flight Center, Huntsville, AL, 2002) predicted that ethanol or methanol would be the most likely to get through the processor, because they are in the humidity condensate and are structurally similar to water. Acetaldehyde and formaldehyde are also possible breakthrough products. Acetone could break through if combustion products enter the humidity condensate, and acetic acid also is a possible early contaminant in the recovered water. Because ethylene glycol is not used in the thermal loops of the ISS, the concerns over its possible leakage into the air, capture in the condensate, and breakthrough of the water purification system are negligible when compared with the situation aboard Mir. The thermal control system of the Russian segment of ISS currently uses a solution of glycerol for heat exchange.

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BASIS FOR THE SWEG FOR TOC

Background on Suggested Water Quality Values for TOC

The limit currently used in ISS operations is 20 mg/L (with formate already subtracted) based on the experience of Russian experts (MORD 2000). This is slightly less than the 25 mg/L limit used in the Mir Phase 1 program. In the past, a standard of 10 mg/L has been proposed for spacecraft for "relatively short crew exposures" (Macler and Cantwell 1993). For the U.S. water processor slated for the U.S. segment of the ISS, the water quality goal has been 0.5 mg/L; however, the origin of that goal cannot be traced to a scientific debate about what the value ought to be. The thinking at the time was that 0.5 mg/L was achievable and would protect against any toxic compounds not removed during urine processing. The value first appeared in a conference report in which it is stated that "if 0.5 mg/L is the maximum allowable TOC, then up to 0.1 mg/L of the organic chemicals present in the final potable water after several recyclings could remain uncharacterized" (Willis 1987).

Our approach to setting a TOC concentration limit includes two facets. The first will be a typical standard defining an upper guideline for taking action if analyses indicate that the guideline has been exceeded. It will be based on the potential breakthrough compounds suggested in Table 10-2. Our second task will be to create a statistically based quality-control guideline that establishes when, if a TOC measurement has unaccountedly risen above the TOC concentrations that preceded it, a response is required. This implies a one-sided test for deviations from the norm.

ESTIMATION OF AN UPPER-LIMIT SWEG FOR TOC

Basically, the task of the WRS is to reduce contaminants in influent water from a TOC concentration of a few hundred mg/L to one that is safe for human consumption. The weight of evidence is that any organic compound that *initially* breaks through the purification beds and increases the TOC concentration is unlikely to pose an immediate threat to crew health. NASA probably will not have the real-time capability to quantify individual pollutants that might break through the WRS, so the TOC measurement will have to serve as a first-line screening parameter for water quality.

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TABLE 10-2 Pollutants in Potable Water from Space Vehicles and Test Beds[a,b]

Pollutant	MSFC Ground Test[c] (average concentration)	JSC Ground Test Phases II, IIa, III[d,e]	Mir Phase 1 Program[d,f]	ISS Expeditions 1-7[d,g]	Exposure Standard: 100-d SWEG (mg/L)	Oral RfD (mg/kg/d) (EPA 2004)
Methanol	0.21	0.27	0.49	0.31	—	—
Ethanol	0.16	—	2.4	16.1	—	—
2-Propanol	0.14	0.18	—	—	—	—
Ethylene glycol	—	—	45.5	—	[23][h]	2
Acetone	0.12	0.03	0.13	0.17	[150]	0.9
Formaldehyde	—	0.02	0.06	0.01	[12]	0.2
Acetaldehyde	—	—	0.05	—	—	—
Caprolactam	—	—	0.03	2.15	[100]	0.5
Lactate	—	1.1	—	0.24	—	—
Oxalate	—	0.41	—	—	—	—
Urea	—	0.30	—	—	—	—
Acetate	—	0.6	1.5	0.14	—	—
Propionate	—	—	—	0.15	—	—
Methyl sulfone	—	0.05	—	0.11	—	—
Phenyl sulfone	—	—	0.08	—	—	—
Dibutyl phthalate	—	—	0.30	—	80	0.1
Di(ethylhexyl) phthalate	—	0.03	0.03	0.05	30	0.02

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N-Butylbenzene sulfonamide	—	—	—	0.11	—	—
Dichloromethane	—	—	0.09[i]	—	40	0.06
Chloroform	—	—	0.18[i]	—	18	0.01
3-*t*-Butyl phenol	—	—	0.11	—	—	—
Cyclododecane	—	—	0.15	—	—	—

[a]Compounds found one or more times at a concentration at or above 0.05. Measurements in mg/L.
[b]A dash signifies that the compound was not measured or detected in water or that no standard has been adopted.
[c]Carter 1997.
[d]Maximum concentration found during test series.
[e]Pierre et al. 2002.
[f]Pierre et al. 1999, Table 4.
[g]Plumlee et al. 2002, 2003; Straub et al. 2004.
[h]Brackets indicate pending SWEGs.
[i]Source was water brought to Mir from the ground.
Abbreviations: ISS, International Space Station; JSC, Johnson Space Center; MSFC, Marshall Space Flight Center; RfD, reference dose.

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The probable breakthrough compounds are small alcohols, aldehydes, and acetone. Of these, formaldehyde is the most toxic with a drinking water exposure level (DWEL) of 7 mg/L (EPA 2004). Ingestion of water contaminated with formaldehyde at a concentration of 7 mg/L for several hundred days appears to pose minimal risk to crew health. The organic carbon derived from this exposure would be 12/30 × 7 mg/L = 2.8 mg/L. A second approach can be to start with the 100-d and 1,000-d SWEGs for formaldehyde of 12 mg/L (see Chapter 7). This is equivalent to 5 mg/L of organic carbon. We rounded up the lower of these numbers to 3 mg/L.

Inspection of Table 10-2 suggests that formaldehyde-contaminated water is the worst-case scenario for elevation of TOC concentration. The chlorine-containing carbon compounds originate from ground sources, and the phthalates come from the holding of the water in plastic pipes. These compounds are extremely unlikely to break through the regeneration system in large quantities. Furthermore, according to SWEG values, formaldehyde is more toxic (potent) than the chlorocarbons or the phthalates. It is possible that the TOC safe concentration limits could change if final values in Table 10-2 differ significantly from those proposed or if new technology comes into use in the space program.

As a comparison to EPA requirements for municipal water supplies, EPA form 5115 is instructive. The final acceptable TOC concentrations are determined in terms of the starting TOC concentrations. Using averages from the starting ranges, the required clean up of water is as follows: a source at 3 mg/L requires a 25% cleanup, a source at 6 mg/L requires a 35% cleanup, and a source at 9 mg/L requires a 40% cleanup. Thus, the product water's TOC concentration would be below 2.25, 3.90, and 5.4 mg/L, respectively. Although the nature of the compounds comprising the TOC will be markedly different in space WRSs, these target values suggest that our TOC safe concentration limit of 3 mg/L is reasonable.

ESTIMATION OF A LIMIT BASED ON CHANGES IN TOC

There clearly are conditions in which the TOC limit is insufficient as an indicator of safe drinking water and in which breakthrough by organic compounds is not impending. For example, if the TOC concentration has been 0.5 ± 0.3 (standard deviation) mg/L, and it suddenly increases to 2.5 mg/L, that could be cause for action. The increase is not necessarily a direct crew health concern; however, it is a condition that if

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left unaddressed, could lead to further degradation in the WRS and, secondarily, to crew health effects.

Statistical process control is an often-used technique to monitor and control technical processes. Basically, periodic samples of the product in question are obtained and analyzed, in this case, for TOC. The TOC values are found to fall within a range that can be defined by statistical properties. By some predetermined criteria, those values can be said to be within the expected random variation inherent in the system and monitoring technology. When a value or values exceed a predetermined criterion, then the system is said to be “out of control,” and an investigation of the cause ensues. The committee recommends that some form of statistical process control be implemented for monitoring TOC concentrations to ensure effective operation of the WRS.

CONCLUSIONS

The upper limit of the standard for a TOC concentration was set at 3 mg/L, assuming that the TOC comes entirely from formaldehyde, which is the most toxic component found at concentrations above 0.05 mg/L in the TOC of potable water. The DWEL for formaldehyde is 7 mg/L, which would contribute 2.8 mg/L to the TOC, and the 100- and 1,000-d SWEGs are 12 mg/L for a contribution of 5 mg/L to the TOC; therefore, taking the lowest of these two numbers, the TOC concentration becomes 3 mg/L (rounded) for ingestions of 100 d. Also it is recommended that a statistical-process control plan be developed to ascertain system performance from TOC measurements.

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11

Zinc and Zinc Salts (Inorganic)

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OCCURRENCE AND USE

Zinc (Zn) is a naturally occurring element found in the earth's crust in most rock-forming minerals. It is also present in significant concentrations in soil near highways (because of emissions and tire wear) and industrial locations such as power plants and factories. In natural waters, zinc exists in several chemical forms. It usually occurs as zinc sulfide, zinc carbonate, zinc chromate, or zinc oxide (ZnO) (Merck Index 1989). Zinc compounds such as zinc chloride ($ZnCl_2$), zinc sulfate ($ZnSO_4$), ZnO, and zinc sulfide are found at hazardous waste sites, and the possibility that they could get into drinking water has been a concern. The acetates, chlorides, and sulfates of zinc are extensively used in the dyeing industry and in common consumer products such as ZnO skin ointments and shampoos. For chemical and physical properties, see Table 11-1.

TABLE 11-1 Chemical and Physical Properties of Zinc and a Few Zinc Compounds[a]

Chemical	Zinc	Zinc Chloride	Zinc Sulfate Heptahydrate	Zinc Oxide	Zinc Acetate
Formula	Zn	$ZnCl_2$	$ZnSO_4 7H_2O$	ZnO	$Zn(C_2H_3O_2)_2$
Molecular weight	66.38	136.29	287.54	81.4	183.46
% of zinc	100%	47.97%	40.5%	80.34%	35.64%
Water solubility	Insoluble	4.32 g/mL at 25 ºC	1.66 g/mL	Insoluble	0.43 g/mL

[a]Data from Merck Index 1989.

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The average zinc concentration in tap water across the United States is 0.245 milligrams per liter (mg/L) (NRC 1980). The highest mean value reported for tap water from galvanized pipes is about 2 mg/L (Sharrett et al. 1982).

Occupational exposure to zinc by means of inhalation occurs extensively in zinc mining, smelting, welding, and the manufacturing of galvanized metals, paints, tires, and certain personal consumer products. $ZnCl_2$ is primarily used in making batteries, zinc silicate in phosphors of cathode ray tubes, and ZnO in the rubber vulcanizing process. Exposure to zinc through drinking water can take place in areas near where these activities occur.

Zinc is an essential food element. Dairy products, grains, meats, fish, and poultry are the richest sources of zinc (Tanner and Friedman 1977). The Recommended Dietary Allowance (RDA) of zinc for non-pregnant women is 12 mg per day (d), and for men it is 15 mg/d. A typical mixed diet provides at least 65-80% of the daily RDA (Bowerman and Harrill 1983). According to the Food and Drug Administration (FDA) Total Diet Study (1991-1997), the mean intake of zinc from food by males between 31 and 50 years (y) of age is 13.38 ± 0.16 mg/d (n = 1,805). Daily intake by women of that age range is 8.51 ± 0.11 mg/d (n = 1,733) (see Appendix E in IOM 2001). Zinc occurs in all living cells as a constituent of metalloenzymes involved in major metabolic pathways (NRC 1989). Zinc controls several enzymes of intermediary metabolism, DNA and RNA synthesis, gene expression, and immunocompetence. Zinc can interact with almost all hormones and plays a significant role in homeostasis of hormones such as thyroid and steroid hormones, insulin, and pituitary hormones like prolactin (Brandao-Neto et al. 1995).

Summary reports on humidity condensates collected from the Mir space station and water recycled from Mir during the years 1995-1998 indicate that zinc was present at concentrations ranging from 1.26 mg/L to 5.3 mg/L in the humidity condensates and at concentrations ranging from 10.4 to 475.0 micrograms (μg) per liter in the processed (recycled) water (Pierre et al. 1999). Although the average concentrations did not exceed the U.S. Environmental Protection Agency's (EPA's) secondary maximum contaminant level (SMCL) of 5 mg/L, zinc was found very frequently in the recycled water. Concerns exist about potential system breakthroughs. This document will be limited to addressing the adverse effects of extraneous zinc that may leach into the drinking water through the water processing system (from distribution lines), the humidity condensate heat exchangers (through corrosion), or as a result of the failure of the ion-exchange resins to remove metal ions completely.

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PHARMACOKINETICS AND METABOLISM

Absorption

The existing studies strongly indicate that absorption of ingested zinc by humans varies widely depending on the type of diet and the presence of dietary components and minerals such as phytates, phosphates, sugars, amino acids, and iron, as well as other metals such as copper and cadmium.

Zinc in animal products has a higher coefficient of absorption than zinc from vegetables. Phytates (IP6 or inositol hexaphosphate) and fiber present in vegetables adversely affect the availability of zinc by reducing its absorption. For example, Sandstrom et al. (1987) determined the absorption of zinc in humans from 60 g meals based on rye, barley, oatmeal, triticale (a cross between wheat and rye), and whole wheat. It was lowest (8.4 ± 1.0%) from oatmeal porridge with a phytic acid content of 600 millimicromoles (mμmoles), and relatively highest (26.8 ± 7.4%) from rye bread meal with a phytic acid content of 100 mμmoles (Sandstrom et al. 1987); thus, absorption of zinc is negatively correlated to phytic acid content. Various numbers from 20% to 40% have been used for the absorption of zinc (NRC 1989). An average zinc intake of 8.6-17.2 mg/d from various diets has been reported (Tanner and Friedman 1977; Holden et al. 1979); this is below the RDA in most cases. After short-term exposures to zinc supplements in the diet, absorption ranged widely from 8% to 81% (Aamodt et al. 1983; Reinhold et al. 1991; Sandstrom 1992). Three days after individuals ingested zinc at 0.05 mg per kilogram (kg) from bread rolls containing different concentrations of proteins, fractional zinc absorption ranged from 8% from low-protein rolls to 26% from high-protein rolls (Hunt et al. 1991), indicating that dietary protein promotes zinc absorption. The fractional absorption of zinc seems to depend on zinc dose. For example, in a group of healthy young men on a constant daily dietary intake of 15 mg/d, when various test doses of zinc were administered, fractional absorption was 81% from a 4.52 mg dose, 67% from a 6.47 mg dose, and 61% from a 24.52 mg dose (Istfan et al. 1983; also see King et al. 2000). A similar phenomenon of changes in fractional zinc absorption in response to changes in the dietary zinc concentrations have been described by several investigators (Wada et al. 1985; Taylor et al. 1991; Lee et al. 1993). Absorption of zinc also seems to depend on the zinc status of the individual. Zinc was absorbed at a higher level by zinc-deficient individuals than by zinc-sufficient individuals (Spencer et al. 1985; Johnson et al. 1988). In a 63-d study conducted with young men, Turnlund et al. (1984) reported that

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zinc absorption from the basal diet was 33.8% ± 2.9%, but it dropped to 17.5% ± 2.5% when 2.3 g of phytate as sodium phytate was added to the basal diet.

Absorption of zinc also seems to depend on the form of the salt. For example, Galvez-Morros et al. (1992) reported that male rats absorbed 40% of labeled zinc from a diet containing zinc as $ZnCl_2$ at 0.81 mg/kg or 8.4% from a zinc-carbonate-containing diet. Zinc uptake from inorganic salts was in the order of sulfate > acetate > chloride > citrate > phosphate (Seal and Heaton 1983). Nevertheless, the total excretion or retention was independent of the salt form. Studies using in vivo ligated intestine and ^{65}zinc indicate that absorption was significantly greater from the duodenum than from the distal portion of the small intestine. In an experiment using an everted sac of rat duodenum and ileum, zinc absorption was shown to be pH dependent. Reducing the pH of the medium from 7.3 to 6.4 decreased absorption from the duodenal sacs.

When a dose of radioactive $ZnCl_2$ was intubated to rats maintained on a diet containing zinc at 40 mg, the maximum radioactivity attained in the whole blood was about 0.09% at 30 minutes (min), 0.045% at 1 hour (h), and 0.01% at 24 h. Liver and pancreas preferentially took up a higher percentage of radioactivity, with a peak uptake at 8 h (Methfessel and Spencer 1973).

A variety of mechanisms have been proposed for the absorption of zinc. It is generally believed that zinc absorption is homeostatically controlled by the zinc content in the intestine and by circulating zinc (Davies 1980; Cousins 1985). Data also indicate that zinc uptake may be partly controlled by a carrier-mediated diffusion mechanism. Cysteine-rich intestinal protein (CRIP), a diffusible intracellular zinc carrier, binds zinc in the mucosa during absorption, a process that seems to be saturable (Hempe and Cousins 1992). Zinc transport in the intestinal lumen is also influenced by metallothionein, which can inhibit zinc absorption by competing with CRIP for zinc (Hempe and Cousins 1991, 1992). It is beyond the scope of this document to discuss the vast amount of literature on various approaches to understanding the absorption mechanism. The interaction of zinc with other metals and the influence of ligands on zinc absorption will be described later.

Distribution

Zinc is present in almost all tissues and body fluids in humans. In blood, zinc is present in erythrocytes (92.4% as a cofactor for carbonic

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anhydrase isoenzymes and superoxide dismutase [SOD]), leukocytes, and platelets. Zinc is found in diffusible and nondiffusible forms in the blood (NRC 1977). About 98% of serum zinc is nondiffusible and is bound to proteins (85% to albumin, most of the remainder complexed with β2-macroglobulin [β2-MG]). Diffusible zinc in blood is associated with albumin and amino acids and not with β2-MG (EPA 1987). Circulating zinc that is tightly bound to α2-macroglobulin in the plasma is nondiffusible and not freely exchangeable with other zinc ligands in serum (Cousins 1985). The range of normal plasma zinc concentrations is 85-110 µg per deciliter (dL). Plasma proteins provide a metabolically active transport compartment for zinc in which about 70% of circulating zinc is loosely bound to albumin in the diffusible form and is freely exchangeable (Cousins 1985). Zinc is also bound to amino acids (primarily histidine and cysteine) as a diffusible form (Henkin 1974). The zinc-amino-acid complex is transported passively across tissue membranes to bind to proteins. An important binding protein in the kidney and liver is metallothionein, although other tissue-binding proteins may be present. The body of a 70 kg normal human male contains 1.4-2.3 g of zinc (see Table 11-2).

High concentrations of zinc are also found in the prostate (100 µg/g wet tissue), semen (100 to 350 picograms [pg] per L) (Hidiroglou and Knipfel 1984), and retina (Bentley and Grubb 1991; Ugarte and Osborne 2001). With age, zinc concentrations increase in the liver, pancreas, and prostate and decrease in the uterus and aorta (Schroeder et al. 1967). Only 3% of zinc was transferred across the perfused placenta and seemed to involve potassium/zinc transport (Aslam and McArdle 1992). Zinc does not accumulate with continued exposure, because the body burden is controlled by homeostatic mechanisms. Studies in animals and humans have shown that the whole-body content of zinc remains constant over a 10-fold range of intakes (Johnson et al. 1993). The homeostatic mechanism acts mainly to adjust the gastrointestinal (GI) absorption and endogenous excretion (Wastney et al. 1986; Walsh et al. 1994; King et al. 2000). Increasing dietary concentrations of zinc were associated with decreasing concentrations of iron in the livers of rats (Yadrick et al. 1989). In the liver, as well as elsewhere, zinc is bound to metallothionein. The greatest concentration of zinc in the body is in the prostate, probably because this organ is rich in the zinc-containing enzyme acid phosphatase (see Klaassen 1996). About 60% of the zinc in the body is located in the skeletal muscle and 30% in the bones (Wastney et al. 1986).

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TABLE 11-2 Typical Tissue Concentrations of Zinc in Normal Human Adults

Tissue	Zinc (μg/g tissue)	Tissue	Zinc (μg/g tissue)
Prostate	100	Pancreas	29
Muscle	54	Spleen	21
Kidney	55	Testes	17
Liver	55	Lung	15
Heart	33	Brain	14
		Adrenals	12

Note: The total body burden (70 kg body weight assumed) of zinc is estimated to be 1.4-2.3 g. The total amount in the skin is estimated to be 0.46 g. In erythrocytes, zinc exists as a cofactor for carbonic anhydrase and SOD.
Source: EPA 1990.

Excretion

Fecal excretion is the predominant route of elimination of zinc after an oral bolus (Davies and Nightingale 1975; Wastney et al. 1986; Reinhold et al. 1991). Only 1-2% is excreted in the urine (Wastney et al. 1986). In normal adults, daily excretion is 300-600 μg/d. A linear excretion of zinc in the feces as a function of dose has been noted (Spencer et al. 1985). Excretion of zinc in the urine also reflects zinc intake (Wastney et al. 1986). Rats receiving zinc as $ZnCl_2$, $ZnSO_4$, zinc phosphate, or zinc citrate at 2.65 mg/kg/d over a 4-d period excreted 87-98% of the intake. Fecal excretion, total excretion, and retention of zinc did not differ for these various zinc forms (Seal and Heaton 1983).

A small amount of zinc has also been shown to be excreted by way of bile as a complex with reduced glutathione, and the transfer from liver to bile occurs by a glutathione-dependent process (Alexander et al. 1981). Low dietary intake of zinc, starvation, and high-protein diets alter the excretion of zinc (Spencer et al. 1976); nevertheless, a homeostatic mechanism maintains the balance by a greater absorption of zinc (Henkin et al. 1975; Hunt et al. 1991).

Interaction of Zinc with Other Metals

Many biologic interactions between trace elements are known to occur, especially when they are present together in the diet. With zinc being an essential trace element in a wide variety of biologic systems,

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interactions of other metals with zinc are very critical for physiologic and pathologic conditions. The trace element interaction of greatest practical significance in human nutrition is the negative effect of excess zinc on copper bioavailability (Festa et al. 1985). One of the most studied modifiers of zinc absorption is the co-absorption of copper. High concentrations of dietary zinc have an antagonistic effect on the absorption of copper, and this phenomenon has been demonstrated in humans and animals. Although dietary intakes of copper and zinc do not interfere with each other's absorption as long their ratio is 1:5 (copper:zinc), higher zinc concentrations in the diet (as with zinc supplements) depress copper absorption, concentrations of tissue copper, and the activity of copper enzymes such as ceruloplasmin and cytochrome oxidase. In zinc-deficient animals, copper concentrations in bones and liver are increased (Roth and Kirchgessner 1977). Excess copper in the diet inhibits zinc absorption from a zinc-sufficient diet, but the effect is relatively minor compared to the effect of excess zinc on copper status (O'Dell 1989). Excess zinc stimulates an increase in the intestinal concentrations of metallothionein, which traps copper because of its high affinity to copper, leading to copper loss when the intestinal cells slough off. Hypocupremia and hypoceruloplasminemia in sickle cell anemia patients who ingest supplementary zinc are well known (Prasad et al. 1978). Similarly, L'Abbe and Fischer (1984a, b) reported that when copper status (as assessed by measuring the concentration of the copper-transport protein serum ceruloplasmin) and activity of copper-zinc SOD were determined in rats fed zinc at 15, 30, 60, 120, or 240 mg/kg in their diet for 6 weeks (wk), the number of animals with low ceruloplasmin increased with increasing doses of zinc. The control diet contained copper at 6 mg/kg and zinc at 30 mg/kg. At 120 and 240 mg/kg of zinc, copper-zinc SOD decreased significantly. The copper-zinc SOD reductions seen in animals fed a high-zinc diet were similar to those in animals fed a low-copper diet. Because the elimination of excess zinc is slow and the intestinal absorption of copper will be affected until excess zinc is eliminated, the slow elimination rate is an important aspect of zinc interactions with other trace elements.

Iron and calcium are important examples of other metals that interact with zinc, because these are frequently used as dietary supplements. Iron inhibited zinc absorption when both were given in inorganic form without food (Solomons and Jacob 1981; Valberg et al. 1984). Metallothionein seems to play an integral part in these interactions. Large amounts of ingested iron (such as from iron supplements) affect the absorption of zinc (Solomons et al. 1983) and may lead to zinc deficiency, which is associated with poor growth, loss of appetite, skin lesions, lack

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of taste and smell, delayed wound healing, delayed sexual maturation, onset of night blindness, impaired memory performance, and impaired immune response. Calcium and zinc have an antagonistic relationship. Experiments in animals indicate that if the intake of calcium is high, the absorption of zinc is decreased, and vice versa (Hanson et al. 1958; Yamaguchi et al. 1983). In vivo, it has been documented that oral administration of zinc to sickle cell anemia patients reduced the number of irreversible sickle cells (Brewer 1979). In this instance, zinc seems to interact with calcium at the red cell membrane by suppressing the calcium-regulating protein calmodulin (Baudier et al. 1983), and this suppresses the formation of irreversible sickle cells.

Interaction of zinc with cadmium results in an increase in the excretion of cadmium when the two elements are administered together. This has been proposed as a mechanism by which zinc protects against cadmium toxicity (Stowe 1976; NRC 1980). Because zinc and cadmium compete for a common transport mechanism, simultaneous administration of zinc and cadmium has beneficial effects on cadmium toxicity (Coogan et al. 1992). Zinc acetate has been shown to prevent cadmium carcinogenesis in the prostate and testes (Waalkes et al. 1989). Similarly, co-administration of zinc with cobalt resulted in a major reduction of cobalt-induced testis tubule damage and degeneration in mice (Anderson et al. 1993). Exposure to cadmium affects the distribution of zinc, leading to preferential accumulation of zinc in liver and kidney and negatively affecting zinc concentrations in other tissues. Because cadmium and zinc additively increase metallothionein induction, they may adversely affect the absorption of other metals.

TOXICITY SUMMARY

Zinc plays an important role in growth and many physiologic functions. It is an essential nutrient, and the RDA values (the estimated amount of zinc required to maintain tissue and the growth and metabolism of the individual) range from 12 mg/d for nonlactating and nonpregnant women to 15 mg/d for adult men. Consumption of concentrations of zinc below the RDA has been reported to lead to loss of appetite, loss of taste and sense of smell, and slow healing of skin sores. Retarded growth and development of reproductive organs and retarded development of offspring have been noted in humans. Zinc is present in blood plasma, erythrocytes, leukocytes, and platelets but is chiefly localized within erythrocytes (where 87% of it is in carbonic anhydrase, the major

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binding site) (Ohno et al. 1985). A vast amount of data pertaining to zinc toxicity is available, and a significant portion of the data is from human case reports and human subject experiments, mainly from zinc-supplementation studies. A significant amount of data pertaining to the oral administration of zinc to rodents is also available. Most studies have addressed changes in hematologic parameters and changes in serum high-density lipoprotein (HDL) cholesterol.

A review of the literature clearly indicates that ingestion of a large amount of soluble zinc compound by humans or animals for an intermediate-to-long duration results in a variety of adverse effects in the GI, hematologic, immunologic, and nervous systems. Some of the key effects reported are decreases in serum HDL cholesterol, hematocrit, hemoglobin, and serum ferritin concentrations, as well as an impact on copper balance, anemia, and lesions in the adrenals, pituitary, and pancreas. Severe GI distress and bleeding were found only in acute cases exposed to high doses of zinc. These effects were not seen with small doses in the long-duration experiments. Occasional renal and reproductive toxicity has also been observed.

Acute Exposures

Callender and Gentzkow (1937) reported that 80% of the two army companies experienced diarrhea and GI distress after drinking limeade prepared in galvanized trash cans. The average dose of zinc ingested was estimated to be about 7 mg/kg. Within 24-48 h after ingesting zinc-contaminated food (2.4-6.8 mg/kg) from galvanized containers, 300-350 persons developed intestinal symptoms such as severe diarrhea with abdominal cramping. About 50% had gross blood in the feces (Brown et al. 1964). In another episode, individuals who had consumed zinc-contaminated alcoholic fruit punch developed a hot taste and dryness in the mouth, nausea, vomiting, and diarrhea between 20 and 90 min post-ingestion. The symptoms resolved in 24 h. In the postacute phase, the individuals reported general discomfort and muscular pain. The estimated dose was 4.6-9.2 mg/kg (Brown et al. 1964). Several studies have reported that zinc ingestion causes GI distress. An individual who had drunk 3 ounces of liquid $ZnCl_2$ (dose not known) immediately suffered throat pain, burning and pain in the mouth, and vomiting. Later, acute symptoms of pancreatitis were noted (Chobanian 1981). A schoolgirl suffered abdominal cramps and diarrhea after she ingested a zinc-sulfate-containing capsule ($ZnSO_4$ at 440 mg/d) prescribed for acne. She suf-

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fered from epigastric discomfort and fainting and also showed serious signs of intestinal bleeding (Moore 1978).

The acute oral LD_{50} (the dose lethal to 50% of test subjects) value for $ZnSO_4$ in rats was reported by Fabrizio (1974) to be 920 mg/kg; acute oral LD_{50} values for $ZnCl_2$ in rats, mice, and guinea pigs are 350, 502, and 200 mg/kg, respectively (Calvery 1942; see also EPA 1990). In a study by Domingo et al. (1988), four zinc compounds (acetate, nitrate, chloride, and sulfate) were administered as a single gavage dose to rats and mice. The LD_{50} values are summarized in Table 11-3.

The majority of deaths occurred during the first 2 d (Domingo et al. 1988). In general, mice seem to be more sensitive than rats to the lethal effects of zinc. In rats and mice, zinc acetate was the most lethal compound tested.

The adrenal cortex is rich in zinc. In animals, zinc deficiency increased plasma 11-hydroxy steroids, and excess zinc decreased the same steroids (Quarterman and Humphries 1979). To understand the effect of excess zinc on adrenal function in humans, 13 subjects (males and females, 20-27 y old) were orally administered zinc (as the sulfate hepta hydrate) at 0.25, 37.5, and 50 mg after a 12-h fast. Plasma cortisol was measured in serial blood samples collected from these subjects for up to 240 min. Each individual served as his or her own control. An acute inhibition of cortisol secretion was observed (Brandao-Neto et al. 1990). Hypothalamus and hypophysis are rich in zinc, and in earlier studies designed to understand the role of zinc in functions mediated by these areas, it had been shown that in vitro addition of zinc to bovine pituitary extracts inhibited the secretion of newly synthesized prolactin (Login et al. 1983; Judd et al. 1984). For this reason, Brandao-Neto et al. (1995) wanted to evaluate the adverse effects of excess zinc on the regulation of prolactin in humans. They reported an inhibition of basal prolactin secretion in 17 normal adult men and women given oral doses of zinc as the sulfate at 0, 25, 37.5, and 50 mg. Serum prolactin was measured at sev-

TABLE 11-3 LD_{50} for Rats and Mice of Four Zinc Compounds

Compound	Rat LD_{50} (mg/kg/d)	Mouse LD_{50} (mg/kg/d)
Zinc acetate	237	86
Zinc chloride	528	605
Zinc nitrate	293	204
Zinc sulfate	623	337

Source: Domingo et al. 1988. Reprinted with permission; copyright 1988, American Academy of Veterinary and Comparative Toxicology.

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eral intervals over 2 h. However, in later studies from the same labortory (Castro et al. 1999, 2002), the authors concluded that in humans, supplementation of oral zinc at 25 mg/d for 3 months (mo) did not change the basal secretion of prolactin. A 2-y-old child who ingested $ZnCl_2$ solution (zinc at 1,000 mg/kg) developed lethargy (Potter 1981). A 17-y-old male ingested about 85 tablets, each with 4 g of zinc gluconate (elemental zinc at 570 mg). He experienced severe nausea and vomiting within 30 min of the ingestion but had no further serious effects such as diarrhea, gastric erosion, esophageal burns, shock, neurologic dysfunction, symptoms of anemia, or hepatic inflammation. His serum zinc concentration was 4.97 mg/dL about 5 h after ingestion (Lewis and Kokan 1998).

Short-Term Exposures (2-10 d)

A 16-y-old boy who ingested 12 g of elemental zinc over a 2-d period (zinc at 86 mg/kg/d) experienced light-headedness, lethargy, staggering gait, and difficulty writing legibly but no apparent GI disturbances (Murphy 1970). Anemia secondary to GI hemorrhage was seen in a case report study of acute exposure to zinc as $ZnSO_4$ at 2.6 mg/kg/d (Moore 1978) given for 1 wk as a medically prescribed treatment for acne.

In rats administered oral doses of zinc at 0.1, 1, or 10 mg/100 g of body weight (zinc at 1, 10, or 100 mg/kg) for 3 d, a significant decrease in femoral calcium was seen with the 100 mg/kg dose (Yamaguchi et al. 1983). These effects were seen as early as 1 d. In addition, a significant decrease in acid phosphatase in the femoral epiphysis was seen in the zinc-treated group. The result that zinc causes a decrease in bone calcium may be very important because it indicates that zinc may trigger bone resorption. This observation is relevant to space missions for which bone resorption has been a concern.

In rats administered zinc as ZnO in their diet at 487 mg/kg/d for 10 d, minor neuron degeneration and proliferation of oligodendroglia and increased amounts of secretory material in the neurosecretory nuclei of the hypothalamus were observed, indicating possible effects of zinc on the central nervous system (Kozik et al. 1980, 1981).

Subchronic Exposures (10-100 d)

Hooper et al. (1980) conducted a study in which 12 healthy nonobese adult males aged 23-35 y old received oral pharmacologic doses of zinc as $ZnSO_4$ at 160 mg/d in capsules (zinc at 2.46 mg/kg/d) for 5 wk. A

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25% reduction (from 40.5 to 30.1 mg/dL) in serum HDL cholesterol at week 5 and at week 7 (2 wk after completion of the study) was noted. Total serum cholesterol levels were unchanged. This study may indicate a lowest-observed-adverse-effect level (LOAEL) of zinc at 2.46 mg/kg/d, although only one dose was used and the effects were seen even 2 wk after the study was completed (Hooper et al. 1980). HDL cholesterol levels returned to normal 11 wk after dosing ceased. There was only a weak relationship between the rise in plasma concentrations of zinc and the fall in HDL cholesterol levels. Similar results were not observed in the double-blinded crossover design study by Samman and Roberts (1988) in women (discussed below).

Chandra (1984) reported that 11 healthy adult males who ingested $ZnSO_4$ as tablets (containing zinc at 150 mg) twice a day for a dose of zinc at 4.4 mg/kg/d for 6 wk showed elevated ($p < 0.05$) serum low-density lipoprotein (LDL) cholesterol and a 32% reduction ($p < 0.001$) in HDL cholesterol (during weeks 4 and 6). Serum copper was not measured. Chemotaxis and phagocytosis of bacteria by polymorphonuclear leucocytes were impaired (Chandra 1984). In addition, the authors reported a decrease in the lymphocyte stimulation response to phytohemagglutinin (PHA). These adverse effects returned to baseline 10 wk after dosing ceased. This study indicates a LOAEL of zinc at 4.4 mg/kg/d for serum lipoprotein profile and immunologic response. In this instance, the changes in the immune system variables may be an important factor because the reversal response to dose cessation was very slow. Zinc plays an important role in the immune system as a cofactor of thymic thymulin and in the lymphocyte response to mitogens. In the above-mentioned study, no effects were seen on the number of lymphocytes or the relative number of T cells. The National Research Council (NRC) committee on spacecraft water exposure guidelines (SWEGs) recognized the various weaknesses in the Chandra study such as a lack of information about volunteer history, the potential for other factors that can influence the observed effects, interpretation flaws, and small sample size. Fischer et al. (1984) reported that in 13 subjects who received two daily doses of zinc as zinc gluconate at 25 mg each (a total of 50 mg/d) for 6 wk, a significant decrease in the enzyme activity of erythrocyte superoxide dismutase (ESOD) activity, indicative of impaired copper status, was observed.

In another study (Samman and Roberts 1988), 47 healthy volunteers (26 women and 21 men) took part in a double-blind crossover trial that lasted 12 wk. The subjects ingested elemental zinc at 50 mg ($ZnSO_4$ at 220 mg) or placebo, three times per day (a total of 150 mg/d) for 6 wk.

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Eighty-four percent of the women (mean age 27 y, n = 26) and 18% of the men (mean age 28 y, n = 21) reported symptoms, which included headaches, abdominal cramps, nausea, loss of appetite, and vomiting. Six female volunteers discontinued the trial, five due to gastric irritations. An average dose of zinc at 2.46 mg/kg/d seems to be a LOAEL for these symptoms. A 24% reduction in the antioxidant activity of ESOD was observed in treated female but not male subjects (Samman and Roberts 1988). The authors concluded that this difference in response might be caused simply by the difference in size between males and females, who received the same total amount of supplemental zinc. This supplementation also resulted in a decrease in LDL cholesterol and a slight decrease in HDL cholesterol in females (n = 26). No changes in hematocrit were observed. In a study by Freeland-Graves et al. (1982), eight healthy adult women per group ingested zinc acetate as a dietary supplement at 0, 15, 50, or 100 mg/d for a dose of 0.45, 1.03, or 1.86 mg/kg/d (plus daily dietary zinc intake at 0.2 mg/kg) for 8 wk. Significant differences in plasma HDL cholesterol were observed only in the group receiving the highest dose (Freeland-Graves et al. 1982). The HDL cholesterol level for the 1.86 mg/kg/d group transiently decreased by 8.4% at week 4 and returned to its normal level at week 6. The decrease in HDL cholesterol coincided with a peak in plasma zinc (at week 4), which declined toward the initial value thereafter. The slight decrease of 5.4% in plasma HDL cholesterol (65 to 59 mg/100 mL) at a does of zinc at 0.45 mg/kg/d was not statistically significant. Thus, the reduction in HDL cholesterol was transient and may not be related to dose. In a 10-wk zinc-supplementation study (Yadrick et al. 1989), 18 women ingested zinc as zinc gluconate (zinc at 50 mg/d [1 mg/kg/d]), and a significant decrease in hematocrit, serum ferritin, and ESOD activity was seen. By the end of 10 wk, the ESOD activity had decreased to 53% of control levels. A similar significant decrease in ESOD activity was also reported in men receiving zinc gluconate for 6 wk (50 mg/d). A decrease in ESOD, which is related to the status of copper, and a significant decrease in serum ferritin and hematocrit values indicated a significant health risk involving the iron status of women (Yadrick et al. 1989).

A 12-wk double-blind study was conducted on white males (Black et al. 1988) to determine the effect of zinc supplementation given orally (zinc as zinc gluconate at 50 mg/d [n = 13] or zinc at 75 mg/d [n = 9]) on serum lipid fractions. Serum was fractionated and cholesterol was determined in various fractions at week 0 and at the end of 2, 4, 6, 8, 10, and 12 wk. Zinc supplements had no more effect on serum total cholesterol, LDL cholesterol, very-low-density-lipoprotein (VLDL) cholesterol, or triglycerides than did the placebo. One-way analysis of variance with

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repeated measures indicated that a significant time-by-treatment effect existed for serum HDL cholesterol: serum HDL cholesterol levels of subjects in the 75 mg/d group were significantly lower (about 15%) at weeks 6 and 12 than the HDL cholesterol concentrations of the placebo group and lower than their own baseline levels. The subjects in the 50 mg/d group had lower serum HDL cholesterol concentrations than the placebo group at week 12. However, statistical analysis of the ratio of HDL cholesterol to total cholesterol or HDL to LDL cholesterol did not show significance for treatment or interaction. For changes in HDL cholesterol, a LOAEL of zinc at 75 mg/d (1.09 mg/kg/d) and a NOAEL of zinc at 50 mg/d (0.85 mg/kg/d) can be identified in this study.

Bonham et al. (2003) evaluated short-term (2 wk) and subchronic (14 wk) effects of zinc supplements (30 mg/d as zinc Chelazome, a zinc glycine chelate) on the immune function and copper status of male subjects (n = 19) with a mean age of 35.6 y. The effect on immune function was evaluated by measuring circulating concentrations of peripheral blood leucocytes (total and differential) and lymphocyte subsets (CD3+, CD19+, and CD3−) in blood samples collected at 0, 2, and 14 wk. The dietary intake of zinc was about 10 mg/d. Copper status was determined by measuring plasma ceruloplasmin oxidase activity and ceruloplasmin concentration in addition to serum copper. Activity of SOD was determined in whole blood. No effect of zinc supplementation on circulating concentrations of leukocytes or lymphocyte subsets or on copper status could be observed. It must be noted that markers of immune status such as phagocytic activity of neutrophils and blastogenic response of cell types to T cells were not measured in this study. The authors also stated that the changes that were evident in the concentrations (number of cells/L) of the lymphocyte subset populations were independent of zinc supplementation. The authors concluded that a total of zinc at 40 mg/d (10 mg from diet and 30 mg from zinc supplement) can be estimated to be without any effect, a NOAEL, in agreement with the tolerance level for zinc recommended by the Institute of Medicine (IOM), Food and Nutrition Board, 2001. But the treatment was for a short duration of only 14 wk, and therefore such a conclusion may be premature.

Animal Studies

Death was reported in mice that consumed zinc as $ZnSO_4$ at 1,110 mg/kg/d in their diet intermittently for 13 wk (Maita et al. 1981). Mortality was also observed in 20% of rats ingesting zinc as zinc acetate at 191 mg/kg/d in drinking water for 3 mo (Llobet et al. 1988).

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Aughey et al. (1977) studied the effects of ingestion of zinc via drinking water on the histology and ultrastructural alterations of the pancreas, adrenal cortex, and the adenocorticotrophic hormone (ACTH)-secreting cells of the anterior pituitary gland in mice. Male and female C3H mice (total n = 150) drank water containing $ZnSO_4$ (0.5 g/L) ad libitum for up to 12 mo. Treated animals were as healthy as controls throughout the study (Aughey et al. 1977). Dietary zinc supplementation did not affect plasma insulin and glucose concentrations. However, histologic examination showed that the zinc-supplemented groups had ultrastructural changes in the individual beta cells of the pancreas, and the cells appeared larger. In the zinc-supplemented mice, the adrenal cortex was thicker than in controls. At 3 mo, hypertrophy of the adrenal cortex and highly positive lipid staining (histochemistry for cholesterol reaction) were seen; at 6 mo or longer, the glomerulosa and reticularis zones of the adrenals also gave strongly positive reactions for lipid staining. Zona fasciculata cells are responsible for the secretion of glucocorticoid hormones such as cortisol. A decrease in cortisol secretion indicates that this area has been damaged. This observation seems to be very similar to that reported in humans by Brandao-Neto et al. (1990). Morphologic changes consistent with hyperactivity were seen in the pituitary in groups that ingested zinc. No significant difference in zinc content between the control and zinc-supplemented groups was observed for the liver, spleen, or skin over a 6-mo observation period (no sex difference was observed with respect to the zinc content of these tissues). Only one dose was used in the mouse study. The dose can be estimated to be zinc at 70 mg/kg/d. This is the only study available that indicates serious adverse effects on beta cells of pancreatic islets, hypertrophy of the adrenal cortex, and hypertrophy of the pituitary. No effects on the pancreas were noted in the 13-wk Maita et al. (1981) study in Wistar rats and ICR mice, even at much higher doses of zinc at 565 mg/kg/d in rats and 1,110 mg/kg in mice. An increase in serum amylase and lipase was observed in C3H mice administered $ZnSO_4$ in drinking water, indicating damage to pancreatic acinar cells (Aughey et al. 1977), which produce amylase, lipase, and other enzymes.

In the 13-wk study of mice that received zinc as $ZnSO_4$ at 1,110 mg/kg/d in the diet, Maita et al. (1981) also reported increased relative and absolute kidney weights in female but not in male mice. The authors identified a LOAEL of 1,110 mg/kg for the hematologic effects (decreased hemoglobin, hematocrit, and erythrocyte concentrations) and a NOAEL of 104 mg/kg/d for both mice and rats for these effects. In an old study, Smith and Larsen (1946) reported that when rats were given zinc carbonate in drinking water for either 5 wk at a dose of 350 mg/kg/d

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or for 6 wk at 500 mg/kg/d, reduction in hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH, hemoglobin amount/red blood cells) and mean corpuscular hemoglobin concentration (MCHC) were reduced. A NOAEL was not identified in these studies.

In a study by Zaporowska and Wasilewski (1992), 2-mo-old Wistar rats of both sexes received $ZnCl_2$ in drinking water at a concentration of 0.12 mg/mL (12 mg/kg/d) for 4 wk. Statistically significant differences were found for less food and water consumption and lower body weight in the treated groups than in the untreated group. Also, a statistically significant decrease in the erythrocyte count and hemoglobin concentration in the peripheral blood was recorded. When these rats received zinc as $ZnCl_2$ at 12 mg/kg/d in drinking water, the percentage of reticulocytes and polychromatophilic erythrocytes increased in the peripheral blood after 4 wk of exposure. No change was observed in the composition of the bone marrow cells (Zaporowska and Wasilewski 1992).

A subchronic study was carried out with young female Sprague-Dawley rats by administering zinc acetate in their drinking water at 0, 47.5, 95, or 190 mg/kg body weight for 3 mo (Llobet et al. 1988). The volume of water consumed and urine excreted by animals in the group dosed with zinc at 190 mg/kg was always lower than these volumes for the other treatment groups and the control group. At this dose, 2 out of 10 rats died. Concentrations of urea and creatinine in plasma were significantly higher for animals in that group. At the end of the study, abnormal histology of the kidneys was seen. It included lesions in the glomerular Bowman's capsule that consisted of flattened epithelial cells as well as proximal convoluted tubules exhibiting desquamation of tubular epithelial cells and pyknotic nuclei. The contribution of decreased water intake to abnormal renal function was noted. A NOAEL of 95 mg/kg/d for this effect in female rats was identified. The hematocrit and hemoglobin concentrations of rats given the highest dose were lower than, but not significantly different from, those of controls. No clear trends of effects as a function of dose were observed for hematocrit or hemoglobin or for the significant increases in serum urea and creatinine at the highest dose.

Chronic Exposures (> 100 d)

A case report (Hoffman et al. 1988) of a woman patient who had three intestinal surgeries for gastric ulcer and ingested a total (as a zinc supplement and zinc in a multivitamin supplement) of $ZnSO_4$ at 520-680 mg/d (zinc at 3.9 mg/kg/d) for 10 mo had decreased concentrations of

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hemoglobin and hematocrit after this chronic oral exposure. Various symptoms related to copper deficiency such as reduction in ceruloplasmin (copper transport protein), hypochromic and microcytic anemia, leucopenia, and neutropenia (Hoffman et al. 1988) were also observed. Similar signs of copper deficiency, including decreased concentrations of plasma ceruloplasmin, were also seen in sickle cell anemia patients given zinc supplements at 150-200 mg/d for 2 y (Prasad et al. 1978). Gyorffy and Chan (1992) reported a case history in which a 57-y-old man who was taking zinc as zinc amino acid chelate at 810 mg for 18 mo presented with hypochromic-microcytic anemia. Serum copper and serum ceruloplasmin were significantly low. When the patient discontinued zinc supplements, his hemoglobin rose to normal levels in about 8 wk. Simon et al. (1988) reported that a 44-y-old man who had been taking zinc as zinc gluconate at 200-300 mg/d for at least 2 y developed anemia and neutropenia and ringed sideroblasts. These effects were secondary to zinc-induced hypocupremia and hypoceruloplasminemia. Similarly, Patterson et al. (1985) reported that a 57-y-old woman who consumed zinc at 450 mg/d for 2 y developed severe anemia, and Hoogenraad et al. (1985) reported that a 23-y-old man who was treated with high doses of $ZnSO_4$ orally for 12 mo had normocytic anemia with granulocytopenia, hypocupremia, and decreased ceruloplasmin; the doses were Zn^{2+} at 90 mg, three times per day for 90 d and 405 mg/d for 3 mo, respectively. Porea et al. (2000) reported that a 17-y-old male developed anemia after taking a zinc preparation to control acne. Although the number of subjects was small and the supplemental doses varied, these studies clearly documented that excess zinc led to serious anemia secondary to copper deficiency. Although these strong data could have been used to derive an acceptable concentration (AC) for long-term exposure, a NOAEL could not be identified for this serious adverse effect.

Because of the small numbers of subjects and dose concentrations and the fact that the subjects were not considered healthy, these data cannot be used to determine a 1,000-d AC even though the resulting adverse effects have been seen in many animal and some human short-term studies.

Animal Studies

Mice that received zinc as zinc oleate at 5,000 ppm (parts per million) in their diet developed severe anemia (based on the postmortem examination) during a 45-wk study. In blood from the orbital sinus of randomly selected mice in the dosed groups, the hemoglobin content was

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only 40% of that of controls. The packed cell volume was also low. The authors did not provide any quantitative data. Furthermore, the investigators reduced the concentration of zinc in the diet every 3 mo to avoid losses from mortality. When $ZnSO_4$ was added to the animals' drinking water at 1,000 ppm (zinc at 190 mg/kg/d) or 5,000 ppm (950 mg/kg/d), no increase occurred in the incidence of hepatoma, malignant lymphoma, or lung adenoma at the end of 45 wk (Walters and Roe 1965). The data are not useful for deriving an AC, because of the uncertainty of the dose and high mortality.

In the study of Aughey et al. (1977) described earlier, C3H mice (male and female) were also exposed to zinc as $ZnSO_4$ at 500 ppm in drinking water for 12 mo (zinc at 80 mg/kg). Between 9 and 12 mo after exposure, the pancreatic islets were found to be larger, and individual cells had a vacuolated appearance and were separated by wide vascular channels. These effects were not seen at 3 mo (Aughey et al. 1977). In addition, in the zinc-supplemented mice, the adrenal cortex was thicker (hypertrophy of the adrenal cortex) compared to that of control animals. Most of the fasciculate cells were hypertrophied, vacuolated, and distended. The histochemistry was weaker at 3 and 6 mo. The ACTH-secreting cells of the anterior lobe of the pituitary gland were also reported to manifest abnormal changes (increase in size and number of granules, with some evidence of increased synthetic and secretory activity). Thus, histologic changes were observed in the pancreas, adrenal cortex, and pituitary of mice given zinc at 80 mg/kg/d in drinking water. Similar pancreatic lesions were reported in rats and mice fed a diet containing $ZnSO_4$ for 13 wk at doses of 450-475 mg/kg/d for rats and 235-245 mg/kg/d for mice (Maita et al. 1981).

Feeding male New Zealand white rabbits (n = 7-8) a diet containing zinc carbonate (zinc at 5,000 µg/g diet; the estimated dose was 175 mg/kg/d) for 22 wk resulted in statistically significant differences in blood hemoglobin (13.55 ± 0.125 g/100 mL in controls and 11.51 ± 0.24 mg/100 mL in the treated group) and in serum copper concentration (0.793 ± 0.121 mg/mL in the control group and 0.274 ± 0.012 mg/mL in the treated group). No changes were reported in the growth rate of these young, recently weaned rabbits (Bentley and Grubb 1991).

Reproductive Effects

The only study on the effect of zinc on human reproduction reported that when pregnant women in the United Kingdom received cap-

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sules containing zinc as $ZnSO_4$ at 0.3 mg/kg/d (elemental zinc at 25 mg/d) during the last trimester, they did not show any change in maternal body weight gain, blood pressure, hemorrhage, or infection (Mahomed et al. 1989). Kumar (1976) orally administered zinc as 2% $ZnSO_4$ at 150 ppm daily to 13 pregnant rats. The diet that these rats received also contained zinc at 30 ppm. Compared with untreated pregnant animals, treated rats had increased resorption of fetuses. In another study of the relationship between development and maternal dietary zinc during gestation and lactation, groups of 10 female Sprague-Dawley rats were maintained on diets containing ZnO at 2,000 ppm (200 mg/kg/d) or 5,000 ppm for 35-38 d (from day 1 of gestation to day 14 of lactation). No malformations were observed, but fetal mortality was seen even in the 2,000 ppm group (Ketcheson et al. 1969). When female rats were exposed to zinc as zinc carbonate at 250 mg/kg/d in their diet for 150 d, there was no reproduction. The NOAEL for this effect was zinc at 50 mg/kg/d (Sutton and Nelson 1937).

Zinc seems to be an important factor in epididymal maturation and in the stabilization of sperm chromatic structure. High concentrations of zinc have been found in the semen and the prostatic fluid of humans, and zinc deficiency results in reduced testicular weight and, in severe cases, sterility. Evenson et al. (1993) studied the effect of excess zinc on sperm chromatin. They fed 3-wk-old male Sprague-Dawley rats (n = 10) experimental diets containing zinc as $ZnCl_2$ at 500 mg/kg/d for 8 wk and observed abnormal structure of the caudal epididymal sperm chromatin. As cell ploidy during spermatogenesis is known to proceed from diploid to tetraploid to haploid, alteration of this progression indicates impaired cell maturation and proliferation (Evenson et al. 1993). However, Maita et al. (1981) found no changes in the testes or ovaries of mice fed zinc as $ZnSO_4$ at 1,100 mg/kg/d for 13 wk.

There were no effects on implantation and no other adverse reproductive effects noted when rats were fed zinc as $ZnSO_4$ at 200 mg/kg/d for 21 d prior to mating, whereas exposure for 21 d during gestational day 0 to the end of the gestational period increased implantation losses (Pal and Pal 1987).

Developmental Effects

The developmental effects of excess zinc have been studied in animals exposed to zinc by oral ingestion before and during gestation and measuring the associated implantations, fetal resorptions, fetal weights, and growth in the offspring.

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Administration of zinc as ZnO at 200 mg/kg/d in the diet to rats 21 d before mating and during gestation resulted in resorption of all fetuses, whereas only 4-25% of fetuses were resorbed if the rats were treated only during gestation (Schlicker and Cox 1968). Depressed growth of the fetal rats was noted at this dose, but zinc at 100 mg/kg/d had no adverse effect on their development. Administration of diet containing zinc as zinc carbonate at 250 mg/kg/d to female rats for 150 d resulted in increased still births. At 50 mg/kg/d there was no effect. In another study (Mulhern et al. 1986), reduced fetal weight, decreased hematocrit, and copper deficiency in the offspring of mice exposed to zinc at 260 mg/kg/d in their diet were observed.

Genotoxicity

Zinc compounds were generally negative in in vitro bacterial reverse-mutation assays with *Salmonella typhimurium* (Thompson et al. 1989) and in in vivo mouse-mediated assays such as the frequency of micronucleated polychromatic erythrocytes in the mouse bone marrow and dominant lethal test (Fabrizio 1974), the *Escherichia coli* assay (Nishioka 1975), the mouse lymphoma forward mutation assay (Amacher and Paillet 1980), and the in vivo rodent somatic and germinal cell cytogenetic assays (Vilkina et al. 1978). Although zinc acetate gave negative results in the *S. typhimurium* mutation assay and in induction of unscheduled DNA synthesis in primary rat hepatocytes, it was positive in the mouse lymphoma assay and in an in vitro cytogenetic assay with Chinese hamster ovary (CHO) cells (Thompson et al. 1989). Human lymphocyte cultures exposed to $ZnCl_2$ at 3×10^{-3} molar (M) to 3×10^{-5} M demonstrated only a weak clastogenic response (Deknudt and Deminatti 1978).

Several in vivo studies have shown positive results indicating genotoxicity. Chromosomal aberrations in bone marrow cells have been observed in rats exposed to zinc as zinc chlorate at 14.8 mg/kg/d in drinking water (Kowalska-Wochana et al. 1988). It has been suggested that the calcium-depleting action of zinc may be responsible for these aberrations (Deknudt and Gerber 1979) because the effects were not seen in mice fed a standard calcium diet. Also, in rats exposed to zinc chlorate at 17.5 mg/kg/d in drinking water, sister chromatid exchanges were observed in the bone marrow (Kowalska-Wochana et al. 1988). Thus, in the bone marrow of both mouse and rat, sister chromatid exchanges and chromosomal aberrations were observed as a result of in vivo exposure

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to zinc salts. Using alkaline single-cell gel electrophoresis (the Comet assay), Banu et al. (2001) reported that single-stranded breaks were induced in the peripheral blood lymphocytes from mice orally administered various doses of $ZnSO_4$ and studied after 24, 48, 72, 96 h, and the first wk posttreatment.

Carcinogenicity

No human data are available that relate cancer to zinc administered orally as zinc supplements or otherwise. When rats were provided with $ZnCl_2$ in their drinking water at 450 ppm (zinc at 13.4 mg/kg/d) for 25 wk and tested for renal lesions, no renal tumors were seen (Kurokawa et al. 1985). Zinc as $ZnSO_4$ was added to drinking water at 1,000 ppm (190 mg/kg/d) or 5,000 ppm (950 mg/kg/d), and mice ingested this for 45 wk. No increase in the incidence of tumors at any of the sites (hepatoma, malignant lymphoma, lung adenoma, or testicular tumors) was noted (Walters and Roe 1965). There was also no evidence of forestomach or glandular epithelial tumors in the treated groups.

Effects on Immune Function

There have been numerous human and animal studies on zinc deficiency and effects on immune functions (Shankar and Prasad 1998). There are only a few reports on the effect of excess zinc ingestion on the immune system. Several studies indicate that zinc supplementation has beneficial effects on susceptibility of human populations to infectious diseases. These effects include reduction of the incidence and duration of acute and chronic diarrhea, incidence of lower respiratory infections, and even the incidence of malaria. Zinc lozenges were shown to decrease the duration of the common cold. It has also been reported that zinc-deficient animals have suppressed immune responses and are highly susceptible to various viral (for example, herpes simplex and Semliki Forest viruses) and bacterial infections such as *Salmonella enteritidis* and mycobacterium tuberculosis (Shankar and Prasad 1998). In general, zinc seems to play a paramount role in normal development and in many effectors of immunity. The effects of zinc deficiency and zinc excess on the immune system and the immune systems relation to plasma zinc concentration has been reviewed by Chandra (1991).

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Spaceflight Effects

In a study using a ground-based spaceflight analog, head-down bedrest, Krebs et al. (1993) found increased fecal excretion and decreased zinc balance 17 wk after bedrest even though the dietary concentration of zinc was sufficient (zinc at 13.8 ± 0.7 mg/d). In an animal study using simulated weightlessness, a decrease in femoral-diaphyseal zinc concentration was reported (Yamaguchi et al. 1991). This decrease may represent the loss of zinc from muscle and bone, where the most zinc is stored. Yamada et al. (1997) reported a significant decrease in zinc concentrations in weight-bearing and non-weight-bearing bones of growing rats after 14 d in space. These investigators also reported abnormal thickening of the endosteal surface of the cortical bone. But on longer spaceflights, zinc excretion seemed to return to normal concentrations (Volpe et al. 2000).

A general overview of the effects of zinc salts is provided in Table 11-4.

RATIONALE

For each exposure duration, the SWEG value will be listed based on the lowest values among the ACs for all the significant adverse effects at that exposure duration. AC values were determined by following the methods for the development of SWEGs (NRC 2000). ACs were calculated assuming a nominal potable water use of 2.8 L (including 0.8 L of water used for the reconstitution of food), in contrast to EPA's reference volume of 2 L of water per day as drinking water. When the end points are adverse hematologic effects or reduction in water consumption, a spaceflight factor of 3 has been used. The most significant hematologic changes resulting from spaceflight are reductions in plasma volume and red blood cell mass—the greatest decreases in hematocrit having been up to 15% after 18- and 63-d Soviet flights and the 28-d Skylab mission (Huntoon et al. 1994). The greatest decreases in hemoglobin concentration were about 25% after a Salyut 4 flight of 96 d. After a Mir flight of 365 d, a 12% decrease was reported, and hemoglobin decreased after 60 d on two Skylab missions (Huntoon et al. 1994). Reduced fluid intake is one of the factors for increased risk for the formation of renal stones during spaceflights (Whitson et al. 1999).

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TABLE 11-4 Toxicity Summary for Zinc Salts by Oral Ingestion

Zinc Compound	Route of Exposure and Dose (mg/kg/d)	Exposure Duration	Species	Toxicity Effects	Reference
Zinc salt (not specified)	Oral ingestion of contaminated food; estimated dose = 2.6-6.8	<1 d	Human	Intestinal symptoms—severe diarrhea, abdominal cramps in 24-48 h	Brown et al. 1964
Zinc salt (not specified)	Contaminated fruit punch; estimated dose = 4.6-9.2	<1 d	Human	Nausea, vomiting, and diarrhea in 20-90 min	Brown et al. 1964
ZnO	Once oral; dose = 6.7	Once	Human	GI disturbance	Callender and Gentzkow 1937
$ZnSO_4$	Oral bolus; dose = 0.5	4 h	Human	Decreased serum cortisol; decreased prolactin secretion	Brandao-Neto et al. 1990
Zinc elemental	Oral dose; dose = 86	2 d	Human	GI distress	Murphy 1970
ZnO	Oral dose; dose = 487	10 d	Wistar rat	Secretory materials in the hypothalamus; minor neuron degeneration	Kozik et al. 1980
Zinc glycine acetate (zinc Chelazome	Oral supplement; dose = 30 mg/d	2 and 14 wk	Human, male	No effect on peripheral blood leukocytes or lymphocyte subsets; no effect on putative indices of copper status	Bonham et al. 2003a, b
$ZnSO_4$	Drinking water; dose = 150	18 d	Pregnant rats (unspecified strain) (n = 13)	Increased resorption of fetuses	Kumar 1976

(Continued)

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TABLE 11-4 Continued

Zinc Compound	Route of Exposure and Dose (mg/kg/d)	Exposure Duration	Species	Toxicity Effects	Reference
$ZnCl_2$	Drinking water; dose = 12	4 wk	Wistar rat, male and female	Decreased hemoglobin and number of erythrocytes	Zaporowska and Wasilewski 1992
$ZnSO_4$	Capsules; dose = 2.46	5 wk	Human, male	Reduction in HDL cholesterol	Hooper et al. 1980
Zinc carbonate	Drinking water; dose = 500	5 wk	Sprague Dawley rat	Decrease in hemoglobin, hematocrit, MCH, and MCHC	Smith and Larsen 1946
$ZnSO_4$	Oral; dose = 4.4	6 wk	Human, male	Reduced HDL cholesterol, elevated serum LDL cholesterol; impaired immune status	Chandra 1984
$ZnSO_4$	Supplement, 3/d; dose = 2.4	6 wk	Human, male and female	Decreased ESOD and ceruloplasmin in females	Samman and Roberts 1988
Zinc gluconate	Gelatin capsules, 2/d; total dose = 0.71	6 wk	Human, male	Decreased erythrocyte copper-zinc superoxide dismutase (affects copper status)	Fischer et al. 1984
Zinc acetate	Dietary supplement (capsule); dose = 0, 0.45,1.03, or 1.86	8 wk	Human, female	Reduced HDL cholesterol only in high-dose group	Freeland-Graves et al. 1982
$ZnSO_4$	Capsules; dose = 0.3	2 mo (8 wk)	Human, pregnant female	No reproductive toxicity	Mahomed et al. 1989
Zinc acetate	Drinking water; dose = 47.5, 91.0, and 191.0	3 mo (9 wk)	Rat, female; strain, Sprague Dawley	Changes in renal pathology; increased plasma creatinine and urea concentrations	Llobet et al. 1988

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Zinc gluconate	Capsules 2/d; dose = 0.83	10 wk	Human, female	Decreased ESOD, hematocrit, and serum ferritin concentrations	Yadrick et al. 1989
$ZnCl_2$	zinc supplement; dose = 0.85 and 1.09	12 wk	Human, male	A trend of decreased HDL cholesterol	Black et al. 1988
$ZnSO_4$	Drinking water; dose = 53 and 565	13 wk	Rat, male and female; strain, Wistar	Decreased hematocrit and white blood cells; pancreatic necrosis (at high dose)	Maita et al. 1981
$ZnSO_4$	Drinking water; dose = 104 and 1,110	13 wk	Mouse, male and female; strain, ICR	Anemia; decreased white blood cells; forestomach ulceration	Maita et al. 1981
Zinc carbonate	Oral dose; dose = 174	22 wk	Rabbit, male; New Zealand white rabbits	Decreased hemoglobin concentrations	Bentley and Grubb 1991
Zinc oleate	Drinking water; dose = 68 (dose was changed every 3 mo)	52 wk (total)	Mouse, sex not specified	Anemia (hemoglobin was 40% of controls)	Walters and Roe 1965
$ZnSO_4$	Medical therapy; dose = 2.9-3.9	10 mo	Human, female; case study (n = 1)	Hypocupremia, hyperchromic and microcytic anemia, leucopenia	Hoffman et al. 1988
$ZnSO_4$	Drinking water; dose = 200	Up to 12 mo	Mouse, male and female; strain, C3H	Hypertrophy of pancreas at 3 mo; vacuolation of islets and adrenal cortex	Aughey et al. 1977

Abbreviations: ESOD, erythrocyte superoxide dismutase; GI, gastrointestinal; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

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The Office of Drinking Water (ODW) of EPA has not established a primary maximum contaminant level (MCL) for soluble zinc salts. However, EPA determined a SMCL of zinc at 5 mg/L for U.S. drinking water based on taste. EPA established an oral reference dose (RfD) of 0.3 mg/kg/d for soluble zinc salts based on a LOAEL of zinc at 59.72 mg/d (corresponding to about 1 mg/kg/d based on the human diet-supplement study). The LOAEL was based on the study by Yadrick et al. (1989), who reported a 47% decrease in the activity of ESOD in adult females after 10 wk of oral ingestion of zinc gluconate capsules. The decrease in ESOD activity in humans seems to reflect the turnover of erythrocytes and the status of copper. The data were also based on decreased hematocrit and serum ferritin. EPA applied a modifying factor of only 3 to the LOAEL of 60 mg/d to arrive at a NOAEL, and they used 60 kg as a nominal body weight for a woman to arrive at an RfD of 0.3 mg/kg/d. EPA stated that a factor of only 3 was used, because of the minimal LOAEL probably meaning that no clinical manifestation occurred at such a dose.

The Agency for Toxic Substances and Disease Registry (ATSDR) did not derive an oral minimal risk level (MRL) for acute or chronic durations, but an MRL of zinc at 0.3 mg/kg/d was established for intermediate-duration exposure by the oral route (ATSDR 1994). This MRL was based on reduced concentrations of ferritin and hemoglobin and reduced ESOD activity reported by Yadrick et al. (1989). This value (zinc at 0.3 mg/kg/d) was accepted as a MRL for chronic duration without applying any time factor.

Recently, IOM's Food and Nutrition Board (of the National Academy of Sciences) derived dietary reference intakes (DRI) for some vitamins and minerals. The board reviewed the existing recommended dietary intakes to derive a tolerable upper intake level (UL) that this board defined as "the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects may increase." zinc was one of the micronutrients evaluated. The RDA for zinc has been set at 11 mg/d for men and 8 mg/d for women (IOM 2001). In deriving the UL for zinc, the board considered the data on the effect of exogenous zinc on reduced copper status in humans (using the change in activity of copper-zinc ESOD as a sensitive indicator of copper status) similarly noted in the studies by Fischer et al. (1984), Samman and Roberts (1988), and Yadrick et al. (1989). Decreased circulating copper concentrations were documented in other studies (IOM 2001). Because of consistent observations in several stud-

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ies, this board used a change in erythrocyte copper-zinc ESOD activity as a credible adverse end point rather than using hematocrit from the Yadrick study, which the National Aeronautics and Space Administration (NASA) has used because of its clinical significance. Using the Yadrick data, the board set 60 mg/d (supplemented zinc at 50 mg and the mean daily dietary intake at 10 mg) as the LOAEL for reduced copper status in female subjects. Similar results were noted by Fischer et al. (1984) were seen in 12 human subjects who were supplemented with zinc at 50 mg/d. Also, Samman and Roberts (1988) showed that ESOD decreased (25%) in women (n = 26) ingesting zinc as $ZnSO_4$ at 150 mg for 12 wk but not in men (n = 21).

Using a LOAEL of 60 mg/d, the board applied an uncertainty factor (UF) of only 1.5 to arrive at a NOAEL. This factor was also used to accommodate interindividual variability. The board justified the use of a UF of only 1.5 by saying that reduced copper status is rare in humans (IOM 2001). A UL was derived by dividing the LOAEL by 1.5, giving a value of 40 mg/d (60 mg/d ÷ 1.5 = 40 mg/d). This included contributions from all sources (diet, water, air, supplements, etc.). See Table 11-5 for a few other agencies' guidelines.

If NASA were to use the value of 40 mg/d for the UL and extend it to calculate an AC for NASA with a nominal volume of water consumption of 2.8 L, then after subtracting a nominal value of 12 mg/d from food (old RDA), the corresponding AC (for 1,000 d) would be

$$(40 \text{ mg/d} - 12 \text{ mg/d}) \div 2.8 \text{ L} = 10 \text{ mg/L}.$$

IOM used data from a subacute study and derived a long-term value without any time factor. The LOAEL-to-NOAEL factor is very low. A prolonged decrease in copper status can result in anemia, as indicated in several studies. Changes in hematocrit concentration, a clinically significant result from the Yadrick study, were not considered. See Table 11-6 for SWEG values.

A review of the literature on the adverse effects of ingesting exogenous zinc (zinc supplements) indicates that data are available from a sufficient number of human studies except for chronic exposures. In certain cases, zinc supplements have been administered as medical interventions. No epidemiologic study has been done to relate the concentration of zinc in drinking water to any adverse effects. Furthermore, the absorption of zinc supplements given as part of the diet is known to vary significantly depending on the nature of the diet (see section on absorption).

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TABLE 11-5 Current Regulations and Guidelines Set by Other Organizations

Organization	Type	Guideline
EPA[a]	MCLG	None established
EPA	MCL	None established
EPA	SMCL (or SDWR)	5 mg/L
EPA	1-d HA[b] (child)	6 mg/L
EPA	10-d HA (child)	6 mg/L
EPA	Longer-term HA[c] (adults)	10 mg/L
EPA	RfD	0.3 mg/kg/d
EPA	Lifetime HA	2 mg/L
EPA	Cancer Group	D[d]
ATSDR (1994)	Acute MRL	None derived
ATSDR (1994)	Intermediate MRL	0.3 mg/kg/d
ATSDR (1994)	Chronic MRL	0.3 mg/kg/d
FDA	Bottled water	5 mg/L
NAS (NRC 1977)	SNARL	5 mg/L
NAS (NRC 1989)	RDA	15 mg/d (men) and 12 mg/d (women)
IOM (2001)	RDA	11 mg/d (men) and 8 mg/d (women)
IOM (2001)	UL	40 mg/d (adults) from all sources

[a]All EPA sources: http://www.epa.gov/waterscience/drinking/standards/dw-standards.pdf.
[b]Legally nonenforceable standards.
[c]This has been dropped from the EPA Health Advisory tables since 2000.
[d]Cancer Group D means "not classifiable as to human carcinogenicity."
Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; EPA, U.S. Environmental Protection Agency; FDA, Federal Drug and Administration; HA, health advisory; IOM, Institute of Medicine; NAS, Nation Academy of Sciences; NRC, National Research Council; MCL, maximum contaminant level; MCLG, maximum contaminant level goal; MRL, minimal risk level; RDA, Recommended Dietary Allowance; RfD, reference dose; SDWR, secondary drinking water regulation; SMCL, secondary maximum contaminant level (replaced by SDWR); SNARL, suggested no adverse response level; UL, tolerable upper intake level.

1-d AC for Ingestion

The accidental ingestion of a very high concentration of zinc-containing food and fluids by humans has resulted in serious GI dis-

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TABLE 11-6 Spacecraft Water Exposure Guidelines for Soluble Zinc Compounds

Duration	mg/L	Toxicity End Point	Source
1 d	11	Impaired immune status and copper status	Bonham et al. 2003a, b
10 d	11	Impaired immune status	Chandra 1984
100 d	2	Reduced hematocrit; impaired immune status	Yadrick et al. 1989; Chandra 1984
1,000 d	2	Reduction in hemoglobin; hypocupremia	Bentley and Grubb 1991

Note: These values represent concentrations for water only, over and above the daily dietary zinc content.

tress with severe nausea, vomiting, and diarrhea (Brown et al. 1964). The doses were estimated to be metallic zinc at 2.4-6.0 mg/kg in one case and 2.4-9.2 mg/kg in another case. The victims recovered from the adverse effects within 24 h. A LOAEL could not be estimated. Because of the uncertainties in the dose, these data were not used. In a case report, a 16-y-old girl was reported to have suffered from epigastric symptoms after exposure to zinc at 2.6 mg/kg/d for 1 wk (Moore 1978). Because only one individual was involved, the data could not be used to derive a 1-d AC. One of the problems in deriving an AC based on GI distress, an important adverse effect, is that none of the short-term or subchronic studies has reported this effect. Brandao-Neto et al. (1990), who studied acute human exposure to zinc, reported that secretion of cortisol and prolactin was inhibited within 4 h after ingestion of zinc as $ZnSO_4$ at 0.5 mg/kg/d. This change indicates that the adrenals and pituitary may be target organs for zinc toxicity. This belief is supported by subchronic animal studies (Aughey et al. 1977; Kozik et al. 1980, 1981). However, later studies reported from the same laboratory could not confirm the effects from similarly designed studies. The effects may have been transient, and peripheral adverse effects resulting from such changes may have been documented. Therefore, for the 1-d AC derivation, the data that can be extracted from short-term zinc supplementation studies in humans can be used as described in the rest of this section.

In a study in which females took zinc as zinc gluconate at 50 mg/d, ESOD, hematocrit, and hemoglobin were measured at 6 and 10 wk. Yadrick et al. (1989) found no statistically significant changes in any of these variables at 6 wk. Effects were seen only 10 wk after supplementation. A

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dose rate of zinc at 0.833 mg/kg/d can be identified as a NOAEL for 1 d or 10 d, because no effects were reported even at 4 wk.

A 1-d AC can be derived as follows:

$$(0.833 \text{ mg/kg/d} \times 70 \text{ kg}) \div 2.8 \text{ L/d} = 21 \text{ mg/L},$$

where
0.833 mg/kg/d = NOAEL;
70 kg = nominal body weight; and
2.8 L/d = nominal water consumption.

The species factor was not needed because this is a human study, and a time factor is not applied because the 2-wk NOAEL will be protective of the 1-d AC.

The second short-term exposure study resulting in data that can be used to identify a NOAEL for 1 d is that of Bonham et al. (2003a, b). In this double-blinded study, groups of 19 healthy men were given an oral supplement of zinc as zinc Chelazome at 30 mg. The authors found no statistically significant differences in the indices of immune status or markers of copper status at the end of 2 wk or 14 wk between these subjects and placebo controls. Before the trial, subjects were extensively screened for blood diseases, liver function, and lipid profiles, and their responses to a lifestyle questionnaire were obtained to help determine their eligibility. A NOAEL of 30 mg/d (zinc at 0.43 mg/kg/d) can be identified.

A 1-d AC can be derived as follows:

$$(0.43 \text{ mg/kg/d} \times 70 \text{ kg}) \div 2.8 \text{ L/d} = 11 \text{ mg/L (rounded)},$$

where
0.43 mg/kg/d = NOAEL;
70 kg = nominal body weight; and
2.8 L/d = nominal water consumption.

No species or time extrapolation factors are needed. The species factor was not needed, because this is a human study, and a time factor is not applied, because the 2-wk NOAEL will be protective of the 1-d AC. Because 0.43 mg/kg/d is a NOAEL for up to 14 wk and used for 1-d AC derivation, no “small n” factor was applied, because it would be overly conservative.

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10-d AC for Ingestion

Hooper et al. (1980) administered $ZnSO_4$ gelatin capsules ($ZnSO_4$ at 440 mg; with meals, elemental zinc at 80 mg/d or 1.14 mg/kg/d) to males for 5 wk, which resulted in a significant decrease (25%) in serum HDL cholesterol, a potential risk factor for arteriosclerosis. The values returned to baseline levels 11 wk after cessation of the dose. Such a short-term change is not likely to be a significant risk factor for a chronic cardiac disease although it took 11 wk for the values to return to baseline. Although a LOAEL (1.4 mg/kg/d) can be identified without a NOAEL, these data were not used for deriving an AC for 10 d, because the change in HDL cholesterol would not be a significant risk factor for chronic cardiac disease.

The second study considered for a 10-d AC was that of Chandra (1984), in which humans (11 males) were given $ZnSO_4$ tablets (zinc as $ZnSO_4$ at 150 mg twice a day, or 4.4 mg/kg/d) for 6 wk. Effects on serum HDL cholesterol (31% decrease in HDL cholesterol and 11% increase in LDL cholesterol) were observed that were very similar to those reported by others. The LDL-HDL cholesterol ratio (the atherogenic index) rose significantly from 2.6 to 4.2 at the end of 6 wk of supplementation. Immune system function, as measured by chemotactic migration and phagocytosis, was also found to be impaired in this study. A dose of zinc at 4.43 mg/kg/d was identified as a LOAEL in this study for both the lipoprotein profile effects and immune system effects—but primarily for the latter. Even though only one dose was used in this study, the effects were significant and supported by other similar investigations.

Thus, a 10-d AC for impaired immunologic variables is derived as follows:

$$(4.4 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 11 \text{ mg/L},$$

where
4.4 mg/kg/d = LOAEL,
70 kg = nominal body weight;
10 = LOAEL to NOAEL extrapolation factor; and
2.8 L/d = nominal water consumption.

The AC calculated from the 4-wk LOAEL will be protective for a 10-d exposure. Therefore, no time factor has been applied.

It must be pointed out that the National Research Council (NRC) committee on SWEGs identified certain weaknesses in the design of the

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study, such as lack of information on volunteer history, other factors that may potentially influence the observed effects, and small sample size. However, in the absence of stronger data, NASA has decided to use the study for 10-d AC derivation.

The second short-term exposure study with data that can be used to identify a NOAEL for 10 d is that of Bonham et al. (2003a, b). When these authors gave groups of 19 healthy men oral supplements of zinc as zinc Chelazome at 30 mg, they found no significant differences between the supplemented groups and placebo controls in the indices of immune status or in the markers of copper status at the end of 2 wk or 14 wk. A NOAEL of 30 mg/d (or zinc at 0.43 mg/kg/d) can be identified for 2 wk. As the NOAEL is valid for up to 14 wk, it is unnecessary to apply a modifying factor of “small n” on this NOAEL while deriving an AC for 10 d.

A 10-d AC can be derived as follows:

$$(0.43 \text{ mg/kg/d} \times 70 \text{ kg}) \div 2.8 \text{ L/d} = 11 \text{ mg/L (rounded)}.$$

100-d AC for Ingestion

Three human-subject studies were identified as candidates for deriving a 100-d AC with similar toxicity end points. In a study reported by Yadrick et al. (1989), 18 female volunteers ingested zinc gluconate capsules for 10 wk (dose of zinc at 0.83 mg/kg/d), and hematocrit, serum ferritin, and copper-zinc ESOD activity were measured at the end of 6 and 10 wk. After 10 wk of supplementation, hematocrit, serum ferritin, and ESOD activity were significantly lower than their pretreatment levels, indicating that iron and copper status can be affected by zinc supplementation. Data were analyzed by the investigators for statistically significant differences using a repeated-measures design. The least significant difference (LSD) was used to compare the effects of time within a treatment.

The data are substantiated by the Fisher et al. (1984) study, in which ingestion of zinc gluconate at 50 mg by healthy men significantly decreased ESOD. Samman and Roberts (1988) showed a decrease in ESOD at 6 wk, but the dose they used was three times higher than that used by Yadrick et al. (1989), who at 6 wk reported a NOAEL of 50 mg/d (or 50 mg/60 kg/d = 0.833 mg/kg/d). Zinc and copper are key elements of the membrane-bound enzyme ESOD. The activity of this enzyme depends on copper status, and therefore, ESOD has been exten-

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sively used in studies concerning effects of micronutrients as a marker for copper status. Several studies have documented that (chronically) reduced copper status has been associated with anemia. In the 10-wk zinc-supplementation study by Yadrick et al. (1989), a dose of zinc at 0.833 mg/kg/d was identified as a LOAEL for changes in hematocrit, a significant toxicologic effect. Although no change in hematocrit was observed at 6 wk (NOAEL for 6 wk), the same dose was identified as a LOAEL at 10 wk.

The 100-d AC was calculated using the 10-wk LOAEL and from extrapolating the data to 100 d using a time-extrapolation factor of 100 d/70 d. In addition, because the experiment data showed no effect at 6 wk, a factor of 3 instead of a default factor of 10 was applied to the LOAEL to get a NOAEL, and this factor will be sufficiently protective. This is also consistent with the lower LOAEL-to-NOAEL factors (3 and below) used by EPA and IOM, as mentioned earlier.

Thus, a 100-d AC based on the change in hematocrit concentrations can be calculated as follows:

$$(0.833 \text{ mg/kg/d} \times 70 \text{ kg}) \div (3 \times 2.8 \text{ L/d} \times [100 \text{ d}/70 \text{ d}] \times 3) = 2.0 \text{ mg/L (rounded from 1.6 mg/L)},$$

where
0.833 mg/kg/d = LOAEL;
70 = nominal body weight;
3 = LOAEL to NOAEL extrapolation factor;
2.8 L/d = nominal water consumption;
100 d/70 d or 1.43 = time extrapolation factor; and
3 = spaceflight safety factor for anemia (changes in hemoglobin).

A 100-d AC based on the change in ESOD activity can be calculated as follows:

$$(0.833 \text{ mg/kg/d} \times 70 \text{ kg}) \div (3 \times 2.8 \text{ L/d} \times [100 \text{ d}/70 \text{ d}]) = 5 \text{ mg/L (rounded)},$$

where
0.833 mg/kg/d = LOAEL;
70 kg = nominal body weight;
3 = LOAEL to NOAEL extrapolation factor;
2.8 L/d = nominal water consumption; and
100 d/70 d or 1.43 = time extrapolation factor.

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In this study, as a result of zinc ingestion, a significant decrease (about 23%) occurred in serum ferritin concentrations, the iron storage protein. Concentrations of serum ferritin have been used clinically to indicate iron status and type of anemia. An AC based on changes in iron status is the same as that calculated for ESOD changes—that is, 5 mg/L.

A second study considered is that of Chandra (1984), in which 300 mg $ZnSO_4$ tablets were given to 11 adult males for 6 wk, and changes were studied at 2, 4, and 6 wk. A significant decrease (32%) in serum HDL cholesterol was reported at 6 wk. LDL cholesterol was significantly increased at 6 wk. The HDL percentile value fell from 43rd to 6th percentile based on population percentile normal values for that age group. Because zinc plays an important role in the maintenance of the immune system, the impaired immune status (as judged by decreased mitogenic response to PHA stimulation) and impaired inflammatory responses (as indicated by the decreased polymorphonuclear migration response to chemotactic migration and impaired phagocytosis of opsonized bacteria) were also documented in this study. Because of its adverse effects on these immune parameters, a dose of zinc at 4.4 mg/kg/d was identified as a LOAEL. The immunologic changes were seen starting at 4 wk.

A 100-d AC can be calculated as follows using a LOAEL of 4.4 mg/kg/d:

$$(4.4 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times [100 \text{ d}/28 \text{ d}]) = 3.0 \text{ mg/L},$$

where
4.4 mg/kg/d = LOAEL;
70 kg = nominal body weight;
10 = LOAEL to NOAEL extrapolation factor;
2.8 L/d = nominal water consumption; and
100 d/28 d or 3.57 = time extrapolation factor.

Another study considered is that of Black et al. (1988), in which groups of young, healthy men were given zinc gluconate capsules at 0, 50, and 75 mg/d. A significant decrease in serum HDL cholesterol was reported as early as 6 wk and continued to be observed until the end of the study (12 wk). This variable usually represents a risk factor for cardiovascular disease. Coronary heart disease (CHD) risk can be estimated using the Framingham CHD risk calculator from the National Heart, Lung and Blood Institute of the National Institutes of Health. Estimation of a 10-y CHD risk, using the worst-case value of HDL cholesterol at the highest dose of zinc at 75 mg/d for 12 wk, did not indicate a significantly

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greater risk for the zinc-supplemented subjects than for subjects who received placebo. Thus, the observed change in serum HDL cholesterol, especially after the relatively short duration of a 100-d zinc supplementation, is not likely to present a significant risk for the development of chronic arteriosclerosis. In addition, a review of data indicated that the direction of the time-response data for the observed changes was not clear. Therefore, the data were not used for deriving an AC for 100 d.

An additional study that can be used to derive a 100-d AC is that of Bonham et al. (2003a, b), described in detail earlier, in which the authors reported no adverse effects on copper status (as determined by ceruloplasmin oxidase activity, whole blood SOD activity, serum ceruloplasmin concentration, and serum copper concentrations) or on two indices of immune status (circulating levels of peripheral blood leucocytes [full blood profile] and lymphocyte subsets) when 19 healthy adult men ingested zinc at 30 mg daily for 14 wk (close to 100 d). The authors did not measure functional markers of immune function status, such as blastogenic response of cell types or phagocytic activity of neutrophils, as was done in the Chandra study. Before they could be included in the study, the subjects underwent extensive screening that included obtaining their responses to a lifestyle questionnaire. The authors were aware of seasonal variations in lymphocyte subsets, and in this study, the changes that were evident were independent of zinc supplementation.

A 100-d AC can be calculated using a NOAEL of zinc at 0.43 mg/kg/d as follows:

$$(0.43 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times [10/\sqrt{19}]) = 5 \text{ mg/L (rounded)},$$

where
0.43 mg/kg/d = NOAEL;
70 kg = nominal body weight;
2.8 L/d = nominal water consumption;
$10/\sqrt{19}$ = uncertainty factor applied to the NOAEL when sample size in a human study is low (NRC 1994, 2000).

No time or species factors are needed.

1,000-d AC for Ingestion

Very few long-term human studies with robust data that can be used to derive a 1,000-d AC are available. One case report describes a

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woman who underwent three GI surgeries for gastric ulcer and was prescribed zinc at 2.9-3.9 mg/kg/d for 10 mo. She developed hypocupremia, hyperchromic and microcytic anemia, and leukopenia (Hoffman et al. 1988). According to several published case reports (see Chronic Exposure >100 d in this document), ingestion of supplemental zinc over a period of 1-2 y resulted in severe anemia (Hoogenraad et al. 1985; Patterson et al. 1985; Simon et al. 1988; Broun et al. 1990; Gyorffy and Chan 1992). Although anemia seems to be a very well-documented adverse effect of chronic zinc ingestion, and although the effects were predicted by indicative observations in subacute studies on animals, these studies could not be used, because of small sample size and lack of a clear NOAEL for this serious adverse effect. These were case studies, and the subjects had no medical conditions before their exposure to zinc.

Data from short-term studies by Black et al. (1988) and the 6-wk study by Chandra (1984), described in detail in earlier sections, could not be extrapolated to 1,000 d with a large time extrapolation factor. Such an extrapolation would result in a very low, unrealistic value that would be far lower than the amount of zinc present in multivitamin tablets used by the public. The study by Black et al. did not identify a clear NOAEL because, although some differences between treated and placebo groups were statistically significant, the effects of placebos varied greatly. An attempt to evaluate a 10-y CHD risk (using the Framingham CHD risk calculator) using the worst-case value of HDL cholesterol at the highest dose of zinc at 75 mg/d for 12 wk did not indicate a risk significantly different from that of the placebo group. One might assume that the risk, if any, of CHD for 1,000 d must then be much lower than the 10-y risk. For this reason, the effect on serum HDL cholesterol was not considered a significant end point for a 1,000-d AC. A 1,000-d value also could not be derived from existing human data that document anemia in subjects therapeutically treated with zinc compounds and in subjects who used zinc supplements (Porter et al. 1977; Hoffman et al. 1988; Prasad 1988; Simon et al. 1988).

Therefore, data from some long-term animal studies were evaluated. In a study by Aughey et al. (1977), male and female C3H mice that ingested zinc as $ZnSO_4$ at 70 mg/kg/d in their drinking water ad libitum for 12 mo exhibited hypertrophy and vacuolation of islet cells of the pancreas, hypertrophy of the zona fasciculata of the adrenal cortex, and histopathologic evidence of increased synthetic and secretory activity of adrenals. Although these effects were prominent at 12-14 mo, they were not seen at 3 mo. Because histochemistry was weakly positive at 6 and 9 mo, a dose of zinc at 70 mg/kg/d can be identified as a LOAEL for 6 mo.

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The limitation of the study is that there are no dose-response data. However, the time-response data were used to derive a 1,000-d AC. A 1,000-d AC was calculated from the 6-mo data as a LOAEL after using a time factor of 1,000 d/180 d. Because tissue damage was demonstrated histopathologically, a factor of 10 was used to derive a NOAEL from a LOAEL.

A 1,000-d AC for hypertrophy of the adrenal cortex and pancreatic and islets was calculated as follows:

$$(70 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 10 \times 2.8 \text{ L/d} \times [1{,}000 \text{ d}/180 \text{ d}]) = 3 \text{ mg/L (rounded)},$$

where
70 mg/kg/d = LOAEL;
70 kg = nominal body weight;
10 = LOAEL to NOAEL extrapolation factor;
10 = species extrapolation factor;
2.8 L/d = nominal water consumption; and
1,000 d/180 d or 5.555 = time extrapolation factor.

Bentley and Grubb (1991) reported that feeding rabbits a diet containing zinc carbonate at 5,000 µg/g (estimated 175 mg/kg/d) for 22 wk resulted in a significant decline in blood hemoglobin and copper concentration. Anemia, possibly secondary to hypocupremia, was also seen. Although the authors had used a 1,000 µg/g diet (studied for only 8 wk) and a 5,000 µg/g diet (fed for 22 wk), they reported the changes in the hemoglobin and copper concentrations only for the highest dose for 22 wk. Although no dose-response data were available, this effect on hemoglobin has been reported by several studies in humans and animals. Therefore, a 1,000-d AC was derived using a LOAEL of 175 mg/kg/d for 22 wk (155 d).

Thus, a 1,000-d AC can be calculated as follows:

$$(175 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 10 \times 2.8 \text{ L/d} \times [1{,}000 \text{ d}/154 \text{ d}] \times 3) = 2 \text{ mg/L (rounded)},$$

where
175 mg/kg/d = LOAEL;
70 kg = nominal body weight;
10 = LOAEL to NOAEL extrapolation factor;
10 = species extrapolation factor;

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2.8 L/d = nominal water consumption;
1,000 d/154 d or 6.5 = time extrapolation factor; and
3 = spaceflight safety factor for anemia (changes in hemoglobin).

EPA determined that an oral RfD of 0.3 mg/kg/d is equivalent to 21 mg/d for a 70 kg adult human. This dose includes sources such as drinking water and diet. If one subtracts 11 mg as the contribution from diet, an acceptable amount from water is 10 mg/d. Using the EPA nominal water volume of 2 L, the ACs will be 5 mg/L (for a human lifetime). A 1,000-d AC of 2 mg/L has been derived by NASA. Although the 1,000-d AC is lower than the EPA guideline, given the fact that NASA's 1,000-d AC also incorporates a safety factor that protects against any spaceflight-induced hematologic effects, the value for spacecraft water derived by NASA from animal studies is consistent with that of EPA.

A summary of ACs and SWEGs for various durations is listed in Table 11-7.

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TABLE 11-7 Summary of Acceptable Concentrations (ACs) for Zinc in Drinking Water

				UFs				AC (mg/L)[a]			
Toxicity End Point	Exposure (mg/kg/d)	Species	Principal Study	To NOAEL	Species	Time Extrapolation Factor	Space-flight Factor	1 d	10 d	100 d	1,000 d
Effects on ESOD, hematocrit, and hemoglobin	NOAEL, 0.833, oral	Human	Yadrick et al. 1989	1	1	1	1	21	—	—	—
Effects on immunologic status and copper status	NOAEL, 0.43	Human	Bonham et al. 2003	1	1	1	1	11	11	—	—
Immune system effects (impaired mitogenic response and chemotactic response)	LOAEL, 4.4	Human	Chandra 1984	10	1	1	1	—	11	—	—
Decrease in hematocrit	LOAEL, 0.833	Human	Yadrick et al. 1989	3	1	1.43	3	—	—	2	—
Decreased ESOD and serum ferritin	LOAEL, 0.833	Human	Yadrick et al. 1989	3	1	1.43	1	—	—	5	—
Effects on immunologic status and copper status	NOAEL, 0.43	Human	Bonham et al. 2003a,b	10/√19	1	1	1	—	—	5	—

(Continued)

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TABLE 11-7 Continued

				UFs				AC (mg/L)[a]			
Toxicity End Point	Exposure (mg/kg/d)	Species	Principal Study	To NOAEL	Species	Time Extrapolation Factor	Space-flight Factor	1 d	10 d	100 d	1,000 d
Effects on immunologic status and copper status	NOAEL, 0.43	Human	Bonham et al. 2003a,b	$10/\sqrt{19}$	1	1	1	—	—	5	—
Hypertrophy of adrenal cortex and pancreatic cells	LOAEL, 70	Mouse	Aughey et al. 1977	10	10	5.55	1	—	—	—	3
Reduction in hemoglobin and in serum copper concentrations (hypocupremia)	LOAEL, 175	Rabbit, male	Bentley and Grubb 1991	10	10	6.5	3	—	—	—	2
SWEG								11	11	2	2

[a]The AC values for water are in addition to the daily contribution of dietary zinc.

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