Spacecraft Water Exposure Guidelines for Selected Contaminants: Volume 1

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

ISBN: 0-309-53074-1, 372 pages, 6 x 9, (2004)

SPACECRAFT WATER EXPOSURE GUIDELINES

FOR SELECTED CONTAMINANTS

VOLUME 1

Subcommittee on Spacecraft Exposure Guidelines
Committee on Toxicology
Board on Environmental Studies and Toxicology
Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
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This project was supported by Contract No. NAG 9-1451 between the National Academy of Sciences and the National Aeronautics and Space Administration. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the organizations or agencies that provided support for this project.

International Standard Book Number 0-309-09166-7 (Book)
International Standard Book Number 0-309-53074-1 (PDF)

Library of Congress Control Number 2004102556

Additional copies of this report are available from:

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500 Fifth Street, NW
Box 285
Washington, DC 20055

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Printed in the United States of America

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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 appears to be 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 subcommittee independently review the draft SWEG documents. In its evaluations, the subcommittee reviews the documents as many times as necessary until it is satisfied that the SWEGs are scientifically justified. This report is the first volume in the series Spacecraft Water Exposure Guidelines for Selected Contaminants. It presents the

SWEGs that have been established for chloroform, dichloromethane, di-*n*-butyl phthalate, di(2-ethylhexyl) phthalate, 2-mercaptobenzothiazole, nickel, phenol, *N*-phenyl-beta-naphthylamine, and silver.

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: Joseph F. Borzelleca, Virginia Commonwealth University; Roy DeHart, Vanderbilt University; David Gaylor, Gaylor & Associates; F. William Sunderman Jr., University of Connecticut; Mark Utell, University of Rochester School of Medicine.

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 George M. Rusch, Honeywell Corporation. 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 (NASA); Raghupathy Ramanathan, Hector Garcia, and Chiu-Wing Lam (all from Wyle Laboratories); and Jean Hampton (University of Texas) for preparing and revising the SWEG documents.

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; Kulbir Bakshi, program director for the Committee on Toxicology; and Kelly Clark, editor. We especially wish to recognize the contributions of project director Susan Martel and project assistants Tamara Dawson and Jessica Brock (through March 2002).

Finally, we would like to thank all the members of the subcommittee for their dedicated efforts throughout the development of this report. In particular, we would like to recognize the longstanding contributions and leadership of Donald Gardner, who chaired the subcommittee from September

1999 to July 2002, and who also chaired the previous Subcommittee on Spacecraft Maximum Allowable Concentrations for almost a decade.

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Chair, Subcommittee on Spacecraft Water Exposure Guidelines

Bailus Walker Jr., Ph.D., M.P.H.
Chair, Committee on Toxicology

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Spacecraft Water Exposure Guidelines for Selected Contaminants

Volume 1

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. 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 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 Subcommittee on Spacecraft Water Exposure Guidelines to address this task, and the subcommittee's first report, *Methods for Developing Spacecraft Water Exposure Guidelines,* was published in 2000 (NRC 2000b). A summary of that report is provided below.

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

of specific tasks during rare emergency conditions lasting 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 as long as 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 space flights and habitation of the ISS, water reclamation, treatment, and recycling is required. Water for long space flights 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 (e.g., by-products 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 (e.g., silver, 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 using condensate from inside the cabin as a source of drinking water could introduce some unwanted substances into the water system.

NASA's current water exposure guidelines are based on standards from the U.S. Public Health Service and the U.S. Environmental Protection Agency (EPA) for public drinking water. Those standards were established to protect the general public, including the elderly, persons with disabilities or compromised immune systems, and infants and young children. Protecting sensitive individuals is necessary and appropriate for the safety of the public health, given the likelihood of lifetime exposures. However, exposure limits for the general public are not necessarily appropriate for spacecraft flight crews. Many of the limits are likely to be overly conservative—much stricter than would be necessary to protect healthy adult astronauts even for several years away from Earth. Other limits could be inadequate—microgravity, increased radiation, or other unique attributes of spaceflight could make astronauts more sensitive to a given contaminant than they would be on Earth. Moreover, water exposure guidance levels are not available for many contaminants that might be found in spacecraft water supplies. Data collected from space-shuttle and Mir missions indicate that organic compounds found in processed water samples are vastly different from the list of target compounds developed by EPA for protection of public drinking water supplies.

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, neurotoxicity, immunotoxicity, reproductive toxicity, genotoxicity, and carcinogenicity.

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 exposures 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; the 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 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 (e.g., 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 carcinogenic and noncarcinogenic risk assessment methods is the benchmark dose (BMD) approach. The BMD is the dose associated with a specified low level of excess health risk, gener-

ally 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. Like the NOAEL and LOAEL, respectively, the $BMDL_{01}$ and $BMDL_{10}$ are starting points 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 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. 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 arrhythmia, 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, nonpregnant adults, the subcommittee 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.

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 the other effects as well.

To ensure that the SWEGs are developed in accordance with the NRC guidelines (2000b), NASA requested that the NRC subcommittee 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 subcommittee is mindful that contaminants will be present as a mixture in drinking water and the potential exists for interactions, the subcommittee was asked to consider each chemical on an individual basis. The subcommittee reviews drafts of NASA's SWEG documents and provides comments

and recommendations in a series of interim reports (see NRC 1999; 2000c,d,e; 2001). The subcommittee reviews 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 subcommittee cannot verify all the data used by NASA. The NRC subcommittee relies on NASA for the accuracy and completeness of the toxicity data cited in the SWEG reports.

This report is the first volume in the series *Spacecraft Water Exposure Guidelines for Selected Contaminants*. SWEG reports for chloroform, dichloromethane, di-*n*-butyl phthalate, di(2-ethylhexyl) phthalate, 2-mercaptobenzothiazole, nickel, phenol, *N*-phenyl-beta-naphthylamine, and silver are included in the appendix of this report. The subcommittee concludes that the SWEGs developed in those documents are scientifically valid values on the basis of the data reviewed by NASA and are consistent with the NRC guideline report. SWEG reports for additional chemicals will be presented in subsequent volumes.

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Appendixes

1 Chloroform

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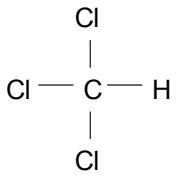
PHYSICAL AND CHEMICAL PROPERTIES

Chloroform is a nonflammable, clear, colorless, volatile and mobile, highly refractive, dense liquid with a characteristic pleasant, non-irritating odor and a slight, sweet taste (see Table 1-1) (ATSDR 1997).

OCCURRENCE AND USE

Chloroform is used as an extractant or solvent for fats, oils, greases, resins, lacquers, rubber, alkaloids, gums, waxes, gutta-percha, penicillin, vitamins, flavors, floor polishes, and adhesives. It is also used as a raw material in the chemical industry for the manufacture of chlorodifluoromethane (Freon 22), resins, and plastics; as a pharmaceutical solvent; as a dry cleaning spot remover; and as an intermediate in the manufacture of dyes and pesticides (ATSDR 1997; ACGIH 1991). In the past, chloroform was used as a general anesthetic, in fire extinguishers, and as a flavoring agent in toothpastes and cough syrups (ACGIH 1991). Trace amounts of chloroform are present in drinking water and in wastewater from sewage treatment plants as a by-product of chlorine treatment to kill bacteria. Trace levels of chloroform are also found almost ubiquitously in the environment.

TABLE 1-1 Physical and Chemical Properties of Chloroform

Formula	$CHCl_3$
Chemical name	Trichloromethane
Synonyms	Chloroform, trichloroform, formyl trichloride, methenyl chloride, methenyl trichloride, methane trichloride, methyl trichloride, NCI-C02686, Freon 20, R-20, TCM
	
CAS registry no.	67-66-3
Molecular weight	119.38
Boiling point	61.3°C
Melting point	-63.2°C
Liquid density	1.485 g/cc
Vapor density	4.36 (air = 1)
Vapor pressure	159 torr at 20°C
Solubility	1 mL dissolves in 200 mL water at 25°C
Odor threshold	2.4 ppm (water); 85 ppm (vapor)
Miscible with alcohol, benzene, ether, petroleum ether, carbon tetrachloride, carbon disulfide, and oils.	

Chloroform was detected in the space shuttle atmosphere in six of 27 missions at levels of 0.002-0.03 milligrams per cubic meter (mg/m^3) (Huntoon 1987; Huntoon 1993) and, in more recent missions, in about 10% of air samples at concentrations in the range of 0.01-0.1 mg/m^3 (James et al. 1994). Small amounts of chloroform are sometimes carried on board the space shuttle as part of mid-deck or module experiments. Drinking water on the International Space Station (ISS) will not be chlorinated, but will be iodinated or treated with silver to kill bacteria. It will be generated from recycled hygiene water, urine, and humidity condensate, and supplemented by water from the shuttle or the Russian *Progress* spacecraft. Thus, it is expected that traces of chloroform may be found occasionally in spacecraft drinking water under normal conditions.

PHARMACOKINETICS AND METABOLISM

Considerable data are available on the uptake, metabolism, and elimination of chloroform in several species. The weight of evidence indicates that chloroform is rapidly distributed throughout the body and that its toxic effects have a threshold that is dependent on the dose rate.

Absorption

Chloroform is rapidly absorbed through the gastrointestinal tract from foodstuffs and drinking water (EPA 1985). The composition and volume of the vehicle in which it is dissolved may affect the rate of absorption of orally administered chloroform. In female $B6C3F_1$ mice, the absorption and tissue dosimetry in blood, liver, and kidneys of a single dose of chloroform administered by gavage was increased in aqueous gavage vehicles compared with corn oil, but in male F-344 rats, the gavage vehicle had minimal effects (Dix et al. 1997). The absorption rate of chloroform in corn oil was decreased at dosing volumes of 10 milliliters per kilogram (mL/kg) compared with 2.5 mL/kg in both rats and mice. In aqueous 2% emulphor, a large volume of liquid was observed in the stomachs of mice at sacrifice, but not in those of rats.

Rate constants for gavage absorption were reported by Corley et al. (1990) to be $K_{aS}(hr^{-1})$, corn oil = 0.6; $K_{aS}(hr^{-1})$, water = 5.0. Absorption of chloroform through the skin is significant (329 μmol/min/cm^2 of skin exposed to the liquid) (EPA 1985).

Distribution

In humans (Smith et al. 1973) and animals (Cohen 1971; Brown et al. 1974a), chloroform absorbed either by inhalation or orally is distributed to all tissues with relative tissue concentrations of body fat > brain > liver > kidneys > blood, as expected due to the lipophilic nature of chloroform. Partition coefficients in humans were reported by Corley et al. (1990) as follows: blood/air = 7.43; liver/air = 17.0; kidney/air = 11.0; fat/air = 280; rapidly perfused tissues/air = 17.0; slowly perfused tissues/air = 12.0.

In mouse studies, the relative distribution among the organs was dependent on the route of administration, the time between dosing and measurement, and the metabolism and covalent binding of metabolites to cellu-

lar macromolecules (Taylor et al. 1974; Brown et al. 1974a). The highest levels were seen in the liver after oral dosing, probably due to a first-pass effect in which most of the chloroform is metabolized by the liver before reaching the general blood circulation.

Excretion

Chloroform was detected in the exhaled air of volunteers exposed to a normal environment, to heavy automobile traffic, or to 2 hours (h) in a dry-cleaning establishment (Gordon et al. 1988). Higher chloroform levels in the breath corresponded to higher exposure levels. The calculated biologic half-time for chloroform in breath was 7.9 h.

Excretion of radioactivity in mice and rats was monitored for 48 h following exposure to ^{14}C-labeled tracer chloroform in chloroform at 10, 89, and 366 parts per million (ppm) in mice or 93, 356, and 1,041 ppm in rats (Corley et al. 1990). In this study, 92-99% of the absorbed radioactivity was recovered in mice, and 58-98% was recovered in rats; percent recovery decreased with increasing exposure. Of the total radioactivity absorbed, the percentages recovered as exhaled ^{14}C-labeled carbon dioxide were 80-85% for mice and 48-85% for rats. After exposure, the fractions recovered as ^{14}C-labeled chloroform were 0.4-8% for mice and 2-42% for rats. The fractions recovered as urinary and fecal metabolites were 8-11% and 0.6-1.4%, respectively, for mice and 0.1% and 0.6%, respectively, for rats. A 4-fold increase in exposure concentration was followed by 50- and 20-fold increases in the amount of exhaled, unmetabolized chloroform in mice and rats, respectively. This indicates that the higher concentrations exceeded the capacity of the body to metabolize chloroform.

Metabolism

The metabolism of chloroform has been studied extensively and is understood fairly well. In humans, approximately 50% of an oral dose of 0.5 g chloroform was metabolized to carbon dioxide (Fry et al. 1972). Metabolism was dose dependent, decreasing with higher exposure. A first-pass effect was observed after oral exposure (Chiou 1975). Approximately 38% of the dose was converted in the liver, and $\leq$17% was exhaled unchanged from the lungs.

In a physiologically based pharmacokinetic (PBPK) modeling study of chloroform, Corley et al. (1990) derived in vivo metabolic rate constants

($V_{max}C$ = 15.7 mg/h/kg, K_m = 0.448 mg per liter [L]) for humans on the basis of experimental results obtained in rats and mice exposed to chloroform by inhalation and enzymatic studies in human tissues in vitro. The order of activity of liver microsomes was hamster > mouse > rat > human. Microsomes obtained from the kidneys of the various species were less active than those obtained from the livers. Virtually no activity could be detected from the three samples of human kidney tissues available; therefore, for the PBPK model, Corley et al. (1990) assumed that activity in human kidney was present at the limit of detection. Their results predicted that the "delivered doses" of chloroform, defined as the milligram equivalents of phosgene bound to macromolecules per liter of liver tissue per day, were about 10-fold lower in humans than in mice and about 5-fold lower in humans than in rats exposed to the same concentrations of chloroform in drinking water. They assume that equivalent levels of macromolecular binding produce equivalent toxicities in target tissues. The relative sensitivities of the three species (mouse > rat > human) predicted by the Corley et al. (1990) PBPK model differ markedly from those predicted by the default assumptions used by EPA. In the absence of experimental data, EPA assumes that equal concentrations in the air or water produce a 10-fold greater risk in humans than in the most sensitive tested species (mice). The Corley et al. data show that humans should have a 10-fold lower risk than mice exposed at equal chloroform concentrations.

Chloroform can be metabolized both aerobically and anaerobically as shown below (Figures 1-1 and 1-2). The production of CO_2 by the aerobic pathway accounts for up to 85% of administered chloroform in mice, 65% in rats, and lesser amounts in humans (50%) and squirrel monkeys (28%) (Brown et al. 1974a; Taylor et al. 1974). In mice, Brown et al. (1974a) found greater levels of radiolabeled chloroform in the kidneys of male mice than in females. Similarly, Culliford and Hewitt (1957) found that chloroform accumulated and metabolized in the renal cortex of males to a greater extent than in females; however, the results may have been influenced by testosterone levels. This effect was not observed in any other species. These species and gender differences in metabolism, distribution, and binding point out the limitations and difficulties in extrapolating studies in lower animals to humans.

Metabolism studies by Pohl (1979) and Stevens and Anders (1981) indicated that chloroform was exhaled from the lungs or was converted to phosgene (Pohl 1979; Stevens and Anders 1981) in the liver and kidneys by cytochrome P-450 (Branchflower et al. 1984; Smith and Hook 1984). Phosgene may react with cellular elements, inducing cytotoxicity to lipids and proteins of the endoplasmic reticulum proximate to the cytochrome P-450.

In phenobarbital-pretreated Sprague-Dawley rats, chloroform treatment yielded a covalent adduct to a single phospholipid, identified as phosphatidylethanolamine, in liver mitochondria (Guastedisegni et al. 1998). It was further demonstrated that chloroform can induce lipid peroxidation and inactivation of cytochrome P-450 in rat liver microsomes under aerobic conditions (DeGroot and Noll 1989). This mechanism may also contribute to chloroform-induced hepatotoxicity in rats, although phosgene and other active metabolites are primarily responsible. The conversion of chloroform to reactive metabolites occurs in nuclear preparations as well as in microsomes (Gomez and Castro 1980). Covalent binding of chloroform to lipids can occur under anaerobic and aerobic conditions, although binding to protein occurs only under aerobic conditions (Testai et al. 1987).

Covalent binding of chloroform metabolites to microsomal protein in vitro was increased by microsomal enzyme inducers and prevented by glutathione (GSH) (Brown et al. 1974b). It was proposed that the reaction of chloroform metabolites with GSH might act as a detoxifying mechanism. Phosgene may combine with two molecules of GSH to form diglutathionyl dithiocarbonate, which is further metabolized in the kidneys (Sipes et al. 1977; Wolf et al. 1977). Chloroform doses that caused liver GSH depletion produced liver necrosis (Docks and Krishna 1976). Furthermore, chloroform has been found to be more hepatotoxic in fasted animals, possibly due to decreased GSH content in the liver (Brown et al. 1974b; Docks and Krishna 1976; Wang et al. 1995).

Evidence that chloroform is metabolized at its carbon-hydrogen bond is provided by experiments that used the deuterated derivative of chloroform (McCarty et al. 1979; Pohl et al. 1980; Branchflower et al. 1984). Deuterated chloroform is one-half to one-third as cytotoxic as chloroform, and its conversion to phosgene is much slower. The results confirmed that the toxicity of chloroform to the liver and kidneys is due primarily to its metabolites (ATSDR 1997).

The final product of the aerobic metabolic pathway of chloroform is carbon dioxide (Fry et al. 1972; Brown et al. 1974a), which is mostly eliminated through the lungs, but some is incorporated into endogenous metabolites and excreted as bicarbonate, urea, methionine, and other amino acids (Brown et al. 1974a). Inorganic chloride ion is an end-product of chloroform metabolism found in the urine (Van Dyke et al. 1964). Carbon monoxide was a minor product of the anaerobic metabolism of chloroform in rats in vitro (Ahmed et al. 1977) and in vivo (Anders et al. 1978; ATSDR 1997).

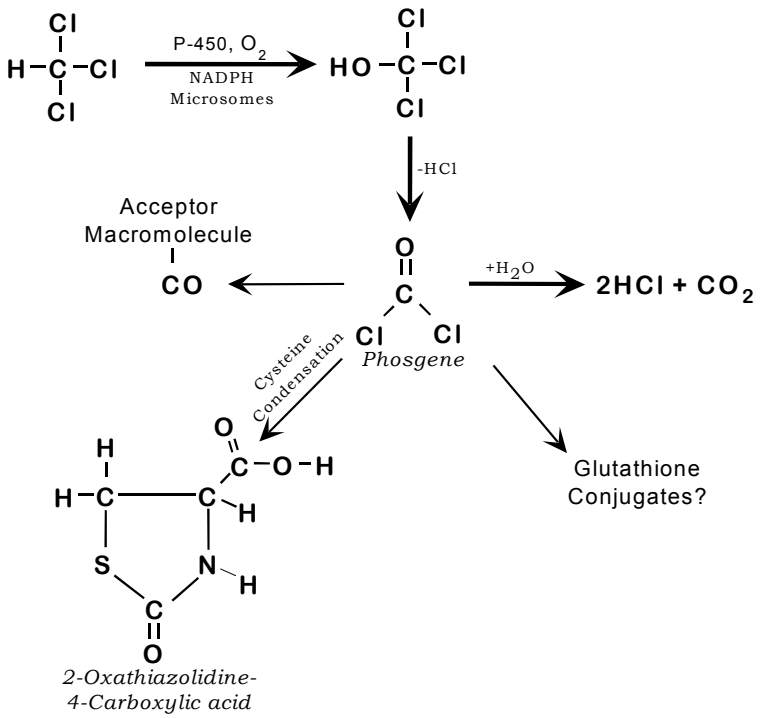


FIGURE 1-1 Major pathway (aerobic). Source: Redrawn from ATSDR 1997.

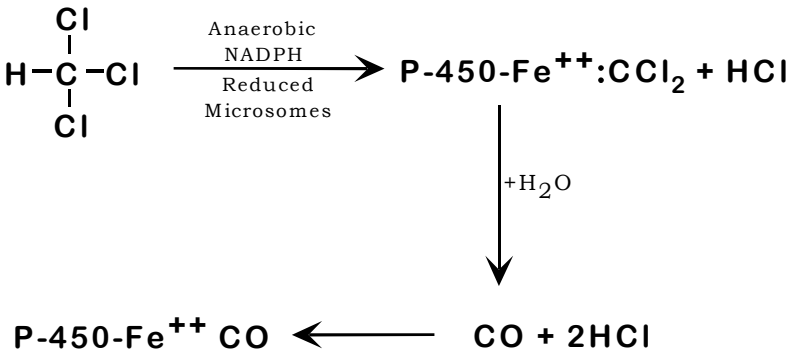


FIGURE 1-2 Minor pathway (anaerobic). Source: Redrawn from ATSDR 1997.

A recent in vitro study of hepatic microsomes in mice indicated that a reductive pathway might play an important role in chloroform hepatotoxicity (Testai et al. 1990). It was demonstrated that radical chloroform metabolites bind to macromolecules (i.e., proteins, lipids), and the process can be inhibited by reduced GSH (ATSDR 1997).

Interspecies differences in the rate of chloroform conversion were observed in mice, rats, and squirrel monkeys. The conversion of chloroform to carbon dioxide was highest in mice (85%) and lowest in squirrel monkeys (28%) (Brown et al. 1974a). Similarly, because of the lower relative rates of chloroform metabolism, ventilation, and cardiac output (per kilogram body weight) in the larger species, physiologically based pharmacokinetic (PBPK) calculations indicated that exposure to equivalent concentrations of chloroform vapor would lead to a lower delivered dose of active metabolites in humans compared with rats; rats would have a lower delivered dose than mice (Corley et al. 1990; ATSDR 1997).

TOXICITY SUMMARY

Although high concentrations of inhaled chloroform vapor have pronounced effects on the central nervous system (CNS), most of which are reversible upon cessation of exposure, there are no reports of CNS effects resulting from exposure to chloroform in drinking water. This is true even at concentrations high enough to render the water unpalatable to very thirsty rodents. This lack of CNS effects is due to a first-pass effect in which most of the chloroform is metabolized by the liver before reaching the general blood circulation. Other reported effects of exposure to high vapor concentrations include cardiac arrhythmias, immune system depression, and, in rats, nasal lesions. None of these effects have been reported for oral exposures. Short-term exposure to high levels by various routes, including oral, causes liver necrosis and kidney degeneration. Long-term exposure to levels high enough to cause cytotoxicity may lead to liver or kidney cancer.

Acute Toxicity (≤1 d)

Hepatotoxicity

The liver has been shown to be the primary toxicity target of ingested chloroform in humans. Ingestion of chloroform at approximately 3,755 mg/kg produced jaundice, liver enlargement and tenderness, increased

levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and lactate dehydrogenase activities, and increased bilirubin levels in an individual who died of chloroform poisoning (Piersol et al. 1933). Autopsy revealed fatty degeneration and extensive centrilobular necrosis.

The hepatotoxicity of chloroform is believed to be due to the production of reactive chloroform metabolites (e.g., phosgene) by cytochrome P-450 and is modulated by hepatic GSH. Brown et al. (1974b) found that exposure of rats to chloroform for 2 h at either 5,000 or 10,000 ppm produced hepatic necrosis and destruction of microsomal enzymes. Pretreatment of rats with phenobarbital to induce microsomal enzyme activity before exposure to chloroform markedly increased the hepatotoxic response to anesthesia. It also produced a 70-80% decrease in hepatic GSH levels compared with uninduced rats in which chloroform exposure resulted in neither depletion of GSH nor in hepatic necrosis at 24 h after exposure (Brown et al. 1974b). Experimental depletion of hepatic GSH by pretreatment with diethyl maleate also resulted in centrilobular necrosis after exposure to chloroform (Brown et al. 1974b).

In cytotoxicity studies at CIIT, freshly isolated hepatocytes cultured from $B6C3F_1$ mice and F-344 rats were exposed to solutions of chloroform for up to 3 h. Concentration-dependent cytotoxicity (lactate dehydrogenase release) was seen in culture at concentrations higher than 1 millimolar (mM) (Ammann et al. 1998). Co-treatment with the cytochrome P-450 inhibitor 1-phenylimidazole prevented both cytolethality and GSH depletion, indicating that metabolism is necessary for chloroform-induced cytotoxicity. These results correlate well with simulations of a physio-logically based dosimetry model for chloroform, which indicated that the livers of mice and rats were exposed to chloroform concentrations up to 5 mM for 3 h after hepatotoxic doses of chloroform (Ammann et al. 1998). The high (>1 mM) concentrations of chloroform necessary to produce hepatotoxicity can be achieved only by bolus dosing, such as gavage. Drinking water exposure results in much lower hepatic chloroform concentrations than bolus gavage and eliminates the hepatotoxic effects (Larson et al. 1994b).

Nephrotoxicity

Unpublished studies at CIIT (B. Butterworth, CIIT, personal commun., Sept. 21, 1998) using mice in which the CYP-450 IIE1 gene had been inactivated showed that the metabolism of chloroform was completely eliminated. Thus, chloroform metabolism in mice is entirely dependent on cyto-

chrome P-450 IIE1 enzyme activity. Because humans have this enzyme in the liver but not in detectable amounts in the kidneys (Corley et al. 1990), it is unlikely that chloroform toxicity to the kidneys would be significant in humans. This is supported by the lack of reported kidney toxicity in humans except in some older case reports of individuals receiving very high doses. In one case, ingestion of chloroform at approximately 3,755 mg/kg produced oliguria after 1 day (d) and increased blood urea nitrogen, creatinine levels, urinary casts, and albuminuria in an individual who died of chloroform poisoning (Piersol et al. 1933). The autopsy revealed epithelial swelling and hyaline and fatty degeneration in the convoluted tubules of the kidneys. Oliguria was observed 1 d after ingestion of chloroform at 2,410 mg/kg in one nonfatal case (Schroeder 1965)

Chloroform induces kidney toxicity in rodents that can be more or less severe than the liver toxicity induced by the same treatment depending on the species and strain. F-344 rats treated by gavage with a single dose of chloroform at 34, 180, or 477 mg/kg in corn oil exhibited a dose-dependent mild to severe necrosis of the proximal tubules of the kidneys (Larson et al. 1993). In contrast, female $B6C3F_1$ mice treated by gavage with a single dose of chloroform at 34, 238, or 477 mg/kg in corn oil exhibited no renal lesions (Larson et al. 1993). The labeling index of the proximal tubule cells was increased 20-fold in rats at 180 mg/kg but only 2-fold in mice at 350 mg/kg. As described above, hepatotoxicity in these same experiments displayed a very different pattern, with only slight to moderate hepatotoxicity in the rats, but dose-dependent centrilobular hepatic necrosis in the mice (Larson et al. 1993).

In certain strains of mice, renal tubular necrosis was reported in 100% of males after exposure to chloroform at $\geq$240 ppm for 2 h (Derringer et al. 1953; Culliford and Hewitt 1957). Mice surviving the exposure were found to have tubular calcifications when examined 12 months (mo) after the exposure. The kidneys of female mice of the susceptible strains and of both male and female mice of other strains were completely unaffected. Females of the susceptible strains became susceptible when treated with testosterone, and immature males and castrated males were resistant to chloroform nephrotoxicity (Culliford and Hewitt 1957). Although this phenomenon is scientifically interesting, it is not a good model for the susceptibility of humans to chloroform nephrotoxicity, because clinical experience with thousands of patients who have undergone chloroform anesthesia (exposure at 8,000-22,000 ppm) suggests a low incidence of nephrotoxicity in humans (Whitaker and Jones 1965; Lieberman 1973; Smith et al. 1973).

Cardiac Effects

Chloroform ingestion is not associated with cardiac toxicity in humans or animals. In a patient who accidentally ingested chloroform at about 2,500 mg/kg, an electrocardiogram showed only occasional extrasystoles and a slight S-T segment depression. After recovery there was no persistent cardiovascular change (Schroeder 1965).

Short-Term Toxicity (2-10 d)

Hepatotoxicity and Reduced Water Consumption

Larson et al. (1994b) at CIIT found no increases in hepatocyte labeling index, nor any macroscopic or histologic changes in the livers of female B6C3F_1 mice given drinking water containing chloroform at 0, 60, 200, 400, 900, or 1,800 ppm for 4 d or 3 weeks (wk). The only observed effect on the liver was reduced eosinophilic staining of the cytoplasm of centrilobular hepatocytes compared with periportal hepatocytes and controls. That was seen at doses of 400 ppm and above in two of five, eight of 10, and four of five mice, respectively, and was transient, being observed only at 4 d of exposure, but not at 3 wk. It is not considered an adverse effect. There was, however, a dose-related reduction in water consumption at chloroform concentrations of 200 ppm and above during the first week of exposure, presumably due to initial taste aversion, with recovery to near control levels for the remainder of the study period. No reduction in consumption was seen at 60 ppm.

In another study, Larson et al. (1994c) found mild degenerative changes in centrilobular hepatocytes of male B6C3F_1 mice after 4 d of treatment with 34 mg/kg/d or 90 mg/kg/d by gavage in corn oil, but these changes were absent at 3 wk of treatment. At higher doses (138 mg/kg/d or 277 mg/kg/d), centrilobular necrosis was observed at 4 d of treatment, and increased severity of necrosis was observed at 3 wk. The labeling index in the livers of mice was increased in a dose-dependent manner at all chloroform doses following 4 d of treatment and in the 277-mg/kg/d dose group following 3 wk of treatment, but declined in the lower-dose groups and was no longer elevated above controls at 3 wk in the 34-mg/kg/d or 90-mg/kg/d dose groups.

Cell proliferation was inhibited in female B6C3F_1 mice given chloroform at 1,800 ppm in drinking water for 5 d, whereas it was enhanced if the

chloroform was administered by gavage in corn oil (Pereira 1994). Mice given chloroform in drinking water and in corn oil by gavage or given corn oil alone by gavage did not have increased hepatic labeling indices (Pereira 1994). Thus, gavage studies in corn oil do not yield results that accurately model the effects of chloroform administered in drinking water.

Nephrotoxicity

In female $B6C3F_1$ mice receiving chloroform in drinking water at concentrations of 0, 60, 200, 400, 900, or 1,800 ppm, a slight increase (no more than twice that of controls) was observed in the labeling index in the kidney in some groups (Larson et al. 1994b). The increases were restricted to the straight portions of the proximal tubules mainly in the outer stripe of the outer medulla. At 4 d of exposure, the labeling index in the cortex was decreased, although there was an increase in the labeling index in the outer medullary region. This trend had disappeared by 3 wk of treatment.

In contrast, treatment of male $B6C3F_1$ mice by gavage in corn oil at somewhat lower daily doses produced marked nephropathy. Larson et al. (1994c) found a dose-related acute tubular necrosis in the kidneys of male $B6C3F_1$ mice after 4 d of treatment by gavage with chloroform at 0, 34, 90, 138, or 277 mg/kg/d in corn oil. After 3 wk of dosing, regenerating tubules were observed in the lower dose groups, while mice treated with 277 mg/kg/d had severe nephropathy characterized by degeneration, necrosis, and regeneration affecting all the proximal tubules. The labeling index in the proximal tubules was increased at all doses after 4 d of treatment. At the end of 3 wk of dosing, the labeling index in the renal cortex had decreased at all dose levels and was no longer significantly different from controls in the 34 mg/kg/d and 90 mg/kg/d groups.

In laboratory animals, susceptibility to chloroform-induced nephrotoxicity varies greatly with species, strain, and gender. This is illustrated in a number of studies involving inhalation exposures in rodents. In BDF_1 mice, Templin et al. (1996a) found degenerative lesions and a 7- to 10-fold increase in the percentage of cells in S phase in kidneys of males, but not females, inhaling chloroform at 30 ppm or 90 ppm for 6 h/d, 5 d/wk, for 2 wk. In the 2-wk exposure groups, 40% of the 30 ppm group and 80% of the 90 ppm group died with severe kidney damage, indicating that both 30 ppm and 90 ppm exceeded the maximum tolerated dose. Male BDF_1 mice exposed to chloroform vapors 6 h/d for 4 d at 0, 0.3, 5, 30, or 90 ppm had a LOAEL (lowest observed adverse effect level) of 30 ppm and a NOAEL

(no observed adverse effect level) of 5 ppm for necrosis of the proximal convoluted tubules, tubule dilation, accumulation of hyaline casts, and focal mineralization of the kidneys (Templin et al. 1996a). Female BDF_1 mice showed no kidney toxicity at any tested dose up to 90 ppm. The NOAEL for male BDF_1 mice inhaling chloroform for 4 d, 6 h/d, was 5 ppm. In contrast, female $B6C3F_1$ mice were more resistant to chloroform nephrotoxicity and had a NOAEL of 100 ppm for a 6 h/d, 7 d inhalation exposure (Larson et al. 1994a). Similarly exposed male F-344 rats had a LOAEL of 30 ppm and a NOAEL of 10 ppm for treatment-induced kidney cell proliferation (Larson et al. 1994a). About 25-50% of the proximal tubules were lined by regenerating epithelium in the kidneys of male F-344 rats (female rats were not tested) and female $B6C3F_1$ mice (male mice were not tested) inhaling chloroform at 300 ppm for 6 h/d for 7 d, but not in those inhaling 100, 30, or 10 ppm (Larson et al. 1994a).

Subchronic Toxicity (11-100 d)

Subchronic exposure to chloroform has been shown to be toxic to the liver and/or kidneys of several species, including humans. High concentrations of chloroform in drinking water have also caused test animals to avoid drinking the water, in some cases to the point of death.

Reduced Water Consumption

Female $B6C3F_1$ mice given drinking water containing chloroform at 0, 60, 200, 400, 900, or 1,800 ppm had a transient dose-dependent depression of body weight due to dose-dependent decreases in water consumption at chloroform concentrations of $\geq$200 ppm (Larson et al. 1994b). The average daily doses (mg/kg/d) for the first 4 d of exposure were 0, 16.0, 26.4, 53.5, 80.9, and 105 mg/kg/d. By 3 wk of exposure, however, the average daily doses had increased to 0, 15.7, 42.7, 82.5, 184, and 329 mg/kg/d (Larson et al. 1994b).

Hepatotoxicity

Female $B6C3F_1$ mice given gavage doses of chloroform at 1, 3, 10, 34, 90, 238, or 477 mg/kg in corn oil 5 d/wk for 4 d or 3 wk had dose-depend-

ent changes, including centrilobular necrosis and markedly elevated labeling index (LI) (Larson et al. 1994b). In a parallel assay, mice given drinking water containing 0, 60, 200, 400, 900, or 1,800 ppm for 4 d or 3 wk had no increase in hepatic LI nor any microscopic alterations in livers except for tinctorial changes (reduced eosinophilic staining of the cytoplasm of centrilobular hepatocytes) evident at 4 d but not at 3 wk (Larson et al. 1994b). Due to initial aversion of the mice to drinking water containing chloroform at ≥200 ppm, the average daily dose (mg/kg/d) for the first 4 d of exposure was reduced (see "Reduced Water Consumption" section).

Cell proliferation was inhibited in female $B6C3F_1$ mice given chloroform at 1,800 ppm in drinking water for 12 d, whereas it was enhanced if the chloroform (263 mg/kg/d) was administered by gavage in corn oil (Pereira 1994). Mice given chloroform in drinking water and in corn oil by gavage or given corn oil alone by gavage did not show increased hepatic labeling indices (Pereira 1994).

Pereira (1994) found that hepatotoxicity in female $B6C3F_1$ mice, as measured by cell proliferation, was increased if the chloroform (263 mg/kg/d) was administered in corn oil by gavage daily for up to 159 d, whereas it was decreased for a similar daily dose (248 mg/kg/d) administered in drinking water at 1,800 ppm. In drinking water, chloroform inhibited cell proliferation when measured at days 5 and 12 of treatment but had no effect when measured at days 33 and 159 of treatment. In contrast, chloroform (263 mg/kg/d) administered in corn oil was toxic to liver cells, producing centrilobular necrosis, swollen hepatocytes, and increased cell proliferation when measured at days 5, 12, 33, and 159 of treatment. These results were confirmed by Larson et al. (1994b) under conditions similar to those used in earlier bioassays that had shown an increased incidence of liver tumors induced by chloroform when administered by gavage in corn oil but not when given in drinking water at similar daily doses (238 mg/kg/d or 477 mg/kg/d in corn oil, 329 mg/kg/d in drinking water).

Bull et al. (1986) reported that male and female $B6C3F_1$ mice given chloroform by gavage at doses of 60, 130, and 270 mg/kg/d for 90 d developed more marked hepatotoxic effects when the chloroform was in corn oil than when it was in an aqueous suspension (2% emulphor).

Rats exposed to chloroform in drinking water at doses of 0.64-150 mg/kg/d for 90 d showed no significant liver toxicity (Chu et al. 1982a). Bull et al. (1986) found no liver effects in mice treated with chloroform at 50 mg/kg/d in drinking water for 90 d. Jorgenson, however, reported that mice given chloroform at 64 mg/kg/d in drinking water for 90 d developed

centrilobular fatty changes that appeared to be reversible (Jorgenson and Rushbrook 1980). Klaunig reported that mice given chloroform at 86 mg/kg/d in drinking water for 1 year (y) developed fatty and hydropic changes, necrosis, and cirrhosis (Klaunig et al. 1986).

Eight male and eight female Beagle dogs per dose group exposed to chloroform at 1, 15, or 30 mg/kg/d in toothpaste capsules 6 d/wk for 7.5 y showed significantly increased SGPT activity in the 30 mg/kg/d group beginning at week 6 and in the 15 mg/kg/d group beginning at week 150 (Heywood et al. 1979).

Rats given drinking water containing chloroform at 500 ppm for 28 d (11 mg/rat/d) had decreased neutrophil counts but no histopathology or hepatic enzyme changes (Chu et al. 1982a). Rats receiving drinking water containing chloroform at 50 ppm (1.3 mg/rat/d) for 28 d had no decrease in neutrophil counts or any other adverse effects (Chu et al. 1982a).

Nephrotoxicity

The effects on the kidneys of chronic exposure to chloroform depend heavily on the gender, strain, and species being exposed as well as the exposure schedule. In a series of studies at CIIT, male F-344 rats and male B6C3F_1 mice exposed to chloroform vapors 7 d/wk for 13 wk had LOAELs for kidney toxicity of 30 ppm, whereas female F-344 rats and female B6C3F_1 mice had NOAELs of 90 ppm (Larson et al. 1996; Templin et al. 1996b). When the exposure schedule was 5 d/wk for 13 wk, the LOAEL increased for male F-344 rats from 30 ppm to 90 ppm but decreased for male B6C3F_1 mice from 30 ppm to 10 ppm (Larson et al. 1996; Templin et al. 1996b).

Thyroid Toxicity

Weanling Sprague-Dawley rats were given drinking water containing chloroform at 0, 5, 50, 500, or 2,500 ppm for 90 d (Chu et al. 1982b). Half the rats were killed at 90 d, and the remaining animals were given tap water for an additional 90 d before sacrifice. In the highest dose group, there was decreased food intake and growth rate and a high incidence of lethality. Another significant finding was mild to moderate thyroid lesions seen only in males at the highest dose, with some recovery within 90 d.

Chronic Toxicity (≥101 d)

Hepatotoxicity and Nephrotoxicity

An individual who ingested 21 mg/kg/d chloroform in cough medicine for 10 y suffered impaired liver function as indicated by increased retention of sulfobromophthalein, but the hepatotoxicity was reversed after cessation of medication (Wallace 1950). In studies of chloroform-containing dentifrice and mouthwash, long-term ingestion of low levels of chloroform has been shown to be without effects in humans (De Salva et al. 1975). Men and women (n = 59) exposed twice daily for 5 y to 1 g of dentifrice containing 3.4% chloroform showed no increases in SGOT, SGPT, BUN, or serum alkaline phosphatase (SAP) levels. Calculations of the amounts ingested assumed that the subjects ingested 25% of the dentifrice yielding exposures of 0.34 mg/kg/ d. Similarly, no hepatotoxicity or kidney toxicity were seen in a subsequent 1 y study of subjects receiving an estimated dose of 0.96 mg/ kg/d, assuming ingestion of 25% (De Salva et al. 1975). Subjects (n = 57) were exposed twice daily to both 1 g of a dentifrice containing 3.4% chloroform and 15 mL of a mouthwash containing 0.425% chloroform.

Torkelson et al. (1976) exposed rats, guinea pigs, and rabbits for 7 h/d, 5 d/wk, for 6 mo to chloroform vapors at 85, 50, or 25 ppm and exposed dogs similarly at 25 ppm. Additional groups of male rats were exposed for 4, 2, or 1 h/d at 25 ppm. In rabbits exposed at 85 ppm, females had cloudy swelling in the kidneys (Torkelson et al. 1976). Similar findings were observed in both male and female rats of the 85 ppm group. At 50 ppm, no adverse effects were found in guinea pigs or rabbits, although in rats the effects were similar to but milder than those at 85 ppm, and female rats were affected less than males. At 25 ppm, male rats exposed for 7 h/d exhibited cloudy swelling of the renal tubular epithelium. Those effects were reversible within 6 wk after cessation of the exposure. No adverse effects were seen in male rats exposed at 25 ppm for 4, 2, or 1 h/d. In female rats exposed 7 h/d at 25 ppm, the relative weights, but not the absolute weights, of kidney and spleen were significantly increased. All other parameters were normal. At 25 ppm, male guinea pigs showed interstitial and tubular nephritis in the kidneys, and female guinea pigs showed foamy vacuolization centrally in the liver and significantly higher absolute and relative kidney weights, contrary to what had been observed at higher concentrations. Rabbits showed only an increase of interstitial and tubular nephritis in males and slight microscopic changes in the lungs, liver, and

kidneys in females. Male dogs exposed at 25 ppm showed no changes, but female dogs exhibited microscopic pathologic changes in the kidneys.

Cardiovascular Effects

No cardiovascular changes were reported in dogs receiving chloroform at up to 30 mg/kg/d in toothpaste capsules for 7 y (Heywood et al. 1979) or in rats and mice chronically treated by gavage with chloroform at 200 mg/kg/d and 477 mg/kg/d, respectively (NCI 1976).

Carcinogenicity

Cancer in Humans

There are no reports of chloroform-induced cancers in individual humans, despite extensive human exposure as a result of its use in the past in industry, as an anesthetic, and as an ingredient in medicinals. Numerous epidemiological studies have been conducted to examine the correlation between the concentration levels of organic compounds (including chloroform) in U.S. drinking waters and increased cancer mortality. A 1981 study of nearly 31,000 subjects in Maryland reported a tendency toward increased rates of bladder cancer in men and liver cancer in women who were supplied with chlorinated surface water at home, but the differences were not statistically significant. A 1992 epidemiology report suggests that consumption of chlorination by-products in drinking water is associated with an increased risk of rectal and urinary bladder cancers (Morris et al. 1992). A 1982 study of Louisiana subjects found an increased risk for rectal cancer, but not for colon cancer, in those using chlorinated Mississippi water (Gottlieb and Carr 1982). A 1984 study showed no correlation between trihalomethanes in drinking water in New York state and colorectal cancer (Lawrence et al. 1984). In contrast, a 1981 study of Wisconsin female cancer mortality (Young et al. 1981) found that colon cancer was significantly associated with chlorination of drinking water. Likewise, a 1997 epidemiology study found a clear dose-response relationship between increasing levels of chlorination by-products in finished drinking water in Iowa and an increased risk of colon cancer in postmenopausal women (Doyle et al. 1997). These epidemiology studies, nevertheless, are of lim-

ited usefulness for assessing the potential carcinogenicity of chloroform in drinking water because they cannot specify which of the chlorination by-products is responsible for the increased cancer incidence.

Cancer in Animals

In animal studies, high doses of chloroform have been shown to induce cancer in the liver and kidneys of mice and rats. Eschenbrenner and Miller (1945) first reported that chloroform was carcinogenic to mice when administered by gavage in olive oil. Since then, numerous animal studies have shown that chloroform can be carcinogenic when given orally. The ability to induce cancer varied with the species, strain, and gender of the exposed animals and with the rate (bolus vs intermittent) at which the chloroform was delivered.

Jorgenson et al. (1985) exposed male Osborne-Mendel rats and female B6C3F_1 mice for 104 wk to chloroform at 0, 200, 400, 900, and 1,800 mg/L in drinking water. Mice exposed to a time-weighted average (TWA) chloroform dose in drinking water of 263 mg/kg/d for 104 wk did not have an increase the incidence of hepatocellular carcinomas and adenomas (Jorgenson et al. 1985). Rats exposed to chloroform at 160 mg/kg/d in drinking water had an increased incidence of kidney tubular cell adenoma and carcinoma, but those exposed at 81 mg/kg/d did not (Jorgenson et al. 1985). Similarly, female Wistar rats exposed for a lifetime to chloroform in drinking water at 200 mg/kg/d had an increased incidence of hepatic neoplastic nodules, and lymphosarcoma was increased in males (Tumasonis et al. 1987).

In studies involving inhalation exposures to chloroform vapors, however, striking differences are seen in the organ specificity between species and between different strains of the same species. Long-term inhalation of high doses of chloroform vapors has been shown to induce kidney cancer in male BDF_1 mice (Yamamoto et al. 1994). In contrast, F-344 rats, both male and female, inhaling chloroform at 10, 30, or 90 ppm for 5 d/wk for 2 y developed no tumors (Yamamoto et al. 1994). Studies were performed at CIIT under conditions used in the Japanese bioassay to elucidate the mechanisms for this lack of carcinogenicity in F-344 rats. Under these conditions, F-344 rats showed only a marginal increase in cell proliferation in the kidneys of males and no treatment-induced histopathology or cell proliferation in the kidneys of females except at a highly toxic dose of 300 ppm, 7 d/wk (Templin et al. 1996b). In BDF_1 mice, however, cancer induc-

tion appeared to correlate with cytotoxicity. Chloroform was found to be cytotoxic to both the liver and the kidneys in BDF_1 mice as well as in $B6C3F_1$ mice; however, BDF_1 mice develop only kidney tumors, not liver tumors. In contrast, $B6C3F_1$ mice develop liver tumors, but no kidney tumors. From this, Templin et al. (1996b) concluded that induced toxicity and regenerative cell proliferation are necessary but not sufficient to induce cancer in a given target organ. In a 1996 study, Larson et al. found a NOAEL of 10 ppm for increases in the labeling indices (LI) of liver cells in female and male $B6C3F_1$ mice exposed to chloroform at 0, 0.3, 2, 10, 30, and 90 ppm for 6 h/d, 7 d/wk, for up to 13 wk and proposed that this should also be a NOAEL for liver cancer in female $B6C3F_1$ mice. In other words, chloroform carcinogenicity should have a threshold if tumorigenesis is dependent on regenerative cell proliferation. This proposal was challenged in a 1998 study by Melnick et al. They argue that tumors can be produced at low doses of other trihalomethanes that do not produce increases in LI (i.e., tumorigenesis is not dependent on regenerative cell proliferation at low doses) and assert that this is therefore true for chloroform also (Melnick et al. 1998). The other trihalomethanes they used to support this contention, however, are DNA reactive, whereas chloroform is not, thus weakening their argument.

In a subsequent paper, Templin et al. (1998) reported a NOAEL of 5 ppm for nephrotoxicity, cell proliferation, and cancer in BDF_1 mice inhaling chloroform vapors for 2 y at concentrations of 5, 30, or 90 ppm for 6 h/d, 5 d/wk.

Male Osborne-Mendel rats exposed to chloroform at 90 mg/kg/d by gavage for 78 wk developed kidney tubular cell adenomas and carcinomas (NCI 1976). Sprague-Dawley rats, however, when exposed to chloroform in toothpaste at 60 mg/kg/d and 165 mg/kg/d by gavage for 80 wk and 52 wk, respectively, did not have an increased incidence of tumors (Palmer et al. 1979).

Eight male and eight female Beagle dogs exposed to chloroform at 1, 15, or 30 mg/kg/d in toothpaste capsules 6 d/wk for 7.5 y had no increase in tumor incidence (Heywood et al. 1979).

Mice exposed by gavage to chloroform at 595 mg/kg/d in oil for 30 d had an increased incidence of hepatomas, while those receiving 297 mg/kg/d did not (Eschenbrenner and Miller 1945). Mice exposed by gavage to chloroform at 1,800 mg/kg/d in oil for 8 wk had no increase in lung tumors (Stoner et al. 1986). Mice exposed at 257 mg/kg/d in drinking water for 52 wk had no increase in tumors (Klaunig et al. 1986).

ICI mice chronically exposed by gavage to chloroform at 60 mg/kg/d in a toothpaste base had an increased incidence of kidney tumors, but those exposed at 17 mg/kg/d did not (Roe et al. 1979). The overall incidence of all tumors, however, was lower in mice receiving the highest dose of chloroform than in controls. No significant differences were seen in the incidence or severity of nephrotoxicity in mice with kidney tumors and those without tumors. Under the same conditions, C57Bl, CBA, and CF/1 mice had no change in the frequency of tumors (Roe et al. 1979). Thus, the significance of the increased incidence of kidney tumors in ICI mice is questionable.

$B6C3F_1$ mice exposed by gavage to chloroform in oil at $\geq$138 mg/kg/d for 78 wk developed hepatocellular carcinomas (NCI 1976). In contrast, $B6C3F_1$ mice exposed to chloroform in drinking water at 263 mg/kg/d for 2 y had no increase in tumor incidence (Jorgenson et al. 1985). Examination of $B6C3F_1$ mice exposed to chloroform in drinking water at up to 1,800 ppm for 4 d or 3 wk revealed no increase in liver cell proliferation (Larson et al. 1994b). In contrast, those given chloroform in corn oil at 238 mg/kg or 477 mg/kg had both centrilobular necrosis and markedly elevated regenerative cell proliferation (Larson et al. 1994c). These studies support the mechanistic-based idea that chloroform's carcinogenicity depends on its capacity to induce necrosis and regenerative cell proliferation. For the liver, Larson et al. (1994c) propose that "the most straightforward risk assessment for chloroform for this tissue would assign no increased cancer risk for dosing regimens that do not induce cytolethality and cell proliferation." The NOAEL for histopathologic changes in liver and kidneys of $B6C3F_1$ mice for chloroform in corn oil was 10 mg/kg/d and for induced cell proliferation was 34 mg/kg/d (Larson et al. 1994c).

Genotoxicity

No data were found on the genotoxicity of inhaled or ingested chloroform in humans.

A number of laboratories have tested chloroform for mutagenicity in *Salmonella* and *E. coli* using a wide range of concentrations with and without metabolic activation. Rosenthal (Rosenthal 1987) critically reviewed these studies and, despite noting some deficiencies in experimental procedures, concluded that chloroform is not mutagenic in bacteria—a conclusion that the current literature still supports (Roldan-Arjona and Pueyo 1993; Pegram et al. 1997).

Tests of chloroform's mutagenicity in various eukaryotes have given mixed results. Callen et al. (1980) obtained only marginal effects in yeast for mitotic gene conversion and crossing over, as well as gene reversion, at chloroform concentrations of 21, 41, and 54 mM for 1 h. Crebelli et al. (1988, 1992) report the induction of aneuploidy by a threshold concentration of 0.16% v/v in the fungus Aspergillus nidulans, but this result is questionable because aneuploidy was not found at 0.20%.

Sturrock (1977) found that chloroform did not cause mutations at the HGPRT locus in Chinese hamster lung fibroblasts exposed to a 1-2.5% solution for 24 h, but no metabolic activation was used. Both negative results (White et al. 1979; Kirkland et al. 1981) and positive results (Morimoto and Koizumi 1983) have been reported for induction of sister chromatid exchanges in human lymphocytes in vitro and mouse bone marrow in vivo, but some aspects of the procedures used preclude reaching definitive conclusions.

In a tightly controlled study of mutation at the thymidine kinase gene in the L5178Y TK+/- mouse lymphoma cell, Mitchell et al. (1988) report mixed results in experiments with and without metabolic activation. The mutant colonies may have resulted from chromosome loss (aneuploidy).

No increase in lacI mutant frequency was seen in hepatocytes isolated from $B6C3F_1$ lacI transgenic mice exposed by inhalation to chloroform at 0, 10, 30, or 90 ppm for 6 h/d, 7 d/wk for up to 180 d (Butterworth et al. 1998a).

No data have been reported that used tests recently designed for unequivocal detection of aneuploidy caused by chloroform, but, like other anesthetics, chloroform can disrupt the microtubules in the spindle of dividing cells. Depolymerization of tubulin is involved in this action (Liang et al. 1983), and low doses of depolymerizing agents can cause one or several chromosomes to come off the spindle, leading to aneuploid daughter cells. Chromosomes not attached to the spindle may form micronuclei, and that is probably the reason there have been reports that chloroform causes small, statistically insignificant increases in micronuclei frequency, interpreted to be chromosome aberrations (e.g., Agustin and Lim-Sylianco 1978; Gocke et al. 1981). A 3-fold increase in the frequency of micronucleated kidney cells was reported by Robbiano et al. (1998) in male Sprague-Dawley rats given a single oral dose of chloroform in corn oil at 4 mmol/kg (476 mg/kg).

Land et al. (1981) found statistically significant increases in the percentages of abnormal sperm heads in mice exposed 4 h/d for 5 d to reagent grade chloroform at 400 or 800 ppm. This may be caused by one or more

mutations, because abnormal sperm head shape in the mouse has been shown to be determined by genes. However, because in the early stages of sperm development (spermatids) cytoplasmic microtubules are still present, it might also be caused by depolymerized tubulin.

In summary, there are no convincing data that chloroform causes gene mutation or chromosome aberrations. It is more probable that it or its metabolic products act on proteins and not on DNA (Mersch-Sundermann et al. 1994) and are, therefore, likely to be aneugenic, but there are not yet definitive studies on that point. If it is an aneugen, it would have a concentration threshold for effect and would show a plateau at higher concentrations.

Reproductive Toxicity

No studies were found regarding reproductive effects in humans after exposure to chloroform. Data concerning the effects of chloroform on fertility in animals are inadequate for an assessment. A 1988 NTP study by Gulati et al. (1988) found that fertility was not affected in either of two generations of mice exposed by gavage to chloroform in corn oil at up to 41 mg/kg/d for 105 d, but at those doses, sperm morphology was not affected. In mice exposed to chloroform by inhalation 4 h/d for 5 d at 400 or 800 ppm, Land et al. (1981) found statistically significant increases in the percentages of abnormal spermatozoa, but no mating studies were done. In contrast, mice receiving five daily intraperitoneal injections of chloroform at 0.025, 0.05, 0.075, 0.1, and 0.25 mg/kg/d showed only nonreproducible, sporadic, small increases in abnormal sperm (Topham 1980).

Developmental and Fetal Toxicity

No studies were found regarding developmental effects in humans after exposure to chloroform. In rats exposed during gestation, chloroform-induced fetotoxicity and teratogenicity (decreased fetal crown-rump length and delayed ossification) were observed by Schwetz et al. (1974), but only at concentrations that produced maternal toxicity, with a LOAEL of 30 ppm. Murray et al. (1979) found increased incidences of cleft palate, decreased ossification, and decreased fetal crown-rump length in rats and an increased incidence of cleft palate in the offspring of mice exposed to chloroform at 100 ppm on days 8 through 15 of gestation.

Embryotoxicity and fetotoxicity were found in pregnant Sprague-Dawley rats exposed for 7 h/d to chloroform at 100 or 300 ppm, but only minor embryo- and fetotoxicity was seen for exposure at 30 ppm on days 6 through 15 of gestation (Schwetz et al. 1974; Baeder and Hofmann 1988). A decreased ability to maintain pregnancy but no significant teratogenicity was observed in CF-1 mice exposed for 7 h/d to chloroform at 100 ppm on gestation days 1 through 7 or 6 through 15 (Murray et al. 1979). When the exposure was on days 8 through 15, however, no decrease was seen in the ability to maintain pregnancy, but a significant increase in the incidence of cleft palate was observed in the offspring.

Interaction with Other Chemicals

Chloroform-induced toxicity can be potentiated by several treatments. Some examples include ethanol, PBBs, ketones, and steroids (EPA 1985). Chemicals such as diethyl maleate, which deplete hepatic GSH, can greatly increase the hepatotoxicity of chloroform (Brown et al. 1974b). Fasting, which also reduces GSH, has a similar enhancing effect on chloroform hepatotoxicity (Brown et al. 1974b).

Pretreating F-344 rats for 3 d with drinking water containing 0.8 mM of chloroform has been shown to enhance the hepatotoxicity of carbon tetrachloride as measured by plasma levels of alanine aminotransferase activity (Steup et al. 1991). Factors that appear to protect against toxicity include disulfiram and high carbohydrate diets (EPA 1985).

RATIONALE

The spacecraft water exposure guideline (SWEG) listed in Table 1-3 for each exposure duration was set on the basis of the lowest value among the acceptable concentrations (ACs) for all the significant adverse effects at that exposure duration. ACs were determined following the guidance of the National Research Council (2000) and were calculated assuming consumption of 2.8 L of water per day. That includes an average of 800 mL/d of water used to prepare or reconstitute food in addition to 2.0 L/d for drinking. The resulting SWEG values differ substantially from limits set by U.S. Environmental Protection Agency (EPA) or the Agency for Toxic Substances and Disease Registry (ATSDR) (Table 1-4) because of differences in the use of safety factors and in the criteria used to define an ad-

TABLE 1-2 Toxicity Summary

Concentration	Exposure Duration	Species	Effects	Reference
Acute exposures (≤1 d)				
34, 180, 477 mg/kg; gavage in corn oil	Single dose	Rat, F-344	Dose-dependent mild to severe necrosis of the proximal tubules of the kidneys; 20-fold increase in LI of proximal tubule cells at 180 mg/kg; slight to moderate hepatotoxicity	Larson et al. 1993
34, 238, 477 mg/kg; gavage in corn oil	Single dose	Mouse, B6C3F_1; female	NOAEL for renal lesions; 2-fold increase in LI of proximal tubule cells at 350 mg/kg; dose-dependent centrilobular hepatic necrosis	Larson et al. 1993
5,000 mg/m^3	1, 2, 3 h	Mouse, C3H	Kidney histopathology in 100% of males and 0% of females; death in 83% of 8-mo-old males and 39% of 2-mo-old males	Derringer et al. 1953
1,200 mg/m^3	2 h	Mouse, CBA	Severe kidney necrosis in males; moderate necrosis in testosterone-treated females; no effects to mild effects in normal females or estrogen-treated males; mild to moderate necrosis in ovariectomized females and castrated males	Culliford and Hewitt 1957
3,300 mg/m^3	2 h	Mouse, WH	Severe kidney necrosis in males; moderate necrosis in testosterone-treated females; no effect in normal females, estrogen-treated or castrated males, or ovariectomized females.	Culliford and Hewitt 1957

Short-term exposures (2-10 d)				
105 mg/kg/d (1,800 mg/L); drinking water	4 d	Mouse, B6C3F$_1$; female	NOAEL for macroscopic changes in liver or kidney, increased hepatic LI, and serum clinical chemistry	Larson et al. 1994b
53.5 mg/kg/d (400 mg/L); drinking water	4 d	Mouse, B6C3F$_1$; female	LOEL for pale cytoplasmic eosinophilic staining of centrilobular hepatocytes compared with periportal hepatocytes (n = 2 of 5 mice)	Larson et al. 1994b
26.4 mg/kg/d (200 mg/L); drinking water	4 d	Mouse, B6C3F$_1$; female	NOEL for pale cytoplasmic eosinophilic staining of centrilobular hepatocytes compared with periportal hepatocytes	Larson et al. 1994b
16 mg/kg/d (60 mg/L); drinking water	4 d	Mouse, B6C3F$_1$; female	NOEL for taste aversion	Larson et al. 1994b
1,800 mg/L; drinking water	5 d	Mouse, B6C3F$_1$; female	Inhibition of hepatic cell proliferation	Pereira 1994
1,800 mg/L; drinking water and 10 ml/kg corn oil	5 d	Mouse, B6C3F$_1$; female	Inhibition of hepatic cell proliferation	Pereira 1994
10 mg/kg/d; gavage in corn oil	4 d	Mouse, B6C3F$_1$; female	NOEL for hepatic histopathology	Larson et al. 1994b
34-277 mg/kg/d; gavage in corn oil	4 d or 5 d/wk for	Mouse, B6C3F$_1$; female	Dose-related acute tubular necrosis and increased LI in kidneys at 4 d; regenerating tubules after 3	Larson et al. 1994c

(Continued)

TABLE 1-2 Continued

Concentration	Exposure Duration	Species	Effects	Reference
	3 wk		wk of dosing at ≤138 mg/kg/d and necrosis of proximal tubules at 277 mg/kg/d	
Short-term exposures (2-10 d)				
34, 90 mg/kg/d; gavage in corn oil	4 d or 5 d/wk for 3 wk	Mouse, $B6C3F_1$; male	Mild degenerative changes in centrilobular hepatocytes after 4 d of treatment that was absent at 3 wk; LI increased (6-fold) in liver after 4 d but returned to baseline by 3 wk; these are considered adaptive effects	Larson et al. 1994c
≥138 mg/kg/d; gavage in corn oil	4 d	Mouse, $B6C3F_1$; male	Hepatic centrilobular necrosis and markedly increased hepatic LI; acute tubular necrosis of kidneys	Larson et al. 1994c
263 mg/kg/d; gavage in corn oil	5 d	Mouse, $B6C3F_1$	Increased hepatic cell proliferation	Pereira 1994
Subchronic exposures (11-100 d)				
329 mg/kg/d (1,800 mg/L); drinking water	3 wk	Mouse, $B6C3F_1$; female	NOAEL for macroscopic changes in liver or kidney, increased hepatic LI, and serum clinical chemistry	Larson et al. 1994b
15.7 mg/kg/d (60 mg/L); drinking water	3 wk	Mouse, $B6C3F_1$; female	NOEL for taste aversion	Larson et al. 1994b

263 mg/kg/d; corn oil	12 d	Mouse, B6C3F$_1$	Increased hepatic cell proliferation	Pereira 1994
1,800 ppm; drinking water	12 d	Mouse, B6C3F$_1$	Inhibition of hepatic cell proliferation	Pereira 1994
1,800 ppm; drinking water and 10 mg/kg corn oil	12d	Mouse, B6C3F$_1$	Inhibition of hepatic cell proliferation	Pereira 1994
11 mg/rat/d (500 ppm); drinking water	28 d	Rat	NOAEL for histopathology and hepatic enxyme changes; LOAEL for decreased neutrophil counts	Chu et al. 1982a
1.3 mg/rat/d (50 ppm); drinking water	28 d	Rat	NOAEL for decreased neutrophil counts	Chu et al. 1982a
263 mg/kg/d; drinking water	4 wk	Mouse, B6C3F$_1$; female	NOAEL for increased incidence of hepatocellular carcinomas and adenomas	Jorgenson 1985
2,500 ppm; drinking water	90 d (+90 d recovery)	Rat, Sprague-Dawley	LOAEL for anorexia, decreased growth rate, increased mortality; LOAEL for thyroid lesions in males, with some recovery at 90 d	Chu et al. 1982b
500 ppm; drinking water	90 d (+90 d recovery)	Rat, Sprague-Dawley	LOAEL for decreased lymphocyte counts in recovery group males	Chu et al. 1982b
263 mg/kg/d; corn oil	33 d, 159 d	Mouse, B6C3F$_1$	Increased hepatic cell proliferation	Pereira 1994

(Continued)

TABLE 1-2 Continued

Concentration	Exposure Duration	Species	Effect	Reference
30 mg/kg/d; toothpaste capsules	6 d/wk for 6 wk	Dog, beagle, n = 16	LOAEL for increased SGPT activity	Heywood 1979
Subchronic exposures (11-100 d)				
595 mg/kg/d; gavage in oil	30 d	Mouse	Increased incidence of hematomas	Eschenbrenner 1945
1,800 mg/kg/d; gavage in oil	8 wk	Mouse	NOAEL for increase in lung tumors	Stoner 1986
Chronic exposures (≥100 d)				
0.34 mg/kg/d; in dentifrice	5 y	Human, n = 59	NOAEL for hepatic and renal effects (SGPT, SGOT, BUN, and serum alkaline phosphatase levels)	De Salva et al. 1975
0.96 mg/kg/d; in dentifrice and mouthwash	1 y	Human, n = 57	NOAEL for hepatic and renal effects (SGPT, SGOT, BUN, and serum alkaline phosphatase levels)	De Salva et al. 1975
21 mg/kg/d; in cough syrup	10 y	Human	Reversible albuminuria, hyaline and granular casts in urine	Wallace 1950
15 mg/kg/d; toothpaste capsules	6 d/wk for 150 wk	Dog, beagle, n = 16	LOAEL for increased SGPT activity	Heywood 1979
30 mg/kg/d; toothpaste capsules	6 d/wk for 7.5 y	Dog, beagle, n = 16	NOAEL for increased tumor incidence	Heywood 1979

200 mg/kg/d; drinking water	Lifetime	Rat, Wistar	Increased incidence of hepatic neoplastic nodules in females; increased incidence of lymphosarcomas in males	Tumasonis 1987
160 mg/kg/d	Lifetime	Rat, Osborne-Mendel	LOAEL for increased incidence of kidney tubular cell adenoma and carcinoma in males	Jorgenson 1985
81 mg/kg/d; drinking water	Lifetime	Rat, Osborne-Mendel	NOAEL for increased incidence of kidney tubular cell adenoma and carcinoma in males	Jorgenson 1985
90 mg/kg/d; gavage	78 wk	Rat, Osborne-Mendel; male	Kidney tubular cell adenomas and carcinomas	NCI 1976
60 mg/kg/d; in toothpaste	80 wk	Rat, Sprague-Dawley	NOAEL for increased tumor incidence	Palmer 1979
165 mg/kg/d; in toothpaste	52 wk	Rat, Sprague-Dawley	NOAEL for increased tumor incidence	Palmer et al. 1979
257 mg/kg/d; drinking water	52 wk	Mouse	NOAEL for promotion of tumors; inhibition of liver and lung tumorigenesis in mice treated with diethylnitrosamine	Klaunig et al. 1986
60 mg/kg/d; in toothpaste by gavage	6 d/wk for 80 wk	Mouse, ICI	Increased incidence of kidney tumors in males but not females	Roe et al. 1979
17 mg/kg/d; in toothpaste by gavage	6 d/wk for 80 wk	Mouse, ICI	NOAEL for increased incidence of kidney tumors in males	Roe et al. 1979

(Continued)

TABLE 1-2 Continued

Concentration	Exposure Duration	Species	Effect	Reference
Chronic exposures (≥100 d)				
60 mg/kg/d; in toothpaste by gavage	6 d/wk for 80 wk	Mouse, C57B1, CBA, CF/1	NOAEL for increased incidence of any tumor	Roe et al. 1979

TABLE 1-3 Spacecraft Water Exposure Guidelines for Chloroform

Duration	Concentration (mg/L)	Target Toxicity
1 d	60	Reduced water consumption
10 d	60	Reduced water consumption
100 d	18	Hepatotoxicity
1,000 d	6.5	Hepatotoxicity

verse effect. ACs were set for reduced water consumption and for hepato-toxicity (see Table 1-5). No ACs were set for CNS effects, nephro-toxicity, thyroid toxicity, carcinogenicity, reproductive toxicity, or developmental toxicity for the following reasons.

CNS Effects

No reports were found of CNS effects in humans or animals caused by exposure to chloroform in drinking water. Most likely that is due to the limited solubility of chloroform in water and a first-pass effect in which most of the ingested chloroform is metabolized by the liver before it can reach the general circulation. Therefore, no ACs were set for CNS effects.

Nephrotoxicity

Because it appears that in humans chloroform-induced kidney toxicity is rare in humans, and the liver is the major target organ for chloroform, ACs that protect against liver toxicity in humans also are protective for kidney toxicity. Therefore, no ACs were set for nephrotoxicity in humans.

Thyroid Toxicity

Despite numerous tests in a variety of species, thyroid toxicity was reported only once, in male rats, and only at the highest dose. No thyroid toxicity has been reported for humans exposed to chloroform. Therefore, no ACs are required to protect humans from thyroid toxicity caused by chloroform exposure.

TABLE 1-4 Drinking Water Standards for Chloroform Set by Other Organizations

Organization	Standard	Amount	Concentration
EPA	MCL	0.003 mg/kg/d	0.1 mg/L (100 ppb) moving annual average
ATSDR	1-14 d MRL	0.3 mg/kg/d	10.5 mg/L
ATSDR	15-364 d MRL	0.1 mg/kg/d	3.5 mg/L
ATSDR	≥365 d MRL	0.01 mg/kg/d	0.35 mg/L

Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry (U.S. Department of Health and Human Services); EPA, U.S. Environmental Protection Agency; MCL, maximum contaminant level; MRL, minimal risk level; ppb, parts per billion.

Carcinogenicity

The weight of evidence indicates that chloroform exposure results in tumors only under conditions that produce treatment-induced cytotoxicity and cell regeneration (Golden et al. 1997). Those effects appear to be necessary but not sufficient for tumorigenesis. Evidence from many studies supports the conclusion that chloroform exposures that do not produce cytotoxicity and cell regeneration will not result in tumorigenesis.

In humans, the organ most sensitive to chloroform toxicity appears to be the liver. ACs that protect against liver toxicity should also protect against carcinogenicity. Thus, no ACs were set for carcinogenicity.

Reproductive and Developmental Toxicity

The clinical significance of Land et al.'s (1981) findings (up to 3.5% morphologically abnormal sperm in mice exposed 4 h/d for 5 d to chloroform at 800 ppm) could not be evaluated because they did not study mating outcomes. Thus, ACs could not be calculated for chloroform's ability to cause changes in sperm morphology. Because NASA policy does not permit pregnant astronauts to fly, ACs also were not calculated for decreased ability to maintain pregnancy, for decreased conception rates in exposed females, or for teratogenic effects.

TABLE 1-5 Acceptable Concentrations (ACs)

			Uncertainty Factors					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
Hepatotoxicity	NOAEL = 0.34 mg/kg/d; in dentifrice twice daily for 1 min for 5 y	Human, n = 59; De Salva et al. 1975	$\frac{\sqrt{n}}{10}$	1	1	1	1	—	—	—	6.5
Hepatotoxicity	NOAEL = 0.96 mg/kg/d; in dentifrice and mouthwash twice daily for 1 y	Human, n = 57; De Salva et al. 1975	$\frac{\sqrt{n}}{10}$	1	1	1	1	—	—	18	—
Hepatotoxicity	NOAEL = 500 ppm; in drinking water	Rat; Chu et al. 1982a	1	1	1	1	1	—	790	—	—
Hepatotoxicity	NOAEL = 329 mg/kg/d; in drinking water	Mouse; Larson et al. 1994b	1	1	1	1	1	8,200	8,200	—	—
Reduced water consumption	NOAEL = 16 mg/kg/d; in drinking water	Mouse; Larson et al. 1994b	1	1	1	1	1	60	60	—	—
SWEG								60[a]	60[a]	18	6.5

[a]If, due to interindividual differences in taste, taste aversion were not an issue for an individual, hepatotoxicity would become the next most sensitive end point and the SWEG values for that individual could be raised to the next lowest AC for hepatotoxicity (8,200 mg/L for 1 d and 790 mg/L for 10 d).

Hepatotoxicity

The ACs for hepatotoxicity for 1-d and 10-d exposures are based on Larson et al.'s (1994b) results in mice given chloroform in drinking water at 0, 60, 200, 400, 900, or 1,800 ppm. The reported liver effects (lighter histologic staining of the cytoplasm of some hepatocytes without detectable functional changes) are considered adaptive rather than adverse, so 329 mg/kg/d would be considered a NOAEL for hepatotoxicity. Due to their initial aversion to drinking water that contained concentrations of chloroform at 200 ppm (mg/L) or higher, the mice consumed less water during the first 4 d than did controls. At the highest dose, 1,800 ppm, the average daily ingested dose was 105 mg/kg/d for the first 4 d but increased to an average of 329 mg/kg/d for 3 wk of exposure. Because the ingested dose for the first 4 d does not reflect the NOAEL demonstrated for the full 3-wk exposure, the 329 mg/kg/d 3-wk NOAEL for hepatotoxicity is used for calculating the 1-d AC. It is converted to a per-person value by multiplying by 70 kg. A factor of 1 is used for interspecies extrapolation because PBPK models for metabolism, absorption, distribution, and toxicokinetics show that humans are no more susceptible, and probably are less susceptible to chloroform toxicity than are rats or mice (see "Metabolism" section and Corley et al. 1990).

$$\text{1-d and 10-d acceptable doses} = 329 \text{ mg/kg/d} \times 70 \text{ kg} = 23{,}000 \text{ mg/d}.$$

Astronauts consume an average of 2.8 L of drinking water per day, so the 1-d and 10-d ACs for hepatotoxicity would be

$$23{,}000 \text{ mg/d} \div 2.8 \text{ L/d} = 8{,}200 \text{ mg/L}.$$

(NOTE: The 8,200 mg/L value exceeds the solubility of chloroform in water at 25°C [7,425 mg/L].)

Another 10-d AC can be calculated using Chu et al.'s (1982a) report of a NOAEL for hepatic enzyme changes in rats exposed to chloroform at 500 ppm (mg/L) in drinking water for 28 d, yielding a dose of 11 mg/ rat/ d. Assuming the rats weighed about 350 g, that would correspond to 31 mg/kg/d. For a 70-kg human, that would correspond to 2,200 mg/d. For a human drinking 2.8 L of water per day, the acceptable drinking water concentration would be 790 mg/L. A factor of 1 is used for interspecies extrapolation because studies comparing the chloroform metabolism rates of human livers vs rat and mouse livers show that they are similar. PBPK

models for absorption, distribution, and toxicokinetics show that humans are no more susceptible, and probably are less susceptible to chloroform toxicity than are rats or mice.

$$\text{10-d AC} = 790 \text{ mg/L}.$$

De Salva et al. (1975) observed no hepatotoxicity in 59 volunteers using toothpaste that contained 3.4% chloroform daily for 5 y (a dose of chloroform at 0.34 mg/kg/d; the only dose tested). A follow-up study by De Salva et al. (1975) at a higher dose (0.96 mg/kg/d) also showed no hepatotoxicity in 57 volunteers using toothpaste that contained 3.4% chloroform and mouthwash that contained 0.425% chloroform daily for 1 y. This calculation assumes ingestion of 25% of the dentifrice—a figure that was reported in the literature for children, not adults. Because adults would probably ingest less of the toothpaste than would children, one could object to the use of this figure; however, it's use probably is justified in this case because the subjects, all adults of various ages, were mentally and/or physically disabled and would likely ingest more of the toothpaste than the average adult. For a 70-kg astronaut, this would be equivalent to ingesting 24 mg or 67 mg chloroform per 2.8 L of drinking water per day for 5 or 1 y, respectively. That corresponds to chloroform concentrations of 8.5 mg/L or 24 mg/L in drinking water, respectively. Only a single dose was tested in each study, and there was no LOAEL dose, so it is not known how much higher the NOAELs could be.

The 100-d AC for hepatotoxicity is based on De Salva et al.'s (1975) 1-y NOAEL adjusted for potential interindividual variability due to the low number (<100) of subjects.

$$\text{100-d AC} = 24 \text{ mg/L} \times \frac{\sqrt{57}}{10} = 18 \text{ mg/L}.$$

The 1,000-d AC for hepatotoxicity is based on De Salva et al.'s (1975) 5-y NOAEL in humans. Because fewer than 100 subjects were tested in determining the NOAEL, the value is adjusted for potential interindividual variability.

$$\text{1,000-d AC} = 8.5 \text{ mg/L} \times \frac{\sqrt{59}}{10} = 6.5 \text{ mg/L}.$$

Reduced Water Consumption

ACs for reduced water consumption for 1-d and 10-d exposures are based on Larson et al.'s (1994b) results in mice given chloroform in drinking water at 0, 60, 200, 400, 900, or 1,800 ppm. At chloroform concentrations of 200 ppm and above, the average daily doses were lower for the first 4 d of exposure than for the entire 3 wk of exposure because of the mice's initial aversion to the drinking water. The 1-d and 10-d ACs are set at the 60 ppm (mg/L) NOAEL for reduced water consumption during the initial exposure period. No adjustment was made for potential species differences in taste aversion.

1-d and 10-d ACs = 60 mg/L.

Spaceflight Effects

Spaceflight is believed to increase the susceptibility of crew members to noncritical cardiac arrhythmias and could amplify the arrhythmogenic effects of chloroform. The blood levels of chloroform that can be achieved by ingesting drinking water are too low, however, to pose a concern for induction of cardiac arrhythmia.

Comparison of SWEG Values with Inhalation Limits for Chloroform

The amount of chloroform to which an individual would be exposed through drinking water at the SWEG values is compared in Table 1-6 with the exposures experienced through inhalation of the recommended spacecraft maximum allowable concentrations (SMACs) for chloroform vapors. The daily amounts ingested using the SWEG values assume consumption of 2.8 L of water per day and 100% absorption. Calculation of the daily amounts that would be absorbed during inhalation of air containing the SMACs for chloroform assumes inhalation of 20 m^3 of chloroform vapor per day and retention of 45% (NRC 2000, pp. 264-306). Forty-five percent is probably low because it was estimated from experiments with humans inhaling much higher concentrations. At concentrations near the SMACs, the retention could approach 100%, and the values in Table 1-6 for milligrams per day at the SMAC values would need to be adjusted.

TABLE 1-6 Comparison of Daily Amounts of Chloroform Exposure Allowable Under SWEGs and SMACs

Exposure	SWEGs			SMACs		
Duration	mg/L	mg/d	Effect	mg/m^3	mg/d	Effect
1 h	—	—	—	10	90	CNS depression
24 h	—	—	—	10	90	CNS depression
1 d	60	120	Reduced water consumption	—	—	—
7 d	—	—	—	10	90	CNS depression, hepatotoxicity, nephrotoxicity, carcinogenicity
10 d	60	120	Reduced water consumption	—	—	—
30 d	—	—	—	5	45	Hepatotoxicity, CNS depression
100 d	18	36	Hepatotoxicity	—	—	—
180 d	—	—	—	5	45	Hepatotoxicity
1,000 d	6.5	13	Hepatotoxicity	—	—	—

Abbreviations: SMACs, spacecraft maximum allowable concentrations; SWEGs, spacecraft water exposure guidelines.

The daily amounts absorbed (mg/d) are comparable for the two routes of exposure. At SMAC values for durations of ≤7 d, inhalation leads to CNS depression, whereas, due to the first-pass effect, similar amounts ingested from drinking water do not reach the CNS because much of the chloroform is metabolized by the liver before it reaches the general circulation.

Comparison of SWEG Values with Standards Set by Other Organizations

The daily amounts ingested using the ACs recommended above and assuming consumption of 2.8 L of drinking water per day and 100% absorption are compared, in Table 1-7, with the drinking water standards set by other organizations.

TABLE 1-7 Comparison of SWEGs for Chloroform with Standards Set by Other Organizations

Organization	Type of Standard	Standard Value (mg/L)	Data Used as Basis For Calculations			Uncertainty Factors Used				Effect
			Reference	NOAEL	Species	To NOAEL	Interspecies	Inter-individual	Exposure Duration	
NASA	1 d SWEG	60	Larson et al. 1994b	16 mg/kg/d	Mouse	1	1	1	1	Taste aversion
ATSDR	1-14 d MRL	10.5	Larson et al. 1994c	26 mg/kg/d	Mouse	1	10	10	1	Hepatotoxicity
NASA	10 d SWEG	60	Larson et al. 1994b	16 mg/kg/d	Rat	1	1	1	1	Hepatotoxicity
ATSDR	15-364 d MRL	3.5	Heywood 1979	15 mg/kg/d	Dog	1	10	10	1	Hepatotoxicity
NASA	100 d SWEG	18	De Salva et al. 1975	0.96 mg/kg/d	Human	1	1	$\frac{\sqrt{n}}{10}$	1	Hepatotoxicity
ATSDR	≥365 d MRL	0.35	Heywood 1979	15 mg/kg/d	Dog	10	10	10	1	Hepatotoxicity
NASA	1,000 d SWEG	6.5	De Salva et al. 1975	0.34 mg/kg/d	Human	1	1	$\frac{\sqrt{n}}{10}$	1	Hepatotoxicity
EPA	MCL; moving annual average	0.1	EPA 1994							

Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; EPA, U.S. Environmental Protection Agency; MRL, minimal risk level; NASA, National Aeronautic and Space Administration; SWEG, spacecraft water exposure guideline.

RECOMMENDATIONS FOR FUTURE RESEARCH

Research is needed to quantitate the organ-specific (liver and kidney) levels of chloroform metabolism in humans, compare them with those in rodents, and elucidate factors, such as glutathione levels, that could modulate the threshold level of chloroform required for toxicity. Once all of that is determined, a PBPK model incorporating those values and addressing both oral and inhalation exposures would be useful.

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2 Dichloromethane

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PHYSICAL AND CHEMICAL PROPERTIES

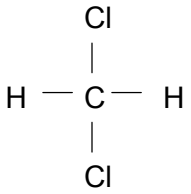
Dichloromethane (DCM) is a nonflammable, clear, colorless, volatile, dense liquid with a mild, sweet, pleasant odor (see Table 2-1) (ATSDR 1998).

OCCURRENCE AND USE

DCM is widely used as an industrial solvent and a paint stripper, and it is used in the manufacture of photographic film and in some aerosol products, including spray paints and other household products. DCM is not used in spacecraft, but it out-gases from nonmetallic materials in spacecraft and can be produced during thermodegradation of chlorine-containing materials, such as polyvinyl chloride plastics. DCM has been detected in the space shuttle atmosphere in 28 of 33 missions from STS-26 to STS-55 at levels of 0.1-1 mg/m^3 (James et al. 1994). DCM vapors would be expected to condense along with water vapors and form "humidity condensate" in spacecraft air-cooling systems. Drinking water on the International Space Station (ISS) is generated from humidity condensate, recycled hygiene water, and urine and is supplemented by water from the shuttle or the

TABLE 2-1 Physical and Chemical Properties of DCM

Formula	CH_2Cl_2
Chemical name	Dichloromethane
Synonyms	Methylene chloride, methylene dichloride
CAS registry no.	75-09-02
Molecular weight	84.9
Boiling point	40°C
Melting point	-95.1°C
Liquid density	1.3182 g/mL at 25°C
Vapor pressure	349 torr at 20°C; 500 torr at 30°C
Solubility	1 mL dissolves in 50 mL of water (2.0% w/v; approximately 20 g/L at 20°C; 16.7 g/L at 25°C)
Taste and odor threshold	9.1 ppm in water; 160-620 ppm vapor in air (most people can detect its odor at ≥300 ppm vapor in air)
Miscible with alcohol, ether, acetone, chloroform, and carbon tetrachloride.	



Russian *Progress* spacecraft. Because the air-to-water partition coefficient for DCM is approximately 6 at 37°C (Gargas et al. 1989), most DCM in spacecraft will be present as vapor in the atmosphere, but it is expected that traces of DCM might be found in the ISS drinking water under normal conditions.

PHARMACOKINETICS AND METABOLISM

Limited data are available on the uptake, metabolism, and elimination of DCM ingested through drinking water by humans or animals. The description below presents data mostly for DCM administered to rodents by gavage in water or corn oil.

Absorption

No quantitative studies were found describing the absorption of ingested DCM in humans, although several case reports of individuals who attempted suicide by consuming DCM provide evidence (in the form of profound CNS depression) that ingested DCM is absorbed in humans.

In mice exposed to DCM at 50 mg per kilogram (kg) by gavage in water, DCM was rapidly absorbed from both the upper and lower portions of the gut, with 75% of the dose absorbed within 10 minute (min) and about 98% of the dose absorbed within 20 min (Angelo et al. 1986a).

Distribution

No studies were found that described the tissue distribution of ingested DCM in humans.

In mice and rats, whole-body autoradiograms were prepared 1 hour (h) after oral gavage with ^{14}C-labeled DCM in corn oil at 100 mg/kg and 50 mg/kg, respectively. The tissues highlighted by their ^{14}C content were the liver, blood, lungs, heart, spleen, bone marrow, salivary glands, and pancreas in mice; and the liver, blood, lungs, kidneys, spleen, brain, salivary glands, intestine, and stomach in rats (Yesair et al. 1977). The liver appeared to be the major site of metabolism. The autoradiograms also indicated that absorption from the digestive tract is relatively rapid. In rats given doses of radioactive DCM at 50-1,000 mg/kg for 14 d, the label was rapidly cleared from all tissues during the 240 min after each exposure, suggesting that DMC and/or its metabolites do not accumulate in any tissues (Angelo et al. 1986a,b).

Excretion

No studies were found that described the excretion of DCM or its metabolites in humans. In mice exposed to DCM at 50 mg/kg by gavage in water, DCM was rapidly eliminated from all tissues examined (Angelo et al. 1986a). DCM elimination was mainly in expired air; 53-61% was excreted as unchanged DCM; 8-23% was excreted as carbon dioxide (CO_2); and 0.5-12% was excreted as carbon monoxide (CO). Within 4 h of dosing, the DCM concentrations in the blood and most of the tissues were below the

limit of detection (<0.05 micrograms [μg] of DCM per gram of tissue) (Angelo et al. 1986a). In rats given oral doses of radiolabeled DCM at 1 mg/kg or 50 mg/kg in water, expired air accounted for 78-90% of the excreted dose in the 48 h following administration (McKenna and Zempel 1981). Radiolabel in the urine accounted for 2-5% of the dose, and 1% or less of the dose was found in the feces (McKenna and Zempel 1981).

Metabolism

At low doses, DCM is almost completely (more than 98%) metabolized to CO and CO_2 in all species studied (rats, mice, hamsters, and humans) (Kirschman et al. 1986). Saturable kinetics are seen between 10 mg/kg/d and 50 mg/kg/d administered orally in both rats and mice. Greater fractions of DCM are expired unmetabolized at or above 50 mg/kg/d (Kirschman et al. 1986). Kirschman et al. (1986) found that metabolism is dose-dependent in mice. They showed a significant change in the proportion of administered DCM that is expired unchanged at doses above 1 mg/kg/d, as shown in Table 2-2 (Yesair et al. 1977; Kirschman et al. 1986).

The discontinuity in the proportion of DCM expired unchanged with increasing dose suggests the involvement of a saturable metabolic mechanism or mechanisms and indicates that doses at or above 50 mg/kg/d may be inappropriate for use in the safety assessment of exposure levels appreciably below those (Kirschman et al. 1986).

DCM is metabolized in mammals by two pathways (Gargas et al. 1986). A portion of ingested DCM is oxidized to CO by cytochrome P-450-dependent mixed-function oxidase enzymes. In humans, a smaller fraction is conjugated by glutathione-S-transferase (GST) to S-chloromethyl glutathione and subsequently to formaldehyde and to CO_2 (Hallier et al. 1994). The S-chloromethyl glutathione conjugate is extremely unstable and short-lived, but if alkylation can occur before the conjugate breaks down, it could lead to mutagenesis (Green 1991). Production of formaldehyde is also believed to be involved in the lung and liver carcinogenicity of DCM seen in mice (Casanova et al. 1996, 1997). The GST pathway is several times more active in mice than in rats or in humans (Green 1991). The distribution of theta glutathione-S-transferase (GSTT1-1), the major enzyme involved in the metabolism of DCM, differs markedly between species (Green 1991). In mice, very high concentrations of mRNA for GSTT1-1 enzyme were found in the centrilobular region and in nuclei in liver parenchyma. In rat and human liver, GSTT1-1 was not localized in specific regions of the liver

TABLE 2-2 Excretion of DCM Equivalents[a]

Dose (mg/kg)	Recovery (% of Dose)					Total Excretion (% of Dose)
	Expired Air					
	CH_2Cl_2	CO_2	CO	Urine	Feces	
0.1	1.7	34.6	34.1	10.4	ND	80.8
1.0	1.9	37.7	21.2	5.0	1.3	67.1
50	36.4	26.2	20.2	2.5	ND	85.3
100	40.5	10.7	3.6	1.4	0.3	56.5
500	55.2	10.5	6.8	1.7	0.05	74.2

[a]Within 24 h of administration of an oral dose of ^{14}C-labeled DCM at 0.1-500 mg in water to $B6C3F_1$ mice.
Abbreviations: ND, not detected.
Source: Data from Kirschman et al. 1986.

or in nuclei. Similar species differences were seen in the lung. These differences in the localization and level of activity of GSTT1-1 correlate with the species differences in the carcinogenicity of DCM. In humans, glutathione-S-transferase theta-1 (GST-T1) is polymorphic; about three-quarters of the population possesses this enzyme activity, and one-quarter lacks it (Nelson et al. 1995). If GST-mediated conjugation is required for carcinogenesis by DCM, then individuals with no measurable glutathione conjugation activity would be expected to have little risk of developing cancer from exposure to DCM (Clewell 1995). Significant ethnic differences in the prevalence of the homozygous deleted genotype of GST-T1 have been reported, with null genotypes seen in the circulating red blood cells of 64% of Chinese, 60% of Koreans, 22% of African-Americans, 20% of Caucasian-Americans, 10% of Mexican-Americans (Nelson et al. 1995), and 38% of Europeans (Premble et al. 1994).

TOXICITY SUMMARY

There are no reports of effects on the central nervous system (CNS) resulting from exposure to DCM in drinking water. High concentrations of inhaled DCM vapor or ingestion of large amounts of DCM as paint stripper have pronounced CNS effects that are reversible upon cessation of exposure. In rodents, hepatotoxicity, rather than CNS depression, is the main

effect of intermediate and long-term exposure to moderate doses of DCM in drinking water. At higher doses in drinking water, mild kidney and hematologic changes have been reported. Inhaled DCM has been shown to be a carcinogen in mice and rats at high doses.

Acute Toxicity (<1 d)

Lethality

Two human fatalities have been attributed to ingestion of DCM. A woman ingested about 300 milliliters (mL) of Nitromors paint remover, which contains DCM as its main ingredient. Other ingredients include methanol, cellulose acetate, triethanolamine, paraffin wax, and detergent. Her death, which occurred about 25 days (d) after ingestion, was attributed to the corrosive effects of the solvent on the intestinal tract rather than to the metabolic consequences of carboxyhemoglobinemia, which peaked at 12.1% 36 h after ingestion (Hughes and Tracey 1993). A 49-y-old man successfully committed suicide by ingesting 300 mL of DCM (Chang et al. 1999). His death occurred 9 d after ingestion, probably due to pulmonary edema complicated by anuria. His COHb levels were 35% at 8 h post-ingestion, 18% at 28 h post-ingestion, 14% at 34 h post-ingestion, 11% at 73 h and 97 h post-ingestion, and 9% at 120 h post-ingestion.

An early report described the case of a man who survived ingesting "between one and two pints" of Nitromors (Roberts and Marshall 1976). Carboxyhemoglobin levels were not measured in that case. Five additional cases have been reported in which patients ingested between 25 mL and 350 mL of DCM and survived after intensive symptomatic and supportive medical treatment (Chang et al. 1999). General signs and symptoms of ingestion of large quantities of DCM in these cases included CNS depression, tachypnea, blistering and ulceration of the GI tract, hemoglobinuria, metabolic acidosis, and gastrointestinal hemorrhage. Hepatic and renal failures were reported in two of six cases. None of these patients developed significant cardiac arrhythmia.

Inhalation of paint stripper vapors containing 80% DCM and 20% methanol was reported to produce delayed and prolonged elevation of carboxyhemoglobin levels in four volunteers (Stewart and Hake 1976). Levels of carboxyhemoglobin peaked about 4 h after a 3-h exposure; values were measured at 6-9%. Stewart and Hale also reported a case of one 66-y-old man who developed symptoms of cardiac infarction 1 h after using

a paint stripper for 3 h in his basement. Two weeks after recovery, he returned to stripping the furniture and was again hospitalized with myocardial infarction. He survived, but 6 months (mo) later, he returned to stripping the furniture, collapsed, and died. Stewart and Hake (1976) showed that simultaneous exposure to methanol extends the biologic half-life of carboxyhemoglobin derived from DCM (Stewart and Hake 1976).

In rats exposed by gavage in water, the LD_{50} (dose lethal to 50% of subjects) of DCM was reported to be 2,100 mg/kg (Kimura et al. 1971), and the LD_{95} for rats exposed by gavage in oil was reported to be 4,382 mg/kg (Ugazio et al. 1973).

CNS Toxicity

DCM vapor inhaled at 1,000 parts per million (ppm) for 1 h produced light-headedness in two of three volunteers and altered visual evoked responses in all three subjects, but no subjective symptoms or objective signs were observed in eight subjects during a 1-h exposure to DCM vapor at 515 ppm (Stewart et al. 1972).

Winneke (1974) reported that a 4-h inhalation exposure to DCM at 300 ppm produced subtle but statistically significant CNS effects in human volunteers (including decreased critical flicker frequency and a decrease in auditory vigilance). Reitz et al. (1997) used Winneke's data in a physiologically based pharmacokinetic (PBPK) model to calculate a brain tissue concentration of DCM at 3.95 mg per liter (L). They extrapolated the inhalation parameters to an exposure to drinking water containing 562 mg of DCM per liter, assuming a 70-kg person consuming 2.0 L/d. The U.S. Agency for Toxic Substances and Disease Registry (ATSDR) (1998) used these data to calculate a minimal risk level (MRL) of 0.5 mg/kg/d after applying an uncertainty factor of 30 (10 for the use of a minimal LOAEL [lowest-observed-adverse-effect level] and 3 for human variability) to the LOAEL of 16 mg/kg/d calculated by Reitz et al. (1997).

Nephrotoxicity

Initial hemoglobinuria and progressive renal failure were seen in a woman who ingested a fatal quantity (300 mL) of paint remover predominantly comprising DCM; acute tubular necrosis was observed postmortem (Hughes and Tracey 1993). Hemoglobinuria was also reported in the case

of a man who survived ingestion of a similar quantity (1-2 pints) of the same paint remover (Roberts and Marshall 1976).

Cardiac and Hematologic Effects

Because DCM metabolizes to CO, increased levels of carboxyhemoglobin (COHb) are observed in exposed humans and animals. Nevertheless, DCM ingestion is not associated with significant cardiac toxicity. Tachycardia (120 beats per minute) and marked hemolysis were observed in a woman who ingested a fatal quantity (300 mL) of paint remover (Hughes and Tracey 1993). Intravascular hemolysis was also reported in the case of a man who survived ingestion of a comparable quantity (1-2 pints) of the same paint remover (Roberts and Marshall 1976). No significant cardiac arrhythmia was found in medical case reports for eight individuals admitted to hospitals after ingesting DCM, despite high carboxyhemoglobin levels (measured at up to 35% in some cases) that remained elevated for several days (Roberts and Marshall 1976; Hughes and Tracey 1993; Chang et al. 1999). It should be noted, however, that in many of those cases, blood oxygen levels were monitored during treatments that often included assisted ventilation with 100% oxygen during portions of the hospital stays. The oxygen treatment was required because of dyspnea and, in some cases, pulmonary edema. Although three of the eight exposed individuals died, the deaths were not attributed to the CO produced by metabolism from DCM, but rather to the corrosive effects of DCM on the GI tract.

NASA previously set exposure limits for inhaled CO based on a maximum blood COHb concentration of 3% (NRC 1994). They reported that 3% COHb would be achieved by a person inhaling CO at 20 ppm for 24 h. Assuming an average minute-volume of 20 L/min over a 24 h period, that would mean

$$20\ \text{L/min} \times 60\ \text{min/h} \times 24\ \text{h/d} \times 20/1{,}000{,}000 \times 1\ \text{mole (mol)}/22.4\ \text{L} = 0.026\ \text{mol/d}.$$

Assuming, as a worst-case scenario, that 100% of the administered DCM were converted to CO, the concentration of DCM in drinking water needed to achieve a blood COHb concentration of 3% for 1 d would be

$$0.026\ \text{mol/d} \times (84.9\ \text{g/mol} \div 2.8\ \text{L}) = 0.78\ \text{g/L} = 780\ \text{mg/L}.$$

Thus, concentrations of DCM in drinking water at 780 mg/L would not be expected to produce clinically significant COHb concentrations.

Short Term Toxicity (2-10 d)

No reports were found of short-term (2-10 d) human or animal exposures to DCM.

Subchronic Toxicity (11-100 d)

Hepatotoxicity

In preparation for a 2-y drinking water study (see Serota et al. 1986a,b), Kirschman et al. (1986) conducted a 90-d study in B6C3F$_1$ mice and F-344 rats ingesting water containing nominal levels of DCM at 0, 0.15, 0.45, and 1.5%. The intakes for these three nominal levels over the duration of the study, calculated based on analysis of DCM concentration and liquid consumption, were 166, 420, and 1,200 mg/kg/d for male rats and 209, 607, and 1,469 mg/kg/d for female rats. For mice, the corresponding values were 226, 587, and 1,911 mg/kg/d for males and 231, 586, and 2,030 mg/kg/d for females. For both rats and mice, the liver was the only target organ noted (Kirschman et al. 1986). At a 30-d interim necropsy, no compound-related effects were found. At the terminal 3-mo necropsy, however, histopathology was found in the liver, including hepatocyte vacuolization, central lobular fatty change, necrosis with fatty change, and pigment deposition. The lowest effect levels were 587 mg/kg/d in mice and 166 mg/kg/d in rats (the lowest tested dose) (Kirschman et al. 1986).

Chronic Toxicity (≥101 d)

Hepatotoxicity

The liver has been shown to be the primary toxicity target of DCM after long-term ingestion. Serota et al. (1986a,b) conducted 2-y drinking water carcinogenicity and toxicity studies in rats and mice administered DCM at target levels of 0, 0, 5, 50, 125, and 250 mg/kg/d in rats and 0, 0, 60, 125, 185, and 250 mg/kg/d in mice. These studies identified the liver as the only

organ showing DCM toxicity, but there were considerable differences in sensitivity between rats and mice (Serota et al. 1986a,b). Doses of 50, 125, or 250 mg/kg/d produced both fatty changes and foci or areas of cellular alteration in the livers of both genders of rats. There was a NOAEL (no-observed-adverse-effect level) of 6 mg/kg/d (actual dose) in both male and female rats. In a parallel study in mice, treatment-related toxic changes were noted in both male and female livers only at the highest dose, with a NOAEL of 185 mg/kg/d in both genders of mice (Serota et al. 1986b).

Decreased Water Consumption and Decreased Weight Gain

In the 2-y study of Serota et al. (1986a), rats of both genders receiving DCM at target dose rates of 125 mg/kg/d or 250 mg/kg/d (actual rates: 131 mg/kg/d and 249 mg/kg/d), but not those receiving target dose rates of 5 mg/kg/d or 50 mg/kg/d (actual rates: 6 mg/kg/d and 55 mg/kg/d), had lower body weights and body-weight gains than controls and lower levels of food and water consumption. The authors considered these effects to be interrelated and attributed to DCM treatment. In a parallel study in mice, no treatment-related effects on body weight or water consumption were observed during the study up to the highest dose, 250 mg/kg/d (Serota et al. 1986b).

Carcinogenicity

Cancer in Animals

Oral DCM ingestion. An elevated incidence of liver tumors were seen in female, but not male F-344 rats receiving DCM at up to 250 mg/kg/d in drinking water for 2 y, but this incidence was within the historical control range (Serota et al. 1986a). Male rats exhibited a lower incidence of both neoplastic nodules and hepatocellular carcinomas than seen in control groups (Serota et al. 1986a). Similarly exposed $B6C3F_1$ mice showed no increase in the incidence of liver tumors (Serota et al. 1986b). Female mice receiving DCM by gavage in olive oil at 500 mg/kg/d for 64 wk showed a slight, but not statistically significant increase in the incidence of mammary tumors (Maltoni et al. 1988).

Inhalation of DCM vapors. Inhalation exposure of $B6C3F_1$ mice (50 mice per gender per dose) to DCM at 2,000 ppm and 4,000 ppm for 6 h/d,

5 d/wk for 2 y significantly increased the incidences of lung and liver tumors in both male and female mice compared with controls (NTP 1986). F-344 rats exposed under the same conditions showed increased incidences of mammary gland tumors in female and, to a lesser extent, male rats (NTP 1986).

No increases in tumor incidence were seen in Sprague-Dawley rats or Syrian golden hamsters exposed by inhalation to DCM at 0, 50, 1,500, or 3,500 ppm for 6 h/d, 5 d/wk for 2 y, but a statistically significant dose-related increase in the number of mammary tumors per tumor-bearing female rat was observed (Burek et al. 1984).

Cancer In Humans

Oral ingestion of DCM. No reports of carcinogenic effects in humans after oral exposure to DCM were found. However, several studies examined the potential for carcinogenic effects from inhalation of DCM vapors during occupational exposures.

Inhalation of DCM vapors. Epidemiology studies have been performed using large cohorts of workers occupationally exposed to DCM in the photographic film base manufacturing industry and in triacetate fiber production. The available data from human epidemiological studies to date provide contradictory evidence concerning DCM's association with cancer of several organs; however, the studies are of limited power, or of only moderate latency since first exposure, or in some instances involve low and possibly ineffective doses. These studies have provided suggestive, but not persuasive evidence of an association between occupational exposure to DCM and increased cancer risk in humans. The studies are summarized below.

Friedlander et al. (1978) conducted a proportionate mortality study and a retrospective mortality study of workers exposed to DCM at a Kodak film manufacturing facility in New York. No statistically significant differences between the two were observed. Hearne et al. (1987, 1990) updated Friedlander et al.'s cohort study and also reported no statistically significant findings for any cause of death. Hearne et al. conducted a second study with a different cohort of 1,311 workers at the same Kodak facility and followed them through 1990. The mean career individual exposure was approximately 40 ppm for 17 y, and the average interval between first exposure and end of follow-up was about 32 y. Total mortality for this cohort

was significantly (22%) lower than the expected mortality, as were mortalities from circulatory diseases and ischemic heart disease. There were no significant elevations in mortalities from any cancers.

In 1978, Ott et al. (1983a,b,c,d) began a retrospective cohort study on a working population at a cellulose diacetate and triacetate plant in Rock Hill, South Carolina. Statistical differences in mortality risk were observed for "all causes," "diseases of the circulatory system," and "ischemic heart disease" in white men. Ott et al. concluded that a potential healthy-worker effect and the low power of their study could not permit them to dismiss the possibility of increased health risks within the working population exposed to DCM. In updates to the Rock Hill study, Lanes et al. (1990, 1993) extended the study of Ott et al.'s cohort through 1990. No excess mortality was observed for ischemic heart disease, but statistically significant excess mortality was observed for cancer of the liver and biliary passages. However, in comparing the probabilities of the observed vs the expected death rates, Lanes et al. estimated that, because no additional deaths from liver or biliary cancer were observed between their first and second studies, it was 21 times more probable that the true standard mortality ratio was 1 rather than 5.75 (the value calculated in their first update study).

Gibbs et al. (Gibbs 1992; Gibbs et al. 1996) studied mortality in another cohort of workers at a different cellulose acetate and triacetate plant in Cumberland, Maryland. The overall mortality rate for the occupational group exposed to high levels of DCM was below the expected rates for the populations of Allegany County, the State of Maryland, and the United States. No significantly elevated incidence of biliary-tract cancer was found. Statistically significant excess mortality was observed from prostate, uterine, and cervical cancers. While the excess of prostate cancers suggested an exposure-response relationship, there were potential confounding factors, and there was no corroboration by other studies.

Tomenson et al. (1996, 1997) studied mortality among 1,785 workers at a cellulose triacetate factory in Brantham, England. The mean career occupational exposure to DCM was 9 y at 19 ppm. In the subcohort of workers exposed to DCM, substantially reduced mortalities were reported compared with national and local rates for all causes and all cancers—liver, biliar-tract, lung, and pancreatic cancers in particular. No in-service mortality due to ischemic heart disease was found in workers with the highest cumulative exposure (800 ppm·y).

A case-control study by Heineman et al. (1994) of 741 men who died of brain or other CNS tumors showed associations with likely occupational exposures to chlorinated aliphatic hydrocarbons, but the associations were

strongest for DCM. The risk of astrocytic brain tumors increased with probability and average intensity of exposure and with duration of employment in jobs involving DCM exposure, but not with a cumulative exposure score.

Green (1997) reviewed evidence supporting the view that, although inhaled DCM causes cancer in mice, DCM does not cause cancer in humans. He proposed that damage to mouse lung Clara cells and increased cell division influenced the development of the lung tumors. He suggested that if the cell of origin of mouse lung tumors is the Clara cell, humans are at less risk of developing lung cancer because humans have proportionally fewer Clara cells than do mice. Green also proposed that the observed species specificity (hamsters did not develop tumors) was a direct consequence of the very high activity and specific cellular (lung and liver cells) and nuclear localization of a theta-class glutathione-S-transferase (GST) enzyme that was unique to the mouse. Specifically, he proposed that the putative carcinogenic metabolite was too reactive to cross the nuclear membrane. DNA damage was not detectable in rats in vivo or in hamster or human hepatocytes (none of which, he claimed, have high levels of GST in their nuclei) exposed to cytotoxic dose levels of DCM in vitro. Thus, mice appear to be unique in their response to DCM. Green therefore proposed that humans are qualitatively different from mice and are not susceptible to DCM-induced carcinogenicity.

Green's views were disputed by OSHA in a 1997 final rule on occupational exposure to DCM (OSHA 1997). OSHA made the following arguments in rejecting Green's views:

- The weight of evidence supports the view that the mechanism of DCM carcinogenesis is through one or more genotoxic metabolites of the GST pathway. Genotoxic metabolites have been shown to cause DNA damage in vitro in cultured CHO cells exposed to DCM in culture medium supplemented with mouse metabolizing enzymes (Graves et al. 1985) (see "Genotoxicity" section below). The active metabolites in those cases were necessarily generated from outside the cells, not just in the cytoplasm of the cells that manifested the DNA damage.
- OSHA is concerned that the target organs in humans may differ from those in mice. Theta isomers of GST occur in human blood and in high concentrations in human bile ducts. The evidence that Clara cells are the cells of origin for mouse lung tumors is weak. Other cell types in the lung, such as the Type II lung cells, also have relatively high metabolic activity and could be the site of origin for lung tumors.

- Most important, great caution should be used in attempting to characterize a difference between species as an absolute qualitative difference. The inability to measure a parameter, such as a concentration of mRNA for GST, does not mean that its value is zero. The allometric prediction is that, on a per-unit-of-tissue basis, humans should have about 7-fold lower activity than mice and about 4-fold lower activity than rats. Given the limit of detection of the assay methods, human metabolic activity (or mRNA levels) only slightly less than the allometric expectation of 7-fold less than mice are often difficult to distinguish from zero. Any interspecies differences are rightly considered first as quantitative rather than qualitative ones. Also, as pointed out by Green (1997), the measured levels of mRNA do not necessarily correlate with levels of GST enzyme activity.
- The available human epidemiological data are of insufficient power to rule out the possibility that DCM exposure causes a low but unacceptable increase in the incidence of tumors in humans.
- The lack of tumors in the rodent drinking water studies can be attributed to the much lower doses administered in those studies compared with the rodent inhalation studies.
- The NTP inhalation bioassay results in mice provide the best and most appropriate toxicologic and statistical data set for calculating the carcinogenic risk of DCM exposures in humans.

The International Agency for Research on Cancer recently reevaluated the data on DCM and classified the chemical as "possibly carcinogenic to humans" (IARC 1999).

Genotoxicity

No data were found on the genotoxicity of inhaled or ingested DCM in humans.

Gocke et al. (1981) found that DCM at concentrations of 125, 250, 500, and 750 microliters (μl) per 9 L of dessicator is mutagenic to *E. coli* strains TA98 and TA100 in the Ames/Salmonella test both with and without metabolic activation (Gocke et al. 1981). Jongen et al. (1981) reported that a 1-h exposure to DCM at 1%, 2%, 3%, and 4% produced a marginal increase in the frequency of sister chromatid exchanges in Chinese hamster V79 cells at all doses, but produced no increase in forward mutations in cultured Chinese hamster ovary cells at doses up to 5% for 17 h and no increase in unscheduled DNA synthesis (UDS) in primary human fibroblasts or V79 cells at DCM doses up to 5% for 1 h.

DCM was reported to be inactive in mouse bone marrow micronucleus tests at doses up to 4,000 mg/kg by gavage in corn oil (Gocke et al. 1981; Sheldon et al. 1987) as well as in both the mouse bone marrow micronucleus and SCE assays by subcutaneous injection of 2,500 mg/kg or 5,000 mg/kg in female $B6C3F_1$ mice (Allen et al. 1990). Nevertheless, inhalation of DCM at 4,000 ppm or 8,000 ppm by female $B6C3F_1$ mice for 10 d resulted in significant increases in the frequencies of SCEs in lung cells and peripheral blood lymphocytes, chromosome aberrations in lung and bone marrow cells, and micronuclei in peripheral blood erythrocytes (Allen et al. 1990). Exposure to DCM at lower concentrations (2,000 ppm) for longer durations (3 mo) produced small but significant increases in lung cell SCEs and micronuclei in peripheral blood erythrocytes (Allen et al. 1990). DCM caused extensive chromosome aberrations and a slight increase in the SCE level in human peripheral lymphocytes, a marginal response in the UDS tests, and a negative response in the cell transformation and point mutation tests in mouse lymphoma L5178Y cells (Thilagar et al. 1984a,b). DCM given by gavage to A1pk:AP rats at 100, 500, or 1,000 mg/kg failed to induce UDS, and inhaled DCM at 2,000 ppm and 4,000 ppm for 2 h or 6 h was negative for UDS activity in the livers of $B6C3F_1$ mice and F-344 rats (Trueman and Ashby 1987). DCM at 2.5, 5, and 10 µl/mL in culture medium containing 0.5% dimethyl sulfoxide (DMSO) was negative for UDS in human lymphocytes cultured for 4 h both in the presence of and in the absence of S-9 metabolic activation mix (Peroco and Prodi 1981). DCM at up to 16 millimolar (mM) did not elicit genotoxicity in the DNA repair test with freshly isolated rat hepatocytes in suspension (Andrae and Wolff 1983).

DCM at 125 mM and 620 mM increased the frequency of recessive lethal mutations in the Basc test in *Drosophila* 2-fold (significant at the 5% level) (Gocke et al. 1981).

Dose-related increases in DNA single-strand breaks were detected in the livers of $B6C3F_1$ mice immediately following a 6-h exposure to DCM at 4,000, 6,000, or 8,000 ppm, but not in mice exposed at 2,000 ppm (Graves et al. 1985). This damage was undetectable 2 h after the exposure, suggesting an active DNA repair process. Similarly, DNA single-strand breaks were detected in whole-lung homogenates taken from mice exposed for 3 h to DCM at 2,000, 4,000, or 6,000 ppm, but not in those taken from mice exposed at 1,000 ppm (Graves et al. 1985). In contrast, no single-strand breaks were observed in DNA from whole-lung homogenates from AP rats similarly exposed to DCM at 4,000 ppm. The DNA of mouse Clara cells incubated 2 h in vitro with DCM at 0, 5, 10, 30, and 60 mM also had single-strand breaks at concentrations of 5 mM and above (Graves et al.

1985). Pretreatment of mice with the glutathione inhibitor buthionine sulphoximine (BSO) caused a decrease in the amount of DNA damage detected, suggesting a GST-mediated mechanism (Graves et al. 1985). DNA damage was also reduced in Clara cells when incubated 2 h in vitro with DCM at 10 mM in the presence of BSO (Graves et al. 1985). In CHO cells, induction of DNA damage depended on exogenous metabolism of DCM by mouse liver S100 fraction (but not microsomes) in the presence of glutathione (Graves et al. 1985). DNA single-strand breaks were not induced in hamster hepatocytes in vitro at DCM concentrations from 5 mM to 90 mM or in eight individual samples of normal human hepatocytes exposed to DCM at similar concentrations (Graves et al. 1985). The ability of DCM to induce DNA single-strand breaks in the three nonhuman species studied parallels the known carcinogenicity of DCM in those species and their greater metabolism of DCM by the GST pathway. That suggests that humans might not be as susceptible to DCM-induced liver cancer, because the mechanism of DCM carcinogenesis is believed to be through genotoxic metabolites of the GST pathway and human metabolic rates for this pathway are much lower than those found in the rat and mouse.

Reproductive Toxicity

No reliable studies were found regarding reproductive effects in humans after exposure to DCM. The limited data available for humans were for inhalation exposures, and the interpretation of the results was uncertain because of confounding factors.

DCM did not induce testicular pathology or reduced fertility in vivo in Swiss-Webster male mice either injected subcutaneously with DCM at 5 mL/kg in corn oil three times a week for 4 wk or inhaling DCM at 100, 150, or 200 ppm for 2 h/d, 5 d/wk for 6 wk (Raje et al. 1988).

In rats, no adverse effects on reproduction were observed at inhaled concentrations up to 1,500 ppm for two generations. Other studies in animals were inconclusive. Existing data suggest that reproductive toxicity is not a major area of concern following exposure to methylene chloride.

Developmental and Fetal Toxicity

No studies were found regarding developmental effects in humans after exposure to DCM in drinking water or by any other route of exposure.

TABLE 2-3 Toxicity Summary

Concentration and Route of Exposure	Exposure Duration	Species	Effects	Reference
Acute exposures (≤1 d)				
16 mg/kg/d (PBPK extrapolation for inhalation at 300 ppm to oral route) (400 mg/L × 2.8 L × 70 kg)	4 h	Human	Decreased flicker frequency	Reitz et al. 1997
Approximately 300 ml paint remover; oral	Bolus	Human, n = 1	Death at 25 d postexposure preceded by corrosion of GI tract and CNS depression	Hughes and Tracey 1993
300 ml; oral	Bolus	Human, n = 1	Death at 9 d post-ingestion concomitant with pulmonary edema, CNS depression, and anuria	Chang et al. 1999
1-2 pints paint remover; oral	Bolus	Human, n = 1	CNS depression, tachypnea, ulceration of GI tract, hemoglobinuria	Roberts and Marshall 1976
25-350 ml; oral	Bolus	Human, n = 5	CNS depression, tachypnea, ulceration of GI tract, hemoglobinuria; no cardiac arrhythmia; hepatic and renal failure in two of six cases; one death	Chang et al. 1999
Subchronic exposures (11-100 d)				
166 mg/kg/d; drinking water	90 d	Rat	Hepatocellular vacuolization; altered clinical chemistry	Kirschman et al. 1986

(Continued)

TABLE 2-3 Continued

Concentration and Route of Exposure	Exposure Duration	Species	Effects	Reference
Subchronic exposures (11-100 d) cont.				
420 mg/kg/d; drinking water	90 d	Rat	NOAEL for kidney effects	Kirschman et al. 1986
1,200 mg/kg/d; drinking water	90 d	Rat	Centrilobular necrosis; increased kidney weight	Kirschman et al. 1986
226 mg/kg/d; drinking water	90 d	Mouse	NOAEL for centrilobular fatty liver	Kirschman et al. 1986
587 mg/kg/d; drinking water	90 d	Mouse	LOAEL for centrilobular fatty liver	Kirschman et al. 1986
Chronic exposures (≥100 d)				
6 mg/kg/d; drinking water	78-104 wk	Rat	NOAEL for hepatocellular foci and fatty changes	Serota et al. 1986a
55 mg/kg/d; drinking water	78-104 wk	Rat	Increased hepatocellular foci and fatty changes	Serota et al. 1986a
55 mg/kg/d; drinking water	78-104 wk	Rat	NOAEL for decreased body weight and decreased food and water consumption	Serota et al. 1986a
131 mg/kg/d; drinking water	78-104 wk	Rat	Decreased body weight and decreased food and water consumption	Serota et al. 1986a
249 mg/kg/d; drinking water	78-104 wk	Rat	NOAEL for hematological effects	Serota et al. 1986a

175 mg/kg/d; drinking water	104 wk	Mouse	NOAEL for hepatic effects	Serota et al. 1986b
236 mg/kg/d; drinking water	104 wk	Mouse	Increased incidence of fatty liver and foci/areas of cellular alteration in liver; NOAEL for hematological effects	Serota et al. 1986b

TABLE 2-4 Spacecraft Water Exposure Guidelines for DCM

Duration	Concentration (mg/L)	Target Toxicity
1 d	40	Reduced water consumption, CNS effects[a]
10 d	40	Reduced water consumption, CNS effects[a]
100 d	40	Reduced water consumption, CNS effects,[a] hepatotoxicity
1,000 d	15	Hepatotoxicity

[a]CNS effects were from inhalation data (Winneke 1974) extrapolated to oral exposures using a PBPK model (Reitz et al. 1997).

No effects on neonatal growth and survival were observed when male and female rats inhaled up to 1,500 ppm (5,205 mg/m^3) for 6 h/d, 5 d/wk over the course of two generations (Wiger 1991). Overall, fetal sensitivity to DCM does not appear to differ from that of adults (Wiger 1991).

RATIONALE

The spacecraft water exposure guideline (SWEG) listed above (Table 2-4) for each exposure duration was set using the lowest value among the acceptable concentrations (ACs) for all of the significant adverse effects at that exposure duration. (See Table 2-5 for guidelines set by other organizations.) ACs were determined following the guidance of the National Research Council (NRC 2000). They were calculated assuming consumption of 2.8 L of water per day. This includes an average of 800 mL/d used to prepare and reconstitute food in addition to 2.0 L/d for drinking. ACs of DCM were set for hepatotoxicity. In humans, DCM-induced kidney toxicity has been reported only after ingestion of near fatal doses. Because the liver is the major target organ for DCM, ACs that protect against liver toxicity in humans also are protective for kidney toxicity. Therefore, no ACs were set for nephrotoxicity in humans.

CNS Effects

Human exposure to inhaled DCM at 300 ppm produced a subtle CNS effect (decreased critical flicker frequency) (Winneke 1974). Reitz et al.

TABLE 2-5 Drinking Water Standards for DCM Set by Other Organizations

Organization	Standard	Amount	Concentration (2 L/d × 70 kg)
EPA[a]	1-h HA	—	10 mg/L for children
EPA[a]	10-d HA	—	2 mg/L for children
EPA[a]	DWEL	0.057 mg/kg/d	2 mg/L
EPA[a]	MCLG (final, 1998)	0 mg/kg/d	0 mg/L
EPA	RfD (oral, 1998)	0.06 mg/kg/d	2 mg/L
ATSDR	0-14 d MRL	0.5 mg/kg/d	17.5 mg/L
ATSDR	15-364 d MRL	NS[b]	NS[b]
ATSDR	365 d MRL	0.2 mg/kg/d	7 mg/L

[a]Set by EPA's Office of Drinking Water.
[b]ATSDR did not derive an oral MRL for exposures of 15-364 d because of an inadequate database.
Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; DWEL, drinking water equivalent level; EPA, U.S. Environmental Protection Agency; HA, health advisory; MCLG, maximum contaminant level goal; MRL, minimal risk level; NS, not set; RfD, reference dose.

(1997) used Winneke's data in a PBPK model to calculate a brain tissue concentration of 3.95 mg/L and extrapolated the inhalation parameters to an exposure to drinking water containing DCM at 562 mg/L, assuming a 70-kg person consuming 2.0 L/d. Reitz et al.'s calculated LOAEL of 16 mg/kg/d was used to calculate an AC assuming consumption of 2.8 L of water per day by a 70-kg person and using an uncertainty factor of 10 to estimate the NOAEL from the LOAEL. Because DCM does not accumulate in the body, this AC would apply for all exposure durations.

$$AC = 16 \text{ mg/kg/d} \times 70 \text{ kg} \div 2.8\text{L} \div 10;$$
$$AC = 40 \text{ mg/L}.$$

Hepatotoxicity

The evidence and logic used to determine the ACs for hepatotoxicity for each exposure duration are documented below.

ACs for hepatotoxicity for 1-d and 10-d exposures are based on Kirschman et al.'s (1986) negative histopathology results at the interim 30-d necropsy of rats and mice given DCM in drinking water for 90 d at concentrations of 0, 0.15, 0.45, and 1.5%. The calculated doses for rats were 166, 420, and 1,200 mg/kg/d (males) and 209, 607, and 1,469 mg/kg/d (females); and the calculated doses for mice were 226, 587, and 1,911 mg/kg/d (males) and 231, 586, and 2,030 mg/kg/d (females). ACs were determined using the NOAEL of 2,030 mg/kg/d in mice necropsied 30 d postexposure. A factor of 10 was used for interspecies extrapolation because, although humans appear to metabolize DCM at a much slower rate than do mice or rats, it is not known if the observed hepatotoxicity is due to metabolites or to the parent compound. If an appreciable fraction of the observed hepatotoxicity is due to the parent compound, the available data do not permit comparison of interspecies differences in susceptibility. Thus, the default factor of 10 was used.

1-d and 10-d acceptable doses = 2,030 mg/kg/d × 70 kg ÷ 10 = 14,200 mg/d.

Astronauts consume an average of 2.8 L of drinking water per day, so the 1-d and 10-d AC in drinking water was calculated to be

$$14{,}200 \text{ mg/d} \div 2.8 \text{ L/d} = 5{,}000 \text{ mg/L}.$$

The 100-d AC for hepatotoxicity is based on Kirschman et al.'s (1986) 90-d LOAEL of 166 mg/kg/d reported for rats. A factor of 10 was applied for extrapolation from a LOAEL to a NOAEL, and a second factor of 10 was applied for extrapolation from rats to humans. Thus, the acceptable dose would be

$$166 \text{ mg/kg/d} \times 70 \text{ kg} \div 10 \div 10 = 116 \text{ mg/d}.$$

Astronauts consume an average of 2.8 L of drinking water per day, so the 100-d AC in drinking water was calculated to be

$$116 \text{ mg/d} \div 2.8 \text{ L/d} = 42 \text{ mg/L}.$$

An AC in drinking water for 1,000 d was calculated using Serota et al.'s (1986a) NOAEL of 6 mg/kg/d for hepatotoxicity reported for rats consuming DCM in drinking water, and an interspecies factor of 10 was applied. The calculation assumed consumption of 2.8 L/d for a 70-kg person.

1,000-d AC = 6 mg/kg/d × 70 kg ÷ 10 ÷ 2.8 L/d;
1,000-d AC = 15 mg/L.

Taste Aversion and Reduced Water Consumption

Using Serota et al.'s (1986a) rat NOAEL of 55 mg/kg/d for reduced water consumption and reduced weight gain in rats treated with DCM in drinking water, the concentration of DCM that did not reduce water consumption in rats can be estimated. Assuming an average rat weight of 200 g and average consumption of 20 mL/d, the 55 mg/kg/d would be achieved at a DCM concentration of

55 mg/kg/d × 0.20 kg ÷ 0.02L = 550 mg/L.

An AC for humans was calculated by applying an interspecies factor of 10.

1-1,000-d AC = 550 mg/L ÷ 10 = 55 mg/L.

Carcinogenicity

Using the lung cancer incidence data from the NTP's 2-y bioassay of DCM in mice and rats as input for a PBPK model, Clewell (2000) calculated the DCM concentrations in drinking water that would yield a cancer risk of 1 in 10,000 in astronauts who consumed 2.8 L/d. The model produced the following results.

1000-d AC = 275 mg/L;
100-d AC = 1,650 mg/L;
10-d AC = 13,000 mg/L; and
1-d AC = greater than the solubility of DCM in water (22 g/L).

Spaceflight Effects

Spaceflight causes a shift of body fluids to the chest with a subsequent reduction in blood volume over the course of several days. The reduced blood volume is believed to contribute to orthostatic intolerance on return to 1-g. DCM at high concentrations in drinking water has been reported to

TABLE 2-6 Acceptable Concentrations (ACs)

End Point	Exposure Data	Species and Reference	Uncertainty Factors				ACs (mg/L)			
			To NOAEL	Inter-species	Exposure Time	Space-flight	1 d	10 d	100 d	1,000 d
Hepatotoxicity	NOAEL = 2,030 mg/kg/d; drinking water; 30 d	Rat (Kirschman et al. 1986)	1	10	1	1	5,000	5,000	—	—
Hepatotoxicity	LOAEL = 166 mg/kg/d; drinking water; 90 d	Rat (Kirschman et al. 1986)	10	10	1	1	—	—	42	—
Hepatotoxicity	NOAEL = 6 mg/kg/d; drinking water; 2 y	Rat (Serota et al. 1986a)	1	10	1	1	—	—	—	15
Taste aversion[a] and decreased water intake	NOAEL = 50 mg/kg/d; drinking water; 2 y	Rat (Serota et al. 1986a)	1	10	1	3	50	50	50	50
CNS effects[b]	LOAEL = 400 mg/kg/d; PBPK extrapolation from inhalation at 300 ppm	Human (Winneke 1974; Reitz et al. 1997; Clewell 2000)	10	1	1	1	40	40	40	40
Carcinogenicity[c]	NTP bioassay, lung tumors; PBPK extrapolation from inhalation at 2,000-4,000 ppm	Rat, mouse (NTP 1986; Clewell 2000)	PBPK	10	PBPK	1	—	13,000	1,650	275
SWEG							40	40	40	15

[a]Decreased palatability was assumed on the basis of decreased water consumption by rats.
[b]Reduced critical flicker frequency.
[c]The calculated 1-d AC was greater thatn the solubility of DCM in water and was therefore not reported.
Abbreviations: CNS, central nervous system; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level.

TABLE 2-7 Comparison of Daily DCM Exposure at the SWEGs and SMACs

Exposure	SWEGs			SMACs		
Duration	mg/L	mg/d	Effect	mg/m^3	mg/d	Effect
1 h	—	—	—	350	3,850	CNS depression
24 h (1 d)	40	112	Taste aversion, CNS depression	120	1,320	CNS depression
7 d	—	—	—	50	550	CNS depression
10 d	40	112	Taste aversion, CNS depression	—	—	—
30 d	—	—	—	20	220	Hepatotoxicity
100 d	40	112	Hepatoxicity, Taste aversion, CNS depression	—	—	—
180 d	—	—	—	10	110	Hepatotoxicity
1,000 d	15	42	Hepatotoxicity, CNS depression	—	—	—

cause a reduction in water consumption, which might exacerbate the normal reduction in blood volume and the resulting orthostatic intolerance.

Comparison of SWEGs to Inhalation Limits (SMACs) for DCM

The amount of DCM to which an individual would be exposed through drinking water at the SWEG values is compared in Table 2-7 (above) with the exposures that would result from inhalation at the recommended spacecraft maximum allowable concentrations (SMACs) for DCM vapors. The SWEG values assume consumption of 2.8 L of water per day and 100% absorption. The daily amounts that would be absorbed during inhalation of air containing the SMACs for DCM vapors assume inhalation of 20 m^3/d and retention of 55% (NRC 1996).

The daily amounts absorbed (mg/d) are comparable for the two routes of exposure, except for exposure durations less than 10 d. Because taste aversion is independent of duration, the ACs will not increase as the exposure duration is reduced from 10 d to 1 d.

TABLE 2-8 Comparison of SWEGs for DCM with Standards Set by Other Organizations

			Data Used for Calculations			Uncertainty Factors				
Organization	Type of Standard	Concentration (mg/L)	Species	LOAEL or NOAEL	Reference	To NOAEL	Interspecies	Inter-individual	Exposure Duration	Effect
NASA	1-d SWEG	40	Rat	50 mg/kg/d	Reitz et al. 1997	10	1	1	1	Taste aversion, CNS depression
ATSDR	1-14 d MRL	17.5	Human	300 ppm, inhaled	Reitz et al. 1997	10	1	3	1	CNS depression
NASA	10-d SWEG	40	Rat	50 mg/kg/d	Reitz et al. 1997	10	1	1	1	Taste aversion, CNS depression
ATSDR	14-364 d MRL	NS								
NASA	100-d SWEG	40	Rat	50 mg/kg/d	Reitz et al. 1997	10	1	1	1	Taste aversion, CNS depression, hepatotoxicity
ATSDR	≥365-d MRL	7	Rat	6 mg/kg/d	Serota et al. 1986a	1	3	10	1	Hepatotoxicity
NASA	1,000-d SWEG	15	Rat	6 mg/kg/d	Serota et al. 1986a	1	10	1	1	Hepatotoxicity

Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; CNS, central nervous system; LOAEL, lowest-observed-adverse-effect level; MRL, minimal risk level; NOAEL, no-observed-adverse-effect level; NS, not set.

Comparison of SWEGs to Standards Set by Other Organizations

The daily amounts ingested using the acceptable concentrations recommended above and assuming consumption of 2.8 L of water per day and 100% absorption are compared in Table 2-8 (above) with the drinking water standards set by other organizations.

RECOMMENDATIONS FOR FUTURE RESEARCH

Research is necessary to establish at what concentration of DCM drinking water becomes unpalatable to adult humans.

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3 Di-*n*-butyl Phthalate

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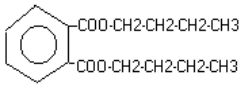
PHYSICAL AND CHEMICAL PROPERTIES

Di-*n*-butyl phthalate (DBP) is a colorless to faint yellow viscous liquid with a slight aromatic odor and a strong bitter taste (see Table 3-1) (NIOSH 1994; Grant 1986).

OCCURRENCE AND USE

DBP is widely used as a plasticizer to give flexibility to manufactured materials. It is not covalently linked to the plastic polymer, so the potential for leaching from the plastic into contained items such as water or food is high. DBP is also used in insect repellants, lacquers, and rocket propellants. During the NASA/Mir program, DBP was found in the recycled water at an average concentration of 14 micrograms per liter (μg/L), with a high of 297 μg/L (Pierre et al. 1999). The humidity condensate from one shuttle flight contained DBP at concentrations of 20-42 μg/L (Straub et al. 1995). In comparison, in approximately 1,500 samples of ground and surface water in Alberta, Canada, the concentrations of DBP averaged 1 μg/L, with a high of 7 μg/L (Chen and Meek 1994). In a summary of data on municipal

TABLE 3-1 Physical and Chemical Properties of DBP

Formula	$C_{16}H_{22}O_4$
Synonyms	Dibutyl phthalate, *o*-benzenedicarboxylic acid, 1,2-benzenedicarboxylic acid, dibutyl ester
	
CAS registry no.	84-74-2
Molecular weight	278.35
Boiling point	35°C
Melting point	340°C
Solubility	0.001% in water at 30°C, soluble in most organic solvents (Lefaux 1968); 9-13 mg/L in water at 20°C (DeFoe 1990)
Density	1.047 at 20°C (WHO 1997)

drinking water from various countries, the maximum concentration of DBP reported was 5 μg/L (WHO 1997).

The presence of DBP in food contained in plastic packaging could affect the amount of DBP ingested by astronauts. Food repackaged by NASA is generally placed in laminated containers that have polyethylene as the inner most component. Polyethylene should not leach any DBP; however, that does not completely preclude the possibility of leaching from packaging in which the food was shipped or in foods that are flown in their original packaging. The amount of food-borne DBP ingested by the crew should be small and certainly no more than that ingested by the general public eating food mostly from commercial packaging.

TOXICOKINETICS

Orally ingested DBP is hydrolyzed to mono-butyl phthalate (MBP) and absorbed by the gut within a few hours. MBP is distributed widely in the body and is further metabolized in the liver by oxidation and conjugation to phthalic acid and MBP glucuronide, respectively. Most elimination from the body is via the urine, with a small fraction eliminated in the feces (Tanaka et al. 1978).

Absorption

Absorption of an oral dose of DBP from the gut is related to its hydrolysis to the monoester derivative (Lake et al. 1977). This was demonstrated by showing the ability of intestinal tissue from rats, ferrets, baboons, and humans to hydrolyze DBP in vitro. The kinetics and extent of absorption of a single oral dose of DBP by pregnant rats depends on the dose. Rats given 500 milligrams per kilogram (mg/kg) of radiolabeled DBP showed peak radioactivity in the plasma in 1-2 hours (h), whereas rats given 1,500 mg/kg had peak plasma radioactivity in 4-6 h (Saillenfait et al. 1998).

Distribution

After an oral dose, DBP and its metabolites are distributed in the blood stream to many organs; however, they are rapidly excreted in the urine and feces. The tissue distribution 24 h after rats were given an oral dose of ^{14}C-labeled DBP at 60 mg/kg showed the following percentages of the dose remaining: intestine, 1.5%; fat, 0.7%; muscle 0.3%; liver, 0.06%; blood, 0.02%; and kidney, 0.02% (Tanaka et al. 1978). Radioactivity was undetectable in the brain, heart, lung, spleen, testes, prostate, and thymus.

After 14 days (d) of administration of DBP at 5 grams (g) per day to pigs in their feed, several tissues were studied for accumulation of DBP (Jarosova et al. 1999). Phthalates were assayed in the liver, kidneys, lungs, brain, heart, muscle, renal fat, and subcutaneous fat. The highest concentrations were found in the muscle and adipose tissue. Similarly, after 14 d of administration of 100 mg/d to chickens in their feed, the muscle, skin, liver, and mesenterial fat were assayed for DBP (Jarosova et al. 1999). The authors report that the distribution in those tissues was uniform. Fourteen and 28 d after the dosing was stopped, both species seemed to have an accumulation of the phthalates in adipose tissue.

Saillenfait et al. (1998) reported the tissue distribution of radioactivity following a single oral dose of ^{14}C-labeled DBP at 500 mg/kg or 1,500 mg/kg to pregnant rats on gestation day 14. The tissues examined were the plasma, kidney, liver, ovary, uterus, placenta, embryo, and amniotic fluid. At the lower dose, radioactivity peaked in each tissue within 1-2 h and declined rapidly, although kidney levels lagged somewhat. Except for plasma, radioactivity on a tissue-weight basis was consistently highest in the kidneys and second-highest in the liver. Radioactivity was almost undetectable in any tissue 48 h after the dose was given. At the higher dose, the tissue distribution was similar to the distribution reported for the lower

dose; however, the maximum levels in the tissues appeared 2-6 h after the dosing and then began a slower decline than that reported for the lower dose (Saillenfait et al. 1998).

Metabolism

Based on studies with intestinal tissue extracts from a variety of species, the initial step in the metabolism of DBP is hydrolysis to MBP and, presumably, *n*-butanol before the dose is absorbed from the gut (see Figure 3-1) (Lake et al. 1977). In rats, the hydrolysis seems to continue, because *o*-phthalic acid has been reported in the urine of rats given an oral dose of DBP (ACGIH 1991). Alternatively, the remaining butyl group in MBP can be oxidized in the liver, or the MBP can be conjugated to form MBP glucuronide (Tanaka et al. 1978; Foster et al. 1982; Saillenfait et al. 1998).

There are distinct differences in the proportion of metabolites produced by different species. Unconjugated MBP is 3-4 times higher in the urine from rats than in the urine from hamsters, even though each species has comparable esterase in the gut (Foster et al. 1982). Both species of animals were treated at a dose of 2 g/kg body weight. However, the authors postulate that the higher level of free MBP may explain the higher susceptibility to testicular toxicity in rats when compared with the susceptibility in hamsters.

The blood disposition of MBP is important because this metabolite is thought to mediate the effects in target tissue. Intravenous doses of DBP in the range of 8-34 mg/kg in rats gave a biphasic blood elimination profile for MBP. This was modeled best with a diffusion-limited, pH-trapping pharmacokinetic model (Keys et al. 2000). Diffusion limited models postulate that the MBP must be in the non-ionic form to move between the blood and tissue compartments. The pH-trapping model postulates that un-ionized MBP is "trapped" as MBP^- in the tissue, thus preventing its movement back into the blood from the tissue until it becomes un-ionized again. This model was able to give reasonably good predictions of blood MBP for approximately 1 d after oral doses of DBP at 43 mg/kg to 857 mg/kg in rats. The biphasic elimination pattern became more obscure as the concentrations were increased.

Elimination

The primary route of elimination in rats is the urine, but the feces is also

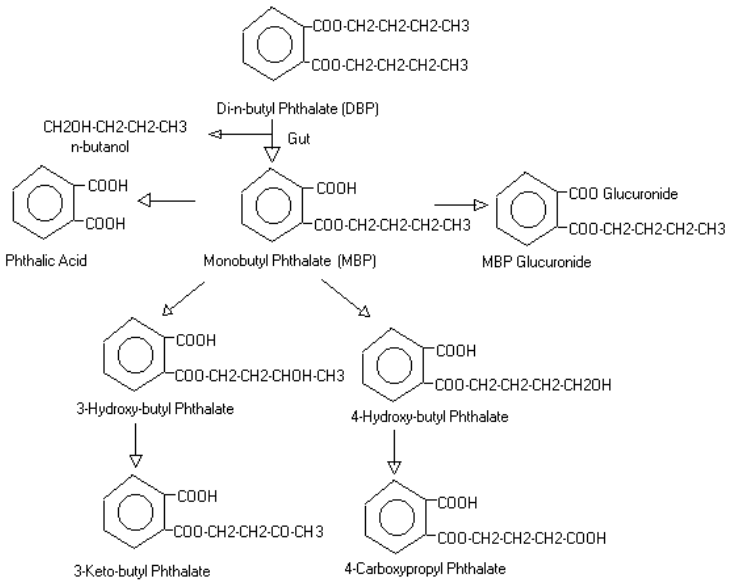


FIGURE 3-1 Metabolism of DBP. Source: ATSDR 1990.

an important route of elimination. Radioactivity from ^{14}C-labeled DBP given to rats orally in 3% dimethyl sulfoxide (DMSO) at a dose of 60 mg/kg was, on average, 94% eliminated in the urine and feces within 24 h of the dose (Tanaka et al. 1978). Of the five animals studied, the fraction in the urine varied from 81% to 98%, and the fraction in the feces varied from 1% to 8%. On average, an additional 6% of the radioactivity was eliminated during the second day after dosing (Tanaka et al. 1978). All of the metabolites shown in Figure 3-1 were found in rat urine except the keto compound; MBP was the predominate metabolite in urine. Hamsters and guinea pigs showed slightly different metabolite profiles in their urine samples (Tanaka et al. 1978).

TOXICITY SUMMARY

The toxicity database focuses on the reproductive and developmental

effects caused by ingestion of DBP. In laboratory animals, the acute and short-term toxicity of the compound is very low by oral ingestion. Well-documented subchronic studies in animals have only recently become available, and no complete reports of a chronic study of DBP could be found. There are no well-documented human oral studies that included a meaningful number of subjects. For the purposes of risk assessment, the best studies are those using an appropriate species, animals of the appropriate age, doses that produce a NOAEL (no-observed-adverse-effect level) or a clear dose response suitable for benchmark dose (BMD) modeling, and a route of administration that is similar to ingestion in water.

Acute Toxicity (1-5 d)

Acute oral data available in the NTP chemical registry indicates toxicities as shown in Table 3-2. The compound is considered mildly toxic by ingestion.

When male rats 3-4 weeks (wk) old were given DBP by oral intubation for 3 d at a dose of 2,000 mg/kg, they had testicular injury and a 24% decrease in testicular weight (Cater et al. 1977). The weight decrease worsened in the 10 d after the treatment ceased. The reduced testicular weight was found in rats given 1,000 mg/kg, but not in those given 500 mg/kg for 4 d; however, there was an 18% loss in testicular weight in rats given DBP at 500 mg/kg for 6 d (Cater et al. 1977). A similar effect was caused on the testes by MBP, but not by *n*-butanol or *o*-phthalic acid. The 4-d NOAEL for testicular effects in young rats was 500 mg/kg/d by bolus oral dose.

A case report of an acute poisoning after ingestion of approximately 10 g of DBP was described and cited by Lefaux (1968). The man developed nausea and vertigo and was taken to the hospital on the day after ingestion. Albumin and blood cells were noted in the urine; the patient was treated and discharged 14 d later with no apparent lasting effects. Whether a person could actually swallow that amount of DBP has been questioned because of its strong and bitter taste (Grant 1986).

Short-Term Toxicity (6-30 d)

The effects of short-term ingestion have been studied by few investigators except for the purpose of understanding the reproductive toxicity, developmental effects, or other specific toxicologic properties of DBP. The

TABLE 3-2 Acute Oral Toxicity of DBP

Type of Dose	Species	Amount
TD_{Lo}	Human	140 mg/kg
LD_{50}	Rat	8,000 mg/kg
LD_{50}	Mouse	5,300 mg/kg
LD_{50}	Guinea pig	10,000 mg/kg

Source: NTP 1991.

reproductive and developmental toxicity of DBP is discussed in specific sections below.

Groups of five F-344 male rats and groups of five female rats were given DBP in their food at concentrations of 0%, 0.6%, 1.2%, or 2.5% for 21 d (CMA 1986). Body weights and food intake were monitored, serum cholesterol and triglyceride were measured (after fasting), and the liver, kidneys, and testes were taken at necropsy for weighing and histopathology. Biochemical parameters indicative of peroxisome proliferation (PP) were assayed in liver tissue. DBP increased the relative liver weights in all DBP-exposed groups; the testes from the high-dose group were 30-40% lower in weight and showed severe atrophy upon histopathologic examination. The report indicates that serum cholesterol and triglyceride were decreased in all treated males and only cholesterol was decreased in all treated females, but the decreases were not dose-related (CMA 1986). Inspection of the data suggests that biologically significant differences related to DBP exposure were not demonstrated by the changes in triglycerides and cholesterol. Evidence of PP was found in the livers from all groups of treated males and in the highest-dose female group. The NOAEL from this study was the lowest dose in the feed, or 0.6%. This was calculated to be equivalent to approximately 620 mg/kg/d. This study was conducted according to good laboratory practices (GLPs) promulgated by EPA.

It is important to note that there is a direct correlation between the time of ingestion of DBP and the magnitude of the hepatomegaly observed in rats. When 3- to 4-wk-old male rats were given DBP at 2,000 mg/kg in corn oil by gavage for 14 d, the relative liver weights of the exposed rats progressively exceeded the relative liver weights of the controls (Cater et al 1977). The fraction by which the exposed rat livers exceeded control rat livers was as follows: after 3 d, 4%; after 7 d, 10%; after 10 d, 11%; and after 14 d, 14%.

Subchronic Toxicity (30-180 d)

Two thorough rodent subchronic feeding studies have been reported—one in F-344 rats and B6C3F$_1$ mice (NTP 1995), and another in Wistar rats (BASF 1992).

Male and female Wistar rats were administered DBP in their food at concentrations of 0, 400, 2,000, and 10,000 parts per million (ppm) over a period of 3 months (mo) (BASF 1992). There were 10 rats per group and gender, and each was evaluated for weight gain, nervous system function, clinical pathology, gross pathology, and histopathology at appropriate times during the study or at the end of the exposure period. At 10,000 ppm (750 mg/kg/d) the changes were as follows: decrease in serum triglycerides and triiodothyronine, increase in cyanide-insensitive palmitoyl-CoA-oxidation in the livers of both genders (indicator of PP), transient decrease in red cell indices in males, increase in serum glucose and albumin in males, and increase in liver weights in both genders (14% in males and 16% in females). No DBP-related changes were detected at the lower concentrations, hence the NOAEL was 2,000 ppm (150 mg/kg/d). This study was conducted according to Organisation of Economic Cooperation and Development (OECD) guidelines, followed good laboratory practices, and was reported in sufficient detail to be useful for setting a health standard.

During a standard 13-wk feeding study, male and female F-344 rats received DBP at 0, 2,500, 5,000, 10,000, 20,000, and 40,000 ppm in their food starting at 5-6 wk of age. Male and female B6C3F$_1$ mice received DBP in their food at half the concentrations received by the rats (NTP 1995). The findings in rats can be summarized as follows: reduced final body weights in male rats receiving 10,000 ppm or higher and in female rats receiving 20,000 ppm or higher; hepatomegaly in male rats at or above 5,000 ppm and in female rats at or above 10,000 ppm; reduced testicular weight in male rats at 20,000 and 40,000 ppm; slight anemia in male rats at or above 5,000 ppm; reduced cholesterol in male and female rats receiving 10,000 ppm or more; reduced triglycerides in all DBP-exposed male rats and in female rats receiving 10,000 ppm or more; elevated peroxisome enzymes in male and female rats ingesting 5,000 ppm or more; and depletion of the germinal epithelium and zinc in testes of male rats given 20,000 ppm or more. If the hypocholesterolemia is not considered an adverse effect, then the NOAEL was 2,500 ppm for F-344 rats. These results and the demonstrated toxic effects of DBP in rats are fully consistent with those reported in the BASF (1992) study.

Several opportunities for BMD calculations were apparent from the subchronic NTP (1995) study. Selected possibilities are shown in Table 3-3.

The bile acid increases in serum reflect cholestasis (alkaline phosphatase was also increased), which is most likely related to the hepatomegaly resulting from PP. Because PP is not known to be inducible in humans by phthalates, a risk assessment of this change was not performed. Kidney weights were increased and were clearly related to DBP exposure; however, despite extensive histopathology, there were no kidney changes noted, so this change was not confirmed as adverse. BMD analyses were conducted on the two indices of testicular injury and on the reduced RBC counts (Table 3-4, Figures 3-2 and 3-3). A NOAEL of 176 mg/kg/d was identified from the NTP (1995) study.

Mice exposed to DBP for 13 wk exhibited many of the same effects reported for rats (NTP 1995). Body-weight gains were reduced for males and females in the 5,000 ppm group and above; liver weights were increased in males and females ingesting 5,000 ppm or above; a slight anemia was found in females ingesting 20,000 ppm; and microscopic changes in the liver were evident in males receiving 10,000 ppm or above and in females receiving 20,000 ppm. The NOAEL for the effects of DBP on mice exposed in this study was 2,500 ppm (353 mg/kg/d for males) (NTP 1995). Because mice were less sensitive than rats, no attempt was made to do BMD calculations using mice data.

Chronic Toxicity (0.5 y to lifetime)

The early work of investigators in the U.S., Germany, and France was summarized by Lefaux (1968). The U.S. investigator (C.C. Smith) gave DBP in food to groups of 10 male rats at concentrations of 0.0%, 0.01%, 0.05%, 0.25%, and 1.25% for 12 mo starting at 5 wk of age. Those concentrations are equivalent to 0, 5, 25, 125, and 600 mg/kg/d, respectively. The three lower doses did not inhibit the growth of the rats; however, the highest dose killed half the rats within the first week. No specific lesions could be found at necropsy. Apparently, the survivors in the high-dose group gained weight at a rate comparable to controls. The animals from all groups that survived for 1 y were subjected to gross necropsy and hematologic studies. Both evaluations were negative in all groups except the highest-dose group. The results of this study suggest a NOAEL of 125 mg/kg/d in rats.

TABLE 3-3 Selected Effects from the 13-wk NTP Study in F-344 Rats

Dose (mg/kg/d)		Bile Acids (μM/L) (F)	Relative Weight Right Kidney (M)	Absolute Weight Right Testis (g) (SE)	Atrophy, Germinal Epithelium, Testes (M)	RBC (10^6/μL) (SE)
Male	Female					
0	0	22.2	3.62	1.459 ± 0.029	0/10	9.15 ± 0.08
176	177	30.8	3.75	1.494 ± 0.023	0/10	9.08 ± 0.12
359	356	35.9[a]	3.91[a]	1.490 ± 0.010	0/10	8.89[a] ± 0.07
720	712	35.2[a]	4.07[a]	1.455 ± 0.017	4/10 (1.8)[a]	8.80[a] ± 0.08
1,540	1,413	40.0[a]	4.27[a]	0.521[a] ± 0.054	10/10 (3.8)[a]	8.24[a] ± 0.16
2,960	2,943	67.6[a]	4.92[a]	0.321[a] ± 0.012	10/10 (4.0)[a]	8.32[a] ± 0.07

[a]Statistically different from controls according to NTP.
Source: Data from NTP 1995.

TABLE 3-4 BMD Analysis of Testicular and Hematologic Lesions[a]

Risk Level	Absolute Weight of Right Testis		Atrophy of Germinal Epithelium		RBC Count	
	10%	1%	10%	1%	10%	1%
BMR	1.3	0.4	0.1	0.01	1.3	0.4
BMD	820	684	652	583	836	611
BMDL	731	591	383	111[b]	397	102[b]
Model	Hill		Probit		Hill	

[a]Based on NTP's (1995) 13-wk study.
[b]Used in Rationale section to estimate an acceptable concentration for humans.
Abbreviations: BMD,benchmark dose; BMDL, lower confidence limit on the BMD; BMR, benchmark response; NTP, National Toxicology Program; RBC, red blood cell.
Source: Data from NTP 1995.

The study in Germany (G. Bornmann) involved gavage administration of DBP to rats at doses of 0.5 mL/kg and 0.25 mL/kg twice a week for 52 wk. Clinical pathology tests were normal; however, a few of the animals developed sarcomas. The French study (E. Le Breton) involved Wistar rats receiving 0.1, 0.3, and 0.5 g/kg in their diets for 3-5 generations. In a complementary study, rats were given the two lowest doses for 21 mo, and the high-dose group was given the DBP-enriched diet for 15 mo. The French study concluded that under the test conditions there were no harmful effects of DBP and that the three doses were equivalent to a 70-kg, moderately active human ingesting 60, 180, and 300 mg of DBP per day, respectively. Even though these tests were not conducted according to modern protocols, they seem to suggest that the chronic oral toxicity of DBP is low.

Genotoxicity

The majority of genotoxicity testing of DBP has given negative results, and that makes sense given that neither of the immediate metabolites of DBP, MBP and *n*-butanol, are mutagenic (WHO 1997). The majority of the DBP studies have been in vitro, and a wide variety of test systems have been employed. The results of those tests have been summarized in review documents, and the reader is referred to those or the original publications for detailed results (WHO 1997; ATSDR 1990). Many of the negative findings with *S. typhimurium* seem to be incompletely reported and should

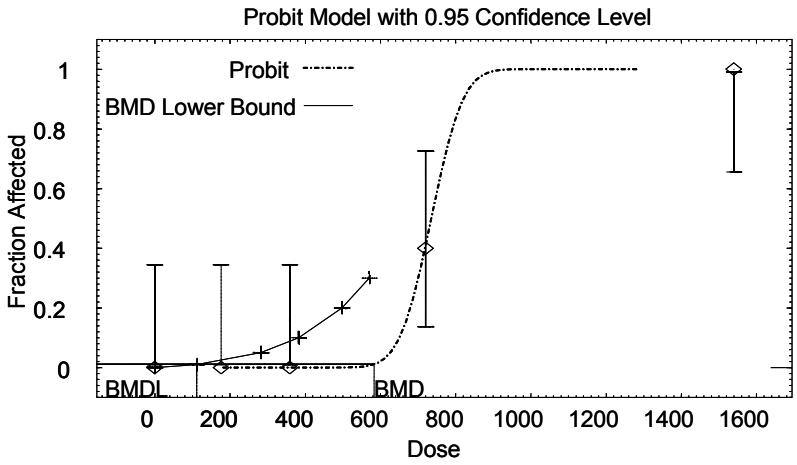


FIGURE 3-2 BMD analysis of the incidence of germinal epithelial atrophy in male rats ingesting DBP for 13 wk ($BMDL_{01}$ = 111 mg/kg/d).

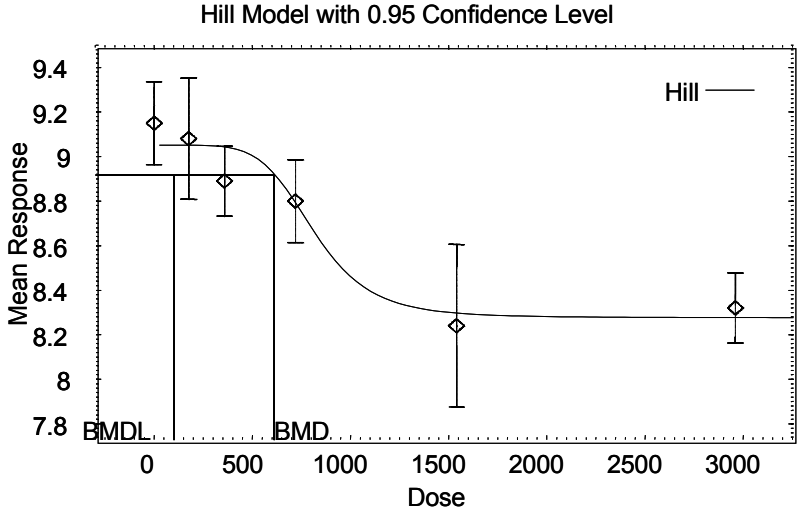


FIGURE 3-3 BMD analysis of RBC data for male rats ingesting DBP for 13 wk ($BMDL_{01}$ = 102 mg/kg/d).

be viewed with caution (WHO 1997). In one of the more thoroughly reported studies, Zeiger et al. (1985) reported negative results for strains TA100, TA1535, TA1537, and TA98 using 100-10,000 μg per plate, with and without activation from S9 (derived from the livers of two induced species). The protocol included repeat assays and positive controls.

The only positive results in vitro have occurred when metabolic activation using the S9 fraction was not used in the test system. Those results have either been very weakly positive and inconsistent with other results, or they have occurred at cytotoxic concentrations where false positives can be expected (Seed et al. 1982; Agarwal et al. 1985; NTP 1995; WHO 1997). For example, Seed et al. (1982) found a 50% increase over baseline in the number of mutants from TA100 strain exposed to DPB in liquid suspension at 0.18 mM without S9 activation; however, there was clear evidence of cytotoxicity at this concentration. Also, in the absence of metabolic activation and at concentrations up to 0.03 mg/mL, an equivocal result was obtained for chromosomal aberrations in a Chinese hamster fibroblast cell line (Ishidate and Ohashima 1977), but other tests of chromosomal effects have been negative. Neither SCEs nor chromosomal aberrations were induced in Chinese hamster ovary (CHO) cells exposed at concentrations up to 27.8 mg/mL (Abe and Sasaki 1977). There was an increase in mutant frequency in L1578Y mouse lymphoma cells under conditions of nonactivation and at concentrations above 46 μg/mL, but that increase occurred at cytotoxic doses (NTP 1995). False positives are expected from such assays at cytotoxic concentrations (WHO 1997).

Reproductive Toxicity

DBP has received considerable study as a reproductive toxicant to male and female rodents, and it has recently been reported to have weak in vitro estrogen-like activity. End points in males and females can be evaluated separately. It is important to NASA that astronauts not incur adverse effects on their reproductive organs during spaceflight; however, NASA does not anticipate that successful reproductive outcomes during flight are plausible. This means that studies in which animals are fed the test substance during gestation are not considered directly relevant for risk assessment in astronauts; however, adverse reproductive performance or pathology at the end of a subchronic or chronic study is relevant to risk assessment for astronauts. It is reassuring that "reproductive-system necropsy data from general toxicity studies can provide a valuable preliminary indication of the likely

reproductive toxicity of the compound under study [when evaluated by using more complex tests]" (Chapin et al. 1998). DBP has been shown to cause reproductive toxicity in male rats, mice, and guinea pigs.

Evidence that DBP could cause serious injury to the testes was not reported from early, prolonged feeding studies. However, in the mid-70s, studies demonstrated profound effects on the testes of rats given only a few doses of DBP at relatively high concentrations (Nikonorow et al. 1973; Cater et al. 1977). The lesion is characterized by decrease in testicular weight, decrease in diameter of the seminiferous tubes, and atrophy of the germinal epithelium. A loss of zinc through urinary excretion and a reduction of zinc in testicular tissue are associated with DBP-induced injury (Gangoli 1982). A study of eight di-*n*-alkyl phthalates showed that those capable of testicular injury were also capable of causing excess urinary excretion of zinc and loss of zinc in testicular tissue (Gangoli 1982). The mouse, rat, and guinea pig are susceptible to DBP-induced testicular injury at 2,000 mg/kg/d for 10 d, but the hamster is not susceptible at that dose.

The lowest dose associated with changes in the adult rat testes is 250 mg/kg/d given by gavage for 15 d (Srivastava et al. 1990a). At two higher doses (500 and 1,000 mg/kg/d), testicular weight was decreased, seminiferous tubes degenerated, and testicular enzymes were changed. At the lowest dose, only a change in one of six testicular enzymes was observed. This was an approximately 20% decrease in acid phosphatase. That study provides an apparent NOAEL of 250 mg/kg/d; however, detailed histopathology data or changes in testicular weights were not given. Changes in sperm counts and acid phosphatase activity were reported and have been used in a BMD analysis (Table 3-5, Figure 3-4). Acid phosphatase was one of six enzymes measured in testes and found to change significantly with DBP administration. The others were sorbital dehydrogenase, lactate dehydrogenase, y-glutamyl transpeptidase, beta-glucuronidase, and glucose-6-dehydrogenase. As discussed below, the changes in acid phosphatase activity are probably adaptive, whereas the reduced sperm counts are adverse.

Based on the NOAELs from the BMD analysis, the reduction in acid phosphatase is a more sensitive end point than the reduction in testicular weights. The question is whether enzymatic changes in the testes are an adverse or adaptive response to DBP exposure. Srivastava et al. (1990a,b) point out that LDH and SDH are associated with postmeiotic cells, GGT and beta G are markers for Sertoli cells, and G6PDH is a marker of spermatogenesis. Acid phosphatase is known to increase as spermatocytes mature to spermatozoa. Relatively small changes (e.g., 20%) in acid phos-

TABLE 3-5 Testicular Changes in Adult Wistar Rats Given DBP by Gavage for 15 d

Dose (mg/kg/d)	Acid Phosphatase Activity in Testes	Sperm Counts (10^6)	BMD Acid Phosphatase			BMD Sperm Counts	
				10%	1%	10%	1%
			BMR	1.3	0.4	1.3	0.4
0	200 (24)[a]	6.2 (0.7)	BMD	211	60	413	147
250	160 (19)[b]	5.8 (1.0)	BMDL	134	39	234	70[c]
500	145 (24)[b]	4.3 (1.1)		Polynomial model		Polynomial model	
1,000	117 (26)[b]	1.9 (1.7)		Homogeneous variance		Homogeneous variance	

[a]Number in parenthesis is the SD, which was calculated from the author's SE and number of subjects.
[b]These outcomes were considered to be statistically significant by the authors ($p < 0.05$). Statistical significance was not indicated for any of the sperm counts.
[c]Used in Rationale section to set an acceptable concentration.
Abbreviations: BMD, benchmark dose; BMDL,lower confidence limit on the BMD; BMR, benchmark response; SD, standard deviation; SE, standard error.
Source: Srivastava et al. 1990a.

phatase in the testes do not seem to affect the production of sperm, which is the functional role of the testes. Hence, the reduced sperm counts, not the reduced acid phosphatase activity (which could be considered a marker of testicular effects), will be considered the adverse effect.

The underlying mechanism of DBP-induced testicular toxicity is unknown; however, it is known that the initial cellular target, according to ultrastructural studies, is the Sertoli cell and that MBP is likely the chemical mediator (Foster 1997). The earliest lesion is cytoplasmic vacuolization of the cells due to enlargement of the cisternae of the endoplasmic reticulum. That might be due to decreased secretion of proteins, disruption of ionic pumps, or changes in cytoskeleton support (Richburg and Boekelheide 1997). The relationship between Sertoli cells and germ cells is complex and intimate. A species-specific number of germ cells, numbering approximately 22 in the rat (Chapin 1997), are associated with and adhere to each Sertoli cell. The mechanism of loss of the germinal epithelium through effects on the Sertoli cells may include the following: disruption of cell-

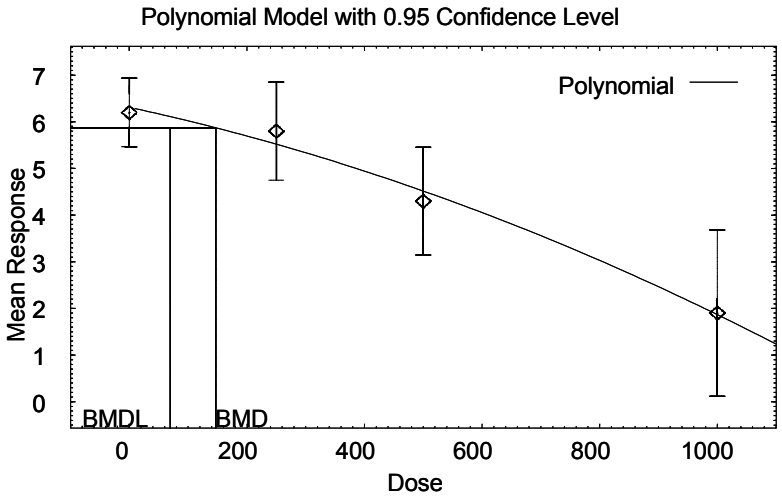


FIGURE 3-4 BMD analysis of sperm counts in rats given DBP by gavage for 15 d ($BMDL_{01}$ = 70 mg/kg/d).

to-cell junctions, loss of intracellular fibers in Sertoli cells, reduced seminiferous fluid secretion due to disruption of Sertoli cell microtubules, disruption of signal transduction (e.g., follicle stimulating hormone receptors on Sertoli cells), failure to provide metabolites (e.g., lactate), or Sertoli-cell directed apoptosis of germinal cells (Richburg and Boekeheide 1997).

DBP has been shown to disrupt androgen-related development of male reproductive indices when administered late in the gestation of rats (Mylchreest et al. 1999). DBP does not elicit the responses expected of an estrogen-like compound in developing females but alters androgen-dependent processes in developing males, so the action is presumed to be anti-androgenic. Even though DBP and its active metabolite, MBP, do not interact with the androgen receptor, the effects seen in vivo are typical of androgen receptor antagonists such as flutamide (Mylchreest et al. 1999). When compared to flutamide, DBP produced a lower incidence of prostrate agenesis and hypospadias and induced intra-abdominal testes rather than inguinal testes. According to Mylchreest et al. (1999), DBP is the only environmental chemical known to have anti-androgenic effects without interacting with the androgen receptor.

According to a continuous breeding protocol, CD-1 mice ingested food containing DBP at 0.0%, 0.03%, 0.3%, and 1.0% for 18 wk (Lamb et al. 1987). According to this protocol, mice were fed the DBP-spiked food for 1 wk. They cohabitated for an additional 14 wk during which DBP was consumed and the pups discarded. The pups were kept for 3 wk and nursed from a mother receiving DBP in her food. During the latter two periods, reproductive function was measured as the number of litters per breeding pair, number of live pups per litter, pup weight, and pup survival. Even though the body weights of the 1.0% males were less than the control body weights, there was not a statistically significant difference in the weights of the testes, epididymis, prostate, or seminal vesicles among the groups (Lamb et al. 1987).

In an NTP continuous breeding study, SD rats were given DBP at 0.1%, 0.5%, and 1.0% in their food; this produced daily intakes of 50, 250, and 500 mg/kg (males) and 80, 380, and 800 mg/kg (females) for the three levels, respectively (Wine et al. 1997). The F_0 generation ingested the DBP-spiked food for 14 wk and produced offspring continuously. The last litter (F_1) received the same food as their parents until they were mated at about 90 d. The number of live pups per litter from the F_0 rats was reduced in all treated groups by 8-17%, and crossover mating demonstrated that this effect was mediated through the females. Sperm parameters and the estrous cycles were not disrupted in any of the groups. The F_1 generation males receiving DBP at 1.0% had a higher incidence (8/10) of degeneration of seminiferous tubules than controls (1/10) or middle-dose animals (3/10). Thus, DBP is a reproductive toxicant in male SD rats when administered at high doses to two generations of animals (Wine et al. 1997).

The results of the NTP study do not yield a firm conclusion as to the NOAEL for reproductive toxicity. The only change observed in the lowest dose F_0 animals was an 8% reduction in the number of live pups produced per litter. In the mid-dose group, a 15% reduction in live pups per litter was observed, and the pup weights were also reduced by 4% (developmental index). The reduced number of live pups was not seen in the crossover breeding experiments or in the second generation; however, the authors attribute this to the greater statistical power of the initial test compared with the follow-up tests. Possible effects on male reproductive organs were indicated by an incidence of three out of 10 F_1 males showing degeneration of the seminiferous tubules at 0.5%. Even though there was a statistically reduced number of live pups produced by the F_0 generation (12.9 in controls and 11.9 in low-dose), this effect was not demonstrated in the F_1 matings (14.0 in controls and 15.5 in low-dose); therefore, the lowest dose to females (80 mg/kg/d) was estimated to be a NOAEL in SD rats.

The ability of DBP and seven other phthalate esters to exhibit estrogenic activity has been evaluated recently using a battery of in vitro and in vivo tests (Zacharewski et al. 1998). Even at doses as high as 2,000 mg/kg, none of the phthalate esters were positive in the two in vivo assays (uterine weights in immature, ovariectomized rats and vaginal epithelial cell cornification in mature, ovariectomized rats). DBP and two other compounds exhibited weak estrogen-receptor-mediated estrogenic activity in the in vitro assays. These results suggest that estrogenic activity might not be involved in the reproductive effects of DBP that have been reported in SD rats (Zacharewski et al. 1998; Wine et al. 1997).

Developmental Toxicity

DBP given in high concentrations to rodents has been shown to be toxic to developing fetuses, but only at concentrations that approach those that are toxic to dams. Data from ATSDR (1990)[1] indicate that the NOAEL in mice is approximately 650 mg/kg/d, whereas the NOAEL in rats is near 60 mg/kg/d for decreased pup weight. In a series of studies published since the ATSDR document was written, Ema et al. (1993, 1997, 2000) showed that pregnant Wistar rats given DBP only during gestation days 7-15 had increased fetal malformations at 630 mg/kg/d, but not at 500 mg/kg/d. They also observed that there may be two discrete response periods for skeletal malformations (days 8-9 and day 15) and that for genital effects in male offspring (undescended testes and reduced anogenital distance), the critical time is gestation days 15-17. When dams were given DBP on gestation days 7-15, maternal body weight gains were statistically below controls for the group given 650 mg/kg/d, but not for the group given 500 mg/kg/d (Ema et al. 1993). When DBP was administered to pregnant rats for only 3 d at 500, 1,000, or 1,500 mg/kg/d, the dams had reduced weight gains at the two highest doses, whereas a reduced anogenital distance was observed in male offspring from all groups, with greatest effect on pups from dams given the DBP on gestation days 15-17 (Ema et al. 2000).

In another developmental toxicity study, CD rats were given oral doses of DBP at 0, 100, 250, and 500 mg/kg/d from gestation day 12 to gestation day 21. F_1 males showed a number of morphologic effects on their reproductive organs, especially at the highest dose (Mylchreest et al. 1999). The only effect seen in the young male rats whose mothers received 100

[1]See ATSDR (1990) Table 2-2.

TABLE 3-6 Toxicity Summary

Dose and Duration	Species	Effects	Reference
Acute Toxicity (1-5 d)			
140 mg/kg, oral	Human, n = 1	Nausea, vertigo, delayed kidney damage	Lefaux 1968
500 mg/kg/d, oral tube, 4 d	SD rat, 3-4 wk old	No testicular effects	Cater et al. 1977
1,000 mg/kg/d, oral tube, 4 d	SD rat, 3-4 wk old	24% decrease in testicular weight	Cater et al. 1977
2,000 mg/kg/d, oral tube, 3 d	Rat, 3-4 wk old	24% decrease in testicular weight	Cater et al. 1977
5,300 mg/kg, oral	Mouse	LD_{50}	NTP 1991
8,000 mg/kg, oral	Rat	LD_{50}	NTP 1991
10,000 mg/kg, oral	Guinea pig	LD_{50}	NTP 1991
Short-Term Toxicity (6-30 d)			
250 mg/kg/d, gavage, 15 d	Adult Wistar rat	Changes in one of six testicular enzymes; no morphological or sperm-count changes	Srivastava et al. 1990a
250 mg/kg/d, gavage, 15 d	Wistar rat, 5 wk old	Changes in five of six testicular enzymes; 5% of seminiferous tubules were shrunken; defective spermatogenesis	Srivastava et al. 1990b
500 mg/kg/d, oral tube, 6 d	SD rat, 3-4 wk old	18% decrease in testicular weight	Cater et al. 1977
620 mg/kg/d, feed, 21 d	F-344 rat, 5 wk old	Increase in liver weights—30% (male), 9% (female); PP	CMA 1986

1,200 mg/kg/d, feed, 21 d	F-344 rat, 5 wk old	Increased liver weights—61% (male), 16% (female); PP; reduced body weight gain; decreased serum cholesterol; relative increase in kidney weight—8% (male)	CMA 1986
2,000 mg/kg/d, oral tube, 10 d	SD rat, 3-4 wk old	54% decrease in testicular weight	Cater et al. 1977
2,100 mg/kg/d, feed, 21 d	SD rat, 5 wk old	Increase in liver weights—86% (male), 61% (female); PP; 60% decrease in relative testicular weight with severe atrophy; 20% increase in kidney weight (male)	CMA 1986
Subchronic Toxicity (30-180 d)			
80 mg/kg/d, feed, 14 wk	SD rat	NOAEL for reproductive toxicity in females	Wine et al. 1997
150 mg/kg/d, feed (2,000 ppm), 3 mo	Wistar rat	NOAEL for broad range of end points	BASF 1992
750 mg/kg/d, feed (10,000 ppm), 3 mo	Wistar rat	Decreased serum triglyceride; PP; transient decrease in RBC indices; increase in liver weights—16% (female), 14% (male); NOAEL for testicular weights	BASF 1992
360 mg/kg/d, feed, 13 wk	F-344 rat, male	Hepatomegaly; slight anemia; reduced serum triglyceride	NTP 1995
720 mg/kg/d, feed, 13 wk	F-344 rat, male	Hepatomegaly; slight anemia; lower cholesterol; reduced triglyceride	NTP 1995

(Continued)

TABLE 3-6 Continued

Dose and Duration	Species	Effects	Reference
1,540 mg/kg/d, feed, 13 wk	F-344 rat, male	Reduced testicular weight; hepatomegaly; slight anemia; lower cholesterol; reduced triglyceride	NTP 1995
710 mg/kg/d, feed, 13 wk	F-344 rat, female	Hepatomegaly; reduced cholesterol and triglyceride; PP	NTP 1995
900 mg/kg/d, feed, 13 wk	$B6C3F_1$ mouse, male and female	Hepatomegaly	NTP 1995
1,600 mg/kg/d, feed, 13 wk	$B6C3F_1$ mouse, male	Microscopic changes in liver	NTP 1995
4,300 mg/kg/d, feed, 13 wk	$B6C3F_1$ mouse, female	Slight anemia; microscopic changes in liver	NTP 1995
Chronic Toxicity (0.5 y to lifetime)			
125 mg/kg/d, feed, 12 mo	Rat, 5 wk old	NOAEL for gross pathology, hematology	Smith 1953
600 mg/kg/d, feed, 12 mo	Rat, 5 wk old	50% died in first week	Smith 1953
500 mg/kg/d, feed, 15 mo	Wistar rat	No apparent effects	Lefaux 1968

mg/kg/d was delayed preputial separation. A NOAEL was not found in the study (Mylchreest et al. 1999). However, in a follow up study, a NOAEL of 50 mg/kg/d was found in male rats; the most sensitive effect was nipple development (Mylchreest et al. 2000).

Spaceflight Effects

The physiologic and biochemical changes that occur during spaceflight would not be expected to increase the sensitivity of astronauts to the toxic effects of DBP on the testes or reproduction; however, astronauts may be more sensitive to hematotoxicants because they loose approximately 10% of their red cell mass within the first few days of spaceflight.

Synergistic Effects

No data were found on DBP synergism with other chemicals.

LIMITS SET BY OTHER ORGANIZATIONS AND COMPARISON TO SWEGS

Differences Between EPA's 1-d Health Advisory and NASA's 1-d SWEG

Both NASA and EPA began with the 500 mg/kg NOAEL for the 4-d exposure reported by Cater at al. (1977). The 1-d EPA health advisory (HA) applies to a child ingesting approximately 1 L of water and weighing 10 kg, whereas the 1-d SWEG applies to a 70-kg adult ingesting 2.8 L of water per day. This accounts for the difference between the HA and the SWEG. The major reason that the HA (50 mg/L) is much lower than the SWEG (1,250 mg/L) is the difference in uncertainty factors. EPA used a factor of 100 (10 for interspecies differences and 10 for intraspecies differences, presumably); only a factor of 10 for interspecies differences was used to estimate the SWEG. There was no intraspecies factor used in the SWEG because the astronaut population consists of healthy adults, so protection of very young persons or ill persons is not required. There is no evidence of genetic differences that would make certain portions of the healthy adult population unusually susceptible to DBP. (See Table 3-7 for SWEGs and Table 3-8 for limits set by other organizations.)

TABLE 3-7 Spacecraft Water Exposure Guidelines for DBP

Duration	Concentration (mg/L)	Target Toxicity
1 d	1,200	Testicular injury
10 d	175	Testicular injury
100 d	80	Hematotoxicity
1,000 d	40	Hematotoxicity

Differences Between the 10-d Health Advisory and the 10-d SWEG

EPA (1992) began with findings from the 1-y rat study by Smith (1953) in which 125 mg/kg/d was the NOAEL. In contrast, NASA began with the data from Srivastava (1990a) on the reproductive toxicity of DBP in rats. A $BMDL_{01}$ of 70 mg/kg/d was estimated for reduced sperm counts using doses from 250 to 1,000 mg/kg/d for 15 d and a polynomial fit of the data (Table 3-5). EPA used parameters for a child (10-kg body weight, 1 L/d drinking water), whereas NASA used adult parameters (70-kg body weight, 2.8 L/d drinking water). Furthermore, EPA used an uncertainty factor of 100, and NASA used an interspecies factor of 10. Hence, EPA's 10-d HA of 10 mg/L is much lower than NASA's 10-d SWEG of 175 mg/L. As noted above, NASA is concerned with protecting healthy adults rather than children and ill adults. There is no reason to expect significant genetic differences in humans that would lead to differences in human responses to DBP.

Differences Between the Long-Term Exposure Standards

The long-term values set by EPA are based on the 1-y rat study reporting a NOAEL at 125 mg/kg/d (Smith 1953). The 7-y HA was calculated as follows:

$$\text{7-y HA} = (125 \text{ mg/kg/d} \times 70 \text{ kg}) \div (100 \times 2 \text{ L/d}) = 44 \text{ mg/L}$$
$$\text{(or approximately 40 mg/L).}$$

The 70-y drinking water equivalent level (DWEL) of 4 mg/L was calculated in the same way (through the reference dose [RfD]) as the HA, except that an uncertainty factor of 1,000 was applied. NASA's 1,000-d SWEG

TABLE 3-8 Standards and Limits for DBP Set by Other Organizations

Organization	Name of Standard	Value	Water Equivalent (mg/L)[a]
EPA	RfD (oral)	0.1 mg/kg/d	3.5
EPA[b]	1-d HA (child)	—	50
EPA[b]	10-d HA (child)	—	10
EPA[b]	7-y HA (adult)	—	40
EPA	70-y DWEL (adult)	—	4

[a]Assumes a 70-kg person consuming 2 L of water per day with no other sources of DBP.
[b]Health advisories published in 1996 by EPA did not include these HAs.
Abbreviations: DWEL, drinking water equivalent level; EPA, U.S. Environmental Protection Agency; HA, health advisory; RfD, reference dose.
Source: Data from EPA 1992.

was also derived from the NOAEL in the Smith (1953) study, except that an exposure time factor of approximately 2.7 was applied, and the uncertainty factor (for possible species sensitivity differences) was only 10, rather than the 100 or 1,000 used by EPA. Based on NOAEL data from the 1-y Smith (1953) study, and applying the spaceflight factor of 3, a 1,000-d AC for hematologic effects was found to be 40 mg/L. A higher volume of water consumption was also used for the SWEG (see below). The 7-y HA of 40 mg/L and the 1,000-d SWEG of 100 mg/L (if the spaceflight factor of 3 is removed) can be viewed as nearly comparable when the exposure times of 7 y for the HA and 1,000 d for the SWEG are considered. The SWEG is 2.5 times the HA, but the HA applies to an exposure time 2.5 times that of the SWEG.

The 100-d SWEG of 80 mg/L was derived from the results of an NTP study (1995) showing a $BMDL_{01}$ for hematologic effects at 102 mg/L (Tables 3-3 and 3-4). Factors of 10 for species differences and 3 for possible susceptibility to reduced red cell mass were included. There is no EPA limit that can be compared directly to this SWEG.

RATIONALE

The toxicity of DBP is low; however, at high doses, effects on the tes-

tes, reproductive function, red blood cells, and livers of rats have been repeatedly demonstrated. The testicular lesion is characterized by changes in enzymes associated with testicular function, vacuolization of Sertoli cells, and a loss of germinal epithelium, which is grossly reflected in decreased testicular weight. Similarly, the changes in the liver involve proliferation of the peroxisomes and increases in related enzymes leading to an increase in liver weight. Liver cancer and earlier changes in the rat liver in response to a peroxisome proliferator such as DBP are extremely unlikely to be an appropriate model for human toxicity. The fundamental reason for this difference appears to be quantitative and possibly qualitative differences in peroxisome proliferator-activated receptor-alpha (Holden and Tugwood 1999). Risk assessment will not be performed on rodent liver lesions, including biochemical lesions associated with cholestasis. A further note is that rats seem to be much more susceptible than primates to the toxic effects of DBP and other phthalates; however, the species extrapolation factor of 10 will be retained for conservatism and because there are few studies involving human subjects. Developmental toxicity was not considered because pregnant astronauts are not expected to fly in the foreseeable future. (See Table 3-9 for summary of ACs.)

Ingestion for 1 d

The most appropriate study from which to derive a 1-d acceptable concentration (AC) is Cater et al. (1977). Young male rats dosed with DBP at 500 mg/kg for 4 d did not show the measurable reduction in testicular weight that was observed for slightly longer exposures and at higher doses. Based on this NOAEL, the 1-d AC for testicular injury was calculated as follows:

$$\text{1-d AC (testicular injury)} = (500 \text{ mg/kg} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times 10);$$
$$\text{1-d AC} = 1{,}250 \text{ mg/L (rounded to } 1{,}200 \text{ mg/L}).$$

This assumes a 70-kg person ingesting 2.8 L of water per day (drinking water and food reconstitution). The factor of 10 accounts for possible differences in species susceptibility between rats and humans. This AC is likely to be conservative because of the use of highly susceptible young male rats, administration of four doses, and the bolus dosing protocol.

TABLE 3-9 Acceptable Concentrations of DBP in Drinking Water

Target Toxicity	Data and Reference	Species and Gender	Uncertainty Factors					Acceptable Concentrations (mg/L)			
			To NOAEL	Species	Micro Gravity	Interindividual	Exposure Time	1 d	10 d	100 d	1,000 d
Reproductive toxicity	500 mg/kg/d, 4 d; NOAEL for testes weight loss; Cater et al. 1977	Young rat, male	1	10	1	1	1	1,200	—	—	—
	$BMDL_{01}$[a] = 70 mg/kg/d, 15 d gavage; reduced sperm counts; Srivastava et al. 1990a	Adult Wistar rat, male	1	10	1	1	1	—	175	—	—
	$BMDL_{01}$[a] = 111 mg/kg/d, 13-wk feed; atrophy germinal epithelium in testes; NTP 1995	Rat, male	1	10	1	1	1.1	—	—	250	—
Hematotoxicity	$BMDL_{01}$[a] = 102 mg/kg/d, 13-wk feed; reduced RBC counts; NTP 1995	Rat, male	1	10	3	1	1.1	—	—	80	—

(Continued)

TABLE 3-9 Continued

Target Toxicity	Data and Reference	Species and Gender	Uncertainty Factors					Acceptable Concentrations (mg/L)			
			To NOAEL	Species	Micro Gravity	Interindividual	Exposure Time	1 d	10 d	100 d	1,000 d
Reproduction	125 mg/kg in food, 1 y; NOAEL; Smith 1953	Rat, male	1	10	1	1	2.7	—	—	—	100
Hematotoxicity	125 mg/kg in food, 1 y; NOAEL; Smith 1953	Rat, male			3			—	—	—	40
SWEG[b,c]								1,200	175	80	40

[a]$BMDL_{01}$ is the lower statistical bound on an exposure level corresponding to an increase of 1% in the probability of an adverse response. For continuous data, the range for adverse effects is considered to be 0.5%, which corresponds to 2.58 standard deviations for the mean response of unexposed individuals.
[b]Limits may exceed solubility limits in pure water.
[c]Guidelines were not set to protect against effects on a conceptus during gestation.

Ingestion for 10 d

The AC to protect against reproductive toxicity was based on the $BMDL_{01}$ value of 70 mg/kg/d (Table 3-5) calculated from reduced sperm counts found in adult rats given 15 daily gavage doses of DBP at 250-1,000 mg/kg/d (Srivastava et al. 1990a). A NOAEL was not indicated by the investigators. The 10-d AC was calculated as follows:

10-d AC (testicular injury) = (70 mg/kg/d × 70 kg) ÷ (2.8 L/d × 10);
10-d AC = 175 mg/L.

This value is expected to be conservative because the DBP was delivered in bolus gavage doses rather than gradually in feed or water. This conclusion is supported by a newly developed PBPK model for DBP and its metabolites that suggests diffusion limitations and pH trapping as mechanisms that limit uptake into human tissues (Keys et al. 2000).

Ingestion for 100 d

Toxicologic end points for testicular atrophy and reduced red blood cell counts were considered in setting the 100-d SWEG.

Reproductive Toxicity

Reproductive toxicity in males was evaluated according to the $BMDL_{01}$ calculations shown in Tables 3-3 and 3-4. The $BMDL_{01}$ for atrophy of the germinal epithelium in rats exposed to DBP in their food for 13 wk was found to be 111 mg/kg/d. The 100-d AC was calculated as follows:

100-d AC (testicular injury) = (111 mg/kg/d ×70 kg) ÷ (2.8 L/d × 10 × 1.1);
100-d AC = 250 mg/L.

The uncertainty factors were 10 for species extrapolation and 1.1 for extrapolation from 90 d to 100 d.

Hematotoxicity

Data from the NTP (1995) subchronic study suggest a slight anemia on

the basis of clinical hematology parameters. A BMD analysis (Table 3-4) of the data using the polynomial model gave a $BMDL_{01}$ of 102 mg/kg/d. This value can be applied to the situation of astronauts as follows:

100-d AC = (102 mg/kg × 70 kg) ÷ (2.8 L/d × 10 × 3 × 1.1);
100-d AC = 77 mg/L (rounded to 80 mg/L).

This incorporates a species extrapolation factor of 10, a time-extrapolation factor of 1.1 for extrapolation from 90 d to 100 d, and a factor of 3 for presumptive astronaut susceptibility to hematotoxicants.

Ingestion for 1,000 d

There are no chronic studies that meet modern standards for quality and completeness of reporting. There are basically two choices: (1) extrapolate from 90-d, subchronic rodent data of high quality (i.e., the $BMDL_{01}$ of 96 mg/kg/d that gave an AC of 70 mg/L), or (2) use data from the 1-y rodent study of Smith (1953), knowing that the latter has important limitations. To avoid the long and uncertain time extrapolations from a 90-d study to a 1,000-d guideline, the latter approach was initially selected. The NOAEL from the rat exposures was 125 mg/kg/d. The 1,000-d AC to avoid presumed reproductive effects was calculated as follows:

1,000-d AC = (125 mg/kg/d × 70 kg × 2.7) ÷ (2.8 L/d × 10);
1,000-d AC = 114 mg/L.

The factor of 10 is for potential species differences and the factor of 2.7 (1,000/365) is to compensate for the exposure time in the study being less than the AC exposure time. This can be rounded to 100 mg/L for reproductive effects; however, an additional factor of 3 was applied for possible interactions between spaceflight effects and hematotoxicity, which was assumed from the hematotoxic findings in the 90-d study (NTP 1995).

1,000-d AC (hematotoxic effects) = 114 mg/L ÷ 3 = 37 mg/L
(rounded to 40 mg/L).

RECOMMENDATIONS

The greatest weakness in the toxicity database is the lack of a chronic

feed or drinking water study conducted according to modern protocols. Conducting such a study in rodents would be of limited value, but a chronic study in primates would be very valuable. Because the chronic studies from Smith (1953) and others were reported in such limited detail, using them as the basis for any standard is risky. Additional studies are needed in primates, including low-exposure studies in human subjects. The diversity and distribution of the peroxisome proliferator-activated receptor-isotypes in tissues from human populations need to be assessed to more confidently ascertain the relevance of rodent toxicity and cancer data.

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4 Di(2-ethylhexyl) Phthalate

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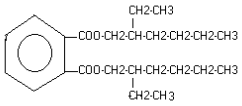
PHYSICAL AND CHEMICAL PROPERTIES

Di(2-ethylhexyl) phthalate (DEHP) is a colorless, viscous liquid with a slight odor. (See Table 4-1 for more details.)

OCCURRENCE AND USE

DEHP became a commercial product in 1933 and is widely used as a plasticizer in many materials, especially polyvinyl chloride (Schmid and Slatter 1985). It is also used in insect repellants, lacquers, and rocket propellants. During the NASA/Mir program, DEHP was found in spacecraft recycled water at an average concentration of 2 micrograms per liter (μg/L), with a high of 28 μg/L (Pierre et al. 1999). The humidity condensate in the shuttle has been found to contain DEHP, which is sometimes called dioctyl phthalate, at concentrations up to 460 μg/L (Straub et al. 1995). By comparison, concentrations up to 3-4 μg/L have been reported in river water in Japan and Europe (WHO 1992). Concentrations in catfish from rivers in the southern United States have been reported to be as high as 3,200 μg per kilogram (kg) (Mayer et al. 1972). Oral exposure of the general population,

TABLE 4-1 Physical and Chemical Properties of DEHP

Formula	$C_{24}H_{38}O_4$
Synonyms	Di(2-ethylhexyl) phthalate (DEHP), dioctyl phthalate, 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester
	
CAS registry no.	117-81-7
Molecular weight	390.56
Melting point	-50°C
Boiling point	387°C
Solubility	45 µg/L in water at 20°C (Leyder and Boulanger 1983); values from 285 µg/L to 360 µg/L have been reported at room temperature (WHO 1992)
Density	0.99 g/mL

primarily from residues in food, has been estimated at 30 µg/kg per day (d), which is approximately 2 milligrams (mg)/d for a 70-kg person (Doull et al. 1999).

The presence of DEHP in food and water contained in plastic could affect the amount of DEHP ingested by astronauts. Food repackaged by NASA is generally placed in laminated containers that have polyethylene as their innermost component. Polyethylene should not leach any DEHP; however, that does not completely preclude the possibility of leaching from the packaging in which the food was shipped or in foods flown in their original packaging. The amount of food-borne DEHP ingested by the crew should be small and certainly no more than that ingested by earthbound people eating food mostly from commercial packaging. Water is not stored in containers made with DEHP.

TOXICOKINETICS

Primary targets for DEHP toxicity are the liver and testes. Effects in the pituitary, thyroid, ovaries, and blood have also been explored. When investigating DEHP's toxicity, it is important to consider significant differences

in species and organ response. The toxicokinetic discussion will focus on the relevance of DEHP's carcinogenic and reproductive effects in rodents to human health. The toxicokinetics of DEHP are remarkably complex and depend on many different factors. Absorption of an oral dose of DEHP is related to the chemical's metabolism to readily absorbed compounds. Its metabolism in the gut is saturable, so the amount of each metabolite absorbed depends on the dose of DEHP. Further metabolism of the absorbed compounds depends on the species and age of the subject.

Absorption

An oral dose of DEHP can be absorbed in the small intestine as unmetabolized DEHP or as mono(ethylhexyl) phthalate (MEHP) and 2-ethylhexanol; the relative proportions of parent compound and metabolites absorbed depend on the dose (Astill 1989). In general, plasma levels peak approximately 1-3 hours (h) after oral exposure. Minimal retention of the compound is observed (NTP 2000). At low doses, most of the DEHP given to rats is hydrolyzed by pancreatic lipase to MEHP; however, above a threshold dose, unmetabolized DEHP is absorbed and reaches the liver (Albro 1986). A larger portion of a low dose is absorbed compared with the portion of a higher dose that is absorbed. For example, the "area under the curve" for DEHP and its metabolites in marmoset blood for the first 24 h after oral administration of DEHP was only doubled when the dose was increased from 100 mg/kg to 2,000 mg/kg (Rhodes et al. 1986). This suggests that the absorption of DEHP and MEHP from the gut are saturable processes.

A key observation regarding absorption of DEHP is that in primates, including humans, much less of an oral dose of DEHP (in milligrams per kilogram of body weight) is absorbed and hydrolyzed to MEHP than in rodents (ICI 1982; Shell 1982; Schmid and Schlatter 1985; Rhoades et al. 1986). For example, at an oral dose of 2,000 mg/kg, marmoset tissues are exposed to approximately the same levels of DEHP and its metabolites as are rat tissues after a dose of only 200 mg/kg (Rhodes et al. 1986). This result may be due to the marmoset's reduced lipase activity in the gut, where absorption of DEHP is facilitated. In a comparative study of monkeys, rats, and mice, Astill (1989) reported that the monkeys hydrolyze substantially less of a gavage dose of DEHP at 100 mg/kg than did either of the rodent species.

Distribution

The tissue distribution of DEHP and its metabolites has received limited study. Rats and marmosets were given 14 massive oral doses of ^{14}C-labeled DEHP at 2,000 mg/kg in corn oil, and selected tissues were removed for study. The distribution of radiolabel in the blood, liver, kidneys, and testes of the rat and marmoset showed a similar tissue pattern; however, the absolute levels in the marmoset tissues were one-fifth to one-tenth of those found in the comparable rat tissues (Rhodes et al. 1986). In another study, rats, dogs, and miniature swine were given unlabeled DEHP at 50 mg/kg for 21-28 d and then given a final dose of ^{14}C-labeled DEHP at 50 mg/kg (Ikeda et al. 1980). Animals were sacrificed 4 h, 1 d, and 4 d later, and the distribution of radiolabel in tissue and body fluids was measured. Of the tissues studied, the labeling was highest in rat liver, dog muscle, and swine fat at the 4-h time point; the pattern of highest labeling changed slightly at 4 d to rat lung, dog muscle, and swine fat (Ikeda et al. 1980). Other tissues studied included the brain and kidneys; however, distribution to the testes was not measured.

Metabolism

The metabolism of DEHP has been studied in great detail, revealing approximately 30 metabolites in various species (ATSDR 1993). Qualitatively, the metabolites are similar in most species studied, but there are important quantitative differences in DEHP metabolism. These quantitative metabolic differences may help explain the interspecies differences in peroxisome proliferation (PP) and in the induction of cancer; however, the data are not completely consistent in implicating specific metabolites. In this section, the metabolism of DEHP will be depicted in summary form, and species differences in the major pathways will be considered. It is these species differences that will have the greatest bearing on the rationale used to develop human exposure limits.

The metabolism of DEHP is summarized in Figure 4-1. In the gut, and in many other tissues, DEHP is hydrolyzed by intestinal lipases to MEHP and 2-ethylhexanol (2EH) so that very little free DEHP, when given in moderate doses, is excreted by any species. Following oral exposure in rodents, the majority of DEHP is absorbed in the form of MEHP because of rapid hydrolysis by gut lipases. The fraction of free MEHP excreted is large in guinea pig urine (72%), intermediate in monkeys and mice urine

(17-18%), low in hamster urine (4.5%), and almost absent in rat urine (Albro 1986; Rhodes et al. 1986; Astill 1989). In a relatively minor pathway, 2EH is oxidized to 2-ethylhexanoic acid (2EHA) and several ketoacids, which are found in human urine (Albro and Corbett 1978). In most species studied, the hydrolysis of MEHP to phthalic acid is not a major pathway. MEHP is hydrolyzed to phthalic acid by enzymes in liver microsomes and is found free in the urine of rodents; hamsters and mice excrete more than rats or guinea pigs (Albro et al. 1982; Albro 1986). In humans and other primates, the portion of conjugated metabolites in urine from side-chain oxidation is 60-65%; guinea pigs, hamsters, and mice have a smaller portion present as conjugates; and rats seem to excrete very little conjugated metabolites in their urine (Albro et al. 1982; Schmid and Schlatter 1985).

Two human volunteers were given 30 mg of DEHP once and 10 mg/d for 4 d. Their urinary metabolites were quantified by gas chromatography and mass spectrometry (Schmid and Schlatter 1985). The major metabolites found in hydrolyzed urine, in decreasing concentration, were IX, V, VI, VII, IV, and I.[1] MEHP was also found in the urine. In the single-dose study, 11-15% of the dose was recovered in the urine in about 50 h, and the vast majority of that was excreted in the first 20 h. When the four repeated doses were given, 15-25% was recovered over 5 d.

The toxicologic significance of the various DEHP metabolites compared with the parent compound differs depending on species and toxic end point. For example, attempts to relate specific metabolites to PP, and presumably to potential induction of liver cancer, have involved several of the many MEHP oxidation products, including 2-ethyl-3-carboxypropyl phthalate (I), 2-ethyl-5-oxyhexyl phthalate (VI), and 2-ethyl-5-hydroxyhexyl phthalate (IX) (Lhuguenot et el. 1985; Mitchell et al. 1985a; Astill 1989). One would hope to find a metabolite that is present at relatively high concentrations in species susceptible to PP and at low concentrations in less susceptible species. Furthermore, in vitro confirmation of the compound's ability to cause PP would be helpful in selecting the proximate metabo-

[1]The designation in Roman numerals is per the convention of Albro et al. (1973,1981,1982), and translates as follows: I, 2-ethyl-3-carboxypropyl phthalate; IV, 2-carboxymethylhexyl phthalate; V, 2-ethyl-5-carboxypentyl phthalate; VI, 2-ethyl-5-oxyhexyl phthalate; VII, 2(2-hydroxyethyl)hexyl phthalate; VIII, 2-ethyl-4-hydroxyhexyl phthalate; IX, 2-ethyl-5-hydroxyhexyl phthalate.

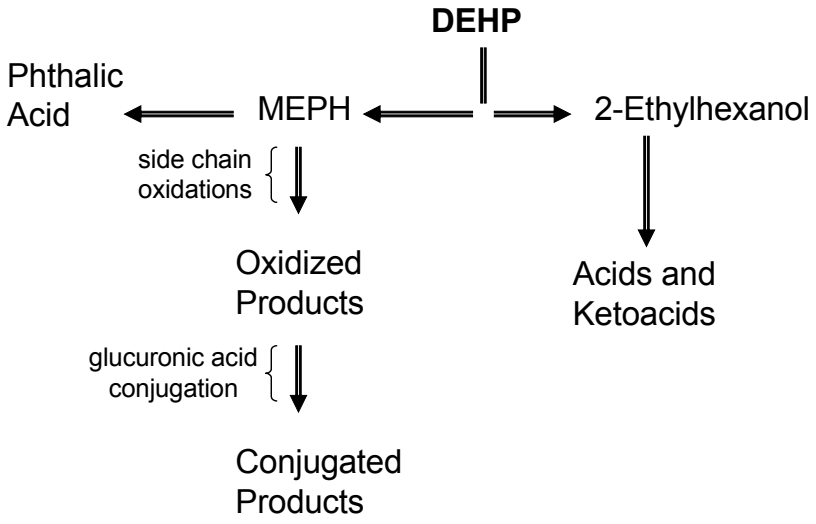


FIGURE 4-1 Overall metabolism of DEHP. See page 123 for details of the oxidation products proposed by Albro et al. (1982).

lite(s). Unfortunately, the search for such a metabolite has resulted in observations that cannot be explained by such a simple approach. In comparing monkeys, rats, and mice, the metabolites found at low concentrations in monkeys and at high concentrations in rats and mice were I and VI (Astill 1989). Compound IX, a precursor of VI, was found in roughly comparable fractions in the three species. Using cultured rat hepatocytes and an enzymatic marker of PP, Mitchell et al. (1985a) found that compound I had little effect on the marker, whereas compounds VI and IX induced the marker approximately 10-fold. The little data available on humans suggest that VI and IX are major metabolites found in urine and I is a minor metabolite found in urine (Albro et al. 1982; Schmid and Schlatter 1985). Humans and other primates are thought to be resistant to PP induced by agents that are capable of inducing peroxisomes in rats (Doull et al. 1999). This might be because of the low level of the peroxisome proliferator-activated receptor-alpha (PPARa) in primate liver (Parkinson 1996). To further complicate the picture, the relationship between liver cancer and PP may not be direct for DEHP. Rather, the ability to induce a persistent increase in replicative DNA synthesis seems to correlate better with cancer induction (Marsman et al. 1988).

The interspecies differences in PP caused by DEHP can be partially explained by proposed differences in high- and low-dose kinetics related to the abundance of PPARa (Holden and Tugwood 1999). The relationship of PP to lipid homeostasis can also be explained by differences in the activation thresholds for the two processes. Below is a diagram (Figure 4-2), adapted from two figures in Holden and Tugwood (1999), modeling how the interspecies differences in PP susceptibility can be understood. A PP compound, such as MEHP, enters the hepatocyte nucleus and causes the heterodimerization of PPARa with another nuclear receptor called retinoid X receptor. This dimmer binds to DNA at the peroxisome proliferator response element, which is a repeat of a TGACCT-like sequence. In rats, this sequence is known to promote acyl CoA oxidase and bifunctional dehydrogenase/dehydratase, which affect the PP process. Similarly, PPARa can activate genes for apolipoprotein and lipoprotein lipase, which control lipid homeostasis. The relative thresholds for activation of these processes (illustrated by the vertical arrow in Figure 4-2) suggest that PP in rats and mice has a much higher threshold in terms of PPARa concentration than does lipid metabolism. The PPARa concentration in human hepatocytes is believed to be regulated upstream of and expressed at only 5-10% of that in rodent hepatocytes; therefore, most humans do not have sufficient PPARa to enable the PP process. These observations are supported by investigations showing lack of PP in humans taking pharmaceutical agents known to induce PP in rodents. Nonhuman primate studies are largely supportive—PP was not observed in marmosets exposed at up to 2,500 mg/kg/d or in cynomolgus monkeys dosed at up to 500 mg/kg/d (Short et al. 1987; Kurata et al. 1998). The DEHP-exposed marmosets, however, had increases in peroxisomal volume. There is some concern that certain people may have sufficient PPARa to exceed the minimum threshold for PP (Holden and Tugwood 1999). This is illustrated by the darkened region on the PPARa arrow in Figure 4-2.

The PPARa knockout mouse model has also provided relevant mechanistic information for other DEHP-induced toxicants. For example, though it is resistant to DEHP-induced hepatic effects, the PPARa knockout mouse has been shown to be susceptible to kidney, developmental, and testicular toxicities (Peters et al. 1997). The role of other PPARs, such as beta or gamma, in mediating organ system toxicity is unclear; however, research has identified specific metabolites of DEHP, such as MEHP, but not not 2EHA, as able to activate PPAR-gamma (Maloney et al. 1999).

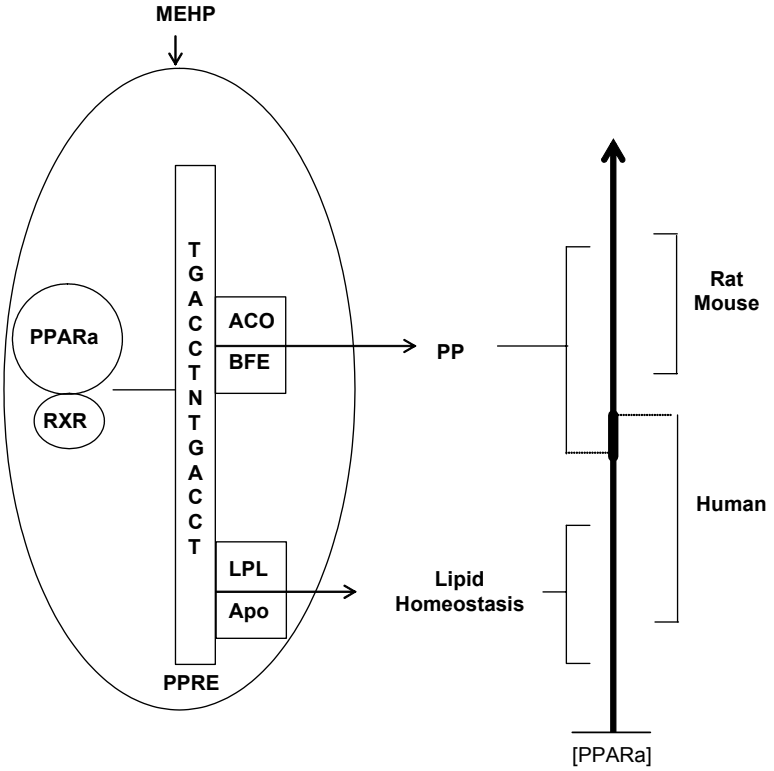


FIGURE 4-2 Nucleus of a hepatocyte showing how PPARa concentration can play a key role in determining species susceptibility to PP compounds such as MEHP and how lipid metabolism is related to PP through DNA activation. Source: Adapted from Holden and Tugwood 1999.

Specific DEHP metabolites have been evaluated for their ability to cause developmental toxicity and/or reproductive toxicity. MEHP is a developmental and reproductive toxicant and is the suspected critical metabolite responsible for testicular toxicity. The evidence for this conclusion is discussed in the reproductive toxicity section. Metabolites 2EH and 2EHA have been shown to produce developmental toxicity in multiple rodent species, but the reproductive effects of those metabolites is less well characterized (NTP 2000). Phthalic acid has also been shown to be a developmental toxicant in rodents but is less potent than other tested metabolites (NTP 2000).

Elimination

The primary routes of elimination of DEHP and its metabolites are the feces and the urine. Two human subjects given a single oral dose of DEHP (30 mg/kg) excreted an average of 13% as urinary metabolites (Schmid and Slatter 1985). Those same subjects excreted an average of 20% after doses of 10 mg/kg/d for 4 d.

A physiologically based pharmacokinetic (PBPK) model was used to predict the elimination of MEHP from the blood of rats given a dose of DEHP or MEHP (Keys et al. 1999). Flow-limited, diffusion-limited, and pH-trapping models were tested for their ability to fit blood elimination profiles. The best predictions from a single model came from the pH-trapping model; however, that does not mean that the other mechanisms are not involved to some extent in the elimination of MEHP from blood. The pH-trapping model postulates that MEHP can diffuse across membranes only in the un-ionized state and that once MEHP enters a cell it converts to $MEHP^-$, which is slowly converted back to MEHP. Thus, the MEHP is trapped in a cell in the ionic form under conditions of suitable pH.

When radiolabeled DEHP was administered intravenously to rats, the elimination of radioactivity in blood was very rapid (Schultz and Rubin 1973). The elimination was at least biphasic, with elimination half-lives of 4.5-9 minutes (min) and 22 min, respectively.

TOXICITY SUMMARY

The toxicity of DEHP has been studied in several species, for many different end points, by various routes of administration, and for a variety of exposure times. The intent of this section is to summarize the studies that might be useful for setting SWEGs for DEHP. For example, only oral studies with a feeding or water protocol were considered, unless only other types of studies were available for a given exposure time or could demonstrate relevant species differences in susceptibility. Oral gavage studies tend to exaggerate the toxic effect of a compound because of the bolus nature of the dose. The primary target organs of DEHP are the testes and liver, at least in rodents. Different species can have important differences in their responses to oral ingestion of DEHP, and absorption of DEHP may depend on the vehicle used to deliver the dose.

Acute Toxicity (1-5 d)

There is a report that attempts to relate the acute toxicity of DEHP from a single dose to toxicity from "chronic" (12-week [wk]) doses (Lawrence et al. 1975). Using intraperitoneal injections 5 d/wk and LD_{50} (dose lethal to 50% of subjects) values as the basis for comparison, these investigators found that DEHP was 28 times more toxic chronically than it was acutely (the LD_{50} for acute exposure was 28 times smaller). This ratio was the highest of any of the eight phthalate esters tested and primarily is due to the low acute toxicity of DEHP compared with the other phthalate esters. The oral LD_{50}s for a single administration of DEHP were 31 g/kg (21-45 g/kg) in male Wistar rats and 34 g/kg (25-46 g/kg) in male rabbits (Shaffer et al. 1945). The authors attribute the deaths, which tended to be delayed for several days after the dosing, to liver and kidney injury. Other oral LD_{50}s have been derived from unpublished data from Union Carbide and include the following: guinea pigs, 26,000 mg/kg; rats, 34,000 mg/kg; and mice, 34,000 mg/kg (Krauskopf 1973). Those LD_{50} values place the acute toxicity of DEHP below that of smaller dialkyl phthalates and above that of larger dialkyl phthalates (Krauskopf 1973).

Repeated administration of DEHP was lethal to rabbits and guinea pigs at 2 g/kg/d when administered for up to 7 d, but the same dose was not lethal to rats and mice (Parmar et al. 1988). Young rats are more susceptible than older rats to DEHP administered over 5 d (Dostal et al. 1987). Wistar rats given a diet containing DEHP at 20,000 ppm for 3 d showed a 34% decrease in serum T4, but there seemed to be a recovery, because the decrease after 10 d was only 15% (Hinton et al. 1986). The reduced T4 was also evident after 21 d of exposure at 10,000 parts per million (ppm). In addition, certain liver enzymes were increased after only 3 d of ingestion of DEHP at 20,000 ppm (Hinton et al. 1986).

In a multi-timed study, Wistar rats were given nominal doses of 50, 200, and 1,000 mg/kg/d in their diets and were sacrificed from 3 d to 9 months (mo) after the start of the dosing (Mitchell et al. 1985b). At sacrifice, the livers were removed and subjected to a number of biochemical, DNA, and histopathologic evaluations. The liver weights of males were increased 26% after 3 d at the highest dose, but the two lower doses did not cause significant hypertrophy until 14 d of ingestion in males. Females were somewhat less susceptible to DEHP-induced liver hypertrophy. Other observations in males ingesting DEHP for 3 d included increased PP (top two doses), transient increased bile flow and damage of the bile canaliculi

(top two doses), increased DNA synthesis (top two doses), and changes in various tissue enzymes (one to three dose levels). In general, females were less susceptible to injury or changes than males. The lowest dose, 50 mg/kg/d, was without adverse effect after 3 d of ingestion.

In another study, after 3 d of consuming food spiked with DEHP at 20,000 ppm, the liver weights of male Wistar rats were increased 46% compared with the liver weights of controls (Mann et al.1985). Light microscopy revealed an increased number of mitotic figures in the liver, and electronmicroscopy of the liver revealed PP. A number of hepatocyte enzymes were also changed after only 3 d of DEHP ingestion at this high level.

Apparently, a single human ingestion of 10 g of DEHP caused gastric distress and catharsis, whereas 5 g did not elicit symptoms (Shaffer et al. 1945).

Short-Term Toxicity (6-30 d)

Observations of toxic effects in this range of exposure times are often made as interim observations during a much longer study. At 2 wk into a 79 wk study, male Wistar rats given DEHP at 2% in their food showed a 60% increase in liver weights and induction of enzymes associated with PP and hydrogen peroxide metabolism (assessed first at 4 wk) (Tamura et al. 1990). In another study, Wistar rats given DEHP at 2% in their food for 1 wk showed severe atrophy of the testes (43% weight decrease) accompanied by high testicular testosterone and low testicular zinc (Oishi and Hiraga 1980). In addition, liver weights increased by 27% and kidney weights decreased by 18% on an absolute organ weight basis (Oishi and Hiraga 1980).

Because most of the studies have involved rat exposures, it is essential to know the relevance of those studies to human exposures to DEHP. In the absence of human data, exposures of other primates can be useful. One such study, by Rhodes et al. (1986), compared the short-term toxicity of DEHP in marmosets and rats. Both species were given 2 g/kg/d as single oral doses in corn oil for 14 consecutive days. Testicular atrophy, hepatomegaly, and reduced body-weight gain were found in the rats; however, only reduced body-weight gain was found in the marmosets. The induction of peroxisomes and associated enzymes was found in rat liver, but not in the livers of the marmosets. Absorption studies suggested that DEHP is not as readily absorbed from the marmoset gut as from the rat gut. Even when the

bioavailability of the DEHP metabolites in target tissue is matched in the two species, the rat seems to respond more readily.

In another study comparing the responses of rats and monkeys, monkeys were found to be much less susceptible to PP induction by DEHP. Male F-344 rats and cynomolgus monkeys were treated with DEHP for 21 d. The average daily dose to specific groups of rats, administered in their feed, was approximately 11, 105, 670, 1,200, or 2,100 mg/kg, whereas the daily dose by gavage to the monkeys was 500 mg/kg (Short et al. 1987). In rats fed 105 mg/kg/d, there were no increases in liver weights, but there were increases in two of three enzymatic markers for PP; at 670 mg/kg/d, liver weights increased 80% (Short et al. 1987). In contrast, monkeys given gavage doses of 500 mg/kg/d showed no evidence of PP.

The study by Mitchell et al. (1985b) included sacrifices at 7, 14, and 28 d after the rats began ingesting DEHP-spiked food at nominal doses of 50, 200, or 1,000 mg/kg/d. By day 14, liver weights among male rats in the low-dose group were 22% above control-group liver weights; male rats in the middle group had liver weights 38% above controls; and male rats in the high-dose group had liver weights 69% above controls. However, at 28 d, only the high-dose group had statistically significant increases in liver weights. Females were less susceptible to the induction of increased liver weights. DNA synthesis was increased in the livers from all groups at 7 d, but was normal in all groups by 14 d. Various enzymes from liver tissue showed different activities from controls at various times after the doses, but the bile canalicular damage seen in male rats at 3 d was gone at 7 d. Based on the increases in liver weights, which were transient at 7-28 d in males exposed at the lowest dose, one can conclude that 50 mg/kg/d produces a marginal adverse effect. The testes from the rats sacrificed at or before 28 d were not examined as part of this study. The "urogenital apparatus" was examined in rats killed after 9 mo of exposure, but the findings were not included in the report (Mitchell et al. 1985b).

A high dose of DEHP (20,000 ppm in the feed) has been used to evaluate the progression of effects induced in Wistar rats after 3-21 d of ingestion (Mann et al. 1985). In terms of weight changes reported in this experiment, the liver was more sensitive than the testes. Large increases (e.g., 140% after 21 d) in liver weight were found at all sacrifice times, whereas the only change in testicular weights was a decrease of 22%, and that was after 21 d. As discussed above, these investigators also report a number of morphologic and biochemical changes in the liver in response to DEHP ingestion, but morphologic and biochemical changes were not evaluated in the testes.

The testes of young rats have been shown to be a target organ for short-term toxicity of DEHP at high doses (Gray and Gangolli 1986). Ad-

ministration of DEHP in corn oil (presumably by gavage) at 2,800 mg/kg/d for 10 d caused seminiferous tubular atrophy and reductions in the weights of seminal vesicles and prostate. These adverse effects occurred in 4- to 5-wk-old rats, but not in 15-wk-old rats. Although this gavage study is not directly useful for risk evaluation, it indicates that age can have a profound effect on susceptibility to DEHP-induced reproductive toxicity. When compared with the results of Mitchell et al. (1985b), this study suggests that in mature rats liver effects occur at much lower doses than do male reproductive effects.

In a study of effects induced in monkeys given 14 consecutive doses of DEHP at 500 mg/kg/d by feeding tube, the findings were negative (Pugh et al. 2000). The test animals were four 2-y-old male cynomolgus monkeys. Animals were evaluated by observation of clinical signs, hematology, clinical chemistry, urinalysis, gross necropsy, and histopathology. There were no detectable effects on the liver, red blood cells, or testes.

Subchronic Toxicity (30-180 d)

The earliest subchronic study of DEHP administered orally was reported almost 60 y ago by Shaffer et al. (1945). Male Wistar rats were fed DEHP at doses equivalent to 0, 0.2, 0.4, 0.9, and 1.9 g/kg/d for 90 d. There was some degree of growth retardation in the three highest-dose groups, but none of the animals died during the study. Hematologic parameters were normal in all groups, and the only histopathologic effect was atrophy and degeneration in the testes of rats from the two highest-dose groups. In addition to the testes, the liver, kidneys, spleen, and heart were examined microscopically. The authors concluded that 0.2 g/kg/d caused no effect, and the only effect at 0.4 g/kg/d was growth retardation of unspecified magnitude. It is surprising, in light of more recent studies, that liver effects were not described.

Two subchronic oral studies have been reported—one in rats, and the other in marmosets. In the first study, Spague-Dawley rats were given DEHP in their diets at 0, 5, 50, 500, and 5,000 ppm for 13 wk (Poon et al. 1997). Liver weights were increased 40% in males and 20% in females in the highest-dose group, but not in any of the other groups. Other changes confined to the highest-dose rats included increased serum albumin in males and females, reduced thyroid follicle size and colloid densities in males and females, decreased serum cholesterol in females, and a 9% average decrease in red cell count in males. Testicular changes in highest-dose rats included minimal to mild seminiferous tubule atrophy (9/10) and mild to moderate

Sertoli cell vacuolization (9/10). In addition, minimal Sertoli cell vacuolization was found in the 500-ppm group (7/10). According to the study authors, the 13-wk NOAEL (no-observed-adverse-effect level) was 50 ppm, equivalent to 3.7 mg/kg/d (males).

It is essential to decide whether the Sertoli cell vacuolization seen in rats that consumed DEHP in food for 13 wk was an adverse effect (Poon et al. 1997). The histopathologic data are summarized in Table 4-2. The authors concluded that the Sertoli cell effects preceded the germ cell effects; therefore, the observation at 500 ppm should be considered an early, adverse effect. That conclusion is difficult to reconcile with the fact that no seminiferous tubule atrophy was observed in the 500-ppm males. Sertoli cells normally contain some vacuoles. Vacuolization of Sertoli cells represents an enlargement of the smooth endoplasmic reticulum (SER) due to alterations in secretion or transport of proteins, disruption of ionic pumps, or changes in the cytoskeleton supporting the SER (Richburg and Boekelhiede 1997). Each Sertoli cell serves a species-specific number of germ cells; in rats, approximately 22 germ cells adhere to each Sertoli cell. Germ cells are lost when the cell-to-cell contacts are lost, and that can lead to release of immature sperm cells. The mechanism of this loss is unknown; however, vacuolization of the Sertoli cells alone seems insufficient to cause loss of germ cells. In agreement with the authors of the study, the subcommittee advises that 3.7 mg/kg/d (50 ppm) be considered the NOAEL. To understand the significance of this pathology, and to understand DEHP's potential reproductive toxicity, it is important to consider the overall weight of scientific evidence that DEHP produces adverse reproductive effects. Over 70 reproductive studies and many good consensus documents discuss this research (NTP 2000). The database includes evidence that DEHP is a reproductive toxicant in male rats, mice, guinea pigs, and ferrets and produces structural changes in the testes, reduced fertility, and altered sperm dysfunction (NTP 2000). Morphologic, functional, and biochemical assessment has shown that the Sertoli cells are cellular targets for adult and pre-adult exposures (NTP 2000). In vitro studies with MEHP exposures in Sertoli-germ cell cultures support the hypothesis that MEHP inhibits proliferation and is a critical player in determining DEHP's testicular toxicity (NTP 2000). Although there are sufficient data to demonstrate that DEHP is a reproductive toxicant in rodents, significant data gaps exist in determining precise dose-response relationships (NTP 2000). The NTP (2000) report used this weight- of-evidence in their assessment and determined that the data supported a NOAEL within the range of 3.7-14 mg/kg/d for male reproductive toxicity caused by oral exposure to DEHP in rats.

TABLE 4-2 Histopathology of Male Rats Fed DEHP[a]

DEHP in Diet (ppm)	Dose (mg/kg/d) (average)	Siminiferous Tubule Atrophy[b]	Sertoli Cell Vacuolization[b]
0	0	1 (0.1)	0
5	0.4	3 (0.5)	4 (0.2)
50	3.7	1 (0.4)	4 (0.5)
500	37.6	0	7 (1.0)
5,000	375	9 (1.5)	9 (2.4)

[a]Study duration was 13 wk.
[b]Average severity of lesions in all tissues examined is shown in parentheses. The first digit indicates the degree of injury (0 = minimal, 1 = mild, 2 = moderate, 3 = severe). Dispersions of lesions were added fractionally to the integer (0.25 = focal, 0.5 = locally extensive, 0.75 = multifocal). For example, 1.25 = mild, focal; and 2.5 = moderate, locally extensive.
Source: Data from Poon et al. 1997.

Although the rodent response to ingested DEHP seems to have been studied thoroughly, the question remains—is the rodent an appropriate model of the human response to DEHP? There are large differences in the absorption and metabolism of DEHP in primates and rodents (Rhodes et al. 1986). According to a recent risk assessment on reproductive toxicity, "Absorption studies, as well as PBPK modeling, suggest that DEHP metabolism to MEHP and its absorption from the human gut and marmoset gut saturates at a low dose relative to that of the rat" (NTP 2000). A subchronic study in marmosets also suggests that rats are probably a poor model of the human response to DEHP. In that study, groups of four male and four female marmosets were administered DEHP via oral catheter at doses of 0, 100, 500, and 2,500 mg/kg/d for 91 consecutive days (Kurata et al. 1998). High-dose males had significantly lower body-weight gains midway through the study, but not at the end of the study. The liver peroxisome number, volume density, morphology, and enzyme activity were unchanged by treatment; however, the mean peroxisome volume increased about 35% in the mid- and high-dose males. According to the authors, measurements associated with liver cytochrome P-450 tended to increase; however, there was no clear dose-response relationship. Atrophic testicular changes reported in rodent studies were not seen in the marmosets, even by electron microscopy. Testicular weights, testicular zinc, and blood testosterone were unchanged in all groups. There were no changes in blood chemistry or organ histopathology associated with DEHP treatment.

Chronic Toxicity (0.5 y to lifetime)

There have been several chronic ingestion studies of DEHP in rodents. Some studies focused on changes in specific organs (e.g., liver), while others attempted to discover the full range of adverse effects caused by DEHP. The discussion below is arranged chronologically, and DEHP-induced neoplastic and non-neoplastic effects are considered separately.

Non-Neoplastic Lesions

The earliest chronic oral study on DEHP came from the same laboratory as the early subchronic study cited above (Shaffer et al. 1945). The chronic study is especially valuable because three species (rats, guinea pigs, and dogs) were exposed, although not for the same length of time. Male and female Sherman rats, beginning at 60 d of age, were exposed to DEHP in their food for 2 y at concentrations that resulted in doses of 0, 0.02, 0.06, and 0.2 g/kg/d (Carpenter et al. 1953). Male and female guinea pigs were given food spiked with DEHP for 1 y; the doses approximated 0, 0.02, and 0.06 mg/kg/d (for the last 10 mo of the study). A few dogs (four controls, four exposed) were given DEHP in capsules at doses of 0.03 milliliters (mL)/kg/d for 19 d and then 0.06 mL/kg, 5 d/wk, for a total of 240 doses. A fairly thorough histopathologic evaluation of the animals was done after necropsy; however, hematology findings, which were negative, were reported only for the rats. Increased liver and kidney weights were reported in the high-dose rats, but no adverse effects were reported from the two lower-dose groups or in any of the guinea pigs or dogs. Despite a few irregularities in the study (e.g., lung infections causing excess mortality in control rats, problems with an unusually high number of litters in F_1 control rats, and liver-weight increases in female guinea pigs that were not dose-related), the authors concluded that the three species are roughly comparable in sensitivity to production of neoplastic lesions following DEHP ingestion, with a NOAEL of 0.06 g/kg/d or higher (Carpenter et al. 1953).

Nikonorow et al. (1973) reported the effects of the administration of DEHP in food to male and female Wistar rats at 0.35% for 12 mo. The rats had decreases in body weights, increases in kidney weights, and liver enlargement. Blood cell counts were unchanged, and the histopathology of liver, kidney, and spleen were normal. This report is difficult to understand because of the different plasticizers and stabilizers studied, the different exposure times, and the incomplete information provided.

A later chronic study, conducted in rats and mice by NTP (Kluwe et al. 1982), was done using concentrations at least 5-fold higher than the study by Carpenter et al. (1953) and revealed significantly different results, including the ability of DEHP to induce liver tumors (see discussion below). F-344 rats ingested DEHP at 0.32 g/kg/d or 0.67 g/kg/d (males) or 0.39 g/kg/d or 0.77 g/kg/d (females) from their food for 103 wk. The mean daily ingestions of DEHP by $B6C3F_1$ mice were 0.67 g/kg/d or 1.32 g/kg/d (males) and 0.80 g/kg/d or 1.82 g/kg/d (females). Only the low-dose female mice showed a statistically significant decrease in survival, and the investigators did not attribute that to DEHP ingestion. The incidence of non-neoplastic lesions in treated male rats and mice was found to exceed the incidence in their respective controls. Pituitary hypertrophy was found in 22 of 49 high-dose male rats (one of 46 controls) and seminiferous tubular degeneration of the testis was found in 43 of 48 high-dose male rats (one of 49 controls). Chronic kidney inflammation was found in 10 of 50 high-dose male mice (one of 50 controls) and seminiferous tubular degeneration was found in seven of 49 high-dose male mice (one of 49 controls). The benchmark dose (BMD) analyses of these data are shown in Tables 4-3 and 4-4 and in Figure 4-3, along with the statistically significant changes.

There was one chronic time point in a study attempting to define the time- and dose-response relationships for male and female Wistar rats administered DEHP in food at 50, 200, and 1,000 mg/kg/d (Mitchell et al. 1985b). The study was focused primarily on changes in the liver. After 9 mo, body weights were lower in the two highest-dose male groups and in the highest-dose female group. Liver weights were from 18% to 40% higher in males of all dose groups and 17% to 31% higher in the two highest-dose female groups. Few changes occurred between the 28-d time point (see subchronic section above) and the 9-mo time point. After 9 mo, thyroid alterations were reported, including basophylic deposits in the colloid and enlargement of the lysosomes. The response of females was qualitatively similar to males, but females were clearly less susceptible to the liver effects caused by DEHP.

In another study focused exclusively on changes in the rat liver induced by DEHP (and other compounds), the relationship between PP and lipid peroxidation was investigated (Lake et al. 1987). Male Sprague-Dawley rats were administered DEHP at 2% in their diets for 2 y. They were then killed, and their livers were studied for various biochemical changes. Two of the three markers of lipid peroxidation showed large increases compared with control values. The study confirms the association between PP and lipid peroxidation; however, the role of enhanced lipid peroxidation in

TABLE 4-3 BMD Analysis of Rat Data Showing Seminiferous Tubule Degeneration[a]

Dose (mg/kg/d)	Incidence	BMD_{01} (mg/kg/d)	$BMDL_{01}$ (mg/kg/d)
0	1/49	285	211
322	2/44		
674	43/48[b]		

[a]A log-logistic model was used.
[b]Statistically significant change level.
Abbreviations: BMD_{01}, benchmark dose corresponding to a 1% risk; $BMDL_{01}$, lower confidence limit on the BMD corresponding to a 1% risk.
Source: Data from Kluwe et al. 1982.

the hepatocarcinogenicity of DEHP remains to be demonstrated (Lake et al. 1987).

In a study focused on the kidneys, Crocker et al. (1988) evaluated the potential for DEHP or residues from artificial kidneys to cause an effect in rats similar to the polycystic kidney disease noted at autopsy in patients who had undergone long-term hemodialysis. Rats (gender and strain unspecified) were administered DEHP at approximately 2 mg/kg (based on a human equivalent dose of 150 mg per 70 kg body weight) three times a week by feeding tube and were killed after 3, 6, 9, and 12 mo. The study suggests that the dose may have been higher because of compression of the usual dialysis patient's exposure time of 5-7 y into a rat's life span, but that is not clear. DEHP apparently caused focal cystic changes in the kidneys of rats killed at the 12-mo time period, and the creatinine clearance was also reduced approximately 50% after 12 mo. However, the reported kidney effects seem inconsistent with reports from other studies in which rats given much higher doses did not exhibit kidney injury. The study also involved an unusually high number of deaths (e.g., four of 20 control animals died). An in vitro study using cultured kidney cells showed that when cells are exposed to MEHP or 2-ethylhexanoic acid, only the former causes a marked toxic effect (Rothenbacher et al. 1998).

In a study focused on the liver effects of DEHP in rodents, Ganning et al. (1991) gave DEHP in food at concentrations of 0.02%, 0.2%, and 2% for 102 wk. The two highest doses caused a reduction in body-weight gain, which was apparent before 24 wk. A number of tissue enzymes were studied for the time course of their changes in response to accumulated exposure

TABLE 4-4 BMD Analysis of Mouse Data Showing Chronic Renal Inflammation[a]

Dose (mg/kg/d)	Incidence	BMD_{01} (mg/kg/d)	$BMDL_{01}$ (mg/kg/d)
0	1/50	525	71
672	2/48		
1,325	10/50[b]		

[a]A Weibull model was used, although a log-logistic model would give the same result. Both models provide an exact fit to the data because there are only three points.
[b]Statistically significant change level.
Abbreviations: BMD_{01}, benchmark dose corresponding to a 1% risk; $BMDL_{01}$, lower confidence limit on the BMD corresponding to a 1% risk.
Source: Data from Kluwe et al. 1982.

to DEHP. Palmitoyl-Co-A dehydrogenase, a marker of PP, increased immediately in the liver homogenates from the highest-dose group; showed rapid increases in the middle-dose group; and showed a slow, near-linear increase to about 100% above the control value by 102 wk. Carnitine-acetyltransferase, a mitochondrial enzyme, showed a pattern of increased activity similar to palmitoyl-Co-A dehydrogenase activity. Other enzymes showed transient spikes in activity during the time course of the study. In animals whose treatment was discontinued after 1 y, all enzyme levels return to normal within 2-3 wk after the end of treatment.These results show the cumulative effects of DEHP ingestion on enzyme activities but also demonstrate the reversibility of the induced changes once the toxicant is withdrawn.

Data from a chronic study in F-344 rats show that aspermiogenesis and possibly hematologic effects resulted from ingestion of DEHP in food (David et al. 2000). The pertinent data and the BMD analysis for reduced red blood cell (RBC) counts is show in Table 4-5 and in Figure 4-4. The data on aspermiogenesis were difficult to use in BMD analysis because of a dose-response reversal in the intermediate doses. However, a $BMDL_{01}$ of 2.2 mg/kg/d was found using a quantal-linear model; this result was used for risk analysis. RBC counts at the highest dose had to be deleted to make the BMD approach work; however, the subcommittee advised against using the RBC data because the results were not considered statistically or biologically significant.

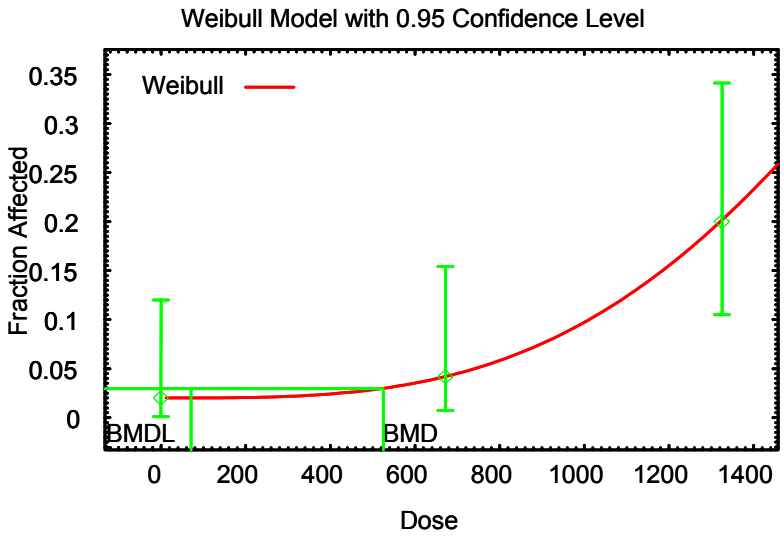


FIGURE 4-3 BMD analysis of the chronic renal inflammation data in Table 4-4. Source: Data from Kluwe et al. 1982.

Neoplastic Lesions

The chronic study by Kluwe et al. (1982) demonstrated an increased incidence of liver tumors in all exposed groups (Table 4-6). The increases were significant when compared either pair-wise with controls or by a trend test. It is interesting that, in male rats, ingestion of DEHP caused a significant reduction in the incidence of neoplasms of the pituitary, thyroid, and testes. For example, the incidences of testicular neoplasms in control, low-dose, and high-dose groups were 47/49, 42/44, and 11/48, respectively (Kluwe et al. 1982).

In a more recent study, the relationship between PP, cell proliferation, and hepatocarcinogenesis was explored. For 104 wk, male and female F-344 rats were fed a diet containing DEHP at 0, 100, 500, 2,500, or 12,500 ppm, and male and female B6C3F_1 mice were fed a diet containing DEHP at 0, 100, 500, 1,500, or 6,000 ppm (David et al. 1999). In a satellite recovery group, the highest-dose animals had treatment discontinued at 79 wk into the study and were evaluated at 104 wk. The total incidences of

TABLE 4-5 Toxic Effects of DEHP in F-344 Rats

Dose (mg/kg/d)	Incidence of Aspermiogenesis	RBC (10^6/μl)	BMD Analysis on Aspermiogenesis
0	37/64	8.91	BMD_{01} = 3.4 mg/kg/d
5.8	34/50	8.38	$BMDL_{01}$ = 2.2 mg/kg/d
28.9	43/55[a]	8.22	Quantal-linear model
146.6	48/64[a]	7.49	
789	62/64[a]	(8.01)	

[a]Incidence considered statistically significant at $p < 0.05$.
Abbreviations: BMD, benchmark dose; BMD_{01}, benchmark dose corresponding to a 1% risk; $BMDL_{01}$, lower confidence limit on the BMD corresponding to a 1% risk; RBC, red blood cells.
Source: Data from David et al. 2000.

hepatocellular neoplasms are given in Table 4-7. Assessment of an enzyme marker of PP at an earlier time point in the study indicates that there was a minimum increase necessary for a significant increase in tumors. The authors concluded that a >70% increase is necessary for rats, and a >300% increase is necessary for mice. That cessation of treatment at 79 wk resulted in a reduction in the incidence of tumors at 104 wk suggests that DEHP has a promoting effect on cells that have been transformed. A dose of 500 ppm (29-36 mg/kg/d) for 104 wk did not increase liver weights, induce PP in the liver, or cause the incidence of liver tumors to increase in rats. For mice, dietary consumption of 100 ppm (19-24 mg/kg/d) did not cause increased liver weights, induce PP, or induce an increased incidence of liver tumors. The authors concluded that a threshold approach to calculating the no-significant-risk dose for human exposure is appropriate (David et al. 1999).

Liver tumors induced by peroxisome proliferators such as DEHP have limited application to human toxicity for two fundamental reasons. First, the absorption of DEHP from the gut in humans is much lower than the absorbtion from the gut in rodents at the high concentrations necessary to induce tumors. This results in a much lower blood level of the proximate metabolite MEHP in humans, and much less of that metabolite is delivered to the target tissue. Second, the response of hepatocytes to a given level of MEHP is less likely to result in PP (and subsequent tumors) in humans because the level of PPARa is much lower than it is in rat hepatocytes (see

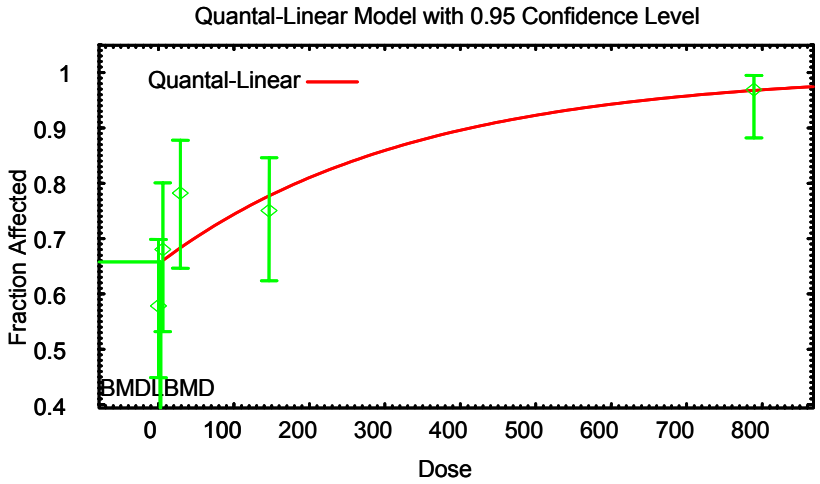


FIGURE 4-4 BMD analysis of aspermiogenesis data given in Table 4-5. Source: Data from David et al. 2000.

Figure 4-2). The importance of PPARa and the role of PP in mediating the DEHP tumor response were demonstrated in PPARa knockout mice exposed to DEHP. None of the mice showed hepatotoxicity or tumor (Ward et al. 1998). On the basis of such mechanistic information, IARC recently changed its classification of DEHP from "possibly carcinogenic to humans" to "not classifiable as to carcinogenicity in humans" (NTP 2000).

Genotoxicity

DEHP and many of its metabolites have been tested for genotoxicity in mammalian-cell test systems, in bacterial mutagenicity test systems, and in non-mammalian eucaryotic systems. The results of that testing have been summarized, and the discussion below will not attempt to repeat that summary (WHO 1992). The goal of this section is to select representative reports in each of the areas of testing and place the overall results into the context of our understanding of the way DEHP induces liver tumors in rodents.

The vast majority of bacterial mutagenicity studies have been negative for DEHP and its metabolites, MEHP, 2-ethylhexanol, and phthalic acid.

TABLE 4-6 Incidence of Liver Neoplasms in Rodents Ingesting DEHP[a]

Species	Gender	Control	Low Dose[b]	High Dose[b]
Rat	Male	3/50	6/49	12/49
	Female	0/50	6/49	13/50
Mouse	Male	14/50	25/48	29/50
	Female	1/50	12/50	18/50

[a]Exposure duration was 103 wk.
[b]The doses vary eith species and gender. For actual doses, see Table 4-8.
Source: Data from Kluwe et al. 1982.

The exception is a report by Tomita et al. (1982) in which *S. typhimurium* TA100 strain showed revertants after DEHP or MEHP exposure and MEHP was positive in *B. subtilis* and *E. coli* mutagenicity assays. DEHP has been evaluated in several species of fungi, and there is only one report of a positive finding (Ashby et al. 1985). Similarly, mammalian cells evaluated for mutagenicity showed positive results in one out of 10 investigations using mouse lymphoma cells (Ashby et al. 1985). Marginally positive results were found in collaborative *Drosophila* mutagenicity testing, but only at specific intermediate concentrations (Ashby et al. 1985).

Generally, methods used to detect DNA interactions, including unscheduled DNA synthesis (UDS) and single-strand breaks, have given negative results for DEHP in mammalian systems (WHO 1992). In vitro results using hepatocytes, CHO cells, and HeLa cells have proven negative for DNA interactions except for an isolated example of UDS, which did not fit a dose-response relationship. The consensus in that case was that DEHP does not cause UDS (Ashby et al. 1985). In vivo studies of rat liver from animals exposed to oral doses of DEHP have likewise been negative. Even under conditions of prolonged administration and induction of peroxisomes,indices of DNA interactions have been negative (Butterworth et al. 1984; Kornburst et al. 1984). One interesting exception is the finding that 8-hydroxydeoxyguanosine was increased in hepatocytes from rats given DEHP at 600 mg/kg/d for 2 wk (Takagi et al. 1990).

Chromosomal changes, such as sister chromatid exchanges, caused by DEHP have been found to be absent in CHO cells. MEHP induced sister chromatid exchanges in three cell lines, but generally at cytotoxic doses (WHO 1992). This cytotoxicity may be due to action of MEHP on cell membranes. DEHP can cause aneuploidy in mammalian cells and in fungi in vitro (WHO 1992).

TABLE 4-7 Incidence (%) of Hepatocellular Neoplasms After DEHP Ingestion[a]

Species	Gender	0 ppm	100 ppm	500 ppm	1,500 ppm	2,500 ppm	6,000 ppm	12,500 ppm	Recovery
Rat	Male	7	10	7	—	17[b]	—	43[b]	33[b]
	Female	0	8[b]	2	—	5	—	31[b]	18[b]
Mouse	Male	11	23	32[b]	42[b]	—	53[b]	—	26[b]
	Female	4	6	11	29[b]	—	63[b]	—	55[b]

[a]Exposure duration was 104 wk.
[b]Significantly different from concurrent control.
Source: Data from David et al. 1999.

A number of in vivo genotoxicity investigations of DEHP have shown negative results. Those include a chromosomal study of bone marrow cells from rats dosed with DEHP for 5 d, a micronucleus assay on blood cells from mice treated for 5 d, and dominant lethal studies in mice (WHO 1992). In most of these tests, metabolites MEHP and 2-ethylhexanol were also found to be negative.

DEHP can stimulate DNA synthesis in rat hepatocytes immediately after dosing, and it has been postulated that this increased cell division rate might increase the probability that a DNA error caused by an endogenous mutagen will become an irreversible mutation (Smith-Oliver and Butterworth 1987). Cells are known to be more vulnerable to irreversible alterations in their DNA when undergoing rapid division (Marx 1990).

Reproductive Toxicity

Certainly the hepatotoxicity of DEHP is its most-studied toxicity aspect; however, the effects of DEHP on the testes have also been thoroughly studied. Some aspects of the testicular toxicity have been discussed above as part of the results from short-term and subchronic rodent bioassays (Poon et al. 1997). Depending on which study is used and how the end points are assessed, the susceptibility of rat livers to PP and rat testes to atrophy by DEHP are roughly comparable in adult animals. However, young male rats are much more susceptible to DEHP testicular injury than are mature rats. For example, in 4-wk-old rats given a 10-d oral treatment of DEHP at 2,800 mg/kg/d, testes, seminal vesicles, and prostates weighed approximately 50% less than the same organs in control rats (Gray and Gangolli 1986). When 15-wk-old rats were given the same treatment, the reductions in the weights of the three organs were all less than 10%, and the weights were not statistically different from the weights of organs from control rats.

MEHP, which is formed when DEHP is absorbed from the gut, is thought to be the metabolite responsible for testicular injury. If MEHP is given by intravenous infusion, the age-related differences in testicular toxicity seen in oral administration studies are not apparent (Sjoberg et al. 1986). The increased toxicity of orally administered DEHP to young male rats may simply be because of their increased ability to absorb DEHP and convert it to MEHP in the process. Furthermore, cocultured Sertoli and germ cells are much more susceptible to germ-cell detachment by MEHP than by metabolites V, IV, and IX of DEHP (Gray and Gangolli 1986). This in vitro, coculture assay correlates well with the in vivo testicular toxicity of a series

of phthalate monoesters (Gray and Gangolli 1986). The extremely limited ability of cultured Sertoli cells to metabolize MEHP to other metabolites also supports the hypothesis that MEHP is the cause of testicular injury (Albro et al. 1989).

Testicular atrophy caused by DEHP varies from one species to another. After reviewing the literature on DEHP-induced testicular atrophy, it was concluded that rats and guinea pigs are the most susceptible to testicular damage, mice are intermediate in susceptibility, and marmosets and hamsters are least susceptible (WHO 1992).

There has been much less study devoted to female reproductive toxicity than to male reproductive toxicity. The reproductive toxicity of DEHP was assessed in the NTP Fertility Assessment by Continuous Breeding protocol. Male and female CD-1 mice were fed chow containing DEHP at 0%, 0.01%, 0.1%, and 0.3% for a 7-d premating period and a 98-d cohabitation period (Melnick et al. 1987). There was complete suppression of fertility at 0.3% and significant reduction at 0.1%. For example, live pups per litter was 10.6 in controls and 5.2 in the 0.01% group. In crossover matings between 0.3% mice and 0% mice, there was a reduced number of live pups per litter whether the DEHP effects were mediated through males or females. DEHP was a reproductive toxicant in both male and female CD-1 mice (Melnick et al. 1987). Weights of both male and female reproductive organs were decreased in animals fed 0.3%, but organs from animals exposed to lower concentrations of DEHP were apparently not evaluated. There were no apparent effects on fertility in the group fed DEHP at 0.01% for 105 d, which, using Table 5 from Tyl et al. (1988), NASA estimates to be approximately 18 mg/kg/d.

DEHP was one of eight commercial phthalate esters tested in a study comparing the results of in vitro and in vivo assays for estrogenic activity. The in vitro assay involved competitive ligand binding to the estrogen receptor, whereas the in vivo assay involved uterotrophic and vaginal cornification assays on ovariectomized SD rats. DEHP was nonresponsive in all assays (Zacharewski et al. 1998). In contrast, Berman and Laskey (1993) have shown that DEHP alters the release of progesterone, testosterone, and estradiol in the culture medium from minced, cultured whole rat ovaries. Ovaries were removed from rats that had been given DEHP at gavage doses of 0 mg/kg/d or 1,500 mg/kg/d for 10 d and were in specific stages of their estrus cycle. The ovaries were cultured for 1 h, and the hormones were assayed from decanted culture medium. The results showed that DEHP altered steroid profiles so that proestrus appeared to be delayed.

The mechanism responsible for DEHP effects on ovarian granulosa cells might be related to the mechanism causing effects on testicular Sertoli

cells. MEHP, the presumptive active metabolite of DEHP, inhibits FSH-stimulated cAMP accumulation in both Sertoli cells and in granulosa cells. The endocrine and paracine support granulosa cells provide to the developing ovum is analogous to the role Sertoli cells play for developing spermatozoa. When MEHP was added at 100 μM to cultured rat granulosa cells, the cAMP produced by FSH stimulation was 40% less than the amount produced by cultured cells without MEHP treatment (Treinen et al. 1990). In addition, the production of progesterone by granulosa cells cultured for 48 h was reduced 30% by treatment with MEHP at only10 micromolar (μM).

Developmental Toxicity

The developmental toxicity of DEHP has been studied in several species and by various routes of administration; however, there are few studies involving the oral exposures. Because absorption in the gut has a major effect on the metabolism of DEHP, the discussion will be confined to oral studies of developmental toxicity.

The developmental toxicity of DEHP has been thoroughly evaluated in F-344 rats and CD-1 mice by administration in food over gestational days 0-20 (rats) and 0-17 (mice). The food concentrations were as follows: 0%, 0.5%, 1.0%, 1.5%, and 2.0% in rats; 0%, 0.025%, 0.05%, 0.10%, and 0.15% in mice (Tyl et al. 1988). In rats, the three highest doses showed maternal toxicity and reduced fetal body weight per litter. The number and percentages of malformed fetuses per litter were not affected by DEHP treatment in any group. In contrast, DEHP caused increased incidences of fetal malformations in mice at the two highest doses, which were also toxic to the dams. However, at 0.05% there were increased incidences of malformations in the absence of maternal toxicity. The NOAELs for embryo-fetal toxicity from this study were 0.025% for mice and 0.5% for rats (Tyl et al. 1988). The NOAEL for developmental effects in CD-1 mice is equivalent to 44 mg/kg/d, which is well above the NOAEL for reproductive effects in the same strain (Melnick et al. 1987).

An NTP panel that reviewed the reproductive and developmental toxicity of DEHP reached a conclusion on the possible health effects in humans at current ambient exposure levels. For the general adult population, the panel expressed "minimal concern that ambient human exposures adversely affect adult human reproduction. This level of concern is not appreciably altered for adults medically exposed to DEHP or MEHP" (NTP 2000). The same panel expressed "concern that [DEHP] exposure may adversely affect

male reproductive tract development in healthy infants and toddlers, and that ambient oral exposures to pregnant or lactating women may adversely affect the development of their offspring." There is a large difference in the doses that are toxic to laboratory animals and those that are encountered by the general human population; however, the unique exposures to DEHP associated with medical devices need further evaluation (NTP 2000).

Spaceflight Effects

DEHP induces a number of toxic effects; however, except for effects on hematologic parameters, spaceflight-induced physiologic changes and the toxic effects of DEHP seem independent. One investigator reported a 9% average decrease in RBC count in male rats ingesting DEHP at 5,000 ppm for 13 wk (Poon et al. 1997).

Synergistic Effects

There are no known chemicals present in spacecraft water that would be expected to increase the toxicity of DEHP. One would expect many of the phthalate esters to be additive in their toxic effects. Loss of red cell mass encountered during spaceflight must be considered when risks from hematologic end points are examined.

LIMITS SET BY OTHER ORGANIZATIONS

Table 4-9 provides a list of the current standards for DEHP. There is considerable range in these values, and that reflects the rapidly expanding database on DEHP toxicity and the evolving understanding of the mechanisms of DEHP toxicity.

Rationales

Drinking Water Standard from the National Research Council

When setting the acceptable daily intake (ADI) value shown in Table 4-9, the National Research Council (NRC) committee noted that DEHP can

TABLE 4-8 Toxicity Summary for Oral Exposures in Drinking Water and Feed

Dose, Route, and Duration	Species	Effects	Reference
Acute Toxicity			
26,000 mg/kg	Guinea pig	LD_{50}	Krauskopf 1973
31,000 mg/kg	Wistar rat, male	LD_{50}	Shaffer et al. 1945
34,000 mg/kg	Rabbit, male	LD_{50}	Shaffer et al. 1945
34,000 mg/kg	Mouse	LD_{50}	Krauskopf 1973
34,000 mg/kg	Rat	LD_{50}	Krauskopf 1973
1,000 mg/kg, feed, 3 d	Wistar rat, male	26% increase in liver weights, damage of bile canaliculi, PP	Mitchell et al. 1985b
200 mg/kg, feed, 3 d	Wistar rat, male	Damage of bile canaliculi, PP	Mitchell et al. 1985b
50 mg/kg, feed, 3-d	Wistar rat, male	No adverse effect	Mitchell et al. 1985b
140 mg/kg	Human, male, n = 1	Gastric distress	Shaffer et al. 1945
70 mg/kg	Human, male, n = 1	No adverse effect	Shaffer et al. 1945
Short-Term Toxicity			
11 mg/kg/d, feed, 21 d	F-344 rat, male	NOEL for changes in PP markers	Short et al. 1987
50 mg/kg/d, feed, 14 d	Wistar rat, male	22% increase in liver weights (gone at 28 d)	Mitchell et al. 1985b
105 mg/kg/d, feed, 21 d	F-344 rat, male	Two enzyme markers of PP were increased; no increase in liver weights	Short et al. 1987

(Continued)

TABLE 4-8 Continued

Dose, Route, and Duration	Species	Effects	Reference
200 mg/kg/d, feed, 14 d	Wistar rat, male	38% increase in liver weights (gone at 28 d)	Mitchell et al. 1985b
500 mg/kg/d, gavage, 21 d	Monkey, male	NOEL for liver weight increases and PP marker increase	Short et al. 1987
500 mg/kg/d, oral tube, 14 d	Monkey, male, n = 4	NOAEL for any effect	Pugh et al. 2000
670 mg/kg/d, feed, 21 d	F-344 rat, male	80% increase in relative liver weight; increases in three markers of PP	Short et al. 1987
1,000 mg/kg/d, feed, 14 d	Wistar rat, male	69% increase in liver weights	Mitchell et al. 1985b
2,000 mg/kg/d, gavage, 14 d	Rat, male, 6-8 wk old	Testicular atrophy, hepatomegaly, reduced body-weight gain, PP	Rhodes et al. 1986
2,000 mg/kg/d, gavage, 14 d	Marmoset	Reduced body-weight gain	Rhodes et al. 1986
1,600 mg/kg/d,[a] feed, 21 d	Wistar rat, male, 5 wk old	140% increase in liver weight; PP; 22% decrease in testicular weight	Mann et al. 1985
1,600 mg/kg/d,[a] feed, 10 d	Wistar rat, male, 5 wk old	80% decrease in liver weight; PP; possible decrease in testicular weight	Mann et al. 1985
2,800 mg/kg/d, gavage, 10 d	SD rat, male, 15 wk old	50% decrease in testicular weight	Gray and Gangolli 1986
2,800 mg/kg/d, gavage, 10 d	SD rat, male, 15 wk old	No decrease in testicular weight	Gray and Gangolli 1986

Subchronic Toxicity			
200 mg/kg/d, feed, 90 d	Wistar rat, male	NOAEL for liver, testicular, and RBC effects	Shaffer et al. 1945
400 mg/kg/d, feed, 90 d	Wistar rat, male	NOAEL for all but growth retardation	Shaffer et al. 1945
900 mg/kg/d, feed, 90 d	Wistar rat, male	Testicular atrophy and degeneration; NOEL for liver effects	Shaffer et al. 1945
4 mg/kg/d, 13 wk	SD rat	NOAEL for any effects	Poon et al. 1997
40 mg/kg/d, 13 wk	SD rat	Mild to minimal Sertoli cell vacuolization in 7/10 rats; NOAEL for other effects, including hematological effects seen at higher doses	Poon et al. 1997
400 mg/kg/d, 13 wk	SD rat	Minimal to mild seminiferous tube atrophy in 9/10; mild to moderate Sertoli cell vacuolization; 9% decrease in RBC count in males; mild thyroid changes; 20-40% liver weight increase, PP	Poon et al. 1997
100 mg/kg/d, oral gavage, 91 d	Marmoset	NOAEL for all end points	Kurata et al. 1998
500 mg/kg/d, oral gavage, 91 d	Marmoset	30% increase in peroxisome volume in males; NOAEL for other end points	Kurata et al. 1998
2,500 mg/kg/d, oral gavage, 91 d	Marmoset	40% increase in peroxisome volume; reduced weight gain; no other changes	Kurata et al. 1998

(Continued)

TABLE 4-8 Continued

Dose, Route, and Duration	Species	Effects	Reference
Chronic Toxicity			
60 mg/kg/d, feed, 2 y	Sherman rat	NOAEL for limited end points	Carpenter et al. 1953
200 mg/kg/d, feed, 2 y	Sherman rat	Increased liver and kidney weights; NOAEL for other end points	Carpenter et al. 1953
60 mg/kg/d, feed, 1 y	Guinea pig	NOAEL	Carpenter et al. 1953
60 mg/kg/d, capsules, 5 d/wk, 240 d	Dogs	NOAEL	Carpenter et al. 1953
320 mg/kg/d, feed, 103 wk	F-344 rat, male	6/49 liver tumors (3/50 controls); NOAEL for testicular effects	Kluwe et al. 1982
670 mg/kg/d, feed, 103 wk	F-344 rat, male	22/49 pituitary hypertrophy (1/46 controls); seminiferous tube degeneration in 43/48 (1/49 controls); 12/49 liver tumors (3/50 controls)	Kluwe et al. 1982
390 mg/kg/d, feed, 103 wk	F-344 rat, female	6/49 liver tumors (0/50 controls)	Kluwe et al. 1982
770 mg/kg/d, feed, 103 wk	F-344 rat, female	13/50 liver tumors (0/50 controls)	Kluwe et al. 1982
670 mg/kg/d, feed, 103 wk	$B6C3F_1$ mouse, male	25/48 liver tumors (14/50 controls); NOAEL for kidney inflammation	Kluwe et al. 1982
1,320 mg/kg/d, feed, 103 wk	$B6C3F_1$ mouse, male	Chronic kidney inflammation (10/50) (1/50 controls); seminiferous tube degeneration in 7/49 (1/49 controls); 29/50 liver tumors (14/50 controls)	Kluwe et al. 1982

800 mg/kg/d, feed, 103 wk	$B6C3F_1$ mouse, female	12/50 liver tumors (1/50 controls)	Kluwe et al. 1982
1,820 mg/kg/d, feed, 103 wk	$B6C3F_1$ mouse, female	18/50 liver tumors (1/50 controls)	Kluwe et al. 1982
50 mg/kg/d, feed, 9 mo	Wistar rat, male	23% increase in liver weight; enzymatic changes marginal	Mitchell et al. 1985b
29-36 mg/kg/d, feed, 104 wk	F-344 rat	NOEL for increased liver weights, PP, liver tumors (incidence in Table 4-3)	David et al. 1999
19-24 mg/kg/d, feed, 104 wk	$B6C3F_1$ mouse	NOEL for increased liver weights, PP, liver tumors (incidence in Table 4-3)	David et al. 1999
5.8 mg/kg/d, feed, 104 wk	F-344 rat, male	NOAEL for aspermiogenesis and all other effects, with the exception of renal effects	David et al. 2000

[a]Amount estimated from the concentration in food.

cause the following toxic effects: liver function changes, reproductive toxicity, developmental effects, and cancer (NRC 1986). The committee started with data on dialysis patients showing that 150 mg/wk (estimated amount) caused no detectable changes in the liver except that after 1 y there were significantly higher numbers of peroxisomes (Ganning et al. 1984). This finding has been rejected as a starting point for risk assessment by others because only one patient showed the increase in liver peroxisomes, and the exposure may have included other peroxisome prolif-erators (ATSDR 1993). For a 70-kg person, this estimated dose equates to an exposure of 0.3 mg/kg/d. The committee applied a safety factor of 10 (presumably converting a LOAEL [lowest-observed-adverse-effect level] into a NOAEL) and an interindividual variability factor of 10 to reach an ADI of 0.003 mg/kg/d. The committee determined that the NOEL for liver function changes was 50 ppm (3.3 mg/kg/d) on the basis of a 7-d feeding study of rats (Morton 1979). To this NOEL the committee applied a safety factor of 1,000, including a factor of 10 to compensate for the shortness of the study, to reach a value of 0.003 mg/kg/d. The committee also looked at a chronic rat study by Ganning et al. (1984) and a rat teratogenicity study by Ruddick et al. (1981), but those led to higher ADIs than did the liver effects end point. The subcommittee noted that the NTP carcinogenesis bioassay produced a 10^{-6} risk (upper 95% confidence) at a lifetime dose of 0.03 mg/kg/d when the generalized multistage model for carcinogenesis was used. This model probably overestimates the cancer risk at low doses, because DEHP seems to be inactive on the liver at lower oral doses (i.e., there is a threshold for cancer).

EPA's Maximum Contaminant Level

It has proven difficult to trace the origin of the EPA's maximum contaminant level (MCL) on DEHP. The appropriate pages of the *Federal Register* (EPA 1992a) contain only a rationale for an MCL goal (MCLG) of zero because DEHP is an animal carcinogen. In a document entitled "Final Drinking Water Criteria Document for Phthalic Acid Esters" (EPA 1992b), an interim value was indicated on the basis of the most sensitive slope of the tumor incidence from the data of Kluwe et al. (1982). Using the upper bound of the cancer risk from ingestion of 2 L of water per day for 70 y, with assumption of linearity, EPA estimated that the risk was 10^{-4} at 0.300 μg/L, 10^{-5} at 0.030 μg/L, and 10^{-6} at 0.003 μg/L.

TABLE 4-9 Standards Set by Government Organizations

Organization	Standard	Value (mg/kg/d)	Water Equivalent (mg/L)[a]	Reference
NRC	ADI	0.003	0.1	NRC 1986
EPA	MCL	—	0.006	EPA 1998
EPA	MCLG	0	0	EPA 1992a
EPA	RfD (oral)	0.02	0.7	IRIS 1993
California		—	0.004	ATSDR 1993
Kansas		—	4.2	
Maine		—	1.2	
Massachusetts		—	0.01	
Minnesota		—	0.04	
Rhode Island		—	1.2	

[a]Assumes a 70-kg person consuming 2 L of water per day and no other source of DEHP.
Abbreviations: ADI, acceptable daily intake; EPA, U.S. Environmental Protection Agency; MCL, maximum contaminant level; MCLG, maximum contaminant level goal; NRC, National Research Council; RfD, reference dose.

EPA's Reference Dose

The reference dose (RfD) for DEHP was based on the 1-y study of guinea pigs given DEHP in food at 0.13% or 0.04% (equivalent to 64 mg/kg/d and 19 mg/kg/d, respectively) (Carpenter et al. 1953). Guinea pigs appeared to be a more sensitive species than the Sherman rats exposed in a second study (Carpenter et al. 1953). From the guinea pig study, 19 mg/kg/d was determined to be a LOAEL for increases in liver weights in females. Factors of 10 were applied for interspecies variation, inter-individual variation, and to compensate for the fact that the exposure was not lifetime. Thus, an RfD of 0.02 mg/kg/d was established. This is equivalent to a water concentration of 0.7 mg/L if all DEHP came from drinking 2 L of water per day. The RfD is not consistent with an MCL of 0.006 mg/L.

Comparison to SWEGs for DEHP

The proposed SWEG for 1,000 d of ingestion is 20 mg/L in water used

for drinking and for reconstituting food (Table 4-10). This was based on BMD) analysis of the aspermiogenesis data from David et al. (2000). The 1,000-d SWEG is well above the water-equivalent chronic RfD of 0.7 mg/L. The 1,000-d SWEG is not consistent with an MCL of 0.006 mg/L.

NTP's Human Reproductive Risk Assessment

An expert review of DEHP health risks provides an important gauge for comparison with the SWEGs presented in this document (NTP 2000). The expert panel expressed minimal concern for the health of general adult population, which they believe is exposed to ambient DEHP at up to 0.03 mg/kg/d (2.1 mg/d for a 70-kg person). Furthermore, the "minimal concern" was not appreciably altered for adults medically exposed to DEHP. Human medical procedures can result in exposures at up to 1,000 mg/y (2.7 mg/d) (NTP 2000, Table 6). Combining the general population exposure and the exposure from medical procedures gives a dose of 4.8 mg/d, which the panel also described as a "minimal concern" (NTP 2000). For persons ingesting 2.8 L/d of DEHP-contaminated water, the concentration in the water could be as high as 1.7 mg/L without exceeding the minimal concern criterion expressed by the panel. This assumes no other significant sources of DEHP exposure. Considering that the medical procedures mentioned in the NTP report could last several years and the normal ingestion period is for an adult lifetime, the 100-d SWEG of 30 mg/L and the 1,000-d SWEG of 10 mg/L are well above the levels discussed by the expert panel, which also considered the equivalent of roughly 2 mg/L ingestion to be of minimal concern (NTP 2000).

RATIONALE

Setting specific human exposure limits for DEHP is difficult because although there is a plethora of data in rodents, sufficient mechanistic data suggest that significant interspecies differences affect the extrapolation of risk to human health. Recent data on the incidence of liver tumors in rats and mice suggest that a linear model of the log-dose vs risk, which was used by EPA (1992), is not applicable. The relevance of liver tumors induced by DEHP to human risk assessment has been critically questioned (Doull et al. 1999). In addition, the limited data available on primates suggest that the rodent is a poor model for hepatic changes caused by DEHP and is perhaps

TABLE 4-10 Spacecraft Water Exposure Guidelines for DEHP

Duration	Concentration (mg/L)	Target Toxicity
1 d	1,800	Gastric upset
10 d	1,300	Testicular injury
100 d	30	Hematotoxicity, testicular injury
1,000 d	20	Testicular injury

also a poor model for testicular effects. Confounding all this is the variation in the target organ sensitivity of different animal models to DEHP-induced toxicity. It could be that the variation in sensitivity is due more to the individual investigator's determination to look for changes in specific organs (liver or testes), rather than a true difference in each model's susceptibility to DEHP. The approach below assumes that the rodent is an acceptable model for hematologic and testicular changes, but not for liver and thyroid changes. The rodent thyroid is thought to be a poor model for the human thyroid (Mc Clain 1992). A risk assessment will also be done for chronic renal inflammation seen in male mice (Kluwe et al. 1982); however, renal toxicity seen in male rats will not be used for risk assessment. Guidelines promulgated by the National Research Council (2000) have been followed to select pertinent data and to analyze it for human health risks.

Ingestion for 1 d

There is one report that ingestion by a human of 10 g of DEHP caused gastric distress, but ingestion of 5 g was without apparent effect (Shaffer et al. 1945). The 5-g dose can be considered a NOAEL. The fact that only a single person was involved is compensated for by the bolus nature of the dose. The same dose administered in water over a day is much less likely to cause gastric distress than a single large dose. The 1-d AC (gastric distress) was calculated as follows:

$$\text{1-d AC} = 5{,}000 \text{ mg} \div 2.8 \text{ L/d} = 1{,}800 \text{ mg/L}.$$

This value is far above any that could be derived from adverse effects on animals.

Ingestion for 10 d

Mann et al. (1985) found evidence of testicular injury in rats ingesting DEHP in their food for 10 d. The paper focused almost entirely on liver effects; however, the testes of rats exposed at approximately 1,600 mg/kg/d weighed only 2.07 g, whereas the control testes weighed an average of 2.28 g. The authors did not consider this difference statistically significant, but there was clearly a difference in weight at 21 d of exposure. Because a decrease in testicular weight is a very insensitive end point for testicular injury, 1,600 mg/kg/d was established as a LOAEL. The 10-d AC for change in testicular weights was calculated as follows:

$$\text{10-d AC} = (1{,}600 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times 10 \times 3) = 1{,}300 \text{ mg/L}.$$

The factors of 10 and 3 are for extrapolation from a LOAEL to a NOAEL and for species extrapolation, respectively. For male reproductive effects, the weight of evidence suggests that adult humans are much less susceptible than rodents to DEHP; therefore, the usual species extrapolation factor of 10 was reduced to 3.

Ingestion For 100 d

The ACs for this time of exposure were set to avoid potential testicular injury and hematotoxicity on the basis of the results of Poon et al. (1997). Rats were fed DEHP in their diets for 13 wk at concentrations of 0, 5, 50, 500, and 5,000 ppm (Poon et al. 1997). Thyroid changes were found in the highest-dosed animals, but rodent thyroids are thought to provide an overly sensitive model of the human thyroid (McClain 1992). Sertoli cell vacuolization was found in seven of 10 of the rats ingesting 500 ppm (38 mg/kg/d, Table 4-2); hence, the NOAEL was set at the next lower dose of 3.7 mg/kg/d. The 100-d AC for potential testicular injury was calculated as follows:

$$\text{100-d AC} = (3.7 \text{ mg/kg} \times 70 \text{ kg}) \div (2.8 \times 3 \times 1.1) = 28 \text{ mg/L}.$$

The factor of 1.1 was used to compensate for the study being only 90 d, whereas the AC is for 100 d. The factor of 3 is for species extrapolation from rodents to humans for male reproductive effects. The data were not suitable for BMD analysis because of the poor fit of the dose-response data by standard approaches and the difficulty in applying the lesion-severity

data in the analysis (see Table 4-2). The subcommittee recommended against attempting a time extrapolation of this value to a 1,000-d ingestion period because good chronic data are available.

A 9% reduction in red cell counts was noted in the group of male rats given DEHP at 5,000 ppm for 13 wk (Poon et al. 1997). The next lower group (500 ppm) did not show a statistically significant change. This amount of DEHP in food is approximately equivalent to 40 mg/kg/d, which was taken as the NOAEL for hematologic effects. The AC for hemotoxicity was calculated as follows:

$$100\text{-d AC} = (40 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times 10 \times 3 \times 1.1) = 30 \text{ mg/L}.$$

The factors of 10, 3, and 1.1 are for interspecies differences, spaceflight effects, and time differences between rat exposures and potential human exposures, respectively.

Ingestion for 1,000 d

Kluwe et al. (1982) reported seminiferous tubular degeneration in F-344 rats and B6C3F$_1$ mice fed DEHP-spiked food for 103 wk. Rats and mice in their respective high dose groups showed the lesion; however, rats in the group fed 320 mg/kg/d did not show the lesion. Using the $BMDL_{01}$ of 211 mg/kg/d (Table 4-3) as a NOAEL (rats were more sensitive than mice), the AC for testicular effects was estimated as follows:

$$1{,}000\text{-d AC} = (211 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L} \times 3) = 1{,}760 \text{ mg/L}.$$

This AC clearly is not consistent with the 28 mg/L estimated for avoidance of testicular effects for a 100-d ingestion period using the 13-wk SD rat data from Poon et al. (1997). Poon et al. (1997) reported mild Sertoli cell vacuolization at 500 ppm (40 mg/kg/d), but minimal to mild seminiferous tubular atrophy was reported only in the highest dose (5,000 ppm, or about 400 mg/kg/d). The subcommittee and the original investigators recommended that 3.7 mg/kg/d be considered the NOAEL in the Poon et al. (1997) study. The difference in the findings is probably related to differences in the depth of assessment of the testicular injury and perhaps also to differences in the inherent susceptibilities of the two strains of rat and in the age at exposure.

Increases in the incidence of bilateral aspermiogenesis were found in F-344 rats exposed to DEHP in their feed for 104 wk (Table 4-5) (David et al.

2000). The study gave a NOAEL of 5.8 mg/kg/d, which results in an AC as follows:

$$1{,}000\text{-d AC} = (5.8 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times 3) = 48 \text{ mg/L}.$$

The NOAEL approach can be compared with the BMD analysis. The dose-response curve reversed at intermediate doses, making the BMD analysis subject to large uncertainty. The resulting $BMDL_{01}$ values spanned a range of several orders of magnitude; however, the three models with the highest *p* values gave consistent, intermediate results. The intermediate $BMDL_{01}$ results were as follows: the logistic model gave 3.0 mg/kg/d, the probit model gave 3.6 mg/kg/d, and the quantal-linear model gave 2.2 mg/kg/d. In this situation, a $BMDL_{01}$ of 2.2 mg/kg/d was used for a conservative risk assessment. That value is somewhat below the range of 3.7 mg/kg/d to 14 mg/kg/d suggested as the NOAEL for reproductive effects in rats (NTP 2000). The AC calculation, using a species extrapolation factor of only 3, was as follows:

$$1{,}000\text{-d AC} = (2.2 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times 3) = 18 \text{ mg/L}.$$

This AC is consistent with the 100-d AC of 28 mg/kg/d derived from the data of Poon et al. (1997) for prevention of Sertoli cell vacuolization.

Chronic renal inflammation was noted in a group of male mice fed DEHP at 6,000 mg/kg in feed for 103 wk, but was not observed in a group fed 3,000 mg/kg in feed (Kluwe et al. 1982). The authors estimate that the mean ingestion of DEHP in the unaffected group was 670 mg/kg/d. A $BMDL_{01}$ of 71 mg/kg/d was established as a NOAEL for kidney effects. The AC for kidney effects was estimated as follows:

$$1{,}000\text{-d AC} = (71 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times 10) = 177 \text{ mg/L}.$$

The factor of 10 is for interspecies differences. Clearly, the kidney is not very sensitive to oral ingestion of DEHP.

Functional Reproductive Toxicity

Aside from the testicular atrophy caused in rodents by DEHP ingestion, a functional impairment has been demonstrated in mice (Melnick et al. 1987). Functional impairment of reproduction was demonstrated in CD-1

TABLE 4-11 Acceptable Concentrations (ACs)

Target Organ	Data and Reference	Gender and Species	Uncertainty Factors				Acceptable Concentrations (mg/L)			
			NOAEL	Species	Exposure Time	Spaceflight	1 d	10 d	100 d	1,000 d
Gastric upset	NOAEL = 70 mg/kg (Shaffer et al. 1945)	Human, n = 1	1	1	1	1	1,800	—	—	—
Testicular injury	LOAEL = 1,600 mg/kg/d, 10 d in feed, decreased testicular weight (Mann et al. 1985)	Wistar rat, male	10	3	1	1	—	1,300	—	—
	NOAEL = 3.7 mg/kg/d, 90 d, Sertoli cell vacuolization, seminiferous tubule degeneration (Poon et al. 1997)	SD rat, male	—	3	1.1	1	—	—	28	—
	$BMDL_{01}$ = 211 mg/kg/d, 103 wk, seminiferous tubule degeneration (Kluwe et al. 1982)	F-344 rat, male	1	3	1	1	—	—	—	1,760
	NOAEL = 5.8 mg/kg/d, 104 wk, aspermiogenesis (David et al. 2000)	F-344 rat, male	1	3	1	1	—	—	—	48
	$BMDL_{01}$ = 2.2 mg/kg/d,[a] aspermiogenesis (David et al. 2000)	F-344 rat, male	1	3	1	1	—	—	—	18

(Continued)

TABLE 4-11 Continued

Target Organ	Data and Reference	Gender and Species	Uncertainty Factors				Acceptable Concentrations (mg/L)			
			NOAEL	Species	Exposure Time	Spaceflight	1 d	10 d	100 d	1,000 d
Hematotoxicity	NOAEL = 40 mg/kg/d, 13 wk, decrease in RBC count (Poon et al. 1997)	SD rat, male	1	10	1.1	3	—	—	30	—
Nephrotoxicity	$BMDL_{01}$ = 71 mg/kg/d, 103 wk, chronic renal inflammation (Kluwe et al. 1982)	$B6C3F_1$ mouse, male	1	10	1	1	—	—	—	177
SWEG[b]							1,800	1,300	30	20

[a]BMD value is extremely sensitive to model selected.
[b]Values may exceed solubility in pure water. SWEGs not set to protect against effects on conceptus during gestation.
Abbreviations: BMD, benchmark dose; $BMDL_{01}$, lower confidence limit on the BMD corresponding to a 1% risk; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; SWEG, spacecraft maximum allowable concentration.

mice at exposures of 0.1% and 0.3% in feed, but not at 0.01%. This ingestion exposure at 0.01% is equivalent to a dose of approximately 18 mg/kg/d and was chosen as a NOAEL. The AC for functional reproductive toxicity was calculated as follows:

$$AC = (18 \text{ mg/kg/d} \times 70 \text{ kg}) \div (2.8 \text{ L/d} \times 10) = 45 \text{ mg/L}.$$

This is applicable to both males and females, because the crossover mating of the mice showed that the effect was mediated through either sex. This AC is higher than those determined from other toxic end points for 100 d or 1,000 d of ingestion, so the calculations for functional reproductive toxicity were not shown in Table 4-11 (above).

RECOMMENDATIONS

The toxicity of DEHP has been broadly studied, especially in rodents; however, the relevancy of rodent studies to human toxicity requires further investigation. Although DEHP has been shown to be a liver carcinogen in rodents, mechanistic studies suggest that this finding is of minimal relevance to human risk assessment. DEHP has been shown to be a developmental and reproductive toxicant in multiple rodent species. In particular, exposure during early development presents a special concern for male reproductive toxicity. However, the purpose of this document is to evaluate relevant risks for occupational exposures for adult, nonpregnant astronauts. Pharmacokinetic studies have suggested significant species differences in absorption kinetics following oral ingestion between humans and rodents. Combined, these findings suggest that the risk assessment for SWEGs have erred on the conservative side. Additional quantitative toxicity data defining the age and kinetic differences in cross-species effects of oral DEHP would help to further refine these assessments.

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5 2-Mercaptobenzothiazole

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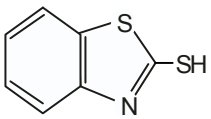
PHYSICAL AND CHEMICAL PROPERTIES

2-Mercaptobenzothiazole (MBT) forms pale yellow monoclinic needles or leaflets and has a disagreeable odor (see Table 5-1) (Dieter 1988).

OCCURRENCE AND USE

MBT is used commercially as an accelerator in the rubber vulcanization process and as a preservative for textile or cordage materials (Dieter 1988). The sodium salt is used as a corrosion inhibitor in petroleum products (Dieter 1988). MBT can also be found in some antifreezes (Kiec-Swierczynska et al. 1999); as a corrosion inhibitor in cutting oils (Fregert and Skog 1962); at 1% in an oil used to release plaster molds from epoxy casts (Wilkinson et al. 1990); in heavy-duty greases, black tire paints, special detergents, and photographic film emulsion (Rudzki et al. 1981); and in fungicides and veterinary medications (Kiec-Swierczynska et al. 1999). MBT has been reported as a contaminant in some injectable solutions of various drugs (digoxin, sodium pentobarbital, epinephrine, lidocaine hydrochloride, mepivacaine hydrochloride, pilocarpine hydrochloride, and dexamethasone

TABLE 5-1 Physical and Chemical Properties of MBT

Formula	$C_7H_5NS_2$
Chemical name	2-Mercaptobenzothiazole (MBT)
Synonyms	Captax, dermacid, mertax, thiotax, 2-benzothiazolethione, 2-benzothiazolyl mercaptan
	
CAS registry no.	149-30-4
Molecular weight	167.25
Melting point	180.2-181.7°C
Specific gravity	1.42 g/cc
Solubility	Insoluble in water; soluble in alcohol, acetone, benzene, and chloroform

sodium phosphate) at concentrations of up to 11.6 micrograms per milliliter (μg/mL) (Reepmeyer and Juhl 1983; Salmona et al. 1984). The MBT contaminate originated from the rubber closures of the single-dose delivery syringe or syringe cartridge, and the amount extracted might be dependant on the level of MBT in the rubber but appeared not to depend on the composition of the solvent for the drug (40% propylene glycol, 10% alcohol, 50% water for digoxin) (Reepmeyer and Juhl 1983). The 2-hydroxyethyl derivative of MBT was found to contaminate blood that came in contact with rubber plunger seals of syringes sterilized with ethylene oxide (Peterson et al. 1981). MBT was found in aqueous extracts of rubber baby bottle nipples (Blosczyk and Doemling 1982; Schweisfurth 1995) and was identified as one of the allergens responsible for tennis shoe dermatitis (Jung et al. 1988).

MBT may enter spacecraft as a component of rubber materials and thus may leach into spacecraft drinking water. Drinking water on the International Space Station (ISS) will be generated from recycled hygiene water, urine, and humidity condensate, and supplemented by water from the shuttle or the Russian spacecraft *Progress*. Because the octanol-to-water partition coefficient for MBT is high (log K_{ow} = 2.41) (Hansch and Leo 1979) and the solubility of MBT in pure water is very low, one would expect that only traces of MBT, if any, would be found in the ISS drinking water under

normal conditions. However, experience on Russia's Mir space station shows that MBT was detected in five of 27 samples of recycled water and six of 28 samples of humidity condensate collected from U.S. missions Mir-18 through Mir-25 at levels of up to 155.7 μg per liter (L) (see Table 5-2).

For ISS, none of the water samples for the first three expeditions has contained MBT at levels above the detection limit (approximately 40 μg/L).

PHARMACOKINETICS AND METABOLISM

No data were found in the scientific literature on human or animal uptake, metabolism, or elimination of MBT ingested in drinking water, probably because MBT is essentially insoluble in pure water. The data described below are predominantly for ^{14}C-labeled MBT ingested in corn oil and, as such, do not distinguish between the parent compound, metabolites, and ^{14}C that has entered the cellular carbon pool.

Absorption

In rats dosed by gavage with ^{14}C-labeled MBT in corn oil, nearly all the radioactivity administered was rapidly excreted in the urine, indicating nearly complete absorption of MBT from the gut (el Dareer et al. 1989). Studies of radiolabeled MBT in guinea pigs showed that it was absorbed through the skin and that abrasion (by tearing off tape stuck on the skin) increased the rate of absorption (Nagamatsu et al. 1979). At 24 and 48 hours (h) after application of ^{14}C- labeled MBT at 5.1 μCi per 3 milligrams (mg) per 0.1 mL in a solution of sodium carbonate (pH = 9) to an area 4 × 4 centimeters (cm) square, 6.14% and 8.39% were excreted in the urine of animals not subject to abrasion and 27.42% and 34.58% were excreted in the urine of animals whose skin was abraded (Nagamatsu et al. 1979).

Distribution

Radiolabeled MBT applied to the shaved skin of guinea pigs was taken up mainly at the application site (15.1%), although small amounts were distributed to the blood (0.063%) and internal organs (0.023%) (Nagamatsu et al. 1979). Among the internal organs, the most radioactivity was found in the thyroid gland. Trace amounts were found in the lungs, kidneys, liver,

TABLE 5-2 Concentrations of MBT in Water Samples from Mir-18 through Mir-25[a]

Source	Number of Samples	Detection Frequency	Concentration Range (μg/L)	Concentration Mean (μg/L)
Recycled water	27	5	ND-41.8	4.2
Humidity condensate	28	6	ND-155.7	10.2

[a]Samples collected by U.S. astronauts on Mir were analyzed by NASA's Water and Food Analysis Laboratory. Sample collection and storage devices were all Teflon and contained no rubber.
Abbreviations: ND, not detected.

spleen, and adrenal glands when examined at both 24 hours (h) and 48 h after application (Nagamatsu et al. 1979).

Similar results were reported by el Dareer et al. (1989) for MBT administered orally to rats. At 8 h after a dose of ^{14}C-labeled MBT at 0.50 mg per kilogram (kg) in corn oil following daily doses at 0.51 mg/kg for 14 days (d), the tissues with the highest concentrations of radioactivity were the kidneys, thyroid, liver, plasma, and whole blood (el Dareer et al. 1989). At 96 h after the dose, a small portion of the administered radioactivity (1.20-1.69%) remained associated with erythrocytes, and most of that was bound to membranes. A time-course study of the levels of radioactivity in the blood showed nearly constant levels in whole blood but rapidly decreasing levels in plasma between 8 h and 48 h after dosing. At 96 h, tissue concentrations were generally low—the highest concentrations were in whole blood and thyroid.

Metabolism and Excretion

Metabolism studies in F-344 rats indicated that the half-life for MBT after administration by gavage was less than 8 h and possibly as short as 4-6 h (CMA 1986). Nearly all (90.7-101%) the radioactivity administered orally to rats as ^{14}C-labeled MBT is rapidly excreted in the urine, and 5.22-9.99% is excreted in feces (el Dareer et al. 1989). Only 0.001% of the administered dose of ^{14}C-labeled MBT was retained at 48, 72, and 96 h. The radioactivity appeared to be covalently bound to erythrocyte mem-

branes. Of the radioactivity excreted in the urine within 8 h following oral administration, none was present as the administered compound (MBT). Only two metabolites were found in rat urine. Initial testing suggested that these polar compounds were probably a thioglucuronide conjugate of MBT (on the basis of acid and enzymatic hydrolysis of the major metabolite) and a sulfonic acid derivative of MBT (on the basis of elution characteristics, UV spectrum, acid and enzyme stability, and facile methylation). No further testing to identify these metabolites was reported. Nagamatsu et al. (1979) reported finding similar metabolites in the urine of guinea pigs; however, they reported a sulfate derivative of MBT, for which el Dareer et al. could find no evidence. At 1 h and 6 h after subcutaneous injection of ^{14}C-labeled MBT into guinea pigs, 66% and 92% of the dose was excreted in the urine (Nagamatsu et al. 1979).

In contrast to the ^{14}C-labeled MBT metabolites, the urinary metabolites of [^{35}S-mercapto]2-mercaptobenzothiazole in rats exposed by intraperitoneal injection were found to consist of conjugates of glutathione (GSH) as well as glucuronic acid and inorganic sulfate (Colucci and Buyske 1965).

MBT also is found as a metabolite after exposure to related substances. MBT is the main urinary metabolite in humans and rats exposed to 2-(thiocyanomethylthio)benzothiazole, a wood preservative and an industrial chemical (Manninen et al. 1996). In rats, MBT, its three conjugates (mercapturate, glucuronide, and sulfate), and its dimer, 2,2'-dibenzothiazyl disulfide (BTDS), were found in the urine after oral administration of N-oxydiethylene-2-benzothiazyl sulfenamide or N-cyclohexyl-2-benzothiazyl sulfenamide (Fukuoka et al. 1995). The S-glucuronide and S-sulfate conjugates were predominantly excreted into the bile. BTDS was also found as a fecal metabolite.

When 2-methylthiobenzothiazole and ^{35}S-labeled GSH were incubated with rat liver homogenates, ^{35}S-labeled S-(2-benzothiazolyl)glutathione and 2-mercaptobenzothiazole were isolated from the reaction mixtures (Larsen et al. 1988). Glutathione-S-transferase appears to be involved in the S-(2-benzothiazolyl)glutathione (GBZ) formation. The evidence indicates that 2-methylthiobenzothiazole is oxidized to its corresponding methylsulphoxide and/or methylsulphone, which become substrates for GSH conjugation. Degradation products identified from the methylthio group were formaldehyde and sulphate. Although sulfur is exchanged in this pathway, which involves oxidation of the methylthio group and GSH conjugation, the net result is an apparent S-demethylation of the methylthio group. Another S-demethylation pathway that does not involve GSH conjugation also functioned in vitro.

TOXICITY SUMMARY

Although the oral toxicity of MBT generally is low, MBT is a moderately potent contact allergen in humans. At high doses, it can cause central nervous system (CNS) depression and, after a lifetime of exposure, possibly kidney toxicity and cancer. However, except for some rare individuals who are exquisitely allergic to MBT, the toxicity of drinking water saturated with MBT is expected to be low because of the low solubility of MBT in pure water.

Acute Toxicity (1 d)

Very few data were found in the literature on adverse effects other than contact dermatitis associated with acute exposures to MBT. The only effects described after acute oral exposures were in animals and included transient, marked CNS depression at high doses and death at very high doses.

Lethality

The oral LD_{50} (lethal dose in 50% of subjects) of MBT in rats was reported to be 3,800 mg/kg (Monsanto Company, unpublished material, as cited in el Dareer et al. 1989). In rabbits dosed dermally, the LD_{50} of MBT was greater than 7,940 mg/kg (Monsanto Company, unpublished material, as cited in el Dareer et al. 1989). An LD_{50} of 1,558 mg/kg was reported in male mice gavage dosed with MBT in a 5% gum arabic aqueous suspension; 1,490 mg/kg was reported in similarly treated female mice; and 3,148 mg/kg was reported in male mice gavage dosed with MBT in corn oil (Ogawa et al. 1989). A review in a National Toxicology Program (NTP) report on MBT stated that reported oral LD_{50} values in mice and rats ranged between 2,000 mg/kg and 3,000 mg/kg, and intraperitoneal LD_{50}s ranged between 100 mg/kg and 400 mg/kg in mice (Dieter 1988).

CNS Depression

MBT appears to produce marked but transient CNS depression (2-4 h duration) in rodents given large oral doses. Mice receiving daily gavage doses of MBT suspended in corn oil at 750 mg/kg or 1,500 mg/kg for 13

weeks (wk) exhibited clonic convulsions and, at 1,500 mg/kg, >50% mortality (Dieter 1988). At 375 mg/kg/d, the mice did not convulse, but exhibited post-gavage lethargy. In a 2-y study, post-gavage lethargy and prostration occurred frequently in rats and mice receiving MBT in corn oil at both 375 mg/kg and 750 mg/kg (Dieter 1988). In another study, no CNS effects were reported in mice consuming MBT in the diet for 20 months (mo) at concentrations up to 1,920 ppm (doses up to 289 mg/kg/d) (Ogawa et al. 1989). Convulsions were seen, however, in mice given single oral doses of MBT at approximately 1,500 mg/kg in 5% gum arabic solution or 3,148 mg/kg in corn oil (Ogawa et al. 1989).

MBT has been shown to biochemically inhibit dopamine beta-hydroxylase in vitro (72% inhibition at 10^{-5} M). This in vitro activity correlated with in vivo studies in which MBT injected intraperitoneally at 300 mg/kg (in 0.25% aqueous methylcellulose) in male CF-1 mice lowered brain noradrenaline levels 60% after 1 h and 2 h while raising brain dopamine levels 24% at 2 h (Johnson et al. 1970). Both noradrenaline and dopamine returned to control levels at 4 h after dosing. Overtly, the activity levels of the treated mice were extremely depressed shortly after dosing and for at least 2 h. After 4 h, the mice appeared normal.

Short-Term Toxicity (2-10 d)

Contact Dermatitis

Allergic contact dermatitis is a syndrome involving immunologic dermal responses (e.g., erythema, eczema) to specific environmental materials. It occurs in two stages: sensitization of naïve individuals (usually requiring moderate to high doses to induce an initial allergic response) and challenge of sensitized individuals (often inducing allergic responses at doses that can be orders of magnitude lower than the sensitizing dose). Allergic contact dermatitis to MBT and its dimer, dibenzothiazyl disulfide, is common and has been reported for exposure to tennis shoes (Jung et al. 1988; Lear and English 1996), releasing-fluid for pottery molds (Wilkinson et al. 1990), antifreeze (Kiec-Swierczynska et al. 1999), and other materials containing MBT. Although MBT has been characterized as a very strong contact allergen in guinea pigs (Maurer et al. 1979), it has been judged a moderate contact sensitizer in humans (Goodwin et al. 1981).

Contact allergens such as MBT are capable of eliciting dermatitis at very low concentrations in sensitized individuals. Although no similar studies were found involving MBT, several studies demonstrated that inges-

tion of another contact allergen (nickel) by sensitive individuals could result in flare-ups of dermatitis (Christensen and Moller 1975; Kaaber et al. 1978; Cronin et al. 1980; Gawkrodger et al. 1986; Veien et al. 1987; Burrows 1992). Nevertheless, none of these reports suggests that oral exposure to contact allergens leads to sensitization of individuals who are not already sensitive (Nielsen et al. 1990). In contrast, there have been some reports (Jordan and King 1979; Santucci et al. 1994) that suggest that long-term ingestion of a contact allergen (nickel) by sensitive individuals can result in desensitization, but those results remain controversial. Because MBT was detected in recycled water and humidity condensate on Mir, it is prudent for NASA to establish exposure limits for MBT in drinking water on ISS to protect any crew members that may be sensitive to MBT.

Lynde et al. (1982) reported the results of patch testing of patients (not further described, but most likely presenting with a suspected allergic dermatitis to an undetermined allergen) with MBT and/or one of two formulations of "mercapto-mix." The purpose of the study was to compare the efficacy of different formulations of screens for the detection of allergies to mercapto compounds. The "old" mix consisted of MBT, N-cyclohexylbenzothiazylsulphenamide, dibenzothiazyldisulphide, and morpholinyl-mercaptobenzothiazole, each at 0.25% in petroleum jelly; the "new" mix eliminated MBT but contained cyclohexylbenzothiazylsulphenamide, dibenzothiazyl-disulphide, and morpholinyl-mercaptobenzothiazole, each at 0.33% in petroleum jelly. Using the old mix, 155 of 5,732 patients (2.7%) were positive to either MBT and/or the mix. Using MBT and the new mix separately, 39 of 2,297 patients (1.7%) were positive to both MBT and the new mix, and 26 of 2,297 (1.1%) were positive to MBT and negative to the new mix.

Screens of allergens (about 50), including MBT, have been used by members of the North American Contact Dermatitis Group for diagnostic patch testing of large numbers of patients presenting with suspected allergic contact dermatitis. The frequency of positive reactions to MBT for 3,440 patients tested between 1996 and 1998 is shown in Table 5-3 along with the results from previous years (Marks et al. 2000). The frequency of MBT-sensitive individuals in the general population presumably is lower, but no data for the general population were found.

Emmett et al. (1994) tested the skin elicitation threshold of MBT for inducing contact dermatitis in a group of 12 MBT-sensitive volunteers. Patches containing 22.7 ± 1.9 mg of petrolatum with MBT at weight concentrations of 1%, 0.316%, 0.1%, 0.0316%, 0.01%, and 0.0032% were applied to the subjects' backs and left covered for 4 d, after which standard

TABLE 5-3 Patch Test Results for MBT

Study Years	% MBT-Positive
1996-1998	1.8
1994-1996	2.1
1992-1994	1.8
1985-1989/1989-1990	2.5

graded readings were recorded by an examiner who was blind to the patch test identities. Patches covered an area of 50 square millimeters ($mm^{)}$ of skin, thus MBT at 0.0032% corresponded to a concentration of 1.45 $\mu g/cm^2$, or 0.73 μg total. The lowest concentrations producing a definite eczematous positive reaction or provocation threshold were 0.01% (in one subject), 0.032% (in one subject), and 0.1% (in five subjects). The potency of MBT relative to nickel, another common contact allergen, can be estimated by comparing these results to those of a previous very similar study of nickel sulfate (Emmett et al. 1988). In a group of 12 nickel-sensitive subjects, the provocation threshold was 0.47 μg (0.01%) when tested in petrolatum. Thus, the potencies of MBT and nickel (in petrolatum) appear to be similar. The provocation threshold for nickel sulfate in aqueous solutions was higher than that for nickel in petrolatum (Emmett et al. 1988).

Wang and Suskind applied preparations of MBT at 0.5%, 2%, 5%, and 10% in petrolatum to the shaved flanks of naïve Hartley albino guinea pigs (350-420 g, 5-6 wk old) under gauze pads for 24 h and examined the sites at 1, 24, and 48 h after removal of the test materials (Wang and Suskind 1988). They reported irritant reactions for MBT at 5% and 10%. The intensity of the 5% reaction was much weaker than that of the 10%. No reactions were seen for the 2% and 0.5% concentrations. In guinea pigs sensitized by pretreatment with MBT at 5% (24 h application, three applications per week, 2 wk) and then challenged with lower concentrations of MBT for 24 h, seven of 10 animals showed mild effects at 2%, two of 10 at 0.5%, and zero of 10 at 0.1% (Wang and Suskind 1988). Although the concentrations required to induce irritation in sensitized guinea pigs were greater than those reported in MBT-sensitive humans, the challenge exposure duration in guinea pigs was only one-fourth (1 d) that in the humans volunteers (4 d). Wang and Suskind also tested three other rubber accelerators and curing agents: morpholine, 4,4-dithiodimorpholine (DTDM), and morpholinyl-mercaptobenzothiazole (MMBT). Of the four compounds

tested, morpholine did not induce any dermatitis reaction, DTDM and MMBT induced erythema and swelling, and MBT induced only erythema.

The studies of Emmett et al., taken together with those of Wang and Suskind, demonstrate that induction of cell-mediated contact hypersensitivity is dependent on dose and duration of exposure as well as the molecular structure of the test chemical. They also show that when sensitized individuals are given a challenge dose of MBT under defined conditions, there are threshold doses below which no reaction will occur, but the magnitude of the threshold dose varies widely between individuals.

Subchronic Toxicity (11-100 d)

Treatment of male and female F-344/N rats by gavage with MBT suspended in corn oil at doses up to 2,500 mg/kg/d for 16 d produced no compound-related gross pathologic effects other than slightly reduced weight gain (Dieter 1988). Similarly treated B6C3F_1 mice that received up to 1,500 mg/kg/d also displayed no compound-related gross pathologic effects other than post-gavage lethargy after day 1, but four of five females that received 1,500 mg/kg/d died before the end of the study, as did four of five males and five of five females that received 3,000 mg/kg/d (Dieter 1988).

Post-gavage lethargy and rough coats were reported for rats and mice treated by gavage with MBT in corn oil at 375 mg/kg/d for 13 wk (Dieter 1988). Five of 10 male mice and seven of 10 female mice that received 1,500 mg/kg/d died before the end of the study, but two of those deaths were related to gavage technique (Dieter 1988). No compound-related gross pathologic effects were reported, but clonic seizures, lacrimation, and salivation were observed in the 750-mg/kg and 1,500-mg/kg groups (Dieter 1988).

Chronic Toxicity (≥101 d)

No-Observed-Adverse-Effect Levels (NOAELs)

Lehman (1965) reported that rats fed a 2-year (y) diet formulated to contain MBT at 12, 37.9, and 120 parts per million (ppm) and dimethyldithiocarbamate at 138, 486, and 1,380 ppm , respectively, and dogs fed the same doses for 1 y, exhibited no significant effects on survival, body-weight

TABLE 5-4 Daily Intake of MBT[a]

Dose (ppm)	Male (mg/kg/d)	Female (mg/kg/d)
30	3.60	3.61
120	14.69	13.52
480	57.90	58.82
1,920	289.40	247.98

[a]Doses from 20-mo study by Owaga et al. (1989) .

gain, hematologic parameters, blood sugar, nonprotein nitrogen values, or histopathology.

In Slc:ddY mice fed a diet containing MBT at 480 ppm or 1,920 ppm for 20 mo (see Table 5-4 for daily doses), Ogawa et al. (1989) reported no treatment-related effects on hematologic parameters (red blood cell [RBC] count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelets, white blood cell counts), blood biochemistry (total protein, albumin, albumin-globulin ratio, blood urea nitrogen, total cholesterol, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase), or organ histopathology of the lungs, liver, or kidneys, including tumor incidence. They did not report any convulsions in mice in the chronic part of their study, whereas convulsions were the primary adverse effect observed during their determination of the LD_{50}s for MBT administered by gavage.

Nephrotoxicity

Ogawa et al. (1989) reported an increased incidence of cell infiltration of the interstitium of the kidneys in male Slc:ddY mice after 20 mo of a diet containing MBT at 480 ppm or 1,920 ppm, as shown in Table 5-5. In female mice, not only was there no increased incidence at either 12 or 20 mo, but the incidence for the zero-dose controls was higher than that for the high-dose males. This may be due, in part, to the small number (5-10) of mice per interim-sacrifice group, although there were 30 mice total per treatment group and 60 per control group.

In a 2-y gavage study for NTP, Dieter (1988) reported an increase in the severity of nephropathy in male F-344/N rats dosed at 375 mg/kg or 750 mg/kg in corn oil for 5 d/wk compared with controls (Dieter 1988):

TABLE 5-5 Incidence of Interstitial Cell Infiltration of the Kidneys of Mice[a]

Gender	Month	0 ppm	30 ppm	120 ppm	480 ppm	1,920 ppm
Male	12	3/10 (30%)	2/5 (40%)	2/5 (40%)	2/5 (40%)	1/5 (20%)
Male	20	1/14 (7%)	1/6 (16%)	1/7 (14%)	4/6 (67%)	5/9 (56%)
Female	12	1/10 (10%)	2/5 (40%)	0/5	0/5	1/5 (20%)
Female	20	9/15 (60%)	2/7 (29%)	5/9 (56%)	3/10 (30%)	2/7 (29%)

[a]Mice were fed MBT for 20 mo.

> Nephropathy, characterized by tubular degeneration and regeneration, was present in all male rats and in more than 75% of the female rats; a severity grade from minimal to severe (1-4) was recorded for each animal. The mean severity of nephropathy was increased in dosed male rats (vehicle control: 2.3 [mild-moderate]; low dose and high dose: 3.4 [moderate-severe]).

Because no severity scores for individual animals or any further details concerning the observed nephropathy were presented in the NTP report, it was not possible to model these data mathematically or determine the statistical significance of the reported increased severity. The type of nephrotoxicity reported by Dieter (tubular degeneration and regeneration) differs from that reported by Ogawa et al. (cell infiltration of the interstitium of the kidney) in male mice. Nyska et al. (1999) reported a possible association between the severity of chronic progressive glomerulonephropathy (CPN) and the incidence of adrenal pheochromocytomas in selected studies of male F-344 rats at NTP.

Carcinogenicity

Cancer in Humans

An epidemiological study to assess any possible increased mortality from cancer associated with exposure to MBT examined mortality trends

for 1,059 full-time, white, male production workers at a rubber chemicals plant in Nitro, West Virginia, during the period between 1955 and 1977 (Strauss et al. 1993). Vital status through 1987 was obtained for 98% of the 1,059 employees eligible for the study. For MBT workers who had concomitant work-related exposures to p-aminobiphenyl (PAB, a known potent bladder carcinogen), Strauss et al. reported finding an excess of bladder cancer (standardized mortality ratio [SMR] = 3,200, 95% confidence interval [CI] = 1,289-6,593). (Note that for this initial report, the SMR of the control group is set as 100, whereas for the update report described in the next paragraph, the SMR of the control group is set as 1.0.) For MBT workers without concomitant exposures to PAB, there were no associations between exposure to MBT and increased rates of most malignant neoplasms. Exceptions included prostate cancer, which was high in all cumulative exposure categories and an unexposed internal comparison group, suggesting that the findings for prostate cancer were related to some extraneous personal or occupational factor that was affecting all groups equally. In addition, the SMR for bladder cancer was increased based on three deaths (SMR = 455, 95% CI = 94-1,328), although these results were too few to evaluate trends by cumulative exposure category. The authors report that possible confounding by cigarette smoking would explain an increase in the SMR only up to 20% for this study population. A major limitation of these results is the small number of deaths from bladder cancer in the cohorts, but this might be improved by updating the study at a later time. SMRs for all other causes of death including heart disease, cerebrovascular disease, and nonmalignant respiratory disease were 100 (not elevated). No clinical or follow-up data were available on renal function or other non-neoplastic disease.

An update that added 9 y of follow-up to the above epidemiological study of 1,059 workers at a rubber chemicals plant in Nitro, West Virginia, reported that exposure to MBT does not seem to increase the risk of most cancers, including cancers of the lungs and prostate (Collins et al. 1999). MBT workers were found to have expected rates of lung (SMR = 1.0, 95% CI = 0.7-1.5) and prostate (SMR = 0.9, 95% CI = 0.2-2.3) cancer. There was an excess of bladder cancer among MBT workers who had definite exposure to PAB (SMR = 27.1, 95% CI = 11.7-53.4) and MBT workers with potential exposure to PAB (SMR = 4.3, 95% CI = 1.4-10.0). There were no deaths from bladder cancer among workers with no exposure to PAB (SMR = 0.0, 95% CI = 0.0-24.7), although only 0.2 deaths were expected.

A study of 2,160 male production workers employed for at least 6 mo

between 1955 and 1984 at a chemical factory in north Wales, of whom 288 were exposed to MBT, found no significant positive trends for risk of death from any cancer with increasing cumulative estimated exposure to MBT (based on job classifications) (Sorahan and Pope 1993). This study found a nonsignificant excess of mortality from bladder cancers in the MBT-exposed subcohort. An update of this study extended the observation period to 1996 and found no significant positive trend for death from bladder cancer increasing with cumulative exposure to MBT (Sorahan et al. 2000). The authors of that study cite the results of the NTP bioassay on MBT and conclude that its results "are uninterpretable. Tumours occurred, but the mortality was generally high and tumour incidences were often not dose related." The authors of the update study of the workers at the Nitro, West Virginia, plant (Collins et al. 1999) also cited the results of the NTP bioassay, stating that "There is some evidence of carcinogenic activity of MBT in one animal species. Rats developed dose related tumours of the adrenal gland but there is no carcinogenic activity in mice." Those authors did not comment on the interpretation of the NTP results.

Cancer in Animals

MBT administered at the maximum tolerated doses did not cause increased tumor incidences in two hybrid mouse strains (C57Bl/6 × C3H/Anf and C57BL/6 × AKR) after 18 mo of chemical administration in the diet in a study conducted at Litton Bionetics Research Laboratories for the National Institutes of Health (Innes et al. 1969). The F_1 generation of hybrids was administered MBT in 0.5% gelatin by gavage at 100 mg/kg from 7 d to 28 d of age and then was fed MBT at 323 ppm ad libitum to maintain approximately the maximum tolerated dose for the remainder of the study (approximately 18 mo).

Mice fed a diet containing MBT at up to 1,920 ppm for 20 mo showed no increase in the incidence of tumors of the lungs, liver, or kidneys (Ogawa et al. 1989).

Lehman also reported no increase in tumor incidence in 10 rats (unspecified strain) of each gender and dose fed diets containing 0, 500, 1,580, and, 5,000 ppm of a formulation containing MBT at 2.4% and dimethyldithiocarbamate at 27.6% (a dietary MBT concentration of up to 120 ppm) for 2 y (Lehman 1965).

In a 2-y study conducted by NTP, rats and mice were treated with technical grade MBT by gavage in corn oil 5 d/wk at 0, 375, and 750 mg/kg in

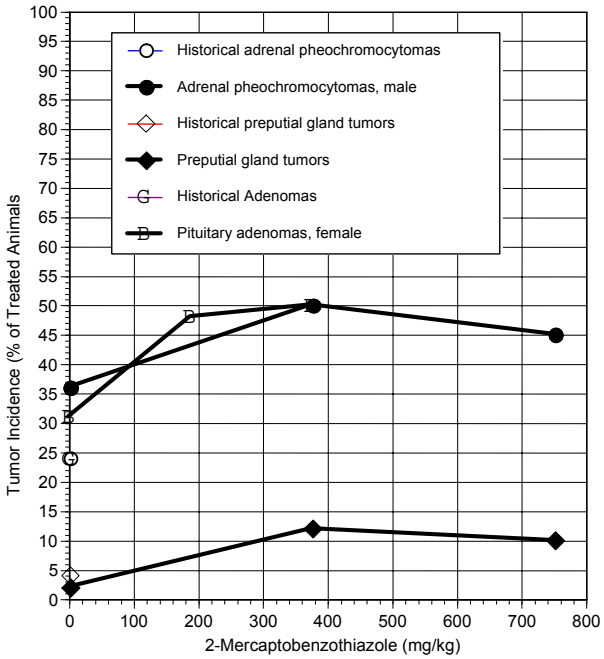


FIGURE 5-1 Carcinogenicity of MBT in rats in an NTP study. Source: Dieter 1988.

male rats and male and female mice and at 0, 188, and 375 mg/kg in female rats (Dieter 1988). The incidences of a variety of tumors were increased in rats dosed with MBT, although some increases were not dose-related. In low-dose male rats, but not high-dose male rats, increased incidences ($p < 0.01$) were observed for mononuclear cell leukemia and pancreatic acinar cell adenomas. Increased tumor incidences with dose related trends ($p < 0.05$) (according to Dieter 1988) in rats included the following: pituitary gland adenomas in females (15/49, 24/50, 25/50); preputial gland adenomas or carcinomas (combined) in males (1/50, 6/50, 5/50); adrenal gland pheochromocytomas or malignant pheochromocytomas in males (18/50, 27/50, 24/49); and adrenal gland pheochromocytomas in females (1/50, 5/50, 6/50).

These tumors were observed at significantly greater incidences ($p \leq 0.05$) in the high-dose groups than in the vehicle controls. Survival of male rats was significantly reduced compared with vehicle controls beginning after week 85 for the low-dose group and after week 83 for the high-dose

TABLE 5-6 Evidence Suggesting MBT Carcinogenicity in Rodents

Type of Cancer	Dose (mg/kg) 0	188	375	750	Historical 0[a] (mean % ± SD)	Evidence for Carcinogenesis
Mononuclear cell leukemia (male rats)	7/50 (14%)	Not tested	16/50 (32%)	3/50 (6%)	202/1,450 (14 ± 8%)	Some
Pancreatic acinar call adenomas (male rats)	2/50 (4%)	Not tested	13/50 (26%)	6/49 (12%)	80/1,381 (6 ± 8%)	Some
Adrenal gland pheochromocytomas (male rats)	18/50 (36%)	Not tested	25/50 (50%)	22/49 (45%)	347/1,442 (24 ± 9%)	Some
Adrenal gland pheochromocytomas (female rats)	1/50 (2%)	5/50 (10%)	6/50 (12%)	Not tested	82/1,443 (6 ± 8%)	Some
Pituitary gland adenomas (female rats)	15/49 (31%)	15/49 (31%)	25/50 (50%)	Not tested	344/1,411 (24 ± 8%)	Some
Preputial gland adenomas and carcinomas (combined) (male rats)	1/50 (2%)	Not tested	6/50 (12%)	5/50 (10%)	65/1,450 (4 ± 4%)	Some
Hepatocellular adenoma or carcinoma (female mice)	4/50 (8%)	Not tested	12/49 (24%)	4/50 (8%)	116/1,489 (7.8 ± 5.56%)	Equivocal

[a]Historical incidence of cancers in untreated rats or mice in NTP studies (mean ± SD).
Abbreviation: SD, standard deviation.
Source: Dieter et al. 1988.

group (except for weeks 94 and 95). Survival of female rats and male mice was somewhat reduced, but not significantly. Survival of the high-dose group of female mice was significantly lower than that of vehicle controls after week 27.

The conclusions reported by NTP for their 2-y studies were as follows:

- There was some evidence of carcinogenic activity of MBT for male F-344/N rats, indicated by increased incidences of mononuclear cell leukemia [NDR], pancreatic acinar cell adenomas [NDR], adrenal gland pheochromocytomas, and preputial gland adenomas and carcinomas (combined).
- There was some evidence of carcinogenic activity for female F-344/N rats, indicated by increased incidences of adrenal gland pheochromocytomas and pituitary gland adenomas.
- There was no evidence of carcinogenic activity of MBT for male $B6C3F_1$ mice dosed with 375 mg/kg or 750 mg/kg.
- There was equivocal evidence of carcinogenic activity for female $B6C3F_1$ mice, indicated by increased incidences of hepatocellular adenomas or carcinomas (combined).

Sorahan et al. (2000) considered the results reported by Dieter (1988) to be uninterpretable. Sorahan et al. state that "Tumours occurred, but the mortality was generally high and tumour incidences were often not dose related." However, they relate that a 1997 German Advisory Committee on Existing Chemicals of Environmental Relevance (BUA) report concluded, on the basis of the Dieter study, that the "possibility of MBT being carcinogenic cannot be ruled out." They conclude that "A reliable scientific assessment of the carcinogenicity of MBT in humans is not possible; the evidence supplied by this study [Sorahan et al. 2000] is of a limited nature and does not modify the BUA conclusion that the possibility of MBT being carcinogenic cannot be ruled out."

Genotoxicity

MBT was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation at doses up to 10,000 μg per plate (Dieter 1988). MBT did not bind to DNA from selected tissues (see Table 5-7) of male and female F-344 rats gavaged with ^{14}C-labeled MBT at 375 mg/kg (Brewster et al. 1989).

TABLE 5-7 Summary of MBT Genotoxicity Studies

Author and Year	Test	Metabolic Activation	Concentrations Tested	Toxicity Test and Lethal Dose	Lowest Effective Dose	Exposure Duration	Overall Evaluation
Dieter 1988 (Zeiger et al. 1987 at EG&G Mason)	Ames mutagenesis TA98, TA100, TA1535, or TA1537	None, 10% hamster S9, 10% rat S9	0, 3.3, 10, 33, 100, 333, 1,000 µg/plate	Slight toxicity at 333 µg/plate (-S9) and 1,000 µg/plate (+S9)	N/A	24 h	Negative except questionable in TA98 + S9
Dieter 1988 (Zeiger et al. 1987 at Case Western Reserve)	Ames mutagenesis TA98, TA100, TA1535, or TA1537	None, 10% hamster S9, 10% rat S9–two trials	0, 10, 33, 100, 333, 1,000, 3,333, 10,000 µg/plate	Toxicity at 1,000 µg/plate	N/A	24 h	Negative
Brewster et al. 1989	DNA binding of ^{14}C-labeled MBT	In vivo in F-344 rats	375 mg/kg gavage	N/A	No DNA binding found in liver, adrenal glands, pituitary gland, pancrease, bone marrow	Gavage, sacrificed after 8 h	Negative
Donner et al. 1983	V79/HGPRT		50-300 µg/ml			4 h	Negative
Pharmakon 1984	CHO/HGPRT mutagenesis	+/-	Up to 300 µg/ml				Negative
Dieter 1988	L5178Y mouse lymphoma mutagenesis	None–two trials	0, 30, 40, 50, 60, 80, 100, 150 µg/ml; 0, 40, 50,	Lethal at 150 µg/ml and 120 µg/ml	N/A	48 h	Negative

			60, 80, 100, 120 µg/ml				
Dieter 1988	L5178Y mouse lymphoma mutagenesis	Yes–three trials	0, 1.25, 2.5, 5, 7.5, 10, 15 µg/ml; 0, 5, 6, 8, 10, 12, 16 µg/ml; 0, 4, 8, 10, 12, 16, 20 µg/ml	No reduction in cloning efficiency at tested doses	15 µg/ml; 5 µg/ml; 12 µg/ml	48 h	Positive
Litton 1985	CHO/SCE	None–one trial	10, 14.9, 19.9, 30.1 µg/ml	No metaphase cells at 24.8 µg/ml	N/A	26 h	Negative
Litton 1985	CHO/SCE	Yes–two trials	0, 99.2, 247.5, 351.6, 401.6, 501.5 µg/ml; 0, 351.6, 401.6, 445.3, 502.3 µg/ml	No metaphase cells at doses greater than 502 µg/ml	501.5 µg/ml; 351.6 µg/ml	26 h	Positive
Litton 1985	CHO/ chromosomal abberations	None–one trial	10, 14.9, 19.9, 30.1 µg/ml	No metaphase cells at 30.1 µg/ml	N/A	8-10 h	Negative
Litton 1985	CHO/ chromosomal abberations	Yes–two trials	351.8, 400.8, 425, 451.0, 500.5 µg/ml; 373.5, 399, 425, 450 µg/ml	No metaphase cells at 500.5 µg/ml and 450 µg/ml	451.0 µg/ml; 373.5 µg/ml	2 h	Positive
Monahan et al. 2000	Swiss mice/ chromosomal abberations	In vivo	0, 480, 960, 1,920 µg per 20 g mouse	N/A	N/A	36 h	Negative

MBT was clearly clastogenic to cultured Chinese hamster ovary (CHO) cells in the presence of rat liver S9 at MBT concentrations of 352-451 μg/mL, inducing aberrations at frequencies comparable to and even exceeding those of the positive control chemical, cyclophosphamide (Dieter 1988). In contrast, zinc MBT was not clastogenic to bone marrow cells in vivo when examined 36 h after intraperitoneal injection into Swiss albino mice at 0, 480, 960, or 1,920 μg per 20-g animal, administered in cotton seed oil (Monahan et al. 2000). MBT increased the frequencies of sister chromatid exchanges (SCEs) in cultured CHO cells at concentrations of 352-502 μg/mL (Dieter 1988; Anderson et al. 1990; Zeiger et al. 1990), as well as mutations at the TK locus of cultured mouse L5168Y lymphoma cells at concentrations up to 100 μg/mL (Dieter 1988).

Reproductive Toxicity

No evidence was found that MBT causes adverse effects on reproduction. In rats fed a diet containing MBT at 0, 12, 37.9, and 120 ppm and dimethyldithiocarbamate at 0, 138, 486, and 1,380 ppm, no cumulative effects on reproduction or lactation performance through the F_2 generation were noted (Lehman 1965). In rats administered MBT at 200 mg/kg by intraperitoneal injection on days 1-15 of gestation (Hardin et al. 1981), there were no chemically related histopathologic effects in maternal tissues, and there was no maternal toxicity.

Developmental and Fetal Toxicity

Aleksandrov (1982) observed embryotoxic effects in outbred white rats (10 rats per group) that received MBT intragastrically at 0 mg/kg or 200 mg/kg in sunflower oil using one of the following two schedules. First, to detect dominant lethal mutations, MBT was given to females before pregnancy at days 1 and 3 of estrus and the females were mated to males treated "twice with an interval of three days." Second, to detect developmental toxicity, MBT was given to females on days 4 and 11 of pregnancy by untreated males (Aleksandrov 1982). Counts were made of the number of corpora lutea in the ovaries and the number of dead and living fetuses in the uterine horns. The fetuses were weighed and measured. Fetal mass was decreased up to 23%. Postimplantation embryonal lethality increased significantly if MBT was administered before but not during pregnancy, indicating possible dominant lethal mutagenesis, but no developmental toxicity.

TABLE 5-8 Toxicity Summary

Concentration, Dose, and Route of Exposure	Exposure Duration	Species	Effects	Reference
Acute Exposures (≤1 d)				
300 mg/kg; intraperitoneal injection	Single dose	Mice	Marked CNS depression (inactivity) and ptosis for 4 h post-dosing	Johnson et al. 1970
1,558 mg/kg; oral suspension in 5% gum arabic	Single dose	Mice, Slc:ddY, male	LD_{50}, convulsions	Ogawa et al. 1989
1,490 mg/kg; oral suspension in 5% gum arabic	Single dose	Mice, Slc:ddY, female	LD_{50}, convulsions	Ogawa et al. 1989
3,148 mg/kg; oral suspension in olive oil	Single dose	Mice, Slc:ddY, male	LD_{50}, convulsions	Ogawa et al. 1989
Subchronic Exposures (11-100 d)				
2,500 mg/kg/d; gavage in corn oil	16 d	Rat (5/gender/ group)	Reduced weight gain; no compound-related gross pathologic effects observed	Dieter 1988
1,500 mg/kg/d; gavage in corn oil	16 d	Mouse (5/gender/group)	Post-gavage lethargy; 4/5 females died before the end of the study, but no males died; no compound-related gross pathologic lesions observed	Dieter 1988

(Continued)

TABLE 5-8 Continued

Concentration, Dose, and Route of Exposure	Exposure Duration	Species	Effects	Reference
375 mg/kg/d	5 d/wk, 13 wk	Rats, mouse (10/gender/group)	Post-gavage lethargy	Dieter 1988
188 mg/kg/d	5 d/wk, 13 wk	Rats, mouse (10/gender/group)	NOAEL for post-gavage lethargy	Dieter 1988
750 mg/kg, 1,500 mg/kg; gavage in corn oil	5 d/wk, 13 wk	Rat, mouse (10/gender/ group)	Lacrimation, salivation, clonic seizure	Dieter 1988
750 mg/kg, 1,500 mg/kg; gavage in corn oil	5 d/wk, 13 wk	Rat, mouse (10/gender/group)	No gross or microscopic pathologic effects, but more than half the mice dosed at 1,500 mg/kg died	Dieter 1988
Chronic Exposures (>100 d)				
289 mg/kg/d; suspension in olive oil	5 d/wk, 20 mo	Mice, Slc:ddY, male	NOAEL for CNS effects	Ogawa et al. 1989
0, 188, 375, 750 mg/kg/d; gavage in corn oil	2 y	F-344/N rat, $B6C3F_1$ mouse (50/gender/group)	Post-gavage lethargy; increased incidences of a variety of tumors; some evidence of carcinogenesis in male and female rats at doses sufficient to accelerate mortality; no evidence in male mice, equivocal evidence in female mice; increased severity of nephropathy in male rats; ulcers and inflammation of the forestomach of dosed	Dieter 1988

			rats; epithelial hyperplasia and hyperkaratosis in male rats	
0 ppm, 323 ppm; in diet	18 mo	Mouse, C57BL/6 × C3H/Anf	NOAEL for carcinogenesis	Innes et al. 1969
0, 12, 37.9, 120 ppm; in diet	2 y	Rat, dog	NOAEL for mortality, body weight changes, hematologic effects, blood sugar and nonprotein nitrogen, reproductive effects, carcinogenicity, and histopathology	Lehman 1965
120 ppm (14.6 mg/kg/d); in diet	20 mo	Mice, Slc:ddY, male	NOAEL for inhibition of body weight gain and nephrotoxicity	Owaga et al. 1989
480 ppm, 1,920 ppm (289 mg/kg/d); in diet	20 mo	Mice, Slc:ddY, male	Inhibition of body weight gain at 1,920 ppm; cell infiltration in the interstitium of the kidneys seen at month 20 at 480 and 1,920 ppm	Ogawa et al. 1989

Hardin et al. (1981) reported finding no fetal toxicity or teratogenesis in groups of 10-15 inseminated Sprague-Dawley rats (250-300 g) administered the maximum tolerated dose (200 mg/kg/d) of MBT in corn oil by intraperitoneal injection on days 1-15 of gestation. Hardin et al. examined uterine contents on day 21 of gestation. The individual fetuses were weighed, measured for crown-rump length, sexed, and examined for externally visible malformation. One-half to two-thirds of each litter was preserved in Bouin's fluid for internal examination by the Wilson method of free-hand razor-blade sectioning, and the balance of each litter was preserved in ethanol for clearing and skeletal staining with alizarin red. No teratogenic effects were suggested for MBT and no treatment-related histopathologic changes were observed in maternal tissues.

RATIONALE

Acceptable concentration (AC) values 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 and 2.0 L/d for drinking. For each exposure duration, the spacecraft water exposure guideline (SWEG) (Table 5-9) was set based on the lowest value among the ACs for all the significant adverse effects at that exposure duration. Drinking water standards set by other organizations are listed in Table 5-10.

Carcinogenicity

No compelling arguments for or against the carcinogenicity of MBT can be made from the available data. The available data from human epidemiological studies show no increased risk of mortality from cancer due to exposure to MBT, but the confounding effect of concomitant exposures to known carcinogen 4-aminobiphenyl does not permit one to rule out a weak contribution by MBT to the observed rate of carcinogenesis. An NTP study of MBT administered by gavage yielded some evidence of carcinogenicity in male and female rats and equivocal evidence of carcinogenicity in female mice. No carcinogenicity was reported in rats, mice, or dogs in the studies of Innes et al. (1969), Lehman (1965), and Ogawa et al. (1989). That could be attributed either to the fact that in those studies MBT was administered

TABLE 5-9 Spacecraft Water Exposure Guidelines for MBT

Duration	Concentration (mg/L)	Target Toxicity
1 d	200	CNS depression
10 d	30	Nephrotoxicity
100 d	30	Nephrotoxicity
1,000 d	30	Nephrotoxicity, carcinogenicity

in the feed rather than by gavage (as in the NTP study) or to the use of doses lower than those used in the NTP study. Assuming that MBT may be weakly carcinogenic, AC values for carcinogenicity were calculated as follows.

The data for the five types of tumors reported in the NTP study were reviewed to determine whether benchmark dose (BMD) methodology could be applied to estimate a NOAEL. Of the five, the data for mononuclear cell leukemia in male rats and for pancreatic acinar cell adenomas in male rats could not be used because, although the low-dose response was more than double the zero-dose response, the high-dose response was less than the zero-dose response. Of the remaining tumor types (adrenal gland pheochromocytomas in male rats, adrenal gland pheochromocytomas in female rats, and pituitary gland adenomas in female rats), the only tumors common to males and females in either species were adrenal pheochromocytomas in rats. Because the low dose for female rats was lower than that for males, the pheochromocytomas incidence data for female rats were used as input for EPA's BMD software, version 1.3.1, to calculate a BMD ($BMDL_{01}$).

The value of the $BMDL_{01}$ (the lower limit of the dose of MBT that would produce a 1% lifetime tumor incidence in female rats),17 mg/kg/d, was used as a point of departure to calculate an AC for carcinogenicity.

The rats in the NTP study were treated 5 d/wk, so the effective weekly dose, divided over 7 d, is

$$17 \text{ mg/kg/d} \times 5/7 = 12 \text{ mg/kg/d}.$$

For a 70-kg astronaut consuming 2.8 L of water, the MBT concentration needed to achieve a dose of 12 mg/kg/d is

$$12 \text{ mg/kg} \times 70 \text{ kg} \div 2.8 \text{ L} = 303 \text{ mg/L}.$$

No species extrapolation factor was used because the epidemiological

data indicate that MBT is, at most, a weak human carcinogen (i.e., humans are probably not more sensitive than rodents to potential MBT carcinogenicity). Thus, the following equation, based on Crump and Howe's 1984 multistage model, with only the first stage dose-related, was used to calculate the exposure concentrations, D, that would yield a tumor risk of 10^{-4} for exposure durations of 10, 100, and 1,000 d:

$$D = \frac{d \cdot (25{,}600)^k \cdot \dfrac{10^{-4}}{risk}}{(25{,}600 - 365 \cdot age)^k - [(25{,}600 - 365 \cdot age) - t]^k},$$

where

d = the concentration during a lifetime exposure (303 mg/L in this case);
25,600 = the number of days in a 70-y human lifetime;
k = the number of stages in the model (1 in this case);
10^{-4} = the acceptable risk level;
age = the minimum age of an astronaut, in years (30 in this case);
t = the exposure duration, in days (10, 100, or 1,000); and
risk = the risk of tumor for lifetime exposure to d (10^{-2} in this case).

This equation yields values of

$D_{1,000}$ = (303 mg/L)(25,600)(1E-2)/1000),
$D_{1,000}$ = (5.0835E13 / 6.0090E11),
$D_{1,000}$ = 80 mg/L;
D_{100} = 800 mg/L; and
D_{10} = 8,000 mg/L.

Dividing the calculated dose values, D, by a factor of 3 to partially protect against the known allergenicity of MBT yields ACs (for carcinogenicity) of

1,000-d AC = 30 mg/L (rounded);
100-d AC = 300 mg/L (rounded); and
10-d AC = 3000 mg/L (rounded).

The available data support the conclusion that if MBT is a carcinogen, it is a weak one. Thus, the risk of tumorigenesis due to ingestion of MBT in drinking water is believed to be negligible on the basis of two arguments.

First, the intermittent exposure by drinking water more closely approximates the exposure pattern in the diet (for which no animal carcinogenesis has been reported) than the bolus exposure by gavage in corn oil (for which some evidence of carcinogenicity has been reported). Second, MBT has very poor solubility in pure water, making it unlikely that sufficient MBT could be ingested in drinking water to result in tumorigenesis.

Allergic Dermatitis

MBT is a known sensitizer for allergic contact dermatitis. However, no data were found in the scientific literature on the effects of exposure to MBT by ingestion in sensitized individuals. Data were found that indicated that ingested nickel, another contact sensitizer with potency similar to MBT (Emmett et al. 1988, 1994), could induce dermatitis in nickel-sensitive patients (Santucci et al. 1988). In another study, orally administered nickel consistently induced dermatitis (pompholyx) in nickel-sensitive subjects only when given in high bolus doses (5.6 mg as $NiSO_4.7H_2O$) (Gawkrodger et al. 1986).

Emmett et al. (1994) showed that in a small population (n = 12) of MBT-sensitive individuals, the lowest concentration of MBT in petrolatum that would induce an observable dermatitis reaction when applied to the skin for 48 h in an occlusive patch was 0.01% (0.1 ppt; 100 ppm). For the conditions of the patch test (22.7 mg of petrolatum-based MBT preparation applied to an area 50 mm^2), that corresponds to 4.5 $\mu g/cm^2$. The highest concentration of MBT to which no reaction occurred in any of the 12 individuals was 0.0032% (0.032 ppt; 32 ppm; 32 mg/L), which corresponds to 1.45 $\mu g/cm^2$.

We must assume that it is possible that a small proportion of astronauts will be already sensitized to MBT before flight. A large range has been reported in the degree of sensitivity of individuals to contact allergens—with a small number of people being extremely sensitive, it is not possible to set an exposure level that will protect all individuals against allergic dermatitis. Thus, to provide at least partial protection against allergic dermatitis, ACs for other toxic end points will be decreased by an arbitrary factor of 3. A larger factor is not warranted because the effect (dermatitis) is not usually severe and can be minimized by pharmacological treatments (e.g., allergy medications, cortisone ointments) that are available in the medical kits on U.S. spacecraft.

Nephropathy

The NTP study of Dieter (1988) reported nephropathy at the end of 2 y in 100% of both control and treated male F-344/N rats, in 75% of the female rats, and in none of the mice. In addition, Dieter reported a treatment-related increase in the severity of nephropathy in male rats. Severity was scored as 1 (minimal), 2 (mild), 3 (moderate), and 4 (severe). The data for the severity ratings for individual animals was not reported. The mean severity rating was the same for rats treated at either 375 mg/kg or 750 mg/kg (3.4 [moderate to severe]), but it was higher than for the vehicle-treated controls (2.3 [mild to moderate]). Nephropathy is common in old rats, and the biologic relevance of such severity data is uncertain. For setting SWEGs, the treatment time was considered lifetime rather than 2 y, because nephropathy occurred only in old age and was seen even in controls. In addition, because there was essentially only a single non-background-effect level, the BMD approach was not used to estimate an AC for this effect. Instead, an AC was calculated by applying uncertainty factors to the lowest tested dose (the LOAEL [lowest-observed-adverse-effect level]) of 375 mg/kg. A factor of 10 was used to estimate the NOAEL from the LOAEL. Other factors include a factor of 3 to partially protect against the known allergenicity of MBT, a weight of 70 kg per crew member, an interspecies extrapolation factor of 10, and a water consumption volume of 2.8 L per crew member per day. Thus,

$$\text{Lifetime AC} = (375 \text{ mg/kg} \times 70 \text{ kg}) \div (3 \times 10 \times 10 \times 2.8) = 30 \text{ mg/L}.$$

Although a standard lifetime is 70 y, and the longest exposure duration for which we currently set SWEG values is 1,000 d, the calculated ACs were not increased by a time factor, because the policy of the subcommittee has been to avoid increasing the AC for exposure durations shorter than the duration for which we have data. This is particularly a problem when data indicating time-dependency are not available. In the absence of other relevant studies on which to base ACs for nephropathy, a conservative approach is to use the 1,000-d AC as the 10-d and 100-d ACs, because it is not clear that the kidneys were examined histologically in the 16-d or 13-wk studies by Dieter. Thus, the ACs for nephropathy are

1000-d AC = 30 mg/mL;
100-d AC = 30 mg/mL; and
10-d AC = 30 mg/mL.

TABLE 5-10 Acceptable Concentrations (ACs)

			Uncertainty Factors					Acceptable Concentrations (mg/L)[b]			
End Point	Exposure Data	Species and Reference	To NOAEL	Interspecies	Exposure Time	Spaceflight	Hypersensitivity	1 d	10 d	100 d	1,000 d
LOAEL for nephropathy	375 mg/kg/d, 2 y, gavage in corn oil	Rat, F0344/N, male (Dieter 1988)	10	10	1	1	3[a]	—	30	30	30
NOAEL for CNS depression	289 mg/kg/d, 20 mo (1,920 ppm in diet)	Mouse, male (Owaga et al. 1989)	1	10	1	1	3[a]	200	200	200	200
$BMDL_{01}$ for carcinogenicity (Pheochromo-cytomas)	12 mg/kg/d, 2 y, gavage in corn oil	Rat, F-344/N, female (Dieter 1988)	—	1	t/25,600	1	3[a]	—	3,000	300	30
SWEG								200	30	30	30

[a]Although it might not be possible to totally and confidently protect the most sensitive individuals against hypersensitivity to MBT (i.e., contact dermatitis), a factor of 3 is used to provide at least partial protection for hypersensitive individuals.
[b]These ACs might not exceed the solubility of MBT in pure water, but the solubility of MBT in water is increased by the presence of other solutes. Also, even at the lowest SWEG concentration of 30 mg/L, it is likely that the water would be too unpalatable to drink, but currently there are no data from which to calculate an AC for taste aversion.

No AC for nephropathy was set for exposure durations of 1 d.

CNS Effects

Clonic convulsions were reported in mice at single gavage doses near the LD_{50} (approximately 1,500 mg/kg, suspension in aqueous media; approximately 3,100 mg/kg, suspension in olive oil) (Ogawa et al. 1989) and in rats and mice treated for 13 wk with MBT in corn oil at approximately 750 mg/kg/d (Dieter 1988). No convulsions were reported in mice during chronic (20 mo) studies at average MBT intakes of up to 289 mg/kg/d in the diet (Ogawa et al. 1989), but post-gavage lethargy at 375 mg/kg was reported by Dieter. An AC was calculated using the highest reported dietary dose as a NOAEL for convulsions or lethargy (Ogawa et al. 1989). The daily intake for mice was adjusted using a factor of 10 for interspecies differences. Thus, for a 70-kg human consuming 2.8 L of water per day,

AC (allergenicity) = (289 mg/kg/d × 70 kg) ÷ (10 × 3 × 2.8 L/d);
AC (allergenicity) = 241 mg/L (rounded to 200 mg/L).

Since recovery from MBT-induced CNS depression has been shown to occur within 4 h in mice, the same AC value is used for all exposure durations.

Spaceflight Effects

Since CNS effects and allergic dermatitis are not known to be affected by spaceflight, no spaceflight factors were applied in the calculation of the ACs.

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6 Nickel

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BACKGROUND

The International Space Station (ISS) will use stainless steel plumbing systems extensively in the wastewater and processed-water distribution lines, the humidity heat exchanger, and the collection and dispenser lines. Nickel is one of the key components of these systems. The lines carry water containing various levels of iodine, which can corrode the lines, and thus nickel can be found in the processed waters. Although the in-line conductivity monitors might be able to flag such leaching, and the ion exchange resins can actively eliminate nickel, high nickel levels can foul these systems. In NASA's ground-based human testing (NRC 2000, Ch. 2) for the development of ISS water-processing hardware, nickel concentrations were found in the processed water through the analysis of archived samples.

Because studies in experimental animals (inhalation and injections) and epidemiological studies have shown that nickel and nickel-containing substances are carcinogenic (cancers of the lung and nasal cavities), and because nickel has been implicated as a cause of chronic dermatitis, it was necessary to assess if an appreciable health risk exists for the presence of nickel as soluble nickel salts in the spacecraft drinking water. Nickel also

TABLE 6-1 Physical and Chemical Properties of Nickel and Soluble Nickel Compounds

CAS registry no.	7440-02-0
Atomic number	28
Synonyms	None
Oxidation states	1, 2, 3, and 4
Nickel chloride (CAS registry no.)	7718-54-9
Nickel oxide (CAS registry no.)	1313-99-1
Structural formulas	Ni, $NiCl_2$, NiO, $NiSO_4$, $Ni(NO_3)_2$, $Ni(CH_3{\cdot}CO_2)_2$
Water solubility	Ni (insoluble); NiO (1.1 mg/L at 20°C); $NiCl_2$ (642 g/L at 20°C)

seems to affect the absorption of iron. The requirement for nickel as an essential trace element for humans is inconclusive.

Nickel is a lustrous hard metal and exists in several salt forms, some soluble and some insoluble in water. The most common soluble forms are the chlorides, sulfates, and the nitrate. This document will discuss the health effects of nickel in drinking water; therefore, only the soluble forms will be discussed. Nickel dust or insoluble forms such as nickel sulfides will not be considered.

Occurrence and Use

Nickel salts are used extensively in electroplating and in the manufacture of alloys, catalysts, and high capacity batteries. Metallic nickel is used in the manufacture of stainless steel. Nickel is present in the earth's crust at 0.018% as the free form but predominantly as sulfides, oxides, arsenides, antimonides, and silicates in ores.

Nickel is very commonly found in surface waters and groundwaters, mostly originating from industrial activities and anthropogenic discharges. In water, nickel mostly occurs as Ni^{2+}. In an aerobic environment, at a pH below 9, nickel will form soluble compounds with hydroxides, carbonate, sulfate, and naturally occurring organic ligands. An EPA survey reported that the concentrations in surface water range from 3.9 micrograms per liter (µg/L) to 672 µg/L,and groundwater levels ranged from 2.95 µg/L to 440 µg/L. Most potable waters contain <40 µg/L of nickel (EPA 1983). Thus,

TABLE 6-2 Physical and Chemical Parameters of Other Nickel Compounds

Form	Molecular Weight	Nickel Content (%)
Nickel	58.69	100
Nickel chloride (monohydrate) ($NiCl_2$)	129.60	45.29
Nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$)	238.00	25
Nickel sulfate ($NiSO_4$)	154.75	37.9
Nickel sulfate hexahydrate ($NiSO_4 \cdot 6H_2O$)	262.8	22.3
Nickel acetate ($Ni(CH_3COO)_2$)	176.80	33.20
Nickel nitrate hexahydrate ($Ni(NO_3)_2 \cdot 6H_2O$)	290.81	20.2

Source: Merck 1989.

several surveys found that community water supplies, surface waters, drinking waters, and tap water samples contained nickel. Nickel may leach into drinking water from plumbing materials. It is important to note that dietary nickel forms a significant portion of the human exposure/body burden. A wide range of nickel intake through diet has been reported (IOM 2001). According to the U.S. Food and Drug Administration total diet study of 1984, the mean adult daily nickel consumption from diet ranges from 74 µg per day (d) to 100 µg/d (Pennington and Jones 1987). On the contrary, a national survey from five Canadian studies during 1986-1988 reported a dietary nickel consumption of 207-406 µg/d for adults (Dabeka and McKenzie 1995).

PHARMACOKINETICS AND METABOLISM

Absorption

The major route of nickel intake is ingestion, although inhalation and dermal absorption are also routes of exposure. Most of the data for ingestion comes from animal studies in which nickel salts were added to the diet. From the available studies it can be inferred that the bioavailability of nickel from dietary sources is different than that from drinking water sources. In general, it has been reported that in animals 1-10% of nickel administered as nickel salts either in the diet or by gavage is absorbed (Sun-

TABLE 6-3 Nickel Levels in Various Processed Water Samples in NASA Investigations

	Nickel (μg/L)		
Sample Source	Minimum	Maximum	Frequency of Occurence[a]
Phase II A (60 d)	1.2	265	44/81
Phase III (90 d)	0	328	47/58
Mir missions			
Mir-18-Mir-25	5.6	157	22/29
Mir water ferried from ground	1.2	79.3	7/7

[a]Found above method quantifiable levels.
Source: Data from NASA-Johnson Space Center Water and Food Analysis Laboratory.

derman 1970). On the basis of urine excretion data, Ho and Furst (1973) reported that female rats given nickel chloride ($NiCl_2$) in dilute acid solution by oral intubation absorbed only 3-6% of doses at 4, 16, or 64 milligrams per kilogram (mg/kg). Sunderman et al. (1989) reported that absorption of nickel from food was much lower than from water in humans (n = 8) who ingested 12, 18, or 50 grams (g) of nickel as nickel sulfate ($NiSO_4$) in food or in water (27% absorption from water and 0.7% from food). The subjects were fasted 12 hours (h) before the dose was administered, and those recieving the dose in water were fasted an additional 3 h after being dosed. The bioavailability of nickel, as measured by serum nickel levels, was similarly elevated in fasted subjects given $NiSO_4$ in drinking water (peak increase within 3 h) but not when nickel was given in food (Solomons et al. 1982). Food constituents such as fibers, phytate, and some metal-ion binding components may bind nickel, influencing the availability. These data indicate that the presence of food significantly decreased the absorption of nickel. Absorption from solutions was higher for more soluble nickel compounds (Ishimatsu et al. 1995). A detailed study by Nielsen et al. (1999) on the kinetics of nickel absorption from food and water and of nickel elimination reported that the maximum nickel levels observed 1 h after ingestion in water were 13 times higher than those observed when nickel was given with food and water. The cumulative amount of nickel excreted increased with an increase in the interval between the meal and nickel administration

in water. From the experimental design and conclusions, it is clear that under normal circumstances, when one ingests food once every 4 h and drinks water intermittently, the existing food in the stomach will affect nickel absorption. In the same study, Nielsen et al. (1999) indicated that women absorb less nickel, and the authors propose that the difference in absorption is due to a gender-related difference in gastric emptying time, which is longer in women than in men.

Nickel is believed to play a role in physiologic processes as a cofactor in the absorption of iron from the intestine. The interaction between nickel and iron occurs only under certain conditions. Nickel increased the absorption of iron from the diet in iron deficient rats (female), but interaction was seen only when dietary iron was in the unavailable ferric form, whereas a mixture of ferrous and ferric sulfates (60% ferric to 40% ferrous) as a supplement to the diet did not elicit any effect (Nielsen et al. 1980). Tallkvist et al. (1994) examined nickel-iron interactions both in vivo after an oral dose and in vitro using isolated perfused jejunal and ileal segments from iron deficient and iron sufficient rats. In both cases, enhanced nickel absorption from iron deficient rats was evident. Furthermore, Tallkvist and Tjalve (1997) observed increased nickel uptake in several tissues of the iron-deficient rats following gastric intubation of nickel. This indicates, at least in part, that nickel is taken up by the same absorptive mechanism for iron in the intestinal epithelium. In a study using monolayers of epithelial coco-2 cells, derived from human colonic adenoma carcinoma in bicameral chambers, Tallkvist and Tjalve (1998) observed that both transportation and accumulation of nickel ($^{63}NiCl_2$) were depressed when the monolayer cultures were loaded with iron, indicating strong interaction between nickel and iron.

However, an important concern is whether increased doses of nickel (by ingestion) will affect iron status (decrease in iron absorption and tissue levels) in subjects with sufficient iron in the diet, eventually resulting in anemia. Nielsen et al. (1979) conducted a fully crossed two-way factorially designed experiment in female SD rats in which the interaction of nickel and iron was studied in rats at varying doses of nickel in the diet (0, 5, and 50 μg/g) for 9 weeks (wk), and the changes in levels hematocrit and hemoglobin levels were reported. The results showed that when iron is present at borderline deficient levels or at normal levels, nickel did not affect the above parameters. Similarly, Oosting et al. (1991) reported that the addition of 0, 3, 50, or 100 μg/kg in the diet to rats had no major impact on iron concentrations in plasma, liver, kidneys, spleen, or femur.

Distribution

The total amount of nickel found in the human body has been estimated to be 86 µg/kg for a 70-kg person (Sumino et al. 1975). Serum nickel levels peaked at 2.5 h to 3 h after ingestion of $NiSO_4$ (Sunderman et al. 1989). In workers who accidentally drank nickel-contaminated drinking water, the serum half-life was 60 h (Sunderman et al. 1988). In short-term and long-term studies of animals administered various soluble nickel salts orally, nickel was found primarily in the kidneys. Whanger (1973) reported that there was a dose-dependent increase in the retention of nickel from the diet when rats were fed diets containing nickel at 0, 10, 50, or 100 mg/kg/d. The relative tissue concentrations were kidneys > lungs > liver > heart > testes (Ambrose et al. 1976; Dieter et al. 1988; Ishimatsu et al. 1995; Whanger 1973.) Oral administration of radioactive $^{63}Ni^{2+}$ to mice resulted in higher accumulation of $^{63}Ni^{2+}$ in the spinal cord than in the cerebellum or frontal cortex (Borg and Tjalve 1989). In control rats, there appeared to be a difference in the levels of nickel in bones between males and female rats—0.53 parts per million (ppm) and <0.096 ppm, respectively (Ambrose et al. 1976). Casey and Robinson (1978) reported that the levels of nickel in the liver, heart, and muscles were similar in human fetuses and in adults. When pregnant rats were fed nickel salts at 1,000 ppm in the diet (50 mg/kg/d), their newborn pups had a total tissue concentration of 22-30 ppm; the levels in decreasing order were bone > spleen > kidneys > heart > intestine > blood > testes, indicating transplacental transfer of nickel (Phatak and Patwardhan 1950, as cited in EPA 1995). When $NiCl_2$ was injected subcutaneously into lactating SD rats, a dose-dependent increase of nickel in milk was observed (Dostal et al. 1989). When nickel at 5 ppm (0.41 mg/kg/d, soluble salt not specified) was added in drinking water, however, the rats did not accumulate significant amounts in tissues (Schroeder et al. 1974).

Metabolism

In humans and animals, serum albumin is the main carrier of nickel in the serum. In human serum, nickel was found to bind to albumin, L-histidine, and alpha-2-macroglobulin (Sarkar 1984). The low-molecular-weight complex of albumin-histidine-nickel can be transported across cellular membranes. Nickel bound to the alpha-2-macroglobulin (nickeloplasmin) pool is considered a nonexchangeable pool (Nomoto and Sunderman 1989). Serum albumin in dogs does not have the specific Ni^{+2} binding site

(Glennon and Sarkar 1982), and hence, most of the nickel in dog serum is not protein-bound. It has been shown that there are large species variations in the proportions of ultrafiltrable and protein-bound serum nickel (Hendel and Sunderman 1972).

Excretion

In humans, most ingested nickel is not absorbed and is eliminated predominantly in the feces. Some of the nickel absorbed from the gastrointestinal tract is excreted in the urine and is associated primarily with low-molecular-weight complexes that contain amino acids. In humans, nickel can also be eliminated through sweat and milk. Sunderman et al. (1989) reported that in humans who ingested 12, 18, or 50 μg of nickel from $NiSO_4$ in food or water, 26% of the dose given in water was excreted in the urine and 76% was excreted in the feces by 4 d post-treatment. Of the dose given via food, only 2% was excreted in the urine and almost all the rest was excreted in the feces. A 40-fold difference was seen in the absorption of nickel from drinking water and from food; absorption was higher from the drinking water. Hansen et al. (1994) reported that excretion of nickel following a single oral dose given to women after an overnight fast was found to decrease with increasing age, suggesting that nickel absorption may decrease with age. Ho and Furst (1973) reported that 1 d after administration of $NiCl_2$ to rats, about 95% was excreted in the feces and about 3-6% in the urine. Similarly, in dogs given $NiSO_4$ in the diet for 2 years (y), only 1% to 3% of the dose was excreted in the urine (Ambrose et al. 1976).

The kinetics of $^{63}Ni^{2+}$ metabolism was studied in rats and rabbits following an intravenous injection of $^{63}NiCl_2$ to develop a compartmental model useful in predicting nickel concentrations under various circumstances (Onkelinx et al. 1973). $^{63}Ni^{2+}$ was rapidly cleared from plasma and serum during the first 48 h after injection and was cleared at a much slower rate from 72 h to 168 h. The model developed was tested and validated in other separate experiments in which $NiCl_2$ was either perfused or injected daily for 14 d (Onkelinx et al. 1973).

TOXICITY SUMMARY

Available evidence indicates that the natural concentrations of nickel present in water, soil, and food do not constitute a threat to humans (NAS 1977). Toxicity studies have demonstrated that significant differences can

exist based on the chemical form of the nickel salt. That is probably because the estimated absorbed fraction of each of the nickel compounds was increased with the increased solubility of the nickel compound (Ishmatsu et al. 1995). Furthermore, although parenteral injections of nickel salts are very toxic, nickel salts have relatively low toxicity in various species of animals when administered orally. Soluble nickel compounds are more toxic than less soluble nickel compounds. For example, although the oral LD_{50}s (doses lethal to 50% of subjects) for less soluble nickel oxide (NiO) and nickel subsulfide (Ni_3S_2) were 4 g/kg, the oral LD_{50} in female rats for $NiSO_4$ was 39 mg/kg, and the LD_{50}s for nickel acetate were 116 mg and 136 mg for female rats and mice, respectively (Haro et al. 1968; Mastromatteo 1986).

The available literature indicates that, following a high oral exposure to nickel in rats and mice, some of the most common effects reported are decline in body-weight gain, reduced water intake, hepatic and renal weight changes, gastrointestinal effects, adverse hematologic effects, and some central nervous system (CNS) effects at high doses. In addition to those effects, allergic reactions (hypersensitivity) to nickel have been reported in individuals with nickel allergic contact dermatitis (ACD).

Acute Toxicity (1 d)

Daldrup et al. (1983, as cited in ATSDR 1997) reported that a 2.5-y-old child died of cardiac arrest after ingesting an oral dose of $NiSO_4$ crystals. The dose was calculated to be 250 mg/kg. In a study that was designed to find the absorption and elimination kinetics of nickel in humans, Sunderman et al. (1989) reported that 7 h after a single dose of nickel as $NiSO_4$ (0.05 mg/kg) in drinking water, a male volunteer developed left hemianopsia for 2 h (loss of sight in the corresponding lateral half of the eye). However, when the dose was lowered in other subjects to 12 µg/kg or 18 µg/kg, that effect was not observed.

Short-Term Toxicity (2-10 d)

During a 2-y nickel-feed study in dogs, Ambrose et al. (1976) observed that during the first 3 d all dogs ingesting nickel at 2,500 ppm in their diet (37 mg/kg/d) vomited, indicating gastrointestinal distress. The concentration of nickel in the diet was raised from 1,500 to 1,700, 2,100, and the target level of 2,500 ppm (37 mg/kg/d) at 2-wk intervals with no further

evidence of emesis. The final dose was 37 mg/kg/d (Ambrose et al. 1976), which was continued for the rest of the 2-y duration.

Acute gastrointestinal effects were also reported by Sunderman et al. (1988). Thirty-two workers in an electroplating factory who accidentally drank water from a water fountain contaminated with nickel suffered from acute gastrointestinal (nausea, vomiting, abdominal discomfort, diarrhea) and neurologic (headache, shortness of breath, giddiness) symptoms. The amount was determined to be 1.63 g/L as $NiCl_2$ and $NiSO_4$. The authors estimated that intake ranged from 0.5 g to 2.5 g in 20 workers who had these symptoms. Symptoms lasted for a few hours and up to 2 d in some workers. Even though boric acid was also present at 68 mg/L in the water, the authors concluded that nickel was the primary cause for the observed effects. In three of 10 workers who were hospitalized, a transient increase in serum bilirubin (indicative of hepatotoxicity), a transient increase in urine albumin (indicative of renal toxicity), and a transient increase in hematocrit were reported.

Other toxic responses to short-term doses of nickel are the allergic dermatologic reactions to nickel ingestion, such as flare-up of eczema and dose-related erythema, seen only in individuals who are already predisposed to nickel ACD. This is discussed in a separate section later in the document.

Subchronic Toxicity (10-100 d)

Weber and Reid (1969) reported significant reductions in body-weight gain, decreased body weight, and signs of hepato- and renal toxicities in animals as a result of daily exposure to nickel via diet or gavage. Nickel as nickel acetate at 0, 1, 100, or 1,600 ppm administered in the diet to male and female mice for 4 wk resulted in decreases in body weight at 100 and 1,600 ppm in females, whereas that change was seen only at 1,600 ppm in males (Weber and Reid 1969). Liver and heart cytochrome oxidase, liver isocitric dehydrogenase, and kidney and heart maleic dehydrogenase were also decreased. A LOAEL (lowest-observed-adverse-effect level) of 143 mg/kg/d for reduction in body weight and reduced enzyme activities was identified.

Weischer et al. (1980) reported that oral administration of nickel as $NiCl_2$ in male rats over a period of 28 d at concentrations of 2.5, 5.0, and 10.0 μg per milliliter (mL) in drinking water (0.38, 0.75, or 1.5 mg/kg/d) resulted in significant dose-dependent hyperglycemia, decreases in serum

urea, and significant increases in urine urea. Increased leukocytes observed at 0.75 mg/kg were not observed at lower doses. Water consumptions were significantly lower than controls at all doses. These effects were noted at extremely small doses compared with other reports; hence, this study was not considered for AC derivation because of lack of confidence in the data.

In weanling rats (n = 24) fed a diet containing nickel as nickel acetate at 100, 500, or 1,000 ppm (10, 50, or 100 mg/kg/d) for 6 wk, significant reductions in weight gain in the mid-dose group and loses of weight in the 100-mg/kg group were noted (Whanger 1973). Reduced blood hemoglobin and packed cell volume (PCV) were also noted. Significant reductions in plasma alkaline phosphatase and in cytochrome oxidase activities in liver and heart were seen in the 100-mg/kg group. Significantly elevated levels of iron in many tissues, particularly in the kidneys and liver, were observed in both the 50-mg/kg group and the 100-mg/kg group. These effects were not seen in the 10-mg/kg group. The influence of nickel on tissue iron levels was not clear.

In a 90-d rat gavage study (American Biogenics Corporation 1988, as cited in IRIS 1996), 30 male and female CD rats received a daily gavage dose of nickel as $NiCl_2$ (hexahydrate) at 0, 5, 35, and 100 mg/d (1.2, 8.6, and 25 mg/kg/d). Various symptoms of toxicity, such as lethargy, ataxia, irregular breathing, hypothermia, salivation, and loose stools, were observed in the 10- mg/kg/d group, and increased mortality (52/60 rats in the high-dose group and 2/60 in the low-dose group) was observed during the regimen. All animals in the 100-mg/kg group and 14 of 60 in the 35-mg/kg group died. Decreased body weights, decreased food intake, and clinical signs of toxicity, including ataxia, lethargy, altered breathing, lower body temperature, and discoloration of the extremities, were observed. Kidney, liver, and spleen weights were lower in the mid-dose and high-dose groups. A dose of 1.2 mg/kg/d appeared to be a NOAEL (no-observed-adverse-effect level) in this study.

Waltschewa et al. (1972, as cited in EPA 1985) reported that daily oral intubations of nickel as $NiSO_4$ at 25 mg/kg for 4 months (mo) in male rats resulted in degenerative cellular changes in the liver and kidneys.

Obone et al. (1999) reported that in adult male Sprague-Dawley rats given $NiSO_4$ (hexahydrate) at 0%, 0.02%, 0.05%, and 0.1%, or 0, 44.7, 111.75, and 223.5 mg/L, respectively (estimated doses of 0, 5, 12.5, and 25 mg/kg/d), in their drinking water for 13 wk, both the absolute and relative liver weights in the 12.5-mg/kg and 25-mg/kg groups were significantly decreased. Total plasma proteins, plasma albumin and globulins, and plasma glutamic pyruvic transaminase activity were significantly decreased

in the highest-dose group. In the splenic lymphocyte subpopulations (T-cells and B-cells), the ratio of CD4 to CD8 cells was suppressed significantly in the highest-dose group (223.5 mg/L). There were also significant decreases (about 20%) in B-cells. A significant decrease in urine volume and an increase in blood urea nitrogen (BUN) were observed at the highest dose. Other nephrotoxic indices, such as excretion of urinary protein, *N*-acetyl-beta-D-glucosaminidase, and gamma-glutamyl transpepeptidase, were unaltered. Biochemical analysis of bronchoalveolar lavage fluid and lung tissue showed some lung damage.

The 1988 American Biogenics Corporation study reported changes in heart weight in rats exposed to nickel as $NiCl_2$ at 8.6 mg/kg/d by the oral route for 91 d. One study indicated that nickel ingestion could be neurotoxic. Nation et al. (1985) fed adult male rats food containing nickel as $NiCl_2$ at 0, 10, or 20 mg/kg/d for a total of 75 d. Fourteen days after the exposure started the animals were trained over 61 d to get their food from a lever-press operand machine. Rats receiving 20 mg/kg/d lever-pressed at a significantly lower rate than the 10-mg/kg/d group and the control group, thus showing behavioral effects. A dose of 10 mg/kg/d (as $NiCl_2$) seems to be a NOAEL, and 20 mg/kg/d a LOAEL, for 75 d.

Chronic Toxicity (≥100 d)

Male albino rats (n = 10, body weight = 80 g, strain not specified) received drinking water containing nickel as $NiCl_2$ at 225 mg/L for 4 mo (estimated dose of about 32 mg/kg/d) (Clary 1975). Significant reductions in body weights, serum lipids, serum cholesterol, urinary calcium, and urine outputs compared with controls were reported in this study. Many of the biochemical changes were secondary to loss in body weight. Water intake was not measured, and the authors assumed that decreased urine output (50% decrease) by the end of 4 mo might be due to decreased water intake. A 50% decrease in urine output is a serious adverse effect, and there is no dose-response data to identify a NOAEL. Because there are other drinking water studies using nickel that provide dose-response data that can be used for AC development, this study was not considered for AC derivation.

Dieter et al. (1988) conducted a study to evaluate tissue disposition, myelopoietic responses, and immunologic responses in female $B6C3F_1$ mice after long-term exposure to nickel as $NiSO_4$ in drinking water at 0, 1, 5, or 10 g/L (0, 44, 108, 150 mg/kg/d) for 180 d. Water consumption, concentration of nickel in blood and tissue, body and organ weights, histopathology, immune responses, bone marrow cellularity and proliferation,

and cellular enzyme activities were determined (Dieter et al. 1988). Mice in the 108-mg/kg/d and 150-mg/kg/d dose groups drank less water than controls; decreases in body and organ weights were seen only in mice in the 150-mg/kg/d dose group, with the exception of the dose-related reductions in thymus weights. Blood nickel concentrations seen at 4 wk and 8 wk were proportional to time and dose; however, those concentrations did not increase substantially in any of the dose groups after 8 wk. The kidney was the major organ of nickel accumulation, and minimal convoluted tubular damage was noted. There was a loss of renal tubular epithelial cells, and hyaline casts were present in the tubules of the 108-mg/kg/d dose group, suggesting protein loss, but not the 44-mg/kg/d dose group. A LOAEL of 108 mg/kg/d and a NOAEL of 44 mg/kg/d for renal effects could be identified. There were dose-related decreases in bone marrow cellularity and decreases in granulocyte macrophage colony forming units (CFUs) and pluripotent stem-cell proliferative response CFUs. Inhibition of certain carbohydrate metabolism enzymes was confined to the fractionated and enriched granulocyte macrophage cell populations, suggesting that these committed stem cells were a primary target of nickel sulfate toxicity. Several immune parameters were evaluated, including spleen cellularity. An observed reduction in the lymphoproliferative response to lipopolysaccharide (with out any change in proliferative response to the mitogen concavalin A), a measure of systemic immunotoxicity, was regarded as secondary to the primary effect of $NiSO_4$ on the myeloid system, because it was the only significant change among a panel of seven immune parameters that were evaluated.

Vyskocil et al. (1994) exposed male and female Wistar rats to drinking water containing nickel as $NiSO_4$ at 100 mg/L for 6 mo. The average nickel intake was calculated to be 6.9 mg/kg/d for males and 7.6 mg/kg/d for females. Kidney weights were significantly increased in the exposed groups. Urinary excretion of albumin increased significantly in female rats, but the increase was marginal (due to wide data variation) in male rats. These results are indicative of adverse effects of nickel on renal function, female rats being more sensitive to the nephrotoxicity of nickel than males. No changes were noted in urine lactate dehydrogenase, N-acetyl-beta-D-glucosaminidase, or beta-2-microglobulin (a marker for renal tubular damage).

Male and female Wistar derived albino rats (25 per gender per group, 56-65 g body weight) were fed diets containing nickel as $NiSO_4$ (hexahydrate) at 0, 100, 1,000, and 2,500 ppm (0, 5, 50, and 125 mg/kg/d) for 2 y (Ambrose et al. 1976). Significant body-weight reductions were observed

in both the male and female rats fed 125 mg/kg/d. Significant increases in heart-to-body weight ratios and decreases in liver-to-body weight ratios were observed at 50 mg/kg/d and 125 mg/kg/d. No significant effects were seen in the 5-mg/kg/d group.

Male and female beagle dogs (three per dose per gender, 6 mo old) maintained for 2 y on diets providing nickel as $NiSO_4$ (hexahydrate) at 0, 100, 1,000, and 2,500 ppm (0, 3, 29, and 70 mg/kg/d) showed various hepatic, renal, and hematotoxic effects. No significant changes were noted in the 3-mg/kg/d and 29-mg/kg/d dose groups (Ambrose et al. 1976). Depressed body-weight gain (40% decrease), lowered hematocrit and hemoglobin levels, and decreased liver-to-body weight ratios and kidney-to-body weight ratios were noted only at the highest dose. Polyurea and increased liver and kidney weights were also noted at the 70-mg/kg/d dose. The authors also reported respiratory system effects in dogs at the highest dose, such as subpleural peripheral cholesterol granulomas, bronchiolectasis, emphysema, and focal cholesterol pneumonia. Microscopic examinations did not reveal any changes in the brains of the dogs for up to 2 y. The principal binding site for nickel to serum albumin is the histidine residue near the amino terminus and is the same in humans, rodents, and bovines (Hendel and Sunderman 1972). Dogs do not have that binding locus. It has been reported that greater than 85% of administered nickel is found in the ultrafiltrable fraction of dog serum albumin. Because this important difference determines the transport and turnover of nickel, extrapolating dog data to humans is questionable.

Schroeder et al. (1974) administered nickel at 5 ppm (average daily dose of 0.41 mg/kg) in drinking water for life (18 mo) to both male and female rats. Significant reductions in body weights were noted. In addition, histopathologic examinations showed an increased incidence of focal myocardial fibrosis.

In a 2-y study, Ambrose et al. (1976) reported increased heart weights without any reported histologic changes of the heart tissue in rats exposed to nickel at 75 mg/kg/d and in dogs given nickel as $NiSO_4$ in the diet at 62.5 mg/kg/d. Similarly, there were no histologic changes in the hearts of rats exposed to nickel in drinking water at 53 mg/kg/d for up to 30 wk (RTI 1988).

Reproductive Toxicity

Although nickel crosses the placenta (Jasim and Tjalve 1986), no spe-

cific reports are available related to adverse effects of nickel on fetal maturation, reproduction, or development in humans. In a 4-mo study, when male rats were intubated with $NiSO_4$ at 25 mg/kg, several testicular changes, such as interstitial cell proliferation, transparent vessel walls, and reduced number of spermatozoa and some testicular enzymes such as steroid 3 beta-dehydrogenase, were seen (Waltschewa et al. 1972, as cited in EPA 1985).

Sobti and Gill (1989) reported increased abnormalities of the heads of spermatozoa 5 wk after mice were treated with a single oral dose of $NiSO_4$, $NiCl_2$, or nickel nitrate ($Ni(NO_3)_2$) at 28, 43, and 23 mg/kg, respectively. $Ni(NO_3)_2$ is the most potent when compared with the sulfate or the chloride salts (which were equipotent). Kakela et al. (1999) conducted a study to determine the reproductive effects of nickel in both female and male Wistar rats in which one or both genders were exposed to nickel at 10-100 ppm as $NiCl_2$ in drinking water. In males (exposed 28 d or 42 d before copulation), $NiCl_2$ induced shrinkage of seminiferous tubules and decreased the number of spermatogonia in the tubules. The number of pregnancies and the number of pups born were reduced. There was a 50% reduction in the fertility index in males drinking water spiked with nickel at 30 ppm (estimated 3 mg/kg/d) for 28 d. In addition, this exposure resulted in an average of only 5.3 live born pups per delivered female in comparison to 10 per controls (females copulated with unexposed males). However, the fertility index in male rats exposed for 48 d was better than in those exposed for 28 d. The reason for that is unclear.

In female rats exposed to nickel as $NiCl_2$ at 10-100 ppm (1, 3, or 10 mg/kg/d) in water for 14, 28, or 100 d before copulation and through pregnancy and lactation, the fertility index was unaffected. Pup mortality during lactation was high (Kakela et al. 1999). There does not appear to be a clear trend of cause-effect relationship, and because only one dose was used, this study was not used to calculate an AC. Mice treated orally with $NiCl_2$ (90.6 mg/kg/d) during gestation days 8-12 did not show any change in the average number of neonates per litter or weights of the neonates (Seidenberg et al. 1986). A multigeneration study of nickel suggested that high oral exposures to nickel salt might adversely affect reproduction. $NiCl_2$ was given in the feed to rats at 0, 22.5, 45, or 90 mg/kg/d (Ambrose et al. 1976). A dose-related increase in the number of stillborn pups and a decrease in the number of weaning pups were seen in the F_1 generation. However, the microscopic examination of testes and ovaries of rats and dogs in the 2-y Ambrose et al. study did not reveal any changes in those tissues.

Developmental Toxicity

There are no reports on developmental effects of nickel in humans. In the three-generation reproduction study in rats by Ambrose et al. (1976) in which nickel was given as $NiSO_4$ in the diet at 0, 12.5, 25, or 50 mg/kg/d, no evidence of teratogenicity was seen in the offspring. A two-generation reproduction toxicity study of female Long-Evans rats given doses of nickel as $NiCl_2$ at 0, 10, 50, or 250 ppm in drinking water (0, 1.33, 6.5, or 32.5 mg/kg) for 11 wk during mating and during two gestation and lactation periods resulted in an increased number of litters with dead pups only at the highest dose during the L1 period (NOAEL of 6.5 mg/kg). During the second generation, significant dose-related increases in the deaths of pups were seen at all doses (Smith et al. 1993). Similar results were reported in the RTI (1988) two-generation reproduction study of rats given $NiCl_2$ in drinking water at 0, 0 , 250, or 500 ppm. In the F_{1a} generation there was a significant decrease in live pups at 500 ppm. In a study on the development of mouse embryos, intraperitoneal injection of a single dose of $NiCl_2$ (hexahydrate) at 20 mg/kg in the preimplantation period resulted in a large frequency of both early and late resorptions, diminished body weights of the fetuses, and diminished sizes of litters (Storeng and Jonsen 1981).

Genotoxicity

Bacterial mutagenicity tests have indicated that nickel is a nongenotoxic agent (Biggart and Costa 1986). In contrast, higher organism cell tests, in vivo and in vitro, have shown that nickel (particulate and soluble compounds) produces a variety of genetic abnormalities: DNA strand breaks, DNA-protein cross-links, nucleotide excision, single gene mutations, instability of DNA repetitive sequences, chromosome aberrations, sister chromatid exchanges, micronuclei, and cell transformation (Coogan et al. 1989; Costa 1991; Fletcher et al. 1994; Snyder 1994; Hartwig 1997; Jackson et al. 1998). Particulate nickel is more mutagenic (as well as carcinogenic) than are nickel compounds (Sunderman 1984).

The primary target molecules for nickel attack appear to be proteins, especially those proteins closely associated with the chromosome and with DNA repair (Lynn et al. 1997). The threshold concentration for nickel-induced mutagenesis is impossible to establish at present because, in most studies, the lowest concentrations used were near those that cause high cytotoxicity. For example, with a typical exposure to $NiSO_4$ in a range of

concentrations at 0.05-0.25 millimolar (mM) or 14.7-73.0 g/mL for 6-24 h, cell survival begins to plummet near the lowest concentrations and reaches near zero at the higher concentrations (Lee et al. 1993; Fletcher et al. 1994). The high cytotoxicity of nickel probably accounts for the relatively low frequency of observed mutations.

Nickel, like ionizing radiation, acts by increasing the levels of endogenous cellular hydrogen peroxide and its short-lived reactive oxygen species (i.e., OH free radicals) (Reid et al. 1994; Lynn et al. 1997). Nuclear protein damage caused by nickel reduces the enzyme activity needed for DNA replication, transcription, recombination, and repair. The demonstrable inhibition of these crucial functions is undoubtedly responsible for much, if not all of nickel's mutagenic and cytotoxic effects, the latter probably due to high mutation load (Jackson et al. 1998).

Pool-Zobel et al. (1994) have found that human nasal and gastric mucosa cells are more susceptible (about 5 times) to the cytotoxic-genotoxic effects of $NiSO_4$ than are cells of those tissues in rats. These data suggest that caution should be exercised in using rat data to estimate nickel risks in humans. Overall, results from genotoxicity studies are "generally consistent with animal carcinogenicity data" (Verma et al. 1999).

Carcinogenicity

Carcinogenic effects of nickel have been well documented in workers exposed occupationally, particularly to nickel subsulfide dust and to nickel oxide. The respiratory system is the primary target for inhaled nickel. In serious cases, histologic changes in the lungs, which include alveolar wall damage and edema in the alveolar space, were reported. Effects included chronic bronchitis, emphysema, and reduced vital capacity. The most common ailment is asthma (Shirakawa et al. 1990), which can result from primary irritation or from an allergic response. Inhalation and intratracheal instillation of Ni_3S_2 in rats resulted in lung tumors. The results with other nickel compounds are inconclusive and appear to depend on the species. For example, although rats showed respiratory tumors when exposed to Ni_3S_2, mice and hamsters did not (Muhle et al. 1992). The National Toxicology Program (NTP 1996) completed three 2-y inhalation carcinogenicity studies with three nickel compounds—two insoluble nickel compounds, Ni_3S_2 and NiO (high temperature), and one soluble nickel compound, $NiSO_4$—in F-344 rats and $B6C3F_1$ mice. The exposure durations were 6 h/d, 5 d/wk for 16 d or 13 wk. When F-344 rats were exposed to Ni_3S_2 at 0, 0.1, or 0.7 mg per cubic meter (m^3) and $B6C3F_1$ mice were exposed to

Ni_3S_2 at 0, 0.4, or 0.8 mg/m^3, there were dose-dependent increases in lung tumors and increased incidence of alveolar and bronchiolar adenomas and carcinomas in male and female rats. There was no evidence of a carcinogenic response in male or female mice, but there were other noncarcinogenic effects such as chronic inflammation of the lungs, focal alveolar epithelial hyperplasia, macrophage hyperplasia, and lung fibrosis (NTP 1996). After exposing rats to NiO at 0, 0.5, 1.0, or 2 mg/m^3 for 2 y, carcinomas and adenomas were seen only in rats exposed at 1.0 mg/m^3 and 2.0 mg/m^3, but these results were not significantly different from controls. There was no evidence of carcinogenicity in male mice; adenomas or carcinomas were seen in female mice even at low doses, without any further increase at high doses. Noncarcinogenic effects described above were also seen. When rats were exposed to nickel as $NiSO_4$ at 0, 0.03, 0.06, or 0.11 mg/m^3 and mice were exposed at 0, 0.06, 0.11, or 0.22 mg/m^3, there was no evidence of carcinogenic activity in either species. There were less pronounced noncarcinogenic effects, less than seen with other insoluble nickel compounds, in both species in both genders.

There has been a lack of agreement among several organizations in the classification of nickel as a potential human carcinogen. Although NTP and IARC have classified nickel and certain nickel compounds as carcinogenic or potential carcinogens to humans (group 1), ACGIH has proposed category A4 (not classifiable as a carcinogen) for the soluble nickel salts. There are no epidemiological reports indicating cancer risk by the oral route. Shroeder et al. (1964, 1974) and Ambrose et al. (1976) could not find any increased incidence of cancer in their study when mice and rats were administered drinking water containing 0.95 mg/kg/d and 0.6 mg/kg/d, respectively, for life. Only intraperitoneal injection of nickel acetate resulted in lung tumors (1.5 lung tumors per mouse [Poirier et al. 1984] and 3/35 or 5/31 lung tumors in rats at 25 mg and 50 mg, respectively [Pott et al. 1992]) (see Table 6-4). Similarly, lung tumors were seen when 50 intraperitoneal injections of $NiSO_4$ at 1 mg each, twice a week, were administered to female Wistar rats (Pott et al. 1992). Several studies in rats did not show even localized tumors when $NiSO_4$ was administered by intramuscular injected (IARC 1990).

Nickel Sensitivity and Allergic Contact Dermatitis

One of the most common effects of nickel exposure is ADC. Contact dermatitis is an allergic response to metallic nickel—a sensitization risk from different types of nickel coatings and alloys. Occupational nickel ACD

TABLE 6-4 Carcinogenicity in Rats After Intraperitoneal Injection of Various Nickel Compounds

Substance	Intraperitoneal Dose (mg) (Number of Injections)	Incidence of Tumors	
		Number	Percent
Nickel chloride	50 (1)	4/32	12.5%
Nickel sulfate	50 (1)	6/30	20.0%
Nickel acetate	25 (1)	3/35	8.6%
Nickel acetate	50 (1)	5/31	16.1%
Nickel carbonate	25 (1)	1/35	2.9%
Nickel carbonate	50 (1)	3/33	9.1%

Source: Data from Pott et al. 1992.

is well known. The five most common causes of ACD in the United States are plant resins, nickel, p-phenylene diamine, rubber chemicals, and ethylene diamine. Contact dermatitis, which results from dermal exposure to nickel, is the most prevalent effect of nickel in the general population. It can result from close contact with costume jewelry and clasps in clothing, eyeglass frames, and wristwatch bands. As evaluated by patch-testing, the prevalence is 7-10% in women and 1-2% in men in the general population. It has been the subject of numerous studies to assess if dietary nickel or ingestion of nickel via water will perpetuate the dermatitis of nickel-sensitive subjects and if sensitization will occur in non-nickel-sensitive subjects.

Several studies have documented that a single oral dose of nickel can result in a flare-up (aggravation) of dermatitis in nickel-sensitive individuals—individuals who had been previously sensitized by dermal contact to nickel. These studies were reviewed in Burrows (1992). The study by Veien et al. (1985) reported that the conditions of 143 patients with vesicular hand eczema (with positive patch test) disappeared in some cases and improved in many cases after 1-2 mo on a diet low in nickel, indicating that ingestion of nickel might be responsible for their condition. In a double-blind study, Christensen and Moller (1975) reported that a single oral dose of nickel at 5.6 mg produced exacerbation in nine of 12 subjects with hand eczema. The same individuals did not react to placebos, as confirmed in several later studies. Five nickel sensitive women were given $NiSO_4$ containing nickel at 0.6, 1.25, or 2.5 mg in 100 mL of water after an overnight fast. Three of five at the lowest dose and five of five at the highest dose exhibited worsening of hand eczema. Erythema was noted in four of five in the highest-dose group. Thus, the lowest single dose resulting in derma-

titis, including erythema on the body, worsening of hand eczema, and a flare-up at the patch-test site, was 0.6 mg/d, or 0.009 mg/kg/d for a 70-kg adult (Cronin et al. 1980). Non-nickel-sensitive individuals were not included in the study. Some studies (Burrows et al. 1981; Gawkrodger et al. 1986) did not observe any reaction exceeding those of placebos, making it hard to interpret results on hypersensitivity. For example, Gawkrodger et al. (1986) reported that in a double-blind randomized study, when 26 subjects who were positive to nickel patch-tests were given either 0.4 mg or 2.5 mg of nickel sulfate ($NiSO_4.7H_2O$) as capsules (also containing lactose) on two successive days for 2 wk, the nickel-induced reactions observed after 3 d did not exceed those seen in placebos. However, when a dose of 5.6 mg was administered, all subjects of this group showed exacerbation of the existing condition. Some of the differences were also due to the conditions of the study and how nickel was administered, as single bolus dose or in the diet for a short-term or long-term period. An analysis by Nielsen (1992) indicates the late reactions in response to provocation in some studies were missed, leading them to conclude absence of a reaction.

Although most of the studies above involved single bolus administrations in water, exposure to nickel via the diet caused similar reactions. Nielsen et al. (1990) fed a diet (oatmeal, soy beans, cocoa) with 5 times the normal levels of nickel (about 0.007 mg/kg/d) to 12 women who had hand eczema and known allergies to nickel for 4 d. An aggravation of hand eczema was found in six of 12 by day 4 after the start of the challenge, and although excess nickel was excreted by 2 d after the last treatment, further exacerbation of hand eczema was observed in 10 of 12 through day 11.

In a double-blind randomized study in which nickel-sensitive subjects were given an oral dose of nickel at 0.5 mg (as $NiSO_4$) two consecutive days in a week (0.007 mg/kg/d) for 4 wk, Jordan and King (1979) did not report flare-up of eczema (one in 10 was positive), suggesting that longer-term oral exposure may even serve to desensitize some individuals. Santucci et al. (1994) observed that in eight nickel-sulfate-sensitive women given increasing daily doses of nickel (0.014-0.3 mg/kg/d) for up to 178 d, hand eczema clinically improved after 1 mo of treatment. Similarly, Gawkrodger et al. (1986) gave three different doses of nickel at 0.4, 2.5, and 5.6 mg (0.006, 0.04, and 0.09 mg/kg, assuming 60 kg as the weight of women) two times a week for 2 wk and found that six of six showed reactions at the highest dose. But all these studies seem to indicate oral doses of nickel do not sensitize individuals who do not have nickel ACD (Nielsen et al. 1999). The pattern of nickel absorption and excretion did not differ from control non-nickel-allergic subjects.

TABLE 6-5 Toxicity Summary (Oral Ingestion)

Nickel Salt	Dose and Route	Duration	Species	Effects	Reference
Acute Exposure (≤1 d)					
$NiSO_4$	250 m/kg; in water	1 d	Human, child (n = 1)	Cardiac arrest	Daldrup et al. 1983
$NiCl_2$ and $NiSO_4$	7.1-35-7 mg/kg; drinking water (also contained boric acid at 65 mg/L)	1 d	Human, workers	Stomach cramps, giddiness, tiredness, renal effects, hematotoxicity	Sunderman et al. 1988
$NiSO_4$	0.0086 mg/kg to 0.036 mg/kg; oral capsule	1 d	Human, nickel sensitive	Dermatitis flare-up	Cronin et al. 1980
$NiSO_4$	0.08 mg/kg; capsule	1 d	Human, female, nickel sensitive	Flare-up at patch-test sites	Christensen et al. 1981
$NiSO_4$	0.050 mg/kg/d; oral gavage	1 d	Human, (n = 1)	Neurological—impaired vision	Sunderman et al. 1989
$NiCl_2$, $NiSO_4$, $NiNO_3$	$NiCl_2$ at 95 mg/kg; $NiSO_4$ at 73 mg/kg; $NiNO_3$ at 72.2 mg/kg; oral bolus	One dose	Mouse	Reproductive effects (sperm-head abnormality; increased bone marrow micronuclei)	Sobti and Gill 1989

Short-Term Exposure (2-10 d)					
$NiSO_4$	0.5 mg twice a day	2 d	Human, female, nickel sensitive (n = 22)	No difference between treated and placebo because of high response to placebo	Burrows et al. 1981
$NiSO_4 \cdot 7H_2O$	0.4 mg/kg/d and 2.5 mg/kg/d; lactose capsules	2 d	Human, female, nickel sensitive (n = 10)	No difference between placebo and treated	Gawkrodger et al. 1986
$NiSO_4 \cdot 7H_2O$	5.6 mg/kg/d; lactose capsules	2 d	Human, female, nickel sensitive (n = 6)	Exacerbation of eczema	Gawkrodger et al. 1986
$NiSO_4$	0.007 mg/kg/d; diet	4 d	Human, female, nickel sensitive	Dermatitis flare-up; erythema	Nielsen 1990
Subchronic Exposures (11-100 d)					
Nickel acetate	143 mg/kg/d, 208 mg/kg/d	4 wk	Mouse	Decreased liver, kidney, and heart enzymes; reduced body weight	Weber and Reid 1969
$NiCl_2$	0.38, 0.75, 1, and 5 mg/kg/d; drinking water	28 d	Wistar rat, male and female	Decreased body weight gain; decreased water consumption, decreased serum urea; increased liver and kidney weights at highest dose	Weicher et al. 1980

(Continued)

TABLE 6-5 Continued

Nickel Salt	Dose and Route	Duration	Species	Effects	Reference
Nickel acetate	0, 10, 50, and 100 mg/kg/d; diet	6 wk	Rat (weanling)	Lowered hemoglobin; PCV; hepatotoxicity	Whanger 1973
$NiCl_2$	0, 10, or 20 mg/kg/d; diet	75 d	SD rat, male, (n = 18)	Decreased lever-press response-behavior in 20 mg/kg/d group; NOAEL was 10 mg/kg/d	Nation et al. 1985
$NiCl_2$	0, 5, 35, and 100 mg/kg/d; gavage	90 d	CD rat, 30 per gender	Ataxia, hypothermia (neuro); decreased body, heart, kidney, and liver weight; increased white blood cells and platelets; death at high dose	American Biogenics Corporation 1988
$NiSO_4$	0, 5, 12.5, and 25 mg/kg/d; drinking water	90 d	SD rat, male, 8 per group	Decreased body weight; reduced subpopulation of spleen lymphocytes and changes in subpopulations of thymocytes; significant increase in BUN and decreased urine volume; no changes in nephrotoxic markers in urine	Obone et al. 1999
$NiCl_2$	0, 10, 30, and 100 ppm (0, 1, 3, or 10 mg/kg); drinking water	14, 28, and 100 d prior to copulation	Wistar rat, 6 per gender per test group	Low fertility index; reduced number of pregnancies, pups born; testicular pathology adverse effects	Kakela et al. 1999
Chronic Exposures (≥100 d)					
$NiSO_4$	25 mg/kg/d; oral intubation	120 d	Rat, male	Liver, kidney, and testicular changes	Waltschewa et al. 1972
$NiCl_2$	225 ppm; drinking water	4 mo	Rat, male (n = 10)	Decreased urine output; metabolic changes; decreased body weight	Clary 1975

$NiCl_2$	53 mg/kg/d; drinking water	24 wk	CD rat, female	Reduced number of offspring and surviving offspring	RTI 1988a,b
$NiSO_4$	0, 44, 108, and 150 mg/kg/d; drinking water	180 d	$B6C3F_1$ mouse, female	Renal tubular damage; decreased bone marrow cellularity; decreased organ weights; reduced water intake	Dieter et al. 1988
$NiSO_4$	6.9 mg/kg/d (m) or 7.6 mg/kg/d (f); drinking water	180 d	Rat	Renal (increased urine albumin)	Vyskocil et al. 1994
Not specified	5 ppm/d for life (0.41 mg/kg/d); drinking water	18 mo	Long Evans rat	Focal myocardial fibrosis (slight increase, 13.3%)	Schroeder et al. 1974
$NiSO_4$	0, 5, 50, 125 mg/kg/d; feed	2 y	Rat, 25 per gender per group	Decreased weight gain, decreased liver weights	Ambrose et al. 1976
$NiSO_4$	3, 29, 70 mg/kg/d; feed	2 y	Beagle dog, 3 per group	Low hematocrit; increased liver weights; polyurea; vomiting	Ambrose et al. 1976

Abbreviations: BUN, blood urea nitrogen; PCV, packed cell volume.

Spaceflight Effects

The physiologic and biochemical changes that occur during spaceflight (Nicogossian et al. 1994) might increase the sensitivity of astronauts to the toxic effects of nickel such as hematologic effects and reduction in water consumption. Reduced plasma volumes and changes in RBC formation (Nicogossian et al. 1994) were observed in crew members from the Skylab and Apollo missions and shuttle missions. Thus, dehydration has been a continued concern for long-duration space missions.

RATIONALE

The following paragraphs provide the rationales for the proposed guidelines for nickel in NASA spacecraft drinking water for 1, 10, 100, and 1,000 d (see Table 6-6 for guidelines set by other organizations). The values listed (see Table 6-7) were set based on acceptable concentrations (ACs) for each duration following the methods for developing spacecraft water exposure guidelines (SWEGs) documented by the National Research Council (NRC 2000). In general, we have applied a spaceflight factor of 3 for certain effects, such as hematologic effects, which are known to be exacerbated by microgravity.

Usually an intraspecies factor is not used because astronauts come from a healthy population, and there is no evidence of healthy people having excess susceptibility to nickel or similar compounds. Nevertheless, a review of the existing literature clearly shows that ingestion of nickel aggravates existing eczema in individuals known to be sensitive to nickel (nickel ACD). However, ingested nickel does not seem to sensitize normal individuals (Nielsen et al. 1999). Though astronauts are not specifically tested for nickel sensitivity, a detailed health questionnaire including questions concerning allergies would reveal such sensitivity. In order to account for any potential hypersitivity to nickel, a factor of 3 has been applied to calculated ACs to at least partially protect astronauts.

Several studies have clearly documented that the presence of food reduced absorption of nickel from the gastrointestinal tract. Sunderman et al. (1989) indicated that 40 times more nickel was absorbed when administered in water compared with administration by food. But, in this study, nickel was administered to subjects who had fasted for 12 h before the dose and fasted for 4 more hours after the intake. The recent work by Nielsen et al. (1999) clearly indicates that, in general, there will always be food in the stomach, and drinking water will be mixed with existing food. When one

TABLE 6-6 Drinking Water Levels for Nickel Set by Other Organizations

Organization	Standard	Level
EPA	MCL	Vacated
EPA	MCLG	Vacated
EPA	1-d HA (child)	1 mg/L
EPA	10-d HA (child)	1 mg/L
EPA	Long-term[a] HA (adult)	1.7 mg/L
EPA	Lifetime HA	0.1 mg/L
EPA	RfD	0.02 mg/kg/d
EPA	DWEL	0.6 mg/L
EPA	Cancer group	D[b,c]
EPA	Cancer risk	NA (10^{-4} cancer risk)
ACGIH[c,d]	Cancer group	Category A4 (soluble nickel salts); not classifiable as a carcinogen
ATSDR	MRL	None set[e]
FDA	Bottled water	0.1 mg/L

[a]The levels that will not cause any adverse carcinogenic effects up to 7 y (10%) of an individual's lifetime.
[b]Group D is considered not classifiable as a carcinogen in animals or humans.
[c]IARC has classified nickel and it's compounds as group 1 carcinogens; NTP (1996) has also concluded that nickel and its compounds can reasonably be anticipated to be carcinogenic.
[d]ACGIH's air standard for soluble nickel compounds is 0.1 mg/m^3 (under revision); OSHA's air standard is 1 mg/m^3 for soluble nickel compounds; and NIOSH's recommended exposure limit (REL) is 0.015 mg/m^3 for metal, soluble, insoluble, and inorganic nickel in air.
[e]ATSDR did not establish a minimal risk level (MRL) because if a value is calculated it will be lower than the mean daily dietary content of nickel and salts.
Abbreviations: ACGIH, American Conference of Governmental Industrial Hygienists; ATSDR, Agency for Toxic Substances and Disease Registry; EPA, U.S. Environmental Protection Agency; DWEL, drinking water equivalent level; MCL, maximum contaminant level; MCLG, maximum contaminant level goal (nonenforcable standard); NA, not applicable; RfD, reference dose.

considers water and food ingested on a continuous basis, as in 10 d, 100 d, or 1,000 d, the difference will be insignificant. If any difference exists, it will be masked by the wide variations in absorptions within a group.

TABLE 6-7 Spacecraft Water Exposure Guidelines for Nickel[a,b]

Duration	Concentrations (mg/L)	Toxicity End Point
1 d	1.7[c]	Myelopoietic system effects and decreased immune function response
10 d	1.7[c]	Myelopoietic system effects and decreased immune function response
100 d	1.7[c]	Myelopoietic system effects and decreased immune function response
1,000 d	0.3	Myelopoietic system effects and decreased immune function response

[a]Dieter et al. (1988) was used to derive all SWEGs.
[b]The values incorporate a safety factor of 3 to partially protect astronauts against the risk of an allergic response to nickel ingestion. These levels do not protect people who are already hypersensitive to nickel.
[c]The AC derived for 100 d was used for 1 d and 10 d without any time-extrapolation factors because of the importance of the effect of nickel on immunologic systems.

No factors were incorporated to account for differences in absorption in the calculation of ACs for water from feed-study data.

A few studies that show definite adverse effects but used only one dose and one time could not be used for AC derivation because a NOAEL could not be identified. For example, mild focal myocardial fibrosis was reported in rats exposed to nickel (form not specified) at 5 ppm in drinking water for 18 mo (Schroeder et al. 1974), but that study could not be used to derive an AC because only one dose was used and it was conducted in a metal-free environment, which might have increased the sensitivity of these animals. (The effect was seen only on 13.3% of animals.) Similarly, Waltschewa et al. (1972, as cited in EPA 1985) reported testicular changes in male rats after 120 d of ingestion of $NiSO_4$ at 25 mg/kg. That study was not used to set SWEGs because a definitive NOAEL could not be identified.

Haber et al. (1998) evaluated the data with benchmark dose (BMD) response modeling and the use of biologic significance in the selection of benchmark response. The authors used oral and inhalation exposure data from several studies to calculate the BMD for nickel. They determined a $BMDL_{10}$ for the oral exposure to nickel (defined by the authors as the lower bound on the dose at which increased probability of an abnormal response is equal to 10%) of 4-5 mg/kg/d on the basis of increased prenatal mortality and immunologic effects. It must be pointed out that in this paper the au-

thors neither derived a reference dose (RfD) nor discussed which uncertainty factors should be used for each of the end points. For modeling they considered the data from two subchronic studies: one by American Biogenics Corporation (1988) in which nickel chloride administered by gavage for 92 d resulted in decreased body weights in males, and another by Dieter et al. (1988) in which exposure of female mice to nickel sulfate in drinking water for 180 d resulted in a decreased lymphoproliferative response (immune system effect). They also modeled data from the chronic $NiSO_4$ dietary toxicity study of Ambrose et al. (1976) in which decreased body weight in female rats was noted after 2 y of nickel-spiked feeding and a two-generation reproduction toxicity study by Smith et al. (1993) in which increased number of litters with dead pups in the first generation was seen in rats exposed to $NiCl_2$ in drinking water. In that study the authors applied a hybrid modeling approach using the Weibull model and the power model. From the results, the authors pointed out that the differences observed in BMD obtained from different studies for the same or similar end points might be due to differences in the chemical form ($NiCl_2$ vs $NiSO_4$) and/or to the mode of oral exposure, such as oral gavages vs diet vs drinking water.

In a later publication, Haber et al. (2000) derived an RfD of 0.008 mg/kg/d for noncancer effects from soluble nickel salts ingested in addition to dietary nickel. In this derivation the authors evaluated data from five studies and analyzed them for deficiencies and strengths. The five studies are as follows: Vyskocil et al. (1994), in which increased albuminuria was reported in rats exposed to nickel via drinking water for 6 mo; Ambrose et al. (1976), in which rats fed nickel for 2 y showed decreased body weights; American Biogenics Corporation (1988), in which reduced body weights in males were observed when nickel was administered to rats via drinking water for 92 d; Dieter et al. (1988), in which mice that ingested $NiSO_4$ via drinking water for 180 d showed thymic atrophy and decreased thymus weights; and Smith et al. (1993), the two-generation reproductive study in which increased death of pups in the second generation was observed in rats exposed to $NiCl_2$ via drinking water at various dose levels. For RfD derivation the authors chose the decreased glomerular function, as evidenced by increased urinary albumin, as the most appropriate and sensitive end point (in the absence of any tubular damage, as measured by urinary beta-2-microglobulin, *N*-acetyl-beta-d-glucosaminidase, and lactate dehydrogenase) reported by Vyskocil et al. (1994). In that study, female rats were more responsive than male rats, and a LOAEL of 7.6 mg/kg/d was identified. A NOAEL was not available because of the single dose. Due to wide variability in controls and some outliers, the changes in male rats were not

statistically significant. The effects were seen at the end of 6 mo, not at the end of 3 mo. Using the LOAEL of 7.6 mg/kg/d from the Vyskocil et al. study for renal effects, and applying a modifying factor of 1,000 based on recommendations described in Dourson et al. (1996), the authors calculated an RfD as shown below. In short, the authors used a factor of 10 for human variability, a factor of 10 for interspecies extrapolation, and a combined factor of 10 for subchronic-to-chronic extrapolation, use of minimal LOAEL-to-NOAEL, and an insufficient database. Thus, the authors calculated the RfD as

$$7.6 \text{ mg/kg/d} \div 1{,}000 = 0.0076 \text{ mg/kg/d}.$$

Using EPA's nominal value of daily water consumption of 2 L/d for a 70-kg person, this value is equivalent to

$$0.0076 \text{ mg/kg/d} \times 70 \text{ kg} \div 2 \text{ L/d} = 0.266 \text{ mg/L (0.27 mg/L when rounded)}.$$

This is entirely from sources of ingestion other than food, because the concentration of nickel in the animal food was not known and was not adjusted in the LOAEL.

It is documented in several studies that exposure to nickel, either in feed or in water, resulted in decreased body-weight gains, in addition to changes in organ weights in some cases. These effects have not been used to set ACs because body- and organ-weight changes are not considered adverse effects. In the following AC calculations, a value of 2 L has been assumed for crew drinking water usage per person per day, instead of a total of 2.8 L (2 L for drinking and 0.8 L for food reconstitution), because the absorption of nickel from food has been reported to be very low.

Ingestion for 1 d

No acute animal toxicity studies at low doses were available for determining a 1-d AC for nickel in water. The short-term studies of Cronin et al. (1980) and Nielsen et al. (1990) on the worsening of hand eczema and erythema in 12 nickel-sensitive women who were challenged with a diet that included low doses of nickel could not be used because they were conducted in only nickel-sensitive subjects.

Sunderman et al. (1989) reported that 7 h after drinking water with a dose of $NiSO_4$ at 0.05 mg/kg, a human volunteer complained of transient

impaired vision for 2 h. This adverse effect or similar symptoms were not seen when the dose was reduced to 18 μg/kg in four other subjects (two men and two women). As these data are from only one subject, they could not be used. Sunderman et al.'s (1988) data on the workers who drank nickel-contaminated water from a water fountain (as both $NiCl_2$ and $NiSO_4$ and also including boric acid) can be used after applying a factor for estimating a LOAEL. Because the data are from 30 subjects who were affected, and the concentrations were determined to be 7.1-35.7 mg/kg (the intake amount), these data could be useful in deriving a 1-d AC. Thus, a 1-d AC can be calculated for neurologic effects (giddiness, weariness, and headache) as well as for transient lowered hematocrit (hematologic effects) and transient serum bilurubin (hepatic effects). Using the LOAEL of 7.1 mg/kg/d and factors of 10 and 3, for LOAEL-to-NOAEL extrapolation and an additional safety factor for nickel sensitivity, respectively, the 1-d AC for a 70-kg person drinking 2 L of water per day is

$$(7.1 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2 \text{ L/d} \times 3) = 8 \text{ mg/L}.$$

Ingestion for 10 d

Whanger (1973) fed weanling rats (OSU brown rats, gender not known, 35 d old, body weights 90 g, six rats per group) a diet containing various levels of nickel acetate (10, 50, or 100 mg/kg/d) for 6 wk. Basal ration contained nickel at 0.21 ppm. Hemoglobin and packed cell volume (PCV) were found to be decreased significantly. These data were used to calculate the 10-d AC using both the NOAEL/LOAEL method and also using the BMD method (using the lower 95% confidence limit on the benchmark dose corresponding to 1% risk, $BMDL_{01}$). BMDs were obtained using the EPA's BMD software, version 1.2, and the polynomial model with the power model on "continuous data." The standard deviations required for processing continous data were derived from the least significant difference (LSD) included in the study by the author. The values from these models were averaged. A summary of these values are in Table 6-8. ACs developed using data from Whanger (1973) appear in Table 6-9. For those ACs, a species extrapolation factor of 10 was used, along with a factor of 3 to account for nickel sensitivity. A spaceflight factor of 3 was used in calculations for the PCV and hemoglobin end points. No time-extrapolation factor was used. All ACs assume 70-kg body weight and 2 L of drinking water per day.

TABLE 6-8 BMD and BMDL Values Calculated from the Whanger (1973) Study (in mg/kg/d)

Parameter	BMD_{01}	$BMDL_{01}$	BMD_{10}	$BMDL_{10}$	LOAEL	NOAEL
PCV	37	11	41	19	100	50
Hemoglobin	27	14.5	47.5	35	100	50
ALP	24	18	31	23	50	10

Abbreviations: ALP, plasma alkaline phosphatase; PCV, packed cell volume.

Ingestion for 100 d

Waltschewa et al. (1972, as cited in EPA 1985) reported that oral intubation of $NiSO_4$ to male rats for 120 d resulted in degenerative changes in liver and kidneys, but their data could not be used because only one daily dose of 25 mg/kg was given, and the initial time when the effects were seen was not indicated. A definite LOAEL or NOAEL is not clear. Similarly, the data on the effects of nickel on spermatozoa and interstitial cell proliferation could not be used. The Sobti and Gill (1989) report on increased abnormalities of the heads of spermatozoa observed in mice 5 wk after treatment with a single oral dose of $NiSO_4$, $NiCl_2$, or $Ni(NO_3)_2$ at 28, 43, and 23 mg/kg, respectively, could not be used because of a lack of experimental details.

100-d AC Based on American Biogenics Corporation (1988)

In the American Biogenics 90-d gavage study, male and female rats (30 per gender) were administered daily doses of $NiCl_2$ at 0, 5, 35, and 100 mg/kg/d. Decreased organ weights, changes in hematologic parameters (increases in white blood cells and platelets), and CNS-related symptoms such as ataxia, lethargy, and altered breathing were some of the clinical signs of toxicity reported. A dose of 5 mg/kg/d was identified as a NOAEL. A species extrapolation factor of 10, a spaceflight factor of 3, and a safety factor of 3 to account for nickel sensitivity were applied to the AC calculation. To extrapolate the 90-d data to the 100-d guideline, a time-extrapolation factor of 100/90 also was applied. The calculation assumes a 70-kg person drinking 2 L of water per day. Thus, the 100-d AC calculated from the American Biogenics Corporation (1988) study is

$$(5 \text{ mg/kg/d} \times 70 \text{ kg}) \div [10 \times (100/90) \times 2 \text{ L/d} \times 3 \times 3] = 1.8 \text{ mg/L}.$$

TABLE 6-9 Summary of ACs from Whanger (1973) Calculated Using NOAELs and BMDs

	AC (mg/L)	
Parameter	NOAEL	BMD
ALP	12	21
PCV	20	4
Hemoglobin	20	6

Abbreviations: AC, acceptable concentration; ALP, plasma alkaline phosphatase; PCV, packed cell volume.

100-d AC Based on the Dieter et al. (1988) Study

Female B6C3F$_1$ mice exposed to nickel as $NiSO_4$ at 0, 44, 108, and 150 mg/kg/d in drinking water for 180 d showed a significant decline in water consumption (32-57%) in the 108-mg/kg/d and 150-mg/kg/d dose groups. Significant decreases in spleen and bone marrow cellularity, decreased bone marrow proliferative response, dose-related decreases in the spleen lymphoproliferative response to a B-cell mitogen, and treatment-related increases in nephrosis in the 108-mg/kg/d and 150-mg/kg/d dose groups were the other significant effects. A dose of 44 mg/kg/d was considered a LOAEL from this study for effects on bone marrow cellularity and mild renal tubular damage. A summary of calculated ACs is presented in Table 6-10. For the AC calculations, a factor of 10 was used for extrapolation from a LOAEL to a NOAEL; a factor of 10 was applied for species extrapolation; a spaceflight factor of 3 was included to account for effects on bone marrow; and a safety factor of 3 was used to account for potential allergic reactions to nickel.

Thymic atrophy accompanied by decrease in lymphocyte-rich thymic cortex was judged moderate to severe. Histopathologic observations were minor and were considered background incidences for the species of this age (Dieter et al. 1988), and hence, ACs were not calculated for some parameters. The BMD analysis and LOAELs and NOAELs for those parameters are available in Table 6-11.

100-d AC Using the Whanger (1973) Study

The data from the 6-wk nickel diet study by Whanger (1973), described

TABLE 6-10 100-d ACs Based on NOAELs and BMDLs Calculated Using Immunotoxicity and Myelotoxicity Data from Dieter et al. (1988)

				ACs (mg/L)	
Parameter	$BMDL_{01}$ (mg/kg)	NOAEL (mg/kg)	LOAEL (mg/kg)	$BMDL_{01}$	LOAEL/ NOAEL Method
Spleen cellularity[a]	63.8	108	150	24.8	42
Lymphoproliferative response to LPS[a]	7.9	NA	44	3.0	1.7
Bone marrow cellularity[b]	9.8	44	108	11.4	1.7
Granulocyte macrophage CFUs[b]	12	NA	44	4	1.7
Stem cell proliferative response CFUs[b]	7.6	44	108	3.0	17

[a]Immune function responses were evaluated by spleen cellularity and lympho-proliferative response to T-cell and B-cell mitogens; in this case only B-cell mitogen LPS data is included.
[b]Myelotoxic responses are represented by bone marrow cellularity and bone marrow proliferative cell response (granulocyte macrophage and stem cell proliferative response CFUs).
Abbreviations: AC, acceptable concentration; $BMDL_{01}$, lower 95% confidence limit on the benchmark dose corresponding to a 1% risk; CFU, colony forming unit; LPS, lipopolysaccharide; NA, not available.

earlier in the document in the 10-d AC section, was also used for deriving a 100-d AC by using a time-extrapolation factor to apply results from 42 d to 100 d. BMD analysis data and NOAELs and LOAELs are available

TABLE 6-11 Other Parameters from Dieter et al. (1988) (mg/kg)

Parameter	BMD_{10}	$BMDL_{10}$	BMD_{01}	$BMDL_{01}$	Ratio of BMD_{10} to BMD_{01}	LOAEL	NOAEL
Thymic atrophy	87	34	27	11	3.22	44	NA
Thymus relative organ weight	21.4	13.3	5.97	3.8	3.58	44	NA
Nephrosis	42	29	13	8.9	3.23	108	44

Abbreviation: NA, not available.

TABLE 6-12 Summary of 100-d ACs Calculated from NOAEL and $BMDL_{01}$ Data from the Whanger (1973) Study

	ACs (mg/L)	
Parameter	NOAEL[a]	$BMDL_{01}$
PCV	8.0	1.8
Hemoglobin	8.0	2.5
ALP	5.0	8.0

[a]Because the $BMDL_{01}$ values are much lower than the experimentally observed NOAELs, ACs calculated based on the NOAEL are the values of choice.
Abbreviations: AC, acceptable concentration; ALP, plasma alkaline phosphatase; $BMDL_{01}$, lower 95% confidence limit on the benchmark dose corresponding to a 1% risk; PCV, packed cell volume.

in Table 6-8. The results of the 100-d AC calculations are reported in Table 6-12 (above). Factors of 10, 3, and 100/42 were included for species extrapolation, sensitivity to nickel, and time extrapolation, respectively. A spaceflight factor of 3 was applied in the calculations for hematologic effects (i.e., PCV and hemoglobin only). The calculations assumed a 70-kg person ingesting 2 L of water per day.

100-d AC Using Obone et al. (1999) Data

Adult male Sprague-Dawley rats were given nickel as $NiSO_4$ (hexahydrate) at 0, 44.7, 111.75, or 223.5 mg/L (0, 5, 12.5, or 25 mg/kg/d) in their drinking water for 13 wk. Several biochemical parameters were measured 24 h after the final dose. Total plasma proteins, plasma albumin and globulins, and plasma glutamic pyruvic transaminase activity were all significantly decreased at 25 mg/kg/d. The significance of these reductions is not clear; therefore, the results for these end points were not used to derive an AC. A dose-dependent and significant decrease in urine volume (75%) and an increase in BUN (80%) were observed in the highest-dose group. There were no differences in BUN in the other dose groups, and there was a steep increase only at the highest dose, which was only 2 times the next lower dose. It should be pointed out that urine *N*-acetyl-beta-d-glucosaminidase, gamma-glutamyltranspeptidase, and urinary protein levels were not affected by nickel ingestion. Lymphocyte subpopulations (T- and B-cells) were suppressed at the 25-mg/kg/d dose. Although the authors concluded that the immune system was a sensitive target, followed by kidneys, the

TABLE 6-13 100-d ACs For Two Parameters from the Obone et al. (1999) Study

Parameter	$BMDL_{01}$ (mg/kg)	NOAEL (mg/kg)	LOAEL (mg/kg)	ACs (mg/L)	
				NOAEL	$BMDL_{01}$
BUN	10.5	12.5	25	13	11
Urine volume[a]	1.12	5	12.5	6.5	1.45

[a]The urine volumes in control rats were very high for a 24-h urine collection. The urine may have been mixed with water from the drinking-water bottle. Because that will affect the BMD curve fit, and due to lack of confidence in the values, the ACs for this end point are not posted in Table 6-15.
Abbreviations: AC, acceptable concentration; $BMDL_{01}$, lower 95% confidence limit on the benchmark dose corresponding to a 1% risk.; BUN, blood urea nitrogen.

dose-response effects on thymocyte subpopulations and spleen lymphocyte subpopulations were not clear. There was a significant decrease in the urine volume compared with controls (36.7 ± 0.76 mL/d for controls vs 9.79 ± 0.7 mL/d at the highest dose). The urine volume in controls was unusually high. The urine samples might have been diluted with water from the drinking-water bottle. Even though the authors measured water consumption weekly, no data was presented or discussed for any change. Only data on BUN and urine volume was used to derive an AC.

One-hundred-day ACs (see Table 6-13, above) were calculated using factors of 10 for species extrapolation and 3 for nickel sensitivity. A time extrapolation factor for 90 d to 100 d also was applied. The calculations assumed a 70-kg person ingesting 2 L of water per day.

100-d AC for Nephrotoxicity Using Vyskocil et al. (1994) Data

Only one dose of nickel (as nickel sulfate) at 6.9 mg/kg/d for males and at 7.6 mg/kg/d for females in drinking water was tested. The measurements were made at 3 mo and at 6 mo, and the 6-mo data show significant changes in urinary albumin excretion in and increased kidney weight in the females (females being more sensitive), so the dose in females can be considered a LOAEL for a 6-mo exposure and a NOAEL for 3 mo. Because there were two durations but only one dose, a BMD calculation was not possible. Using the NOAEL of 7.6 mg/kg/d, and applying factors of 10, 3, and 1.11

for species extrapolation, nickel sensitivity, and time extrapolation from 90 d to 100 d, respectively, the 100-d AC for potential nephrotoxic effects (increased albuminuria) was calculated as follows:

$$(7.6 \text{ mg/kg/d} \times 70 \text{ kg}) \div [10 \times 2 \text{ L/d} \times 3 \times (100/90)] = 8 \text{ mg/L}.$$

As usual, the calculation assumes a 70-kg person ingesting 2 L of water per day.

100-d AC for Behavioral Effects

Adult male rats were fed $NiCl_2$ at 0, 10, or 20 mg/kg/d via a 10 g daily food ration. Following 14 d of exposure, animals were trained over a period of 61 d to lever-press for food on a VI-2 operant training schedule while continuing to ingest the indicated daily doses of nickel (Nation et al. 1985). Rats in the 20-mg/kg/d group lever-pressed at a significantly lower rate than those in the 10-mg/kg/d and control groups, thus indicating behavioral effects. A LOAEL of 20 mg/kg/d and a NOAEL of 10 mg/kg/d were identified. As the authors presented the data in a graphic form, numerical data for BMD calculation were not available. Applying a factor of 10 for species extrapolation, a factor of 3 for nickel sensitivity, and a factor of 1.333 for time extrapolation from 75 d to 100 d, the 100-d AC for behavioral effect was calculated as follows:

$$(10 \text{ mg/kg/d} \times 70 \text{ kg}) \div (10 \times 2 \text{ L/d} \times 3 \times 1.333) = 9 \text{ mg/L}.$$

Ingestion for 1,000 d

A 13% increase in focal myocardial fibrosis was observed in a life-term study of rats ingesting nickel at 5 ppm as $NiCl_2$ in drinking water for 18 mo (Schroeder et al. 1974). However, the experiments were done in a metal-free environment, which may have made the animals more sensitive. These data could not be used due to the lack of dose- and time-response data.

Two 2-y drinking water studies were considered for calculating the 1,000-d ACs. They are two-generation and three-generation reproductive and developmental studies using $NiCl_2$ and $NiSO_4$ in drinking water, respectively. Microscopic examination of ovaries and testes of rats and dogs that

received $NiSO_4$ and $NiCl_2$ by the oral route for 2 y did not indicate any abnormality (Ambrose et al. 1976; American Biogenics Corporation 1988). NASA has not considered using developmental toxicity data for determining SWEGs.

The only other long-term study, a 2-y feeding study in rats and dogs, is that of Ambrose et al. (1976). Rats of both genders (25 per gender per group) were given $NiSO_4$ (hexahydrate) at 0, 100, 1,000, and 2,500 ppm (0, 5, 50, and 125 mg/kg/d). Although significant depression in growth, decreases in body weights, and decreases in liver- and heart-to-body weight ratios were noted at the 1,000-ppm and 2,500-ppm doses, no such effects were noted at the 100-ppm dose. A parallel study in dogs indicated that at higher doses hemoglobin and hematocrit values tended to be lower, and the dogs showed lung and bone marrow lesions. NASA has not used the dog data, because nickel is not bound to albumin in dogs the way it is in humans and rodents. The pharmacokinetics, and hence the toxicologic mechanisms, may be different in dogs.

1,000-d ACs Based on Dieter et al.'s (1988) 180-d Study

Dieter et al.'s (1988) 180-d drinking water study was used after applying a time factor. In that study, female $B6C3F_1$ mice were given $NiSO_4$ at 0, 44, 108, and 150 mg/kg/d in drinking water for 180 d. Significant changes in the immune system, decreases in water consumption, decreases in bone marrow cellularity, mild renal tubular damage, and thymic atrophy were significant toxic effects noted at the two highest doses. A 1,000-d AC was calculated using a time-extrapolation factor of 5.555 (1,000/180). Factors of 10 and 3 were applied for species extrapolation and nickel sensitivity, respectively. A spaceflight factor of 3 was added to the calculations for hematologic end points. A LOAEL-to-NOAEL factor of 10 was included in calculations that used the LOAEL. The calculations assumed a 70-kg person ingesting 2 L of water per day. (See Table 6-15 for ACs.)

The most sensitive parameters seem to be the lymphoproliferative response and the granulocyte macrophage colony forming response—both end points give a 1,000-d AC of 0.3 mg/L.

TABLE 6-14 Acceptable Concentrations (ACs)

		Factors Used					ACs (mg/L)			
Toxicity End Point	Exposure Data and Reference	LOAEL to NOAEL	Species	Exposure Time	Space-flight	Nickel Sensitivity[a]	1 d	10 d	100 d	1,000 d
Neurological (giddiness, weariness, and headache);	Human, LOAEL = 7.1 mg/kg/d; Sunderman et al. 1989	10	1	1	1	3	8			
Decreased PCV	$BMDL_{01}$ = 11 mg/kg/d; Whanger 1988	1	10	1	3	3		4		
Decreased proliferative response (GM-CFU and SC-CFU)	LOAEL = 44 mg/kg/d; Dieter et al. 1988	10	10	1	3	3	1.7[b]	1.7[b]	1.7	
Neurological effects, hematological effects	Rat, NOAEL = 5 mg/kg/d; American Biogenics Corporation 1988	1	10	100/90	3	3			1.8	
Decreased PCV	$BMDL_{01}$ = 11 mg/kg/d; Whanger et al. 1988	1	10	100/42	3	3			1.8	
Increased BUN	$BMDL_{01}$ = 10.5 mg/kg/d; Obone et al. 1999	1	10	100/90	1	3			11	

(Continued)

TABLE 6-14 Continued

Toxicity End Point	Exposure Data and Reference	Factors Used					ACs (mg/L)			
		LOAEL to NOAEL	Species	Exposure Time	Space-flight	Nickel Sensitivity[a]	1 d	10 d	100 d	1,000 d
Renal effects (albuminuria)	NOAEL = 7.6 mg/kg/d; Vyskocil et al. 1994	1	10	100/90	1	3			8	
Behavioral effects	NOAEL = 10 mg/kg/d; Nation et al. 1985	1	10	100/75	1	3			9	
Effects on immunological and myelopoietic systems	Mouse, LOAEL = 44 mg/kg/d; Dieter et al. 1988	10	10	1,000/180	3	3				0.3
SWEGs							1.7	1.7	1.7	0.3

[a]This column incorporates a factor of 3 to partially protect individuals who might already be allergic to nickel or have nickel ACD.
[b]Because of the risk of adverse effects on the immunological system, the derived 100-d AC will be adopted for 1 d and 10 d.
Abbreviations: BUN, blood urea nitrogen; GM-CFU, granulocyte macrophage cell forming units; PCV, packed cell volume; SC-CFU, stem cell proliferative response cell forming units.

TABLE 6-15 1,000-d ACs Calculated for Various End Points Using Data from the Dieter et al. (1988) Study

Parameter	$BMDL_{01}$ (mg/kg)	NOAEL (mg/kg)	LOAEL (mg/kg)	ACs (mg/L)	
				$BMDL_{01}$	NOAEL
Spleen cellularity	63.8	108	150	4.5	7.5
Lymphoproliferative response to LPS	7.9	NA	44	0.55	0.3
Bone marrow cellularity	9.8	44	108	0.7	3.08
Granulocyte macrophage CFUs	12	NA	44	0.8	0.3
Stem cell proliferation CFUs	7.6	44	108	0.5	3

Abbreviations: AC, acceptable concentration; $BMDL_{01}$, the lower 95% confidence limit on the benchmark dose corresponding to a 1% risk; CFU, colony forming unit; LPS, lipopolysaccharide.

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7 Phenol

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PHYSICAL AND CHEMICAL PROPERTIES

Phenol is a colorless to white solid when pure; it is hygroscopic. Phenol has a sickening-sweet and tarry characteristic odor. Humans can detect phenol at about 40 parts per billion (ppb) in the air and 8 milligrams per liter (mg/L) in water (Amoore and Hautala 1983). The physicochemical properties of pure phenol are listed in Table 7-1 (ACGIH 1991).

OCCURRENCE AND USE

Phenol is used mainly in the manufacture of phenolic resins and a variety of chemicals and drugs (ACGIH 1991). It is also used as a disinfectant, slimicide, deodorant, and sanitizer. Dilute solutions of phenol (1-2%) are used medicinally in antipruritic skin preparations (Remington 1985, p. 1315). Phenol has been identified in automobile exhaust and in cigarette smoke (ATSDR 1998). In the International Space Station (ISS), phenol was detected at 15 micrograms (μg)/L in a regenerated potable water sample (SVO-ZV) collected aboard on Jan 10, 2001, during Expedition 1 (ISS-5A); phenol was not detected (<4 μg/L) in the samples collected at various times (September 2001 to November 2002) during Expeditions 2-5 (Plumlee et al. 2002, 2003).

TABLE 7-1 Physical and Chemical Properties of Phenol

Formula	C_6H_5OH
CAS registry no.	108-95-2
Synonyms	Carbolic acid, phenic acid, phenylic acid, phenyl hydroxide, hydroxybenzene, oxybenzene
Molecular weight	94.11
Boiling point	182°C
Melting point	43°C
Vapor pressure	0.35 mmHg at 25°C
Solubility in water	1 g/15 mL
pKa	10 at 25°C

PHARMACOKINETICS AND METABOLISM

Absorption and Distribution

Phenol is rapidly absorbed by all routes of exposure (Deichmann and Keplinger 1981), as illustrated by the observations that symptoms of acute toxicity occurred within minutes after phenol administration (Pullin et al. 1978) and that a man died 10 minutes (min) after he spilled phenol over his body (Gottlieb and Storey 1936, as cited in NIOSH 1976). Humphrey et al. (1980) reported the half-life of absorption to be 5.5 min in rats that were injected with doses of phenol (2.5 mg per kilogram [kg] or 25 mg/kg labeled with a radiotracer) directly into the small intestines. A similar study by Kao et al. (1979) showed that 2 hours (h) after injection into the small intestine, about 77% of the dose was recovered in the urine. When radiolabeled phenol (0.01 mg/kg) was given to three human subjects in food or drink, about 90% of the radioactivity was recovered in the urine, which was collected for 24 h (Capel et al. 1972).

When rats were each given a large oral dose of phenol (207 mg/kg, one-half an LD_{50} [dose lethal to 50% of subjects]) containing a radiotracer, liver was found to have the highest specific radioactivity of all the tissues examined at every sampling time interval (0.5-16 h post-treatment), and it accounted for about 42% (range 29-56%) of the dose (Liao and Oehme 1981). Only 0.3% of the administered dose remained in tissues 16 h after dosing. Diechmann and Witherup (1944) showed that in rabbits that died 15 min after an oral phenol dose (500 mg/kg), the total phenol concentration

in liver was twice that in the blood. For those that died or were killed after 82 min, the concentrations in the liver were less than those in the blood. These observations indicate that the liver takes up a large fraction of phenol when the compound is administered in large doses.

When small doses of phenol are given to animals, the liver does not take up more phenol (per unit tissue) or contain more phenol metabolites than other tissues. Power et al. (1974) reported that in rats treated with radiolabeled phenol (10 μCi orally or peritoneally), whole-body autoradiograms showed that radioisotope levels in the liver did not, at any time, exceed those in the blood. The authors further showed that neither phenol nor its metabolites were concentrated in the liver (see "Metabolism" section below for more information).

In an inhalation study in which human test subjects were exposed to phenol at 6-20 mg per cubic meter (m^3) through a gas mask, the lung uptake of phenol was 60-80%, as determined by concentration differences between inhaled and expired air (Piotrowski 1971).

Metabolism

The metabolic fate of phenol was studied in three human test subjects, each of whom was given a single oral dose (radiolabeled phenol at 0.01 mg/kg); 77% of the radioactivity was recovered in the urine as phenyl sulfate and 16% was recovered in the urine as phenyl glucuronide. A total of about 1% was identified as quinol (1,4-dihydroxybenzene) sulfate or glucuronide (Capel et al. 1972). In the same report, Capel et al. also documented that phenol conjugates were the major metabolic products in 18 animal species treated with phenol orally or intraperitoneally (25 mg/kg). Using liver preparations, Campbell and Van Loon (1987) and Bock et al. (1988) showed that phenol was conjugated with sulfate by cytosolic phenol sulfotransferases or with glucuronic acid by glucuronyltransferases. These results revealed that, if glucuronic acid and sulfate are not depleted, and the conjugating enzymes are not saturated, little phenol is metabolized to quinol or other P-450 metabolic products.

When isolated mouse livers were perfused with phenol, hepatic effluent was found to contain phenol, phenyl glucuronide, phenyl sulfate, hydroquinone, and hydroquinone glucuronide (Hoffmann et al. 1999). When mouse liver microsomal preparation (free of phase II conjugation enzymes) was used, phenol was found to be metabolized, presumably by P-450, to hydroquinone (87.5%), catechol (5%), and trihydroxybenzene (1.2%) (Scholosser et al. 1993). The same authors also found that liver microsomal

preparations from mice metabolized about twice as much phenol (chiefly to hydroquinone) as those from rats.

Like phenol, dihydroxybenzenes (catechols), which potentially are P-450 metabolic products of phenol, are conjugated by sulfotransferases or glucuronyltransferases. The processes of conjugating phenol and dihydroxybenzenes with sulfate or glucuronic acid are metabolically competitive. The relative amounts of sulfate and glucuronide conjugates depend on the abundance of sulfate, glucuronic acid, and the conjugation enzymes as well as the kinetic parameters of the two in a given animal species. Because the conjugates are much more water-soluble than the parent compound, conjugation by either pathway enhances the elimination and excretion of phenol.

The role of liver in phenol metabolism in animals given low levels of phenol was investigated. Using isolated and washed rat gut preparations (free of debris), Powell et al. (1974) perfused the gut with physiologic buffer containing 10 μCi of ^{14}C-labeled phenol with or without 10 mg of unlabeled phenol. After 2 h of perfusion, 50-78% of the phenol was recovered in the serosal medium. At the end of the experiment, all phenol recovered from both serosal and mucosal media essentially was in the form of conjugates (5% phenyl sulfate and 95% phenyl glucuronide). No unchanged phenol was detected. These results showed that the gut was capable of metabolizing phenol and that all of the phenol was conjugated.

The role of the gut in phenol metabolism was further investigated. Powell demonstrated that only phenol conjugates were found in the plasma of blood collected from the portal vein of rats whose intestines were perfused in situ with phenol. Their postulation that the liver does not play a major role in metabolism of low doses of phenol was further supported by examination of the extent of phenol conjugation in hepatectomized rats. Radiolabeled phenol (5 mg/kg or 10 mg/kg) was given by intravenous injection to intact control rats and to test rats whose livers, spleens, and intestines were removed. Recovery of radioactivity from the urine of test and control animals over 3 h was essentially the same. Urine samples from both groups of rats contained phenol conjugates; the test rats had a higher proportion of phenol glucuronide in their urine than did control rats. From the results of these three experiments, Powell et al. (1974) concluded that liver is not essential in phenol detoxification and that phenol ingested in the diet is absorbed into the bloodstream from the gut essentially as phenol conjugates. This observation is consistent with the findings of Casidy and Houston (1984), who studied in vivo capacity of hepatic- and extrahepatic-enzyme phenol conjugation over a 35-fold dose range; the capacity of the intestinal conjugating enzymes was found to be remarkably high, whereas that of hepatic enzymes was readily saturable. At low doses of phenol (less

than 1 mg/kg), the capacity of intestinal and hepatic conjugation was comparable, but at higher doses (greater than 5 mg/kg), the capacity of intestinal enzymes far exceeded that of enzymes in the liver.

Elimination

In a study of three human subjects who ingested single doses of ^{14}C-labeled phenol, phenyl sulfate and glucuronide accounted for a total of 90% of the urinary radioactivity. A trace amount (<1%) of ^{14}C was eliminated as sulfate or glucuronide conjugates of 1,4-dihydroxybenzene (Capel et al. 1972). Similar results were observed in two female rhesus monkeys given single oral doses of ^{14}C-labeled phenol (50 mg/kg, 10 μCi per animal); the recovery of radioactivity in urinary phenyl sulfate and phenyl glucuronide was approximately 65% and 35%, respectively. In contrast, squirrel and capuchin monkeys eliminated phenol mostly as phenyl glucuronide, with phenyl sulfate as a minor metabolite (Capel et al. 1972). When ^{14}C-labeled phenol (63.5 nanomoles [nmol]) was administered orally to rats, Hughes and Hall (1995) observed that less than 1% of the radioactivity remained in the body 72 h after the administration; the amounts in the liver, muscle, skin, fat, and blood were 0.2%, 0.08%, 0.07%, 0.02%, and 0.02% of the administered dose, respectively. Phenyl sulfate was found to be the major metabolite in urine.

In an inhalation exposure study, eight human subjects were exposed (through a face mask) to phenol at 6-20 mg/m^3 for 8 h (including two 0.5-h breaks), and the total pulmonary absorption of phenol was estimated from inspired and expired air (Piotrowski 1971). The recovery of phenol (phenol and metabolites) in urine collected for 24 h averaged 100% (84% to 114%). The concentrations of phenol in the urine samples reached a peak value 0.5 h after the exposure ended. The concentration then decayed exponentially, and urinary phenol concentrations returned to pre-exposure levels within 16 h after the exposure ended. Excretion followed first-order kinetics with an elimination half-life of 3.5 h.

TOXICITY SUMMARY

Phenol is rapidly absorbed into the body by all routes of exposure. It is moderately toxic at high bolus doses, but is low in toxicity when doses are administered gradually and do not overwhelm detoxification by conju-

gation. When phenol is ingested in drinking water, a route that allows gradual and steady intake of phenol and, subsequently, detoxification in the gut, the toxicity is relatively low. When a large oral dose of phenol is ingested, some of the absorbed phenol presumably enters the blood without being conjugated in the gut. Casidy and Houston (1984) showed that hepatic conjugating enzymes were readily saturable. Phenol, if not conjugated, could cause a spectrum of neurologic symptoms, such as twitching, tremors, lethargy, and convulsions, and histopathologic changes in liver, kidneys, spleen, thymus, and other organs. The histopathology is likely to due to the P-450 metabolites of phenol (such as hydroquinone).

Acute (≤1 d) and Short-Term Exposures (2-10 d)

Human Studies

Ingestion of phenol has been documented in numerous reports of suicide or attempted suicide. Stajdubar-Caric (1968) reported that a woman died within an hour after ingesting 10-20 g of phenol. On the basis of this information, and assuming that 10 g of phenol was ingested and the woman weighed 70 kg, Bruce et al. (1987) estimated that the dose ingested was 140 mg/kg.

Gottlieb and Storey (1936, as cited in NIOSH 1976) reported a case of a 32-year-old man who spilled a solution of phenol over his scalp, face, neck, shoulders, and back; the victim died in 10 min. The phenol caused coagulation necrosis of the skin and congestion of the lungs, liver, spleen, and kidneys.

Cardiac arrhythmias were reported in 39% (21/54) of patients who underwent chemical exfoliation of the face and neck simultaneously and in 22% (22/100) of patients who had their faces and necks treated 24 h apart; the preparation contained approximately 50% phenol (Gross 1984). Information about the total amount of phenol applied to each patient was not provided. Phenol concentrations in serum of blood collected at unspecified times after application of the exfoliator ranged from 4.4 mg/L to 323 mg/L. If one is to assume that the average concentration of phenol in whole-body tissue (whole-body - bone = 70 kg - 12.3 kg = 57.7 kg) was the same as that in the serum, the patients received a dose of 3.6-266 mg/kg per application.

When small doses of phenol are applied to skin, no overt toxicity is observed. Ruedeman and Dechmann (1953) observed no clinical symptoms in 20 adult humans who received one to four applications, each containing

1 g of phenol in 50 g calamine lotion or in 21 g camphor-liquid petrolatum. The applications, covering over 75% of the skin, were separated by periods as short as 90 min or as long as 3 weeks (wk). Thus, a subject weighing 70 kg would receive 14.3 mg/kg per application, assuming total absorption.

A retrospective cohort study was conducted in 1,352 Korean households exposed to phenol in drinking water from a contaminated reservoir. On March 13, 1991, an industrial plant spilled 30 tons of phenol, contaminating a river that fed water to a reservoir. Significantly more phenol-associated symptoms were reported by the exposed individuals compared with symptoms reported by the residents of a nearby unexposed area (39.6% vs 9.4%) (Kim et al. 1994). The symptoms were sore throat, gastrointestinal illness (such as nausea, vomiting, diarrhea, or abdominal pain), dark urine, and skin rash. During the accident, more people in the exposed group also experienced a peculiar taste or odor (92% vs 34.3% in the control group). The accident was not reported to the local government, and the Korean water authorities continued water chlorination until consumers reported that their water had a bad taste. Analysis of water samples collected on March 16, 17, and 18 showed that the samples had phenol concentrations of 0.05, 0.05, and <0.01 mg/L, respectively. On March 19, the concentration of chlorophenols in tap water was 0.085 mg/L. The authors presumed that by the time phenol concentrations in the water were measured, the peak phenol concentration had already passed. The authors pointed out that to humans, the tastes and odors of some chlorophenols are 100 to 1,000 times stronger than those of phenol.

Phenol is the active ingredient in Cepastat lozenges. Regular and extra-strength Cepastats contain 14.5 mg and 29 mg per lozenge, respectively (PDR 1997). Cepastat lozenges can be taken once every 2 h, not to exceed 300 mg or 10 lozenges per day, according to SmithKline Beecham (Pittsburgh, PA). Thus, an adult taking the maximum allowable number of Cepastat lozenges would consume about 145-290 mg of phenol a day.

Animal Studies

Flickinger (1976) estimated that for rats, the oral LD_{50} of phenol would be 650 mg/kg. Deichmann and Witherup (1944) reported an LD_{50} of 530-540 mg/kg for Wistar rats that were given an aqueous solution with phenol at 10% or less; when a 20% solution was given, the LD_{50} was 340 mg/kg.

The acute and subacute toxicities of phenol were evaluated in groups of F-344 rats (eight rats per group). The rats were given phenol orally in

single doses at 0, 12, 40, 120, or 224 mg/kg or in doses of 0, 4, 12, 40, or 120 mg/kg daily for 14 consecutive days (Berman et al. 1995). In the 1-d study, two rats of the 224-mg/kg group died, and four of the remaining animals had necrosis or atrophy of the spleen, thymus, or kidneys. The incidences of hepatic necrosis in the 0-, 12-, 40-, and 120-mg/kg groups were 0/8, 0/8, 1/7, and 2/6, respectively. In the 14-d study, all eight rats in the 120-mg/kg group died. The incidences of necrosis or atrophy of spleen or thymus in the 0-, 4-, 12-, and 40-mg/kg groups were 0/8, 0/8, 1/8, and 2/8, respectively. Renal lesions were seen in three rats in the 40-mg/kg group, whereas controls had no renal lesions.

At sublethal toxic doses, phenol given intraperitoneally produced myoclonus or myoclonic convulsions characterized by short-last muscular jerks with no evidence of prolonged tetanic activation of the muscles (Angel and Rogers 1972). The time to the start of the response was 2 min. The temporal course of the convulsive effect was exponential decay. The CD_{50} (convulsive dose) of phenol in mice was 1.04 millimoles (mmol)/kg (97.9 mg/kg). The CD_{50}s for some of the possible metabolites of phenol were also determined. For catechol, resorcinol, and quinol (1,2-, 1,3-, and 1,4-dihydroxybenzene), the CD_{50}s were found to be 0.92, 0.90, and 0.38 mmol/kg, respectively. Liao and Oehme (1981) also observed tremors of muscles around the eyes, followed by convulsion and coma, in rats dosed with phenol at 207 mg/kg (half of the oral LD_{50} dose).

Central nervous system (CNS) symptoms were also seen after dermal application of molten (pure) phenol at 500 mg/kg to 35% to 40% of the body surface of three pigs (Pullin et al. 1978). Within 5 min after the phenol application, excessive salivation, nasal discharge, respiratory distress, twitching, and tremors were observed. These signs were immediately followed by lethargy, cyanosis, convulsion, and coma; death (two pigs) occurred about 95 min after the exposure.

Subchronic (11-100 d) and Chronic Exposures (>100 d)

Human Studies

An accidental spill of 37,900 L of phenol (100%) from a rail car occurred in a rural area of southern Wisconsin on July 16, 1974. The incident subjected the nearby residents to phenol exposure from contaminated well-water (Baker et al. 1978, as cited in ACGIH 1991). Phenol concentrations in water samples collected 7 d after the spill from the two nearest wells (on

either side of the railroad track) were 0.2 mg/L and 3.2 mg/L. Tests of water samples collected from the six nearest wells during the last week of July and all of August showed phenol concentrations ranging from 15 mg/L to 126 mg/L. Most families continued to drink their well-water for several weeks after the spill, until an unusual taste or odor developed. On November 26, 1974, EPA proposed an emergency phenol standard of 0.1 mg/L as temporarily acceptable for human consumption. Several persons living near the spill site had mouth sores, skin rash, nausea, and diarrhea in late July. In late October, physical examination and clinical chemistry analysis of several local families revealed no significant abnormalities.

A retrospective study was conducted by investigators from the Centers for Disease Control and Prevention, the EPA Water Supply Research Laboratory, and the Wisconsin State Department of Health and State Laboratory. The study, including evaluation of medical records, water-intake history, and blood and urine chemistry analyses, was conducted 7 months (mo) after the spill (Baker et al. 1978, as cited in ACGIH 1991). Study subjects were divided into three groups on the basis of phenol concentrations in the wells and distances from the spill site: groups 1 (>0.1 mg/L), 2 (0.1 to 0.001 mg/L), and 3 (no phenol; 1.9 kilometers [km] away) consisted of 39, 61, and 58 subjects, respectively. Significantly more people in group 1 complained of diarrhea, mouth sores, dark urine, and burning mouth than in groups 2 and 3. The authors defined phenol-related illness as having two of these four symptoms between July 1, 1974, and February 23, 1975. Groups 1, 2, and 3 reported 17, 5, and 2 cases of illness, respectively. The average duration of illness was 2 wk. Symptoms in members of group 1 occurred primarily in July and August; those in members of groups 2 and 3 occurred randomly throughout the 8-mo period. The difference in symptoms between groups 2 and 3 was not statistically significant, so those two groups were combined as the control group. Members of group 1 had significantly more frequent complaints of bad-tasting or bad-smelling water during July and August than did their neighbors. Members of all groups were given a physical examination 8 mo after the spill. Results of the blood test (for liver enzymes, etc.) and urinalysis revealed no statistically significant difference among the test groups. The authors concluded that "the illness appears to have had no long-term sequelae and to have occurred only in those exposed to more than 0.1 mg/L of phenol in water" (Baker et al. 1978, as cited in ACGIH 1991). The symptoms reported by subjects exposed to <0.1 mg/L and by the control group are shown in Table 7-2. The symptoms that were related significantly to phenol in drinking water

TABLE 7-2 Symptom Distribution (%) in Subjects Exposed to Phenol in Contaminated Water

Symptoms	Group 1[a]	Groups 2 and 3[b]
Vomiting	15.4	13.9
Diarrhea	41.0[c]	13.5
Headache	23.1	16.1
Skin rash	35.9	22.6
Mouth sores	48.7[c]	12.6
Parethesia or numbness	13.2	8.4
Abdominal pain	23.1	11.8
Dizziness	21.1	9.3
Dark urine	17.9	3.4
Fever	15.4	10.9
Back pain	20.5	11.0
Burning mouth	23.1[c]	6.8
Shortness of breath	10.3	6.7

[a]Phenol concentration >0.1 mg/L; n = 39.
[b]Phenol concentration <0.1 mg/L; n = 61 for group 2; n = 58 for group 3.
[c]Significantly greater than controls, $p < 0.01$, Fisher's exact test.
Source: Baker et al. 1978, as cited in ACGIH 1991.

were those produced by phenol in the alimentary tract. Those observations indicate that at those low concentrations guts that have normal activity of conjugating enzymes allow no free phenol to enter the bloodstream.

Animal Studies

The subchronic toxicity of phenol was determined in groups of 10 B6C3F$_1$ mice and 10 F-344 rats of each gender exposed to drinking water containing phenol at 0, 100, 300, 1,000, 3,000, or 10,000 parts per million (ppm) for 13 wk (NCI 1980). Gross and microscopic histopathologic examination of tissues or organs showed no changes attributable to the phenol consumption. Unfortunately, the amounts of phenol consumed by these animals were not reported. For risk assessment, one would need to know

how much phenol was consumed daily (mg/kg/d) by the animals; to calculate phenol consumption, one would need to know the amount of water consumed daily and the body weights of the animals.

The National Cancer Institute (NCI) report documents that water consumption by mice in the 3,000-ppm and 10,000-ppm groups was only 60% and 20%, respectively, of that of the controls. Male and female rats of the 10,000-ppm group consumed 50% and 33% less than controls (NCI 1980). The decrease in water consumption by these high-dose animals likely was due to the unpleasant taste of phenol. The amounts of water consumed by the animals (including controls) in this study were measured, but they were not reported. Fortunately, water consumption by control (untreated) rodents of the same species and age were reported in several 13-wk subchronic toxicity studies and 2-y carcinogenesis studies conducted by the National Toxicology Program (NTP). Table 7-3 shows the 13-wk average daily water consumption by rats and mice of either gender from the NTP studies.

If we assume that in the 13-wk NCI phenol study the control animals and the animals whose water consumption was not affected by the phenol treatment consumed the same amount of water as the control animals in the NTP 13-wk studies listed in Table 7-3, and if we also take into account the reduction of water consumption in the high-dose groups, then it is possible to estimate the 13-wk average amounts of daily water consumption in all the phenol-treated groups, which is shown in Tables 7-4 and 7-5. The NCI 13-wk phenol study reported the body weights of all groups; that information is needed for dose estimation. In the control groups, the initial mean body weights (10 per group) were 109 (male rats), 91 (female rats), 21 (male mice), and 18 g (female mice); the final mean body weights were 323, 182, 27, and 22 g, respectively. If we assume the 13-wk average body weight equals the initial body weight plus the final body weight less the initial body weight divided by two, then the 13-wk average body weights of the phenol-treated groups can be calculated and are shown in Tables 7-4 and 7-5. Phenol treatment did not significantly (<5%) affect the body weights, except those of the 10,000-ppm groups.

The concentrations of phenol in the drinking water of the NCI rat and mouse studies were 0, 100, 300, 1,000, 3,000, or 10,000 ppm (NCI 1980). Using the estimated 13-wk average water consumption and 13-wk average body weights (Tables 7-4 and 7-5) described above, one can calculate the average daily phenol consumption in the NCI study. The highest-dosed male and female rats are estimated by NASA to have consumed 532 mg/kg/d and 908 mg/kg/d, respectively (Table 7-4); the corresponding values

TABLE 7-3 Daily Water Consumption (mL) by Control Animals Reported by NTP[a]

	F-344 Rats				$B6C3F_1$ Mice			
	Male		Female		Male		Female	
NTP Toxicity Report Series	13 wk	2 y	13 wk	2 y	13 wk	2 y	13 wk	2 y
NTP 1992a	20.9		15.5		4.8		6.9	
NTP 1993a	21.2		15.6		4.5		6.3	
NTP 1993a	21.2		17.9		6.7		8.7	
NTP 1993a	22.3		18.8		5.1		6.2	
NTP 1993b	17.9		16.3		4.8		5.6	
NTP 1996a					5.3		6.5	
NTP 1996b	22.3		17.2		5.1		7.5	
NTP 1991a	23.5	22.3	22.7	19.8				
NTP 1991b	23.6	21.4	20.7	20.0				
NTP1992b	22.4	25.8	17.1	17.8	4.6	5.0	4.9	4.8
NTP 2000	21.9	24.9	16	17.2	5.6	5.9	6.5	5.5
NTP 2001	17.9	22.3	22.7	19.8	3.1	3.7	3.0	2.7
Average water consumption (mL/d)	21.4	23.3	18.2	18.9	5.0	4.9	6.2	4.3

[a]NTP 13-wk toxicity and 2-y carcinogenicity studies.

for mice are 444 mg/kg/d and 700 mg/kg/d (Table 7-5). The EPA Office of Water Health Advisories (EPA 1993) also calculated the daily doses of phenol consumption for these NCI studies (which are also included in Tables 7-4 and 7-5 for comparison), and reported the values for those animals to be 1,350 mg/kg/d (male rat), 1,350 mg/kg/d (female rat), 2,000 mg/kg/d (male mouse), and 2,000 mg/kg/d (female mouse) (EPA 1993). The Office of Water Health Advisories provided no information about how the data were calculated and did not account for decreased water consumption by high-dosed animals (e.g., mice in the 10,000-ppm groups consumed 80% less water than the control mice) or gender differences in body weight and water consumption. Calculations that do not account for those factors overestimate daily phenol consumption. Despite consuming relatively large daily doses of phenol in drinking water, the 13-wk animals showed no gross

TABLE 7-4 Estimated Daily Dose in Rats Consuming Phenol in Drinking Water for 13 wk

Data Description	Male Rats					Female Rats				
Phenol Concentration in water (ppm or mg/L)	100	300	1,000	3,000	10,000	100	300	1,000	3,000	10,000
Water consumption relative to controls (%)	100	100	100	100	50	100	100	100	100	67
Water consumed daily (ml)[a]	21.9	21.9	21.9	21.9	11.0	16.9	16.9	16.9	16.9	11.3
Estimated average body weight (g)[b]	215.5	216.0	216.0	210.5	197.5	138.5	138	137.5	136.5	124.5
Phenol consumption estimated by NASA (mg/kg/d)	10.2	30.4	101.4	312.1	557.0	14.3	43.0	144.0	435.2	1,100.4
Phenol consumption calculated by EPA (mg/kg/d)[c]	13	38	135	375	1,350	13	38	135	375	1,350

[a]Control value from Table 7-3; other values were calculated on the basis of a decrease in water consumption documented in the NCI (1980) report.
[b]Estimated 13-wk average body weight = initial body weight + [(final body weight - initial body weight) ÷ 2].
[c]EPA Office of Water Health Advisories (EPA 1993).
Source: Data from NCI 1980.

TABLE 7-5 Estimated Daily Dose in Mice Consuming Phenol in Drinking Water for 13 wk

Data Description	Male Mice					Female Mice				
Phenol Concentration in water (ppm or mg/L)	100	300	1,000	3,000	10,000	100	300	1,000	3,000	10,000
Water consumption relative to controls (%)	100	100	100	60	20	100	100	100	60	20
Water consumed daily (ml)[a]	5.0	5.0	5.0	3.0	1.0	6.2	6.2	6.2	3.7	1.2
Estimated average body weight (g)[b]	25.5	25.0	25.5	24.5	22.5	19.5	20	20.5	20.0	20.0
Phenol consumption estimated by NASA (mg/kg/d)	19.6	60.0	196.1	367.3	444.4	31.8	93.0	302.4	555.0	600.0
Phenol consumption calculated by EPA (mg/kg/d)[c]	20	60	200	600	2,000	20	60	200	600	2,000

[a]Control value from Table 7-3; other values were calculated on the basis of a decrease in water consumption documented in the NCI (1980) report.
[b]Estimated 13-wk average body weight = initial body weight + [(final body weight - initial body weight) ÷ 2].
[c]EPA Office of Water Health Advisories (EPA 1993).
Source: Data from NCI 1980.

or microscopic histopathology. The highest-dose male and female rats and mice showed only reductions in water consumption and body weight (Tables 7-4 and 7-5).

NCI (1980) also conducted a 2-y study in which groups of 50 B6C3F_1 mice and 50 F-344 rats of each gender were provided drinking water containing phenol at 0, 2,500, or 5,000 ppm. No clinical signs related to phenol consumption were observed in either species at any time during the study. Histopathologic study was conducted on all major organs as well as on bone, bone marrow, spleen, lymph nodes, the entire alimentary tract, sex organs, reproductive and urinary tracts, and the pituitary, adrenal, thyroid, parathyroid, and other endocrine glands. No histopathologic lesions in either species were attributed to phenol exposure. Water consumption in low- and high-dose mouse groups decreased to about 75% and 55% of that of the controls, respectively; the corresponding values for rats were 90% and 80%. Unfortunately, the amounts of water consumed by the control animals were not reported. The average amounts of water consumed daily by control animals can be estimated if we assume that these rodents consumed the same amounts of water as the controls in NTP bioassays. Using the averaged values shown in Table 7-3 for the control animals in the NCI phenol study, and taking into consideration that water consumption in phenol-treated groups decreased compared with the control group, we can calculate the amounts of water consumed daily by all the animals in the NCI study. The calculated values are shown in Table 7-6. The body weights in Table 7-6 were estimated from the growth curves in the NCI phenol report. The body weight and water consumption estimates allow the calculation of daily doses of phenol for the rodents exposed for 2 y; those data are also shown in Table 7-6. Included in the table are the phenol doses calculated by the EPA Office of Water Health Advisories (EPA 1993). Again, EPA did not take into consideration the decreased water consumption in the phenol-treated groups. This NCI study showed that exposing rats to phenol in drinking water at doses as high as 336 mg/kg/d (calculated by NASA) and exposing mice to doses as high as 450 mg/kg/d for 2 y produced no clinical signs or histopathology except decreased body-weight gain in the high-dose groups.

An unpublished study was conducted by Dow Chemical Company (Dow Chemical, unpublished material, 1944, as cited in Bruce et al. 1987) on rats gavaged for 6 mo with phenol (50 mg/kg or 100 mg/kg; 136 doses). Changes in liver and kidneys in the high-dose groups were slight and slight to moderate, respectively. Renal damage in low-dose animals was slight.

TABLE 7-6 Estimated Daily Dose in Rodents Consuming Phenol in Drinking Water for 2 y

Data Description	Male Rats			Female Rats			Male Mice			Female Mice		
Phenol concentration in water (ppm or mg/L)	0	2,500	5,000	0	2,500	5,000	0	2,500	5,000	0	2,500	5,000
Water consumption relative to controls (%)	100	90	80	100	90	80	100	75	55	100	75	55
Water consumed daily (mL)[a]	23.3	21.0	18.9	18.2	14.6	15.1	4.9	3.7	2.7	4.3	3.2	2.37
Estimated average body weight (g)[b]	400	390	380	250	250	225	34	32	30	28	27	27
Phenol consumption estimated by NASA (mg/kg/d)	0.0	135	249	0.0	146	336	0.0	289	450	0.0	296	439
Phenol consumption calculated by EPA (mg/kg/d)[c]	0	313	625	0	313	625	0	500	1,000	0	500	1,000

[a]Control values estimated from several similar National Toxicology Program (NTP) studies.
[b]Estimated from the growth curves in the NCI report.
[c]EPA Office of Water Health Advisories (EPA 1993).
Source: Data from NCI 1980.

Carcinogenicity

In the above-mentioned 2-y NCI drinking water study, the carcinogenic potential of phenol was evaluated in both rats and mice (NCI 1980). The incidences of leukemia and lymphoma in male rats of the 0-, 2,500-, and 5,000-ppm groups were 18/50, 31/50, and 25/50, respectively; the corresponding incidences for the female rats were 16/50, 15/50, and 12/50. Statistical tests showed that neoplastic incidence was significantly higher only in the low-dose male rats, not in the high-dose male rats. No statistically significant increases in any form of neoplasm were observed in female rats of either dose or phenol-treated mice of either gender. Under the conditions of the study, NCI concluded that phenol was not carcinogenic for rats or mice.

Reproductive and Developmental Toxicity

Heller and Pursell (1938, as cited in ATSDR 1988, p. 50) conducted a multi-generation developmental study in which rats were given drinking water containing phenol at 0-12,000 ppm for periods of up to five generations. All animals in any generation exposed at 15-5,000 ppm were normal with respect to growth, reproduction, and appearance of litters. Growth retardation of pups was observed in rats treated with 7,000 ppm; many litters from parents exposed at 8,000 ppm and above died (see Table 7-7).

In a study in which pregnant mice were given phenol at 0, 70, 140, or 280 mg/kg orally on gestation days 6 through 15, the increases in incidence of tremor, ataxia, lethargy, and irritability were statistically significant in the highest-dose group (Jones-Price et al. 1983). In that group (35 mice), four dams died from treatment; clinical signs included tremor, ataxia, lethargy, irritability, and weight loss. Fetuses borne by the highest-dose group had reduced body weights. The increase in incidence of cleft palate was not statistically significant. With this test protocol, phenol produced maternal and fetal toxicity, but not teratogencity. Based on the absence of maternal and fetal toxicity, 140 mg/kg/d was the highest NOAEL (no-observed-adverse-effect level).

In a similar study in CD-1 rats (20-22 rats per group) gavaged with phenol at 0, 30, 60, or 120 mg/kg/d on gestational day 6 to 15, no statistically significant signs of maternal toxicity were observed (Jones-Price et al. 1983). A significant proportion of litters with resorption sites were found in the low- and mid-dose groups, but not in the high-dose group. Thus, the rate of resorption was not dose-related. Average live fetal body weight

TABLE 7-7 Summary of Toxicity in Rodents Consuming Phenol in Drinking Water

Concentration	Exposure Duration	Species	Toxicity	Reference
4.7 mg/L	4 wk	CD-1 mice	No changes in spleen cellularity; no changes in differential white blood cell counts; no differences from controls on all three immunotoxicologic assays	Hsieh et al. 1992
19.5 mg/L	4 wk	CD-1 mice	No changes in spleen cellularity; no changes in differential white blood cell counts; fewer spleenic IgM-producing cells, less circulating IgM specific to sheep red blood cells	Hsieh et al. 1992
95.2 mg/L	4 wk	CD-1 mice	No changes in spleen cellularity; no changes in differential white blood cell counts; fewer spleenic IgM-producing cells, less circulating IgM specific to sheep red blood cells, less lymphoproliferative response to three of four mitogens	Hsieh et al. 1992
100 ppm (10-15 mg/kg/d, rats; 20-32 mg/kg/d, mice)	13 wk	Rats (20), mice (20	No effects on body and organ weight; no histomorphologic alterations	NCI 1980
300 ppm (30-43 mg/kg/d, rats; 60-93 mg/kg/d, mice)	13 wk	Rats (20), mice (20)	No effects on body and organ weight; no histomorphologic changes	NCI 1980

(Continued)

TABLE 7-7 Continued

Concentration	Exposure Duration	Species	Toxicity	Reference
1,000 ppm (101-144 mg/kg/d, rats; 193-302 mg/kg/d, mice)	13 wk	Rats (20), mice (20)	No effects on body and organ weight; no histomorphologic alterations	NCI 1980
3,000 ppm (310-435 mg/kg/d, rats; 367-555 mg/kg/d, mice)	13 wk	Rats (20), mice (20)	No effects on body and organ weight; no histomorphologic alterations; water consumption decreased by 40% in mice	NCI 1980
10,000 ppm (577-1,100 mg/kg/d, rats; 444-600 mg/kg/d, mice)	13 wk	Rats (20), mice (20)	No histomorphologic alterations; in rats, water intake decreased 30-50%, body weight gain was 16-26% less than controls; in mice, water intake decreased 80%, body weight gain was 12-17% less than controls	NCI 1980
2,500 ppm (135 mg/kg/d [m], 146 mg/kg/d [f])	2 y	Rats, 50 per gender	No clinical signs or pathologic changes except increased incidence of leukemia in males; water consumption decreased by 10%	NCI 1980
2,500 ppm (290 mg/kg/d [m], 296 mg/kg/d [f])	2 y	Mice, 50 per gender	No clinical signs or pathologic changes; no statistically significant in increases in any form of cancer; water consumption decreased by 45%	NCI 1980
5,000 ppm (250 mg/kg/d [m], 336 mg/kg/d [f])	2 y	Rats, 50 per gender	No clinical signs or pathologic changes; no statistically significant increases in any form of cancer; water consumption decreased by 20%	NCI 1980

5,000 ppm (450 mg/kg/d [m], 439 mg/kg/d [f])	2 y	Mice, 50 per gender	No clinical signs or pathologic changes; no statistically significant increases in any form of cancer; water consumption down by 45%	NCI 1980
100 ppm (100 mg/L)	5 generations	Rats	No effect on growth and reproduction	Heller and Pursell 1938
500 ppm	5 generations	Rats	No effect on growth and reproduction	Heller and Pursell 1938
1,000 ppm	5 generations	Rats	No effect on growth and reproduction	Heller and Pursell 1938
3,000 ppm	3 generations	Rats	No effect on growth and reproduction	Heller and Pursell 1938
5,000 ppm	3 generations	Rats	No effect on growth and reproduction	Heller and Pursell 1938
7,000 ppm	2 generations	Rats	Growth retarded in young	Heller and Pursell 1938
8,000 ppm	2 generations	Rats	Growth retarded, many litters died	Heller and Pursell 1938
10,000 ppm	1 generation	Rats	Young not cared for, reproduction retarded; some offspring died at birth	Heller and Pursell 1938

TABLE 7-8 Acute Toxicity of Phenol When Administered by Routes Other Than Drinking Water

Dose	Route	Exposure Duration	Species	Results	Reference
Nonlethal					
5 g (70 mg/kg)	Oral	Single dose	Human	Survived	Willhard 1886
15 g (210 mg/kg)	Oral	Single dose	Human	Survived	Model 1889
50 g (710 mg/kg)	Oral	Single dose	Human	Survived	Geill 1888
53 g (750 mg/kg)	Oral	Single dose	Human	Survived	Bennett et al. 1950
12 mg/kg	Oral	Single dose	F-344 rat	No effect on liver, kidneys, spleen, or thymus	Berman et al. 1995
40 mg/kg	Oral	Single dose	F-344 rat	One of seven had liver lesions; no effect on kidneys, spleen, or thymus	Berman et al. 1995
120 mg/kg	Oral	Single dose	F-344 rat	Incidences of lesions in liver, kidneys, spleen, and/or thymus were 1/6, 0/8, and 1/7, respectively	Berman et al. 1995
98 mg/kg	IP	Single dose	Mice	Convulsion in 50% of treated animals	Angel and Roger 1972
207 mg/kg	Oral	Single dose	Rats	Tremor of eye muscles, convulsion, coma	Liao and Oehme 1981
Lethal					
10-20 g (140-290 mg/kg)	Oral	Single dose	Human	Died	Stajdurhar-Caric 1968
~15 g (210 mg/kg)	Oral	Single dose	Human	Died	Kronlein 1873
25-30 g (360-430 mg/kg)	Oral	Single dose	Human	Died	Geill 1888

90 mg/kg	SC	Single dose	Cats	LD_{50}	Tollens 1905
100 mg/kg	Oral	Single dose	Cats	LD_{50}	Macht 1915
160 mg/kg	IV	Single dose	Rabbits	LD_{50}	Deichmann and Witherup 1944
220 mg/kg	Oral	Single dose	Rats	Two of eight died	Berman et al. 1995
300-500 mg/kg	SC	Single dose	Mice	LD_{50}	Tollens 1905
400-600 mg/kg	Oral	Single dose	Rabbits	LD_{50}	Deichmann and Witherup 1944
450 mg/kg	SC	Single dose	Rats	LD_{50}	Deichmann and Witherup 1944
500 mg/kg	Dermal	Single dose	Pigs	Two of three died	Pullin et al. 1978
500 mg/kg	Oral	Single dose	Dogs	LD_{50}	March 1915
530 mg/kg	Oral	Single dose	Rats	LD_{50}	Deichmann and Witherup 1943
500-600 mg/kg	IP	Single dose	Rabbits	LD_{50}	Deichmann and Witherup 1944
500-600 mg/kg	SC	Single dose	Rabbits	LD_{50}	Tollens 1905
600 mg/kg	Oral	Single dose	Rabbits	LD_{50}	Clarke and Brown 1906
650 mg/kg	Oral	Single dose	Rats	LD_{50}	Flickinger 1976
680 mg/kg	SC	Single dose	Guinea pigs	LD_{50}	Duplay and Cazzin 1891

(Continued)

TABLE 7-8 Continued

Dose	Route	Exposure Duration	Species	Results	Reference
850 mg/kg	Skin	Single dose	Rats	LD_{50}	Flickinger 1976
2,500 mg/kg	Skin	Single dose	Rats	LD_{50}	Deichmann and Witherup 1943
160 mg/kg/d	Oral	Gestation days 6-15	F-344 rats	One of six died	Jones-Price et al. 1983
200 mg/kg/d	Oral	Gestation days 6-15	F-344 rats	One of six died	Jones-Price et al. 1983
250 mg/kg/d	Oral	Gestation days 6-15	F-344 rats	Five of seven died	Jones-Price et al. 1983
275 mg/kg/d	Oral	Gestation days 6-15	CD-1 mice	Two of 13 died	Jones-Price et al. 1983
300 mg/kg/d	Oral	Gestation days 6-15	CD-1 mice	Five of 20 died	Jones-Price et al. 1983
320 mg/kg/d	Oral	Gestation days 6-15	F-344 rats	Seven of eight died	Jones-Price et al. 1983
320 mg/kg/d	Oral	Gestation days 6-15	CD-1 mice	Seven of eight died	Jones-Price et al. 1983

Abbreviations: IP, intraperitoneal; IV, intravenous; LC_{50}, dose lethal to 50% of subjects; SC, subcutaneous.

TABLE 7-9 Subchronic and Chronic Toxicity Summary of Phenol Administered by Routes Other Than Drinking Water

Dose	Route	Exposure Duration	Species	Effects	Reference
Subchronic Exposures (11-100 d)					
4 mg/kg/d	Oral	14 d	F-344 rats	No effects on liver, kidneys, spleen, or thymus	Berman et al. 1995
12 mg/kg/d	Oral	14 d	F-344 rats	One of eight rats had necrosis or atrophy of spleen or thymus	Berman et al. 1995
40 mg/kg/d	Oral	14 d	F-344 rats	Incidences of lesions in liver, kidneys, spleen and/or thymus were 1/8, 3/8, and 2/8, respectively	Berman et al. 1995
120 mg/kg/d	Oral	14 d	F-344 rats	All subjects (8) died	Berman et al. 1995
Chronic (>100 d)					
50 mg/kg	Gavage	136 doses over 6 mo	Rats	Slight kidney damage	Dow Chemical, unpublished material, 1944
100 mg/kg	Gavage	136 doses over 6 mo	Rats	Slight to moderate kidney damage, slight change in liver	Dow Chemical, unpublished material, 1944

TABLE 7-10 Reproductive and Developmental Effects of Phenol

Dose	Route	Exposure Duration	Species	Effects	Reference
20 mg/kg	IP	Gestation days 9-11 or 12-14	Pregnant rats	No gross anomalies; no difference in fetal weight	Minor and Becker 1971
63 mg/kg	IP	Gestation days 9-11 or 12-14	Pregnant rats	No gross anomalies; no difference in fetal weight	Minor and Becker 1971
200 mg/kg	IP	Gestation days 9-11 or 12-14	Pregnant rats	No gross anomalies; fetuses from mothers treated on gestation days 12-14 gained 10% less weight	Minor and Becker 1971
70 mg/kg/d	Oral	Gestation days 6-15	Pregnant CD-1 mice	No maternal or fetal toxicity	Jones-Price et al. 1983
140 mg/kg/d	Oral	Gestation days 6-15	Pregnant CD-1 mice	No maternal or fetal toxicity	Jones-Price et al. 1983
280 mg/kg/d	Oral	Gestation days 6-15	Pregnant CD-1 mice	Tremor, ataxia, lethargy, irritability, weight loss; four of 35 dams died; fetal weight was reduced	Jones-Price et al. 1983
30 mg/kg/d	Oral	Gestation days 6-15	Pregnant CD rats	No maternal or fetal toxicity	Jones-Price et al. 1983
60 mg/kg/d	Oral	Gestation days 6-15	Pregnant CD rats	No maternal or fetal toxicity	Jones-Price et al. 1983
120 mg/kg/d	Oral	Gestation days 6-15	Pregnant CD rats	No maternal toxicity; fetal weight decreased	Jones-Price et al. 1983

Abbrevation: IP, intraperitoneal.

decreased in a dose-related manner, but only that of the high-dose group was significantly below the average weight of controls. On the basis of the results of this study, 60 mg/kg/d is considered to be the highest NOAEL.

In a teratology study, Sprague-Dawley rats were injected intraperitoneally with phenol at 0, 20, 63, and 200 mg/kg on gestation days 9-11 or 12-14. In fetuses from dams treated on days 12-14, body weights were lower than those of controls. No fetal death or gross anomalies were attributed to phenol treatment (Minor and Becker 1971).

Immunotoxicology

Phenol is a metabolite of benzene, an immunotoxic chemical. The immunotoxicologic potential of phenol was evaluated in male CD-1 mice exposed to drinking water containing phenol at 0, 4.7, 19.5, and 95.2 mg/L for 4 wk (Hsieh et al. 1992). White blood cell and differential white blood cell counts and spleen cellularity were not affected by phenol treatment. Compared with the controls, mice of the two high-dose groups had significantly less spleenic IgM antibody-producing cells (plaque-forming cells) and circulating IgM antibodies specific to sheep erythrocytes (Table 7-7). When mitogens (liposaccharide, pokeweed, phytohemagglutinin, or concavalin A) were incubated with spleenic lymphocytes isolated from the mice, lymphocytes from only the highest-dose group produced significantly less proliferative response (assayed by incorporation of tritiated thymidine) to three of the four mitogens when compared with those isolated from control animals.

Comparative Toxicity of Phenol by Different Means of Administration, Routes of Dosing, and Durations of Exposure

The toxicity data on rodents administered phenol in drinking water or as bolus gavage doses are shown in Table 7-11. Results of all three phenol-in-drinking-water studies (13-wk NCI study, 2-y NCI carcinogenesis bioassay, and a multi-generation developmental toxicity study) showed that the toxicity of phenol ingested in drinking water was much lower than that of phenol given in bolus oral doses (see Table 7-11). For example, in the 13-wk study, when phenol was given in drinking water at 10,000 ppm—557 mg/kg/d and 1,100 mg/kg/d in male and female rats and 444 mg/kg/d and 600 mg/kg/d in male and female mice—no histopathologic lesions were found in any organs, and no clinical toxicity signs were found except de-

TABLE 7-11 Comparative Toxicity of Phenol Administered by Gavage or in Drinking Water

Concentration	Exposure Duration	Species	Effects	Reference
Administered by Gavage				
4 mg/kg/d	14 d	F-344 rat	No effects on liver, kidneys, spleen, or thymus	Berman et al. 1995
12 mg/kg/d	14 d	F-344 rat	One of eight rats had necrosis or atrophy of spleen or thymus	Berman et al. 1995
40 mg/kg/d	14 d	F-344 rat	The incidences of lesions in liver, kidneys, spleen and/or thymus were 1/8, 3/8, 2/8	Berman et al. 1995
50 mg/kg/d	136 doses over 6 mo	Rat	Slight kidney damage	Dow Chemical, unpublished material, 1944
100 mg/kg/d	136 doses over 6 mo	Rat	Slight to moderate kidney damage, slight change in liver	Dow Chemical, unpublished material, 1944
120mg/kg/d	14 d	F-344 rat	All rats died (n = 8)	Berman et al. 1995
207 mg/kg/d	Single dose	Rat	Tremor of eye muscles, convulsion, coma	Liao and Oehme 1981
160 mg/kg/d	Gestation days 6-15	F-344 rat	One of six died	Jones-Price et al. 1983
200 mg/kg/d	Gestation days 6-15	F-344 rat	One of six died	Jones-Price et al. 1983
250 mg/kg/d	Gestation days 6-15	F-344 rat	Five of seven died	Jones-Price et al. 1983

275 mg/kg/d	Gestation days 6-15	CD-1 mouse	Two of 13 died	Jones-Price et al. 1983
300 mg/kg/d	Gestation days 6-15	CD-1 mouse	Five of 20 died	Jones-Price et al. 1983
320 mg/kg/d	Gestation days 6-15	F-344 rat	Seven of eight died	Jones-Price et al. 1983
400 mg/kg/d	Gestation days 6-15	CD-1 mouse	Two of five died	Jones-Price et al. 1983
530 mg/kg	Single dose	Rat	LD_{50}	Deichmann and Witherup 1943
650 mg/kg	Single dose	Rat	LD_{50}	Flickinger 1976
Administered in Drinking Water				
100 ppm (10-15 mg/kg/d, rats; 20-32 mg/kg/d, mice)	13 wk	Rat (20), mouse (20)	No effects on body and organ weights; no histomorphologic changes	NCI 1980
300 ppm (30-43 mg/kg/d, rats; 60-93 mg/kg/d, mice)	13 wk	Rat (20), mouse (20)	No effects on body and organ weights; no histomorphologic changes	NCI 1980
1,000 ppm (101-144 mg/kg/d, rats; 196-302 mg/kg/d, mice)	13 wk	Rat (20), mouse (20)	No effects on body and organ weights; no histomorphologic changes	NCI 1980
3,000 ppm (310-435 mg/kg/d, rat; 367-555 mg/kg/d, mice)	13 wk	Rat (20), mouse (20)	No effects on body and organ weights; no histomorphologic changes; decreased water consumption	NCI 1980

(Continued)

TABLE 7-11 Continued

Concentration	Exposure Duration	Species	Effects	Reference
10,000 ppm (577-1,100 mg/kg/d, rats; 444-600 mg/kg/d, mice)	13 wk	Rat (20), mouse (20)	No histomorphologic changes; decreased water consumption and body weight	NCI 1980
2,500 ppm (135 mg/kg/d)	2 y	Rat, male	No clinical signs or pathologic changes except increased incidence of leukemia or lymphoma (31/50 vs 18/50 controls); decreased water consumption	NCI 1980
2,500 ppm (146 mg/kg/d)	2 y	Rat, female	No clinical signs or pathologic changes; no statistically significant increase in any form of cancer; decreased water consumption	NCI 1980
2,500 ppm (290 mg/kg/d [m], 296 mg/kg/d [f])	2 y	Mouse, 50 per gender	No clinical signs or pathologic changes; no statistically significant increase in any form of cancer; decreased water consumption	NCI 1980
5,000 ppm (250 mg/kg/d [m], 336 mg/kg/d [f])	2 y	Rat, 50 per gender	No clinical signs or pathologic changes; no statistically significant increase in any form of cancer; decreased water consumption	NCI 1980
5,000 ppm (450 mg/kg/d [m], 439 mg/kg/d [f])	2 y	Mouse, 50 per gender	No clinical signs or pathologic changes; no statistically significant increases in any form of cancer; decreased water consumption	NCI 1980
100 ppm	5 generations	Rat	No effect on growth and reproduction	Heller and Pursell 1937
500 ppm	5 generations	Rat	No effect on growth and reproduction	Heller and Pursell 1937

1,000 ppm	5 generations	Rat	No effect on growth and reproduction	Heller and Pursell 1937
3,000 ppm	3 generations	Rat	No effect on growth and reproduction	Heller and Pursell 1937
5,000 ppm	3 generations	Rat	No effect on growth and reproduction	Heller and Pursell 1937
7,000 ppm	2 generations	Rat	Growth of young retarded	Heller and Pursell 1937
8,000 ppm	2 generations	Rat	Growth retarded, many litters died	Heller and Pursell 1937
10,000 ppm	1 generation	Rat	Young not cared for, reproduction retarded, some offspring died at birth	Heller and Pursell 1937

crease in body-weight gain and water intake (NCI 1980). However, when phenol was given in bolus gavaged doses, three of eight F-344 rats had kidney lesions after they were given phenol at 40 mg/kg/d for 14 d, whereas all eight rats given phenol at 120 mg/kg/d for up to 14 d died (Berman et al. 1983).

As discussed above, Casidy and Houston (1984) demonstrated that the gut has a high capacity for phenol conjugation. Thus, when phenol is ingested in food or drinking water, it enters the gut gradually and steadily, and the absorbed phenol is conjugated in the intestinal mucosal cells. The cells have time to replenish sulfate or glucuronic acid. The conjugates are very water soluble and bulkier than the parent compound. Conjugation generally prevents the compound from being taken into the cells and usually enhances urinary excretion of the xenobiotics. If cellular sulfate or glucuronic acid is not depleted and the enzymes are not saturated, the length of time humans or animals drink phenol-contaminated water is not likely to influence the outcome of toxicity. That would explain the observation that, regardless of the exposure duration, phenol toxicity in rats consuming phenol in drinking water is determined primarily by the phenol concentration in the drinking water (Table 7-11).

In contrast, when phenol is given in a large bolus dose, it depletes sulfate and glucuronic acid and/or saturates the conjugation mechanism. Free phenol is then absorbed into the blood, resulting in toxicity to the CNS, liver, kidneys, spleen, and/or thymus (Berman et al. 1983). Casidy and Houston (1984) demonstrated that the conjugating enzymes in liver were readily saturable; phenol can be metabolized by cytochrome P-450 in liver and other organs to quinone and semiquinone, which may be more toxic than the parent compound. The toxicity of phenol given this way also depends on both the dose and exposure duration. As illustrated in the study by Berman et al., all eight rats survived when they were given single gavage doses of phenol at 120 mg/kg; however, all eight rats died when that dose was administered daily for up 14 d (Table 7-11). Based on the information on phenol metabolism and pharmacokinetics, one could conclude that phenol given as a bolus dose by any route would be much more toxic than if it was administered in drinking water.

Genotoxicity

Because phenol is a metabolite of benzene and benzene is carcinogenic, phenol has been extensively tested for mutagenicity. Table 7-12 summarizes the results of in vivo mutagenicity studies on phenol. Phenol given

TABLE 7-12 Genotoxicity of Phenol In Vivo

Dose (mg/kg) and Route	Species	End Point	Result	Reference
40, 80, 120; intraperitoneal	Mouse	Micronuclei (bone marrow)	Negative	Marrazzini et al. 1994
40, 80, 160; intraperitoneal	Mouse	Micronuclei (bone marrow)	Negative	Barale et al. 1990
265, intraperitoneal	Mouse	Micronuclei (Bone marrow)	Positive	Ciranni et al. 1988
265, intraperitoneal	Mouse	Micronuclei (fetal liver)	Positive	Ciranni et al. 1988

by intraperitoneal administration at doses up to 160 mg/kg did not produce micronuclei in mice. However, when the dose was 265 mg/kg, the same laboratory showed that phenol produced micronuclei (Table 7-12). These high doses, which overwhelmed the detoxification mechanism, have little value for assessing the genotoxicity of phenol ingested in drinking water. The results of in vitro mutagenicity tests will not be discussed here, because in vitro test systems do not model the detoxification role (conjugation) of the gut or other organs. Interested readers can find the in vitro data summarized in the ATSDR Toxicological Profile for phenol (ATSDR 1998).

Interaction of Phenol with Iodine

On the space station, reclaimed water will be treated with iodine. Phenol in water is known to react with chlorine to form 2-chlorophenol (2-CP), 4-CP, 2,4-di-CP, 2,6-di-CP, and 2,4,6-tri-CP (Kim et al. 1994). Phenol can also react with bromine in water to form ortho- and/or para-brominated phenol (Morrison and Boyd 1974, p. 502). It also can be iodinated in an anhydrous solvent yielding 2,4,6-triiodophenol (Fieser and Fieser 1976, p. 503). However, information about possible reactions between phenol and iodine in water could not be found.

RATIONALE

The SWEGs for 1 d, 10 d, 100 d, and 1,000 d are listed in Table 7-13. The rationales for those values are presented in this section. An astronaut

TABLE 7-13 Spacecraft Water Exposure Guidelines for Phenol

Duration	Concentration (mg/L)	Target Toxicity
1 d	80	Local gastrointestinal effects
10 d	8	Local gastrointestinal effects and taste
100 d	4	Local gastrointestinal effects and taste
1,000 d	4	Local gastronintestinal effects and taste

consuming 2.8 L of water (in drink and in food) containing phenol at the 1-d SWEG of 80 mg/L would consume 224 mg of the compound per day. As discussed above, the extra-strength Cepastat lozenge, an over-the-counter sore-throat medication, contains 29 mg phenol per lozenge; a patient taking the maximum allowable 10 lozenges daily would ingest 290 mg of phenol. Phenol at 80 mg/L will make water unpalatable, but will not pose a toxicologic concern. Phenol has a detectable smell at 8 mg/L (Amoore and Hautala 1983); NASA would allow crew to consume water that had a detectable smell of phenol at 8 mg/L for 10 days, but no longer. Thus, the SWEGs for 100 d and 1,000 d are set at 4 mg/L. Standards set by other organizations are listed in Table 7-14. EPA set 1-d, 10-d, long-term, and lifetime health advisories (HAs) at 6, 6, 4, and 4 mg/L, respectively. To set those HAs, EPA used the findings that no maternal or fetal toxicity occurred in CD-1 mice given phenol in bolus doses at 60 mg/kg/d by gavage on gestation days 6 to 15 (Jones-Price et al. 1983). NASA wonders why the results from the more relevant NCI studies and the multi-generation study in which phenol was ingested in drinking water were not used for setting exposure limit of phenol in drinking water. EPA used safety factors different from those used by NASA. The NASA spacecraft water exposure guidelines (SWEGs) for phenol were derived in accordance with guidance developed by the National Research Council (NRC 1998). The SWEGs are set by choosing the lowest values among the acceptable concentrations (ACs) identified in the literature.

In the section "Comparative Toxicity of Phenol by Different Means of Administration, Routes of Dosing, and Durations of Exposure," it is pointed out that when administered in drinking water, phenol is much less toxic than it is when given in a bolus dose by gavage or by any other route. Data are available from several good studies in which rodents consumed phenol in

TABLE 7-14 Exposure Limits Set by Other Organizations

Agency	Exposure Type	Exposure Concentration	Rationale and Comments	Reference
EPA[a]	1-d HA	6 mg/L	RfD = 60 mg/kg/d ÷ 100; HA = RfD × 10 kg ÷ 1 L/d	EPA 1993
	10-d HA	6 mg/L	RfD = 60 mg/kg/d ÷ 100; HA = RfD × 10 kg ÷ 1 L/d	EPA 1993
	Long-term HA	6 mg/L	RfD = 60 mg/kg/d ÷ 100; HA = RfD × 10 kg ÷ 1 L/d	EPA 1993
	Lifetime HA	4 mg/L	RfD = 60 mg/kg/d ÷ 100; HA = RfD × 70 kg ÷ 2 L/d	EPA 1993
	ADI (criterion level)	3.5 mg/L	NOAEL = 0.1 mg/kg/d; ADI = 0.1 mg/kg/d × 70 kg ÷ 2 L/d	EPA 1980
	Water quality criterion (organoleptic)	0.3 mg/L	Study identified test subjects who could detect (taste) phenol in water at 0.3 mg/L	EPA 1980

[a]EPA's RfD (reference dose) uses 60 mg/kg/d becuase it was the highest dose that produced no materal and fetal toxicity in a study in which CD-1 mice were given phenol on gestation days 6-15 (Jones-Price et al. 1983); for the 1-d, 10-d, and long-term HA, EPA used body weight and consumption parameters of a child (10-kg child ingesting 10 L of water per day); for the lifetime HA, EPA assumed a 70 kg person ingesting 2 L of water per day.
Abbreviations: ADI, acceptable daily intake; EPA, U.S. Environmental Protection Agency; HA, health advisory; RfD, reference dose.

drinking water. Therefore, phenol exposure by other routes or by bolus oral doses will not be considered in setting phenol exposure limits in spacecraft drinking water.

When groups of CD-1 mice (five per group) were exposed to drinking water containing phenol at 0, 4.7, 19.5, and 95.2 mg/L for 4 wk, Hsieh et al. (1992) observed that the highest-dose group had significantly fewer spleenic IgM antibody-producing cells (plaque-forming cells) and circulating IgM antibodies specific to sheep erythrocytes. When mitogens were incubated with spleenic lymphocytes isolated from the mice, the proliferative response to three of four mitogens (assayed by incorporation of tritiated thymidine) of lymphocytes from the highest-dose group was significantly lower than the response of lymphocytes isolated from control animals. However, white blood cell and differential white blood cell counts and spleen cellularity were not affected by the phenol treatment. NCI (1980) also did not find any effects on bone marrow, spleen, lymph nodes, or thymus in 200 F-344 rats and 200 B6C3F_1 mice treated with phenol in drinking water at concentrations of 2,500 ppm or 5,000 ppm for 2 y. In the NCI study, rats and mice were exposed for their lifetime to phenol in drinking water at daily doses 50 times higher than the highest-dose level in Hsieh et al.'s study. Survival rate and life-span were not affected by phenol treatment. The effects seen by Hsieh et al. will not be considered for setting SWEGs.

ACs Based on Data From Humans

As discussed above, the phenol spillage incident in Wisconsin resulted in phenol concentrations in the six nearest wells at 15-126 mg/L (average 80 mg/L). Most families continued to drink their well-water for several weeks after the spill until an unusual taste or odor developed. Some of the residents had mouth sores, nausea, and diarrhea. Those are local irritating effects of phenol in the alimentary canal. If we accept that these symptoms are relatively mild, the concentration of 80 mg/L could be used as AC for 1 d. An astronaut consuming 2.8 L water (in drink and in food) containing 80 mg/L phenol would consume 224 mg of the compound. As discussed above, the extra-strength Cepastat lozenge, an over-the-counter sore-throat medication, contains 29 mg of phenol per lozenge; a patient taking the maximum allowable 10 lozenges per day would ingest 290 mg of phenol. A 1-d SWEG of 224 mg of phenol would be less than the amount in 10 lozenges.

The AC for 10 d was set by applying a safety factor of 10 (from LOAEL [lowest-observed-adverse-effect level] to NOAEL) to the average concentration of 80 mg/L of phenol found in the wells; therefore, the AC is set at 8 mg/L. Because humans can detect phenol at 8 mg/L water (Amoore and Hautala 1983), the ACs for 100 or 1,000 d are further reduced to 4 mg/L. This concentration is the same as EPA's lifetime health advisory limit on phenol in water (4 mg/L).

ACs Based on Data from NCI Rodent Studies

When rats and mice were given drinking water containing phenol at 1,000 ppm for 13 wk, tissues and organs showed no gross or microscopic histopathologic effects attributable to the phenol consumption. In this study, the male and female rats consumed 101 mg/kg/d and 144 mg/kg/d, respectively; the corresponding values for mice were 196 mg/kg/d and 302 mg/kg/d. The exposure level of 101 mg/kg/d is considered to be the NOAEL. As pointed out above in the section on phenol metabolism, if cellular sulfate or glucuronic acid is not depleted and the phenol conjugation enzymes are not saturated, the amount of time that humans or animals drink phenol-contaminated water is not likely to influence the outcome of toxicity. Therefore, an AC was set for all exposure durations. A species-extrapolation factor of 10 was applied to obtain a human NOAEL; the calculation assumes a 70-kg person ingesting 2.8 L of water per day. The AC was calculated as follows:

$$AC = 101 \text{ mg/kg} \times 70 \text{ kg} \div 10 \times 2.8 \text{ L} = 253 \text{ mg/L}.$$

NCI (1980) also conducted a 2-y study in which groups of 50 B6C3F$_1$ mice and 50 F-344 rats of each gender were provided drinking water containing phenol at 2,500 or 5,000 ppm. No clinical signs or histopathologic lesions attributed to phenol consumption were observed in either species. A statistically significant increase in the incidence of leukemia or lymphoma was observed only in male rats of the low-dose group. NCI does not consider phenol to be a carcinogen. All groups of mice and rats ingesting phenol had lower water consumption rates than controls.

After adjusting for the decrease in water consumption, phenol consumption in the 2,500-ppm groups was found to be 135 mg/kg/d and 146 mg/kg/d for male and female rats and 289 mg/kg/d and 296 mg/kg/d for male and female mice. As discussed previously, bad-tasting water is con-

TABLE 7-15 Acceptable Concentrations (ACs)

		Uncertainty Factors			ACs (mg/L)			
Exposure Data	Species and Reference	Species	Exposure Time	LOAEL to NOAEL	1 d	10 d	100 d	1,000 d
NOAEL = 101 mg/kg; 13 wk; drinking water	Rats (NCI 1980)	10	1	1	253	253	253	253
LOAEL for water consumption = 135 mg/kg; 2 y; drinking water	Rats (NCI 1980)	10	1	10	—	—	—	34
LOAELfor minor gastrointestinal symptoms = 80 mg/L (residents drank well-water containing ~15-126 mg/L)	Humans (Baker et al. 1978)	1	1	10	80	8	4	4
SWEGs					80	8	4	4

sidered undesirable for long-term consumption. Therefore, 135 mg/kg is considered a LOAEL. A factor of 10 was used to extrapolate LOAEL to NOAEL, and a species-extrapolation factor of 10 was applied to obtain a NOAEL for humans of 34 mg/L. The 1,000-d AC was calculated as follows:

$$1{,}000\text{-d AC} = 135 \text{ mg/kg} \times 70 \text{ kg} \div 10 \times 10 \times 2.8 \text{ L} = 34 \text{ mg/L}.$$

AC Summary Table and SWEGs

The ACs derived from various toxicity end points are summarized in Table 7-15 (above). The SWEGs are set by choosing the lowest values among those ACs.

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8 *N*-Phenyl-beta-naphthylamine

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PHYSICAL AND CHEMICAL PROPERTIES

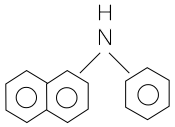
N-phenyl-beta-naphthylamine (PBNA) is a light tan or gray compound produced as flakes or powder. (See Table 8-1 for summary of physical and chemical properties.)

OCCURRENCE AND USE

Background

PBNA is manufactured from beta-naphthol and aniline (Scott 1962, pp. 73-74). PBNA has been used in rubber industries as an antioxidant to increase resistance to heat and cracking in natural and synthetic rubbers and in latexes (ACGIH 1991, pp. 1211-1213). It has also been used as an antioxidant in various greases and lubricating or transformer oils. PBNA has been employed as a stabilizer in industrial applications such as silicone enamels (Kehe and Kouris 1965), as a catalyst, as a polymerization inhibitor, and as a vulcanization accelerator. PBNA has been used in the production of dyes and as a component of rocket fuels since the mid-1950s (Mossberg 1976). It has also been used in surgical plasters (Brzezicka-Bak

TABLE 8-1 Physical and Chemical Properties

Formula	$C_{16}H_{13}N$
Synonyms	PNA, *N*-phenyl-2-naphthylamine, 2-napthalenamine, *N*-phenyl, N-(2-naphthyl) aniline, 2-anilinonaphthaline, beta-naphthylphenylamine, Agerite powder, Neozone-D, Antioxidant 116
	
CA S registry no.	135-88-6
Specific gravity	1.24
Molecular weight	219.29
Melting point	108°C
Boiling point	395.5°C
Solubility	Insoluble in water (as pure powder); soluble in alcohol, ether; soluble in water at parts per million level (Vine et al. 1984)
Vapor pressure	8.3×10^{-6} mmHg (at 25°C)

1973) and in tin-electroplating baths. Commercial grade PBNA in the United States has been reported to contain, as a contaminant, 20-30 milligrams per kilogram (mg/kg) of the human bladder carcinogen beta-naphthylamine (BNA) (IARC 1974). In Japan, commercial grade PBNA contains aniline, 2-naphthol, and BNA. Levels of BNA contamination in commercial PBNA in the United Kingdom are reportedly reduced to less than 1 mg/kg in at least one commercial product (Veys 1996). In 1976, PBNA was nominated and selected for toxicology and carcinogenesis studies by the National Toxicology Program (NTP) because of its large annual production, widespread human exposure, and structural and possible in vivo metabolic similarity to the known human urinary bladder carcinogen BNA (NTP 1988). Domestic production of PBNA in the early 1970s was 1.4 to 2.2 million kg per year (y) (ACGIH 1991, pp. 1211-1213). PBNA is no longer used in the United States.

Detection in Spacecraft

PBNA has been detected in multiple humidity-condensate and regener-

ated-water samples of the Mir space station. Its origin is unknown. The Mir-18 and Mir-19 missions demonstrated PBNA concentrations ranging from 0.3 micrograms per liter (μg/L) to 13.1 μg/L in four samples of regenerated water (Pierre et al. 1996). PBNA was detected at concentrations ranging from 0.3 μg/L to 0.5 μg/L in humidity-condensate samples collected in a series of water tanks during the Mir-20 mission. A concentration of 75 μg/L was detected in humidity condensate via use of the Russian atmospheric-condensate sampler during that same mission (Pierre et al. 1996). There was no PBNA detected in ground supply water used in the Mir-18 or Mir-20 missions. PBNA was detected in four regenerated water samples from the Mir-21 mission at concentrations ranging from 0.2 μg/L to 11.7 μg/L (Pierre et al. 1997). It was also detected in four humidity-condensate samples at concentrations ranging from 4.8 μg/L to 55.5 μg/L. It was not detected in stored (launched from the ground) water samples from that mission. It is noted that BNA was detected at 0.5 μg/L in one of three hot regenerated water samples collected from Mir-19 (Pierre et al. 1996). BNA was not found when evaluated in other spacecraft water samples.

PHARMACOKINETICS AND METABOLISM

In Vivo PBNA Conversion to BNA

It has been reported that PBNA is dephenylated to the bladder carcinogen BNA in rats, dogs, and humans by an undefined metabolic process. Several studies have been conducted to support that theory, and they are summarized in Table 8-2. Kummer and Tordoir (1975) conducted a study in which 19 volunteers orally ingested PBNA containing an impurity of BNA at 0.8 parts per million (ppm), which was equated to 8 nanograms (ng) (0.008 μg) per 10 mg dose of PBNA. Based on the BNA contamination of ingested PBNA, and the amounts of BNA found in the urine samples of the volunteers, PBNA was determined to be at least partially metabolized to BNA in humans. Seven of 19 human subjects, six of whom were nonsmokers (BNA is a component of tobacco smoke), demonstrated otherwise unexplainable amounts of BNA in their urine following PBNA ingestion. Dogs given a single dose of a commercial grade of PBNA at 5 mg/kg were found to have BNA in 24-hour (h) urine samples at 3-4 μg (Batten and Hathway 1977). Laham and Potvin (1983) described experimental results indicating the metabolism of PBNA to BNA in Sprague-Dawley rats. A study conducted at the Southern Research Institute (SoRI) in 1986 for the National

TABLE 8-2 Conversion of PBNA to BNA in Various Species

Dose	Exposure Duration	Species	Conversion	Reference
10 mg, contaminated with BNA at 0.8 ppm (oral ingestion)	Single dose	Human, n = 19	0.4-3.0 μg BNA (24-h urine sample) in seven subjects (six nonsmokers); detection limit, 0.5 μg/500mL	Kummer and Tordoir 1975
30 mg, contaminated with BNA at 0.8 ppm (oral ingestion)	Single dose	Human, n = 1	10 μg BNA (12-h urine sample); detection limit, 0.5 μg/500 mL	Krummer and Tordoir 1975
40 mg, contaminated with BNA at 0.8 ppm (inhalation, 15-20 min)	Single dose	Human, n = 4	3-8 μg BNA (24-h urine sample); detection limit, 0.5 μg/500 mL	Krummer and Tordoir 1975
50 mg, contaminated with 0.7 μg BNA (ingestion)	Single dose	Human, n = 2	3-4 μg BNA (24-h urine sample); detection limit not stated	Moore 1977
5 mg/kg, PBNA or ^{14}C-labeled PBNA, commercial grade (gelatin capsule)	Single dose	Dog, n = 9 (4 male, 5 female)	0-10 μg BNA in urine; detection limit, 0.5 μg/500 mL	Batten and Hathway 1977
400 mg (27 mg/kg), 5 d/wk, and then 5 mg for 1 d (gelatin capsule)	4 wk	Dog, n = 8, (4 male, 4 female)	0-7 μg BNA in urine; detection limit, 0.5 μg/500 mL	Batten and Hathway 1977

(Continued)

TABLE 8-2 Continued

Dose	Exposure Duration	Species	Conversion	Reference
50 mg/rat/d (200 mg/kg), purified PBNA (gavage)	Single dose	Sprague-Dawley rat, male, groups of 5	0.37 μg BNA in urine at 0-24 h (16 μg PBNA); 0.40 μg BNA in urine at 24-48 h (4 μg PBNA); detection limit, 0.5 μg/mL	Laham and Potvin 1983
100 mg/rat/d (400 mg/kg), purified PBNA (gavage)	4 d	Sprague-Dawley rat, male, groups of 5	2.6 μg BNA in urine at 0-24 h (16 μg PBNA); 7.3 μg BNA in urine at 24-48 h (110 μg PBNA); 5.4 μg BNA in urine at 48-72 h (278 μg PBNA); 7.3 μg BNA in urine at 72-96 h (415 μg PBNA); detection limit, 0.5 μg/mL	Laham and Potvin 1983
300 mg/kg (high), 180 mg/kg (low), 99% pure PBNA (ad libitum feed)	8 d	F-344 rat, male, 5 per dose, 8 wk old, 158-178 g	No detectable BNA; detection limit, 2 μg/mL	SoRI 1986[a]
280 mg/kg (high), 170 mg/kg (low), 99% pure PBNA (ad libitum feed)	7 d	F-344 rat, male, 3 per dose, 13 wk old, 203-229 g	High-dose group: BNA excretion at 2.8-10 μg/d (following acid hydrolosis of urine); Low-dose group: BNA excretion at ≤0.87 μg/d; detection limit, 0.05 μg/mL	SoRI 1986[a]

[a]The mg/kg concentrations were derived by NASA.

Institute of Environmental Health Sciences determined that BNA detected in the urine of male F-344 rats only indicated an impurity in the 99% pure PBNA test compound. Following acid hydrolysis for improved sensitivity, urine samples were observed to contain 2.8-10 μg of BNA. From the above studies it appears that humans, dogs, and some rat species metabolize orally ingested PBNA to BNA. The SoRI (1986) and Laham and Potvin (1983) studies demonstrate that better measurements of BNA are obtained using techniques that minimize degradation of the amine in urine samples, such as the collection of urine under dry ice or the use of liquid nitrogen for immediate freezing of the collected sample. Both Batten and Hathway (1977) and Laham and Potvin (1983) utilized heptafluoro derivatives of PBNA and BNA to optimize detection of the amines by gas chromatography.

The purity of tested PBNA, and especially the amount of BNA contamination, can effect experimental results relative to the demonstration of BNA excretion in an animal species. The Kummer and Tudoir (1975), Moore (1977), Laham and Potvin (1983), and SoRI (1986) studies provide statements regarding the purity and/or BNA contamination of the tested PBNA. Batten and Hathway (1977) offer no assertion regarding the amount of BNA contaminant in tested PBNA, but indicate that a commercial (nonpurified) grade of PBNA was used. Laham and Potvin (1983) state that tested PBNA in their study was purified (percentage not given) through treatment with ethanol and charcoal; however, BNA contamination is not addressed. In the absence of verifiable BNA contamination levels (Laham and Potvin 1983; Batten and Hathway 1977), it may be suggested, but not confirmed, that BNA observed in the urine of tested species originated as an impurity in the tested PBNA rather than as an in vivo metabolite.

BNA is excreted in the feces and urine of both rats and dogs (Boyland and Mason 1966) following subchronic dietary administration. Previous investigations have shown that it is the metabolites of BNA that are responsible for its carcinogenicity. Primary BNA carcinogenic metabolites 2-naphthylhydroxylamine (BNHA) and 2-amino-naphthylsulfate were not detected (detection limit of 50 ng) in the urine samples of dogs fed a single dose of PBNA at 5 mg/kg or in the urine of dogs fed multiple doses at 27 mg/kg for 4 weeks (wk) (Batten and Hathway 1977). Neither BNA nor its carcinogenic metabolite BNHA were detected (detection limits not given) in mammalian hepatic microsomal preparations incubated with 0.5 millimolar (mM) PBNA (Anderson et al. 1982).

Batten and Hathway (1977) applied the Druckrey and Kupfmuller equation $d \times t \times n = k$ (d is the daily dose in mg/kg, t is the time between treatment initiation and tumor formation in months, and n is a small positive

integer), which describes the dose-effect and time relationships in animals subjected to daily dosing with a chemical. Assuming that this equation can be employed when BNA is produced as a metabolite of PBNA ingestion, Batten and Hathway calculated that the time that would have to elapse for tumors to be formed when 1,500 ng of BNA (average amount of BNA found in all PBNA-treated animals) was the daily exposure dose would be 31 y, thus exceeding the lifespan of the species. They concluded that BNA generated from metabolism of PBNA is at such low levels that any alleged dephenylation of PBNA in vivo does not produce carcinogenic risk.

Absorption

No data on the absorption of PBNA have been found. Laham and Potvin (1983) suggest that PBNA is absorbed through the gastrointestinal tract as demonstrated by slow excretion over several days as either unchanged PBNA or as BNA in both the feces and urine of orally dosed Sprague- Dawley rats administered a single dose (50 mg per rat) of PBNA (Table 8-3).

Distribution

There are no data on the distribution of orally ingested PBNA. Laham and Potvin (1983) suggest that PBNA is stored in rat tissues during repeated administration; however, no data have been found to support that suggestion.

Excretion

PBNA is apparently excreted as a free amine, as the dephenylated metabolite BNA, as free and conjugated hydroxylated metabolites of either PBNA or BNA, or as epoxides (Laham and Potvin 1983). The primary route of excretion is the fecal route. Dogs dosed intragastrically with a single dose of ^{14}C-labeled PBNA at 5 mg/kg demonstrated excretion of >90% of the radioactivity from the body within 3 days (d) (Batten and Hathway 1977). Excretion occurred principally via the biliary/ fecal route (amount not given). PBNA was excreted in the urine for 3 d following a single dose (50 mg) by oral administration to male Sprague-Dawley rats (Table 8-3) (Laham and Potvin 1983). No PBNA was detected after 72 h.

TABLE 8-3 Urinary Excretion of PBNA and BNA in Male Sprague-Dawley Rats Following a Single Oral Dose of PBNA[a]

Time Interval After Dose (h)	PBNA (μg)	BNA (μg)
0-24	15.96	0.37
24-48	3.93	0.40
48-72	Trace	0.35
72-96	Not detected	0.28

[a]50 mg per rat (200 mg/kg assuming a 0.25-kg rat); detection limit was 0.5 μg/mL.
Source: Laham and Potvin 1983.

Following multiple-dose administration (100 mg/kg/d), PBNA was detected in rat feces in substantial amounts 5 d after the last administration. Increasing urinary concentrations of PBNA and BNA at 0-96 h after the first dose are presented in Table 8-4 (Laham and Potvin 1983).

Metabolism

Anderson et al. (1982) conducted an in vitro study on the hepatic microsomal metabolism of and macromolecular binding to PBNA in seven mammalian species, including male Sprague-Dawley rats, male B6C3F$_1$ mice, a male rhesus monkey, male Syrian Golden hamsters, a human, a male beagle, and a female pig. The relative abilities of the tested species to metabolize PBNA decreased in the order of hamster, mouse, rat, monkey, dog, human, pig (Table 8-5). Rates of metabolism were determined by colorimetric assay indicating formation of hydroxylated species. Hydroxyarylamine concentrations were calculated on the basis of changes in absorbtion at 535 nm.

Anderson suggests that metabolism of PBNA may occur via oxidation by cytochrome P-450 mixed function oxidase systems to various hydroxylated metabolites. Two metabolites formed by all seven animal species were 6-hydroxy-*N*-phenyl-2-naphthylamine (6-OH-PBNA) and 4'-hydroxy-*N*-phenyl-2-naphthylamine (4'-OH-PBNA). These (and other) metabolites were identified by high performance liquid chromatography (HPLC) and mass spectral analysis, with nuclear magnetic resosance (NMR) confirmation. The ratio of 6-OH-PBNA to 4'-OH-PBNA formation varied between species: In mouse, rat, and monkey, the ratio was 0.4; in man and dog, the ratio was 1.0; and in hamster, the ratio was 1.5. Induction of cytochrome

TABLE 8-4 Urinary Excretion of PBNA and BNA in Male Sprague-Dawley Rats After 4 d of Oral Administration[a]

Time Interval After First Dose (h)	PBNA (μg)	BNA (μg)
0-24	20.92	2.62
24-48	110.64	7.28
48-72	277.91	5.36
72-96	415.37	18.48

[a]100 mg per rat per day (400 mg/kg/d assuming a 0.25-kg rat); detection limit was 0.5 μg/mL.
Source: Laham and Potvin 1983.

P-450 in rats by pretreatment with phenobarbital or 3-methylchol-anthrene caused an increase in the rate of formation of hydroxylated species (Table 8-6). Rat liver microsomes incubated with PBNA in the presence of cytochrome P-450 inhibitors resulted in reduced production of the hydroxylated metabolites by approximately 50%. The ratio of 6-OH-PBNA and 4'-OH-PBNA formation varied with species, with cytochrome P-450 induction by 3-methylcholanthrene, and with cytochrome P-450 inhibition by DPEA (Table 8-7). This infers that epoxidation of PBNA occurs through distinct cytochrome P-450 systems. There is no information available on the specific cytochromes responsible for PBNA metabolism.

TABLE 8-5 Relative Rates of Microsomal Metabolism of PBNA by Various Species[a]

Species	Rate (nmol/min per mg protein) ± SD
Pig	0.4 ± 0.2
Human	0.6
Dog	0.9 ± 0.2
Monkey	1.2 ± 0.4
Rat	1.3 ± 0.1
Mouse	1.9 ± 0.1
Hamster	3.5 ± 0.5

[a]Rates were determined by colorimetric assay.
Abbreviations: nmol, nanomoles; SD, standard deviation.
Source: Anderson et al. 1982.

TABLE 8-6 Induction of PBNA Metabolism in Rat Hepatic Microsomes[a]

Pretreatment	Rate (% of control) ± SD
Control	100 ± 13
3-Methylcholanthrene	300 ± 13
Phenobarbitol	150 ± 8

[a]Rates were determined by colorimetric assay.
Abbreviation: SD, standard deviation.
Source: Anderson et al. 1982.

Figure 8-1 illustrates the conjectured avenues of metabolic degradation of PBNA. Anderson et al. (1982) hypothesize that epoxidation of the naphthyl or the phenyl group of PBNA occurs via cytochrome P-450 oxidation, followed by isomerization to yield the respective 6-OH-PBNA and 4'-OH-PBNA metabolites. Alternatively, *N*-hydroxylation of PBNA could occur, followed by acid-catalyzed decomposition to produce a delocalized nitrenium-carbonium ion, which would then solvolyse, leading to the formation of the same hydroxylated metabolites. However, the *N*-hydroxylation pathway is unlikely. For either argument, Anderson et al. suggest that 4'-OH-PBNA can undergo oxidation to form benzoquinone and BNA. Anderson et al. (1982) demonstrated oxidative dephenylation of 4'-OH-PBNA to produce BNA and 1,4-benzoquinone (Figure 8-1) by treating the hydroxyarylamine with an excess of hydrogen peroxide in sodium phosphate. These avenues of PBNA metabolism are purely conjectural, as many of the proposed metabolic species (bracketed in Figure 8-1) were not iso-

TABLE 8-7 Ratio of Principal Metabolites of PBNA Formed in Microsomal Incubations When Pretreated with DPEA[a]

	Ratio (6-OH-PBNA/4'-OH-PBNA)		
	Rat Microsomes		
DPEA (mM)	Control	MC-treated[b]	Human Microsomes
0	0.30	0.25	0.35
0.1	0.43	0.80	0.82
0.5	0.62	1.09	1.04

[a]Pretreated for 3 min.
[b]Rat microsomes subjected to the cytochrome P-450 inducer 3-methylcholanthrene.
Source: Anderson et al. 1982.

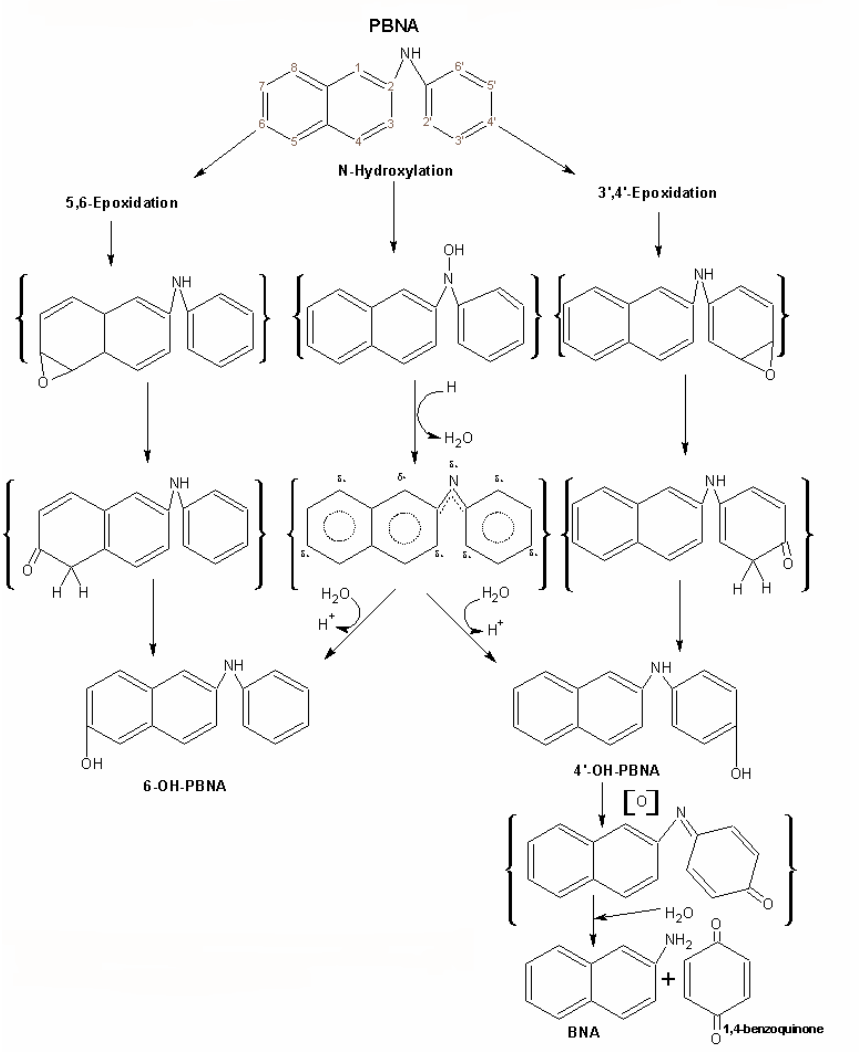


FIGURE 8-1 Conjectured metabolism of PBNA by microsomal enzymes via cytochrome P-450. Bracketed species were not isolated. Source: Anderson et al. 1982.

lated in the experiment. Also, the BNA was produced only by artificial (nonphysiologic) means.

^{3}H-PBNA microsomal incubations were examined by HPLC for detection of BNA and its primary hydroxylation product, 2-amino-1-naphthol

(detection limit not given). However, neither BNA nor 2-amino-1-naphthol was detected in microsomal incubations.

Anderson et al. (1982) also demonstrated a time-related linear increase in protein binding occurring parallel to the formation of the above metabolites by microsomal metabolism of PBNA. This finding suggests that metabolites of PBNA, perhaps epoxides, are involved in macromolecular binding.

TOXICITY SUMMARY

There are no studies that address toxicity resulting from ingestion of PBNA in drinking water for humans or nonhumans. Toxicity studies have been conducted for orally administered PBNA by means of intragastric or dietary ingestion. Loss of body weight and increased liver-to-body weight ratios were seen in short-term, subchronic, and chronic rodent toxicity studies (NTP 1988). The kidney is the primary target organ for adverse effects of orally ingested PBNA. Nephropathic lesions were observed in both F-344N rats and B6C3F_1 mice. Other adverse effects include gastrointestinal disturbances, evidence of immunotoxicity and reproductive toxicity, and hematopoietic irregularities. A primary concern of PBNA exposure is that it is converted in vivo to the bladder carcinogen BNA, and can hence initiate cancerous lesions. Epidemiological studies of workers in rubber tire factories presented no evidence that occupational exposure to atmospheric PBNA increased incidence of bladder tumors, although inhalation of PBNA mixed with other chemicals was associated with increased incidences of other cancers among workers. No studies have been conducted to determine carcinogenicity of PBNA to humans exposed via oral ingestion.

Acute Toxicity (1-5 d)

An oral LD_{50} (dose lethal to 50% of subjects) of 8,730 mg/kg has been established for rats administered PBNA orally in a vegetable oil suspension (Kelman 1964). Unspecified vascular changes in the liver, lungs, and brain occurred as a result of venous congestion in rats administered this dose. Kelman also established an oral LD_{50} of 1,450 mg/kg for mice given PBNA in an oil suspension. Evidence of central nervous system (CNS) depression was observed and disturbance of liver function was indicated by depressed concentrations of hippuric acid. The strain and gender of the animals and

the purity of the PBNA were not specified in either study. There are no human studies that address acute toxicity from PBNA administered orally.

Short-Term Toxicity (6-30 d)

The NTP elected to evaluate PBNA for its toxicologic and carcinogenic potential as an industrial chemical. Groups of five female and five male F-344N rats and B6C3F_1 mice were used in a 14-d study (NTP 1988). Rats were fed 98% pure PBNA via ad libitum diet at doses of 330, 660, 1,400, 3,250, and 7,900 mg/kg (males) and 380, 820, 1,680, 4,120, and 7,800 mg/kg (females). Mortality rates were significant for both male and female rats at the higher doses—three of five males and four of five females that received the highest doses died prior to the end of the study. Arched backs, rough coats, and diarrhea were observed in males that received PBNA at 1,400 mg/kg or higher and in females that received 4,100 mg/kg or higher. B6C3F_1 mice received PBNA at 380, 740, 1,480, 2,960, and 6,150 mg/kg (males) and 450, 1,000, 1,900, 4,000, and 7,620 mg/kg (females). No compound-related clinical signs of toxicity were observed in this trial. Rats apparently were more susceptible to adverse effects of oral ingestion of PBNA than were mice.

It is noted that mice in the 14-d NTP (1988) study received PBNA at up to 7,620 mg/kg via ad libitum diet and demonstrated no evidence of clinical toxicity, whereas Kelman (1964) established an LD_{50} for mice ingesting PBNA at 1,450 mg/kg via oil suspension. These differing responses may be attributed to the strains of mice used in the experiments (the strain of mice in Kelman's study is not stated), the vehicle used for ingestion of PBNA, the purity of the PBNA (purity of PBNA was not given in the Kelman study), or the animal or laboratory husbandry employed in the studies.

Subchronic Toxicity (30-180 d)

In 13-wk feed studies, groups of 10 male and 10 female F-344N rats were fed 98% pure PBNA via ad libitum diet at 200, 400, 800, 1,800, and 6,800 mg/kg (males) and 300, 600, 1,200, 2,800, and 8,300 mg/kg (females) (NTP 1988). Rough coats were observed in rats that received 1,800 mg/kg. Liver-to-body weight ratios in dosed animals were observed to be substantially higher than those in control animals. The final mean body

weights of rats that received the highest doses were 60% lower than those of controls in males and 44% lower than those of controls in females. B6C3F_1 mice fed 800, 1,400, 3,300, 5,200, and 13,000 mg/kg (males) and 1,000, 1,900, 4,300, 6,400, and 17,000 mg/kg (females) were also observed to have substantially higher liver-to-body weight ratios compared with control groups. Renal injury was the primary toxic response to PBNA in male and female rats and mice. Increased incidences of nephrotoxic lesions were observed in both species at higher dose levels.

Four of 10 male rats died at peak dosage (6,800 mg/kg) 4 wk into the study. Nine of 10 female rats died at a peak dosage (8,300 mg/kg) 4 wk into the study. Two of 10 male mice died at peak dosage (13,300 mg/kg) and seven of 10 female mice died at peak dosage (17,000 mg/kg) at varying times prior to the completion of the study.

Hematologic Effects

In the 13-wk NTP (1988) oral feed study, hematopoietic hypoplasia or atrophy of the femoral bone marrow was seen in 7 of 10 male rats and in 8 of 10 female rats at the maximum doses of PBNA, 6,800 mg/kg and 8,300 mg/kg, respectively (NTP 1988). Two of 10 females also demonstrated these hematological effects at a dose of 2,800 mg/kg. Evidence that these lesions caused specific hematopoietic deficiencies such as erythropenia was not presented, nor was a quantifiable description of the hematopoietic effects offered in the study. For these reasons, hematologic effects were not used in establishing an acceptable concentration (AC) for PBNA ingestion.

Gastrointestinal Effects

In a study conducted to examine the induction of bladder cancer by aromatic amines, Syrian Golden hamsters were dosed twice per week via gavage with technical-grade PBNA in arachadis oil at 75 mg/kg for approximately 10 wk (Green et al. 1979). Hamsters exhibited hemorrhagic enteritis, diarrhea, and stomach ulcers. The majority (exact number not given) of the animals perished within a 10-wk period. Green et al. attributed the high mortality to the inability of the hamsters to tolerate this dose. Other possible explanations include the use of a technical grade of PBNA as the test chemical or the quality of animal care.

Reproductive Toxicity

Testicular hypospermatogenesis was demonstrated in two of 10 male F-344N rats that received the maximum dose of PBNA, 6,800 mg/kg, in the 13-wk NTP feed study (NTP 1988).

Immunotoxicity

Lymphoid degeneration of the thymus occurred in 4 of 10 male F-344N rats and in 7 of 10 females at the maximum doses of 6,800 mg/kg and 8,300 mg/kg, respectively, in the 13-wk toxicity study by NTP (1988). Lymphoid depletion of the spleen also occurred at the above doses in 2 of 10 males and 6 of 10 females.

Nephrotoxicity

F-344N rats exposed to PBNA in the NTP (1988) 13-wk feed study were observed to have chemical-related nephropathies characterized by renal tubular epithelial degeneration and hyperplasia, with the occurrence of reddish-brown granulated material and degenerating leukocytes within the dilated tubules. The nephropathic lesions occurred at increased incidence and severity in female rats receiving PBNA at 1,200 mg/kg or greater doses. Male rats demonstrated lesions at doses of 1,800 mg/kg (4/10) and 6,800 mg/kg (7/10). Dose-related nephropathies were also observed in $B6C3F_1$ mice that received PBNA at 1,900 mg/kg or greater in the 13-wk NTP feed study. Lesions included cortex dilation, epithelial necrosis, and tubular epithelial regeneration.

Chronic Toxicity (0.5-3 y)

PBNA was administered in the diet (ad libitum) to F-344N rats and $B6C3F_1$ mice for 2 y (NTP 1988). Doses given to rats were 103 mg/kg (low dose) and 225 mg/kg (high dose) for males and 118 mg/kg (low dose) and 261 mg/kg (high dose) for females. Male mice received 500 mg/kg (low dose) and 1,000 mg/kg (high dose). Female mice received 450 mg/kg (low dose) and 900 mg/kg (high dose). Rats treated at low doses were

observed to have mean body weights 12-15% lower than controls, and rats treated at high doses had mean body weights 16-31% lower than controls. Mean body weights of low-dose mice were comparable to or within 7% of controls, while mean body weights of high-dose mice were 5-13% lower than controls. Survival rates of dosed mice were comparable to those of controls. Dosed male and female rats had greater survival rates than those of the control animals; this was attributed to the lower body weight of dosed rats.

Nephrotoxicity

The kidney was the target organ for toxic effects in the 2-y feed study by NTP (1988), a finding consistent with previous observations that aromatic amines have a high potential for producing kidney lesions (NTP 1988). Male rats received 103 mg/kg as a low dose and 225 mg/kg as a high dose. Female rats received 118 mg/kg as a low dose and 261 mg/kg as a high dose. Male mice received 500 mg/kg (low dose) and 1,000 mg/kg (high dose), and female mice received 450 mg/kg (low dose) and 900 mg/kg (high dose). Chemical-related non-neoplastic nephropathic lesions, karyomegaly, and hyperplasia occurred in the kidneys of both rats and mice that received the high doses of PBNA. Chemical-related kidney lesions in male rats consisted primarily of acute suppurative inflammation of the renal tubules. Suppurative inflammation of the kidneys occurred in 80% (40/50) of the high-dose male rats, in 64% (32/50) of the low-dose male rats, and in 16% (8/50) of the control rats. This inflammation could possibly be attributed to an infectious process among the experimental animals; therefore, its implications are examined with caution.

Kidney lesions were more extensive and severe in female rats than in males. Kidney mineralization and calculi, necrosis of the renal papilla, tubular atrophy, epithelial hyperplasia, hydronephrosis, atrophy, and multifocal fibrosis were observed at increased incidences in high-dose (261 mg/kg) female rats.

Nephropathic observations in high-dose (900 mg/kg) female mice included karyomegaly, occurring primarily in the convoluted tubules of the renal cortex, tubular regeneration, thickened basement membrane, dilated tubules containing granular casts, and mononuclear cell infiltrates. Tubular cell hyperplasia was observed in two high-dose female mice.

Carcinogenicity

Syrian golden hamsters were administered technical-grade PBNA in arachidis oil by gavage at 37.5 mg/kg twice per week for life (approximately 1.8 y). No chemical-related neoplastic growths were observed. Tumorigenic results were comparable between dosed and control animals, with tumor incidences of 11% for PBNA-treated animals and 14% for control animals receiving only arachidis oil. Other observed macroscopic and microscopic alterations were comparable to controls used in the experiment (Green et al. 1979).

In NTP's 2-y study, there was no evidence of carcinogenic activity for male or female F-344N rats fed diets containing PBNA at 103 mg/kg (low dose) and 225 mg/kg (high dose) for males and 118 mg/kg (low dose) and 262 mg/kg (high dose) for females (NTP 1988). The absence of carcinogenicity in rats in that study might be related to the limited ability of this species strain to metabolize PBNA to the carcinogen BNA or its carcinogenic metabolites; however, PBNA metabolites were not evaluated in the study (NTP 1988). Kidney neoplasms observed in both rats and mice were not significantly different from the historical incidence of tumors in NTP studies, with the exception of two tubular adenomas found in the high-dose female mice. Other carcinomas found in the study were determined to be unrelated to PBNA administration. Equivocal evidence of carcinogenic activity, indicated by the occurrence of two rare kidney neoplasms, a tubular cell adenoma and a tubular cell adenocarcinoma, was seen in high-dose (900 mg/kg) female $B6C3F_1$ mice. Incidences of other neoplasms found in that study were within the historical control range of the laboratory in which the studies were conducted (NTP 1988).

Refined PBNA (refined via repeated dissolution in 98% ethyl alcohol and recrystallization) was administered by gavage to male Wistar rats at 160 mg/kg and 320 mg/kg for 12 months (mo) (Wang and Wang 1981). Twenty- seven of 57 dosed rats developed carcinomas of the lungs, kidneys, prostate, and pancreas. (Six of 43 oil-injected control animals developed these predominant tumors.) There was no information given regarding distinction of results on the basis of dose. In contrast to the 1988 NTP chronic (2-y) study in which PBNA was administered via ad libitum diet, rats in the Wang and Wang (1981) study received gavage (intragastric) administration of PBNA. Absorption characteristics differ between dietary and bolus dosage of a chemical and can affect the toxicologic consequences of chemical ingestion.

No bladder tumors were observed in female dogs fed PBNA at 540 mg, 5 d/wk in food, for 4.5 y (Gehrmann et al. 1949). Male and female mice were given commercial (nonpurified) PBNA at 46 mg/kg by gavage for 3 wk followed by 18 mo of PBNA administration via ad libitum feed at 500 mg/kg (Innes et al. 1969). Dosed animals had increased tumor incidences in an uncertain range. The results of this study were inconclusive, and the authors cited a need for additional statistical evaluation and/or experimentation for adequate interpretation of the findings.

In another study, male and female Sprague-Dawley rats given 600 mg/kg intragastrically twice per week for life demonstrated no neoplastic growths (Ketkar and Mohr 1982). Tumors observed in treated animals (and not seen in controls) were considered isolated observations or within commonly occurring spontaneous tumor incidences for a normal population of the animals. Tumor incidence data revealed a 20% tumor incidence for treated male rats and a 3% incidence for treated female rats. Control animals, which received only arachidis oil, were observed to have a tumor incidences of 60% and 75% for male and female rats, respectively.

An epidemiological study of workers in Shanghai concluded that an excess incidence of lung cancer in certain workshops of rubber tire factories might have resulted from exposure to PBNA aerosols (Wang et al. 1984). The average concentrations measured in air samples in the "mixing" areas of one rubber factory ranged from 0.404 mg per cubic meter (m^3) to 0.535 mg/m^3. Concentrations in a second factory, where there was a lesser incidence of cancer among workers, averaged from 0.082 mg/m^3 to 0.140 mg/m^3. Wang et al. concluded that although experimental results suggest that PBNA might be carcinogenic to the lungs via inhalation, those results are inconsistent with the results of a separate in situ animal study that indicated that PBNA was not the sole cause of excess cancer incidences of the lungs or other organs. It is appropriate that Wang et al. also concluded that further epidemiological investigations of other materials in the factories, including other rubber additives, would be valuable.

The epidemiological study conducted at a Midlands tire factory in the United Kingdom concluded that exposure to and use of PBNA did not increase the incidence of bladder tumors in the exposed workforce at the factory under normal operating conditions (Veys 1996). The study, undertaken to assess bladder cancer morbidity, compared the medical follow-up through 1990 of 3,038 male rubber workers employed beginning January 1950 with the 2,577 men employed beginning January 1951 (less the 461 men engaged in 1950). Antioxidants used in rubber manufacture contained

BNA at 2,500 ppm; those antioxidants were withdrawn from factory use in 1949. Workers employed beginning January 1950 were exposed to the residuals of those antioxidants. Workers employed beginning January 1951 were exposed to PBNA (at 0.34-6.7 mg/m^3) and other antioxidants containing trace amounts of BNA (20-50 ppm). Observed bladder tumors in factory workers were compared with national and regional rates of bladder tumor incidence. Based on national and regional incidence rates, a small excess of bladder tumors was observed in the cohort employed beginning January 1950. This excess was considered the result of "spillover risk" from residuals of carcinogenic antioxidants used prior to 1950. There was no excess bladder tumor incidence in factory workers employed starting January 1951, supporting the conclusion that there was effective removal of occupationally hazardous antioxidants from the work environment at the end of 1949.

It is noted that Veys (1996) presented his study only in respect to bladder cancer, whereas Wang et al. (1984) included cancers detected in multiple body organs. Wang et al. asserted that other factory aerosols could be responsible for the increased cancer incidences.

Reproductive Toxicity

No data were found relating to the reproductive toxicity of PBNA in humans. Impaired reproductive function, indicated by desquamation of spermatogenic epithelium, was reported in white male rats fed diets containing industrial-grade PBNA at 100 mg/kg for 18 mo (Shumskaya et al. 1971). Shumskaya et al. observed that when dosed males were mated with healthy females, the resulting embryos perished twice as often as embryos resulting from mating between control males and healthy females. Further quantitative or qualitative descriptions of reproductive toxicity were not presented. In the NTP (1988) 2-y rodent feed study, ovarian and uterine suppurative inflammation and abscesses of other organs were observed in 15 of 50 female mice given the low dose (450 mg/kg) and in 19 of 50 female mice fed PBNA at the high dose (900 mg/kg) (NTP 1988). However, 10 of 50 control female mice also demonstrated these abscesses. The inflammations were not specified as chemical-related events, and may have resulted from infection that subsequently compromised both the kidneys and ovaries in the mice. These inflammations are not useful as end points for establishing PBNA ACs because of the uncertainty of their origin.

Developmental Toxicity

No data were found on the developmental toxicity of PBNA.

Genotoxicity

PBNA was evaluated as having a structural alert for DNA reactivity (potential genotoxic carcinogenicity) because of its carcinogenic subunit, BNA (Ashby et al. 1989). Per evaluation of results from a battery of genotoxicity tests disposed by the NTP, PBNA is ostensibly a rather weak clastogen and mutagen at elevated concentrations, extended exposure times, and in the presence of metabolic activation (Table 8-8). Dimethyl sulfoxide (DMSO) was the solvent of choice for those studies, and the purity of the tested PBNA was 99% or better.

PBNA has been detected in both reclaimed-water and humidity-condensate samples from the Mir space station. The data from Mir-21 are representative of the concentrations that were found at roughly equal intervals over the duration of the mission. Five sequential samples of reclaimed water had concentrations of 0.2, 0.2, 0, 6.3, and 11.7 μg/L. Four samples of humidity condensate had concentrations of 15.3, 4.8, 15.3, and 55.5 μg/L.

The data on PBNA presented in Table 8-8 show that it is a weak, if not questionable, genotoxic compound at nontoxic concentrations. Levels of PBNA detected in spacecraft regenerated-water or humidity-condensate sample levels did not exceed 55 μg/L, or 0.055 μg per milliliter (mL). Because test concentrations are several orders of magnitude above spacecraft contaminant levels and yield mainly negative results, there should be minimal risk of PBNA genotoxicity for spacecraft crew members.

Health and Occupational Standards

The International Agency for Research on Cancer (IARC) has classified PBNA a Group 3 (not classifiable as to its carcinogenicity to humans) chemical (IARC 1987). IARC has concluded that there is limited evidence that PBNA is a carcinogen to animals. The Occupational Safety and Health Administration (OSHA) has not set a PEL (permissible exposure limit) for PBNA (ACGIH 1991, pp. 1211-1213). The National Institute for Occupa-

TABLE 8-8 Summary of PBNA Genotoxicity Studies[a]

Reference	Test	Metabolic Activation	Concentration[b]	Exposure Duration	Toxicity Test/ Lethal Dose	Lowest Effective Dose	Overall Evaluation
Zeiger 1990; NTP 1988 (DMSO concentration not given)	Ames mutagenesis (TA100, TA1535, TA97, TA98)	None	0, 0.1, 0.3, 1, 3, 6, 10, 33, 100, 166, 333 μg/plate	24 h	No toxicity data		Negative
		10% hamsters					Negative
		30% hamsters					Negative
		10% S9 rat					Negative
		30% S9 rat					Negative
Anderson et al. 1990 (DMSO ≤ 1%)	CHO ABS assay	None	0, 2.97, 9.9, 29.7 μg/mL	8 h	No toxicity data		Negative
		S9 rat		2 h			Negative
	CHO SCE assay	None	0, 1.4, 3.4, 11.4 μg/mL	~26 h			Negative
		S9 rat	0, 3.4, 11.4, 34.1 μg/mL	~30 h		11.4 μg/mL	Weakly positive
Sofuni et al. 1990 (DMSO concentration not given)	CHO/CHL ABS assay						
	CHO cells	None	3, 9.9, 29.7 μg/mL	8 h	Toxic at 49.5 μg/mL		Negative
		S9		2 h			Negative
	CHL cells	None	0, 30, 60, 90 μg/mL	6 h	Toxic at 30 μg/mL (48 h)		Negative
		S9		6 h		30 μg/ml	Weakly positive

Myhr et al. 1990 (DMSO at 1%)	L5178Y MOLY at tk locus (forward mutation via induction of TFT resistance	None	0-32 µg/mL (7 concentrations)	4 h	Toxic at 40 µg/mL (4 h)		Inconclusive, negative (one trial each)
		S9 rat	0-13 µg/mL (9 concentrations)	4 h		10 µg/mL	Positive (two of three trials)

[a]PBNA was supplied by the National Toxicology Program at a purity of 99% or better for all studies.
[b]At a concentration of zero, only DMSO (solvent) was administered.

TABLE 8-9 Toxicity Summary[a]

Concentration	Exposure Duration	Species	Effects	Reference
Acute Exposures (1-5 d)				
1,450 mg/kg in vegetable oil suspension by gavage	Single dose	Mouse, n = 10, 18-21 g	LD_{50}	Kelman 1964; IARC 1992
8,730 mg/kg in vegetable oil suspension by gavage	Single dose	Rat, n = 6, 170-200 g	LD_{50}; venous congestion leading to acute liver, lung, brain, and vascular changes; CNS depression; disturbance of liver function indicated by depressed concentrations of hippuric acid (approximately 50%)	Kelman 1964; IARC 1992
Short-Term Toxicity (6-30 d)				
330-7,900 mg/kg[b] (m), 380-7,800 mg/kg[b] (f), in feed	14 d	F-344N rat, 5 per gender, 7-8 wk old	Males at ≥1,400 mg/kg and females at ≥4,120 mg/kg exhibited arched backs, rough coats, and diarrhea; 660 mg/kg was established as a NOAEL in males, 1,680 mg/kg was established as a NOAEL in females	NTP 1988
380-6,150 mg/kg[b] (m), 450-7,620 mg/kg[b] (f), in feed	14 d	B6C3F_1 mouse, 5 per gender, 8 wk old	No clinical signs of toxicity	NTP 1988
Subchronic Exposures (30-180 d)				
1,750 mg/kg by gavage	30 d	Rat, n = 10	Lethargy, somnolence, diminished appetite; granular degeneration in liver cell plasma; destruction of renal glomerules	Kelman 1964

75 mg/kg twice per week for life, by gavage	10 wk	Syrian Golden hamster, 40 per gender	Majority died within 10 wk; hemorrhagic enteritis, stomach ulcers; diarrhea; no chemical-related tumors	Green et al. 1979
200-6,800 mg/kg[b] (m), 300-8,300 mg/kg[b] (f), in feed	13 wk	F-344N rat, 10 per gender, 7-8 wk old	Incidence of nephropathic lesions in males: control, 0/10; 200 mg/kg, 0/10; 400 mg/kg, 0/10; 800 mg/kg, 0/10; 1,800 mg/kg, 4/10; 6,800 mg/kg, 7/10 Incidence of nephropathic legions in females: control, 0/10; 300 mg/kg, 0/10; 600 mg/kg, 0/10; 1,200 mg/kg, 2/10; 2,800 mg/kg, 7/10; 8,300 mg/kg, 8/10 In females, hematopoietic hypoplasia was present at 2,800 mg/kg and a NOAEL was established at 600 mg/kg; in males, testicular hyposmermatogenesis, lymphoid degeneration of the thymus, and lymphoid depletion of the pleen were present at 6,800 mg/kg and a NOAEL was established at 800 mg/kg	NTP 1988
800-13,300 mg/kg[b] (m), 1,000-17,000 mg/kg[b] (f), in feed	13 wk	$B6C3F_1$ mouse, 10 per gender, 8 wk old	Incidence of nephropathic lesions in males: control, 0/10; 800 mg/kg, 0/10; 1,400 mg/kg, 0/10; 3,300 mg/kg, 7/10; 5,200 mg/kg, 10/10; 13,000 mg/kg, 10/10 Incidence of nephropathic lesions in females: control, 0/9; 1,000 mg/kg, 0/10; 1,900 mg/kg, 2/10; 4,300 mg/kg, 3/10; 6,400 mg/kg, 9/9; 17,000 mg/kg, 8/8. NOAELs were set for males and females at 1,400 mg/kg and 1,000 mg/kg, respectively	NTP 1988

(Continued)

TABLE 8-9 Continued

Concentration	Exposure Duration	Species	Effects	Reference
Chronic Exposures (0.5-3 y)				
103 mg/kg (low), 225 mg/kg (high) in males; 118 mg/kg (low), 261 mg/kg (high) in females; in feed	103 wk	F-344N rat, 50 per gender, 7-8 wk old	Non-neoplastic kidney lesions, more severe in females; kidney suppurative inflammation in high-dose males; kidney mineralization, papillar necrosis, tubular atrophy, multifocal fibrosis, epithelial hyperplasia, and hydronephrosis in high-dose females; NOAEL set at 103 mg/kg	NTP 1988
500 mg/kg (low), 1,000 mg/kg (high) in males; 450 mg/kg (low), 900 mg/kg (high) in females; in feed	103 wk	$B6C3F_1$ mouse, 50 per gender, 8 wk old	In females: kidney karyomegaly in high-dose group; nephropathy in high-dose group; incidence of ovarian, uterine suppurative inflammation was 10/50 (control), 15/50 (low dose), and 19/50 (high dose); equivocal evidence of carcinogenicity in the high-dose group with formation of two rare kidney tumors (tubular cell adenoma and tubular cell adinocarcinoma)	NTP 1988
20 mg/kg in feed	18 mo	White rat, male, n = 40	No significant toxic effects	Shumskaya et al. 1971
100 mg/kg in feed	18 mo	White rat, male, n = 40	Decreases in urinary hippuric acid and adrenal ascorbic acid; decreased urinary function; reproductive function impairment; increased urinary protein	Shumskaya et al. 1971

46 mg/kg by gavage; approximately 500 mg/kg in feed	3 wk; 18 mo	Mouse, hybrid groups (C57BL/6 × C3H/Anf and C57BL/6 × AKR), 18 per gender	Results inconclusive; increased tumors in uncertain range	Innes et al. 1969
37.5 mg/kg by gavage twice per week	life (to 1.8 y)	Syrian Golden hamster, 40 per gender	Results similar to controls; no neoplastic growth attributed to PBNA	Green et al. 1979
540 mg/d (36 mg/kg) in gelatin capsule	4.5 years	Dog, female, n = 3	No bladder tumors	Gehrmann et al. 1948
600 mg/kg by intragastric administration twice per week	life (129 wk)	Sprague-Dawley rat, 40 per gender	No evidence of toxicity	Ketkar and Mohr 1982
160 mg/kg and 320 mg/kg, partially purified PBNA, by gastric intubation, 5 times per week	12 mo	Wistar rat, male	Gastrocecal ulceration; lung, kidney carcinoma	Wang and Wang 1981

[a]All exposures in the studies included were by the oral route.
[b]Doses were described in parts per million—NASA derived mg/kg values.

tional Safety and Health (NIOSH) considers PBNA to be a potential occupational carcinogen. NIOSH recommends that all occupational carcinogens be limited to the lowest feasible concentration. There are no set recommended exposure limit/immediately dangerous to life or health (REL/IDLH) levels for PBNA by NIOSH. The American Conference of Governmental Industrial Hygienists (ACGIH) has evaluated PBNA as an A4 (not classifiable as a human carcinogen) chemical with no recommended Threshold Limit Value (TLV) (ACGIH 1998, p. 55). There are no further regulatory agency health or occupational standards for PBNA.

RATIONALE

There were no toxicity studies in which PBNA was administered in drinking water to human or nonhuman species. Therefore, data used to establish acceptable concentrations (ACs) for PBNA are those involving oral ingestion via ad libitum feed. This lends some uncertainty to the process of deriving spacecraft water exposure guidelines (SWEGs) for PBNA, because absorption of PBNA administered intragastrically or in the diet can vary from absorption of PBNA ingested in drinking water. ACs were formulated for each principal adverse affect, nephrotoxicity and gastrointestinal toxicity, observed following exposure of various animal species to PBNA. Calculation of ACs includes the assumption that each crew member will use 2.8 L of water per day and has an average body weight of 70 kg. SWEG values were derived based on the lowest obtained AC for each principal adverse effect. AC values were determined following guidance set by the National Research Council (NRC 2000).

PBNA is stated to be soluble in water at the parts-per-million level (Vine et al. 1984). The established guidelines for 1- and 10-d exposures (Table 8-10) clearly exceed PBNA water solubility. These guidelines afford crew members protection from gastric irritation. All of the ACs for PBNA are presented in Table 8-11.

Ingestion for 1 d

There are no reported human or nonhuman studies for acute toxicity to PBNA. Short-term toxicity studies conducted by NTP demonstrated a NOAEL (no-observed-adverse-effect level) of 660 mg/kg for gastrointestinal effects (diarrhea) observed at 1,400 mg/kg or more in male F-344N rats receiving 98% pure PBNA for 14-d in feed (NTP 1988). A species extra-

TABLE 8-10 Spacecraft Water Exposure Guidelines for PBNA

Exposure Duration	Concentration (mg/L)	Target Toxicity
1 d	1,600	Gastrointestinal toxicity
10 d	1,600	Gastrointestinal toxicity
100 d	500	Nephropathic lesions
1,000 d	260	Nephropathic lesions

polation factor of 10 was applied in addition to the assumption of a 70-kg person ingesting 2.8 L of water per day. The AC for 1 d was calculated as follows:

$$\text{1-d AC} = (660 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 1{,}650 \text{ mg/L}.$$

Ingestion for 10 d

The AC for a 10-d exposure period was derived on the basis of the 14-d NTP study using the NOAEL of 660 mg/kg for gastrointestinal disturbances in male F344N rats.

$$\text{10-d AC} = (660 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d}) = 1{,}650 \text{ mg/L}.$$

Ingestion for 100 d

Chemical-related nephrotoxicity was demonstrated in NTP's 13-wk studies in F-344N rats and $B6C3F_1$ mice. A dose-related increase in the severity of nephrotoxic response occurred in females of both species (NTP 1988). Nephropathic lesions were observed at 1,900 mg/kg doses in mice. Nephropathic lesions observed at 1,200 mg/kg in female rats were not seen at the next lower dose of 600 mg/kg, hence a NOAEL of 600 mg/kg was designated. A species extrapolation factor of 10 and a time factor of 1.1 (for extrapolation from 91 d to 100 d) were applied to the AC calculation along with the assumption of a 70-kg person ingesting 2.8 L of water per day. The 100-d AC for nephrotoxicity was calculated as follows:

$$\text{100-d AC} = (600 \text{ mg/kg} \times 70 \text{ kg}) \div (10 \times 2.8 \text{ L/d} \times 1.1);$$
$$\text{100-d AC} = 1{,}300 \text{ mg/L (rounded)}.$$

TABLE 8-11 Acceptable Concentrations (ACs)

End Point	Exposure Data	Species and Reference	Uncertainty Factors				ACs (mg/L)			
			To NOAEL	Interspecies	Time	Spaceflight	1 d	10 d	100 d	1,000 d
Gastrointestinal toxicity	NOAEL = 660 mg/kg	F-344N rat (NTP 1988)	1	10	1	1	1,600[a]	1,600[a]	—	—
Nephrotoxicity	NOAEL = 600 mg/kg	F-344N rat (NTP 1988)	1	10	1.1	1	—	—	1,300	—
Nephrotoxicity	$BMDL_{01}$ = 226 mg/kg	F-344N rat (NTP 1988)	1	10	1.1	1	—	—	500	—
Nephrotoxicity	NAOEL = 103 mg/kg	F-344N rat (NTP 1988)	1	10	1	1	—	—	—	260
SWEGs[b]							1,600	1,600	500	260

[a]Lower estimate for calculated AC (1,650 mg/L).
[b]PBNA is soluble in water at the parts-per-million level (Vine et al. 1984), so the SWEG values listed are above water solubility levels for PBNA.
Abbreviation: $BMDL_{01}$, benchmark dose at 95% confidence interval and 1% benchmark response.

TABLE 8-12 BMD Calculations for Incidences of Nephropathy[a]

	Models						
	Gamma	Logistic-Log	Multistage	*Probit-Log*	Quantal-Linear	Quantal-Quadratic	Weibull
BMD_{10}	1,028	1,019	861	*1,013*	431	861	1,032
$BMDL_{10}$	586	639	508	*604*	258	661	551
P-value	0.94	0.94	0.7	*0.97*	0.38	0.95	0.89
BMD_{01}	499	473	266	*571*	41	266	402
$BMDL_{01}$	135	151	58	*226*	24	204	105
P-value	0.94	0.94	0.7	*0.97*	0.38	0.95	0.89

[a]Based on the NTP (1988) 91-d exposure of female F-344N rats via ad libitum feed.
Abbreviations: BMD, benchmark dose; BMD_{01}, BMD corresponding to a 1% risk; BMD_{10}, BMD corresponding to a 10% risk; $BMDL_{01}$, lower 95% confidence interval on the BMD corresponding to a 1% risk. $BMDL_{10}$, lower 95% confidence interval on the BMD corresponding to a 10% risk;

BMD calculations for the 100-d AC were derived using data from the NTP (1988) study in which female F-344N rats were fed doses of PBNA at 300, 600, 1,200, 2,800, and 8,300 mg/kg for 13 wk. (Incidences of nephrotoxicity were 0/10, 0/10, 0/10, 2/10, and 7/10 at those respective doses.) A dose-related increase in the severity of nephropathic lesions was observed in the rats. Nine of 10 rats receiving the highest dose, 8,300 mg/kg, perished; consequently, this dose was not included for derivation of the BMD. The probit-log model was selected as the most appropriate model based on the generated *p* value of 0.97. BMD calculations at the 95% confidence interval and at a benchmark response of 1% resulted in a 500 mg/L AC for nephrotoxicity. (BMD results are presented in Table 8-12, above.)

100-d AC = (226 mg/kg ($BMDL_{01}$ or NOAEL) × 70) ÷ (10 × 2.8 L/d × 1.1);
100-d AC = 500 mg/L (rounded).

Ingestion for 1,000 d

The 1,000-d AC was calculated on the basis of the 2-y NTP (1988) feed studies in which F-344N rats were observed to have non-neoplastic kidney lesions at the maximum doses of PBNA, 225 mg/kg for male rats and 261 mg/kg for female rats. Lesions were not observed at the lowest dose of 103 mg/kg in the male species or 118 mg/kg in the female species.

1,000-d AC = (103 mg/kg × 70 kg) ÷ (10 × 2.8 L/d);
1,000-d AC = 260 mg/L (rounded).

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9 Silver

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PHYSICAL AND CHEMICAL PROPERTIES

Silver is a white, lustrous, ductile, malleable metal (see Table 9-1 for a list of properties). It reacts with dilute nitric acid and hot sulfuric acid. Silver forms several inorganic and a few organic salts, including silver chloride, silver fluoride, silver iodide, silver nitrate, silver acetate, silver sulfide, silver perchlorate, silver benzoate, and silver diethyl dithiocarbamate (see Table 9-2).

OCCURENCE AND USE

Silver, a transition metal, is a rare element that naturally occurs in the earth's crust, both in pure form and as an ore with lead and copper. Soil concentrations vary by geological location. Silver has also been reported in the air; in sea, well, and surface waters (originating from natural resources and from industrial waste); and in finished public drinking water supplies (Durfor and Becker 1964; Kopp and Kroner 1967). A median concentration of silver at 2.2 micrograms per liter (μg/L) (range 0.3-5 μg/L) in finished water supplies has been reported in the United States (Kopp and Kroner 1967).

TABLE 9-1 Physical and Chemical Properties

Formula	Ag
Chemical name	Silver
Synonyms	Argentum, shell silver, silber (German), silver colloidal (Stokinger 1981)
CAS registry no.	7440-22-4
Molecular weight	107.87
Atomic number	47
Melting point	960.5°C
Density	10.5 g/cm^3 at 20°C
Units	1 ppm in water = 1 mg/L in water
Solubility	Metallic silver is practically insoluble in hot and cold water; it is soluble in fused alkali hydroxides; most silver salts have limited solubility in water; low solubility depends on pH and chloride concentrations (0.1-10 mg/L)

Silver and silver salts have been extensively used in making jewelry, table silverware, coinage, solder, high capacity batteries and conductors, and dental alloys. It is used extensively in photographic processing. Silver also has some use as an antibacterial agent in water treatment (Merck 1989). Pharmaceutical preparations used for the treatment of warts and burns contain silver. Silver nitrate has also been used as a prophylaxis against opthalmia neonatorum. Silver acetate has been used in chewing gums and lozenges as a smoking deterrent. High concentrations of silver were found in the blood and urine of subjects who consumed silver acetate lozenges (Macintire et al. 1978; East et al. 1980). The use of silver in medical equipment and devices has been a major area of research in dentistry and medicine (e.g., silver amalgam vs mercury amalgam and antimicrobial efficacy and biocompatibility of silver-coated central venous catheters, prosthetic valves, and silver impregnated collagen cuffs to decrease infection in tunneled catheters).

Human exposure to silver usually occurs by inhalation of silver-containing dust in the environment or by dermal contact to jewelry or photographic materials containing silver. Silver has been the primary agent used to disinfect potable water processed from humidity condensate in the Russian Mir space station. Silver will also be used in the humidity-conden-

TABLE 9-2 Physical Properties of Silver Salts

	Silver Nitrate	Silver Acetate	Silver Lactate	Silver Chloride	Silver Fluoride
Formula	AgNO3	CH3COO.Ag	Ag(CH3CH (OH)COO)	AgCl	AgF
Molecular weight	169.89	166.92	214.97	143.32	126.88
% Silver (w/w)	63.5	64.63	54.78	74.65	85.04
Solubility in water	122 g/100 mL at 0°C	1 g/100 mL at 0°C	1 g/15 mL at 0°C	1.93 mg/L at 25°C	1.82 g/100 mL at 15°C

Source: Merck 1989.

sate water-processing assembly in the Russian service module (SM) to support the crew during the early phases of assembly of the International Space Station (ISS). The Russian and U.S. crew members aboard the early assembly missions of the ISS will consume water containing silver at about 0.5 milligrams (mg)/L. Moreover, silver will be added electrolytically in the Russian water supplies carried to the ISS via *Progress* resupply vehicles during the ISS assembly phase. The concentrations of silver in the archived water samples from the cold and hot water galleys of various Mir missions ranged from 8 μg/L to 670 μg/L, although the target concentration was 500 μg/L. That probably indicates that the mechanism of silver addition did not work reliably, or there was a silver demand in the system after it had been added. During the Mir missions, when U.S. astronauts lived in the Mir space station for 3-6 months (mo), the fuel-cell water transferred from the shuttle was deiodinated and silver was added as silver salts to support the crew drinking water requirements. The residual iodine precipitated some of the silver, which caused very low silver concentrations in some samples. The common salts that were used to maintain silver in solution were formate and fluoride. If the crew uses water recovered from the humidity condensate, the forms of the silver salts will depend on the salts of calcium and magnesium added as mineralizing agents to improve the organoleptic properties. Because that has been proprietary, the exact forms are not known.

PHARMACOKINETICS AND METABOLISM

The bioavailability of silver appears to depend on whether it is metallic silver or one of the various silver salts. The available data on the toxicity of silver focus primarily on its bioaccumulation in aquatic organisms and its potential toxicity to humans.

Absorption

In an occupational setting, silver can be absorbed readily through inhalation of silver dust or dermal exposure to photographic processing chemicals. It also has been absorbed after ingestion of colloidal forms (Hill and Pillsbury 1939; Newton and Homes 1966; Dequidt et al. 1974, as cited in ATSDR 1990).

Data on absorption was estimated from the excretion kinetics of radioactive silver after administration by the oral route. Furchner et al. (1968) determined the body burden (by measuring whole-body radioactivity using a gamma-ray detector) and retention of silver at various times after administering doses of ^{110m}Ag (as the nitrate) via the intravenous and oral routes in female RF mice, male Sprague-Dawley rats, beagle dogs, and *Macacca mulatta* monkeys. It was reported that the body burden (based on whole-body monitoring) was higher when silver was administered intravenously rather than orally and was proportionately higher as a function of species size. One must note that the calculated doses (in milligrams per kilogram [mg/kg]), which were based on the specific activity of the radioactivity administered, varied widely from species to species. It is not known how that would have affected the relative amounts absorbed. On the basis of the cumulative excretion by the second day after oral ingestion, which was between 90% and 99% of the orally administered dose, the authors concluded that gut absorption was very low. The dog appeared to retain the greatest percentage of the dose, and the authors explained that the phenomenon was related to gastrointestinal transit time (8 hours [h] in mice and rats and about 24 h in dogs, monkeys, and humans). The much longer intestinal transit time resulted in higher absorption in dogs compared with the other species studied. By extrapolating the parabolic relationship between body weights and estimated equilibrium factors from small animals to humans, the authors estimated 4% retention of silver in humans.

A much higher level of silver retention was estimated from a case history study of silver poisoning associated with antismoking lozenges (Respa-

ton) that contain 6 mg of silver acetate (Macintire et al. 1978). A 47-y-old woman with a 2-y history of blue-gray discoloration of neck and face (argyria) reported the onset of discoloration after the use of 32 lozenges per day for 6 mo. The woman was given an oral dose of silver acetate labeled with silver tracer ^{110m}Ag (4.4 μCi and 10 mg of ammonium chloride, as in the Respaton formulation). Retentions of silver measured by whole-body counter at 1, 2, 8, and 30 weeks (wk) were 21%, 20%, 19%, and 18.7% of the total radioactive count measured 20 minutes (min) after the dose (normalized as 100%) (also see East et al. 1980). There was almost no excretion after 1 day (d). The blood level 2 h after administration was very low, and based on the whole-blood volume, the total amount in blood represented only 1.8% of the administered dose. The effect of ammonium chloride in the formulation on the retention and absorption of silver in this instance is not known. Prior to the tracer dose, the total body burden of silver was estimated by neutron analysis to be about 6.4 ± 0.2 grams (g). However, East et al. (1980) reported that such high-level constant retention after an initial drop was not consistent with the biological half-life of 5 d for the retention of whole-body ^{110m}Ag and the half-lives of 30, 15, and 10 d in bone, liver, and kidneys, respectively, as reported by the International Commission on Radiological Protection. That implies that there should be insignificant retention at 30 wk. In addition, according to East et al. (1980), other investigations believed that the use of the lozenges did not result in any significant level of absorption of silver. That indicates that with repetitive doses, the overall body retention might be higher, perhaps due to the saturation of the only biliary excretion pathway, resulting in increased distribution to tissues and poor excretion. Hence, the high percentage of retention could be a gross overestimate of what might result from chronic small doses. This level of retention is much higher than that derived by Furchner et al. (1968) for humans. It has to be noted that a different silver salt was used in those reports. A summary of oral absorption is presented in Table 9-3.

In a case of accidental inhalation exposure of one worker to dust containing radioactive ^{110m}Ag from an experimental nuclear reactor, radioactivity was found in the liver and feces even after 200 d (Newton and Holmes 1966). This strongly indicates that silver could be absorbed through the lungs into the systemic circulation. Whole-body radiation monitoring during the first 155 d revealed wide areas of radioactivity, and 25% of it was located in the liver. Silver also was found in measurable concentrations in the blood of workers in a silver oxide/silver nitrate manufacturing plant, indicating exposure through inhalation (Rosenman et al. 1979).

Similarly, Armitage et al. (1996) reported that the blood silver levels ranged from 0.1 μg/L to 23 μg/L in 98 occupationally exposed workers

TABLE 9-3 Interspecies Differences in the Retention of Silver After an Oral Dose[a]

Species	Form	Dose Retained	Reference
Mouse	Silver nitrate	<1%	Furchner et al. 1968
Rat	Silver nitrate	<2%	Furchner et al. 1968
Monkey	Silver nitrate	<5%	Furchner et al. 1968
Dog	Silver nitrate	<10%	Furchner et al. 1968
Human	Silver acetate	18% of initial retention[b]	Macintire et al. 1978; East et al. 1980

[a]Estimated from cumulative excretion at day 2. The animal data were obtained after only tracer doses of silver nitrate. Doses were very small (mg/kg) and were different for each species.
[b]Data from one argyric human who ingested silver acetate from lozenges for over 2 y. The formulation also contained ammonium chloride.

involved in bullion production, cutlery manufacture, and silver reclamation. When colloidal silver was administered to Wistar rats orally at 1.68 g/kg for 4 d or 0.42 g/kg for 12 d, about 2-5% of the dose was absorbed (Dequidt et al. 1974, as cited in ATSDR 1990). In another study by Scott and Hamilton (1950), it was found that when carrier-free radioactive silver (<1 μg; 1 μCi) was intragastrically administered to rats, 99% of the dose was eliminated in the feces and 0.18% was eliminated in the urine within 4 d. The total tissue distribution of the radioactivity was about 0.84% of the dose. The results indicated very little absorption.

Distribution

Reports strongly indicate that silver is distributed to almost all organs of the body after exposure. Rats given silver nitrate in drinking water (0.15% or 8.8 millimolar [mM] of silver) for 5 wk showed deposition of silver granules in the kidneys (Moffat and Creasey 1972; Creasey and Moffat 1973). Similarly, in Sprague-Dawley rats that received silver nitrate at various concentrations (6, 12, and 24 mM of silver) in drinking water for 60 wk, silver accumulated in the basement membranes of the glomerulus, colon, liver, thyroid, urinary bladder, and prostatic acini (Walker 1971). Although the rats were restored to silver-free water, the deposited silver did not diminish even 10 wk after silver salt ingestion. Maffat et al. (1973) reported that when silver nitrate was given to three rabbits and 10 rats as a

0.15% solution (8.8 mM of silver) in drinking water, silver was found in the medullary interstitial tissue and in the interstitial cells (which showed signs of degeneration) in both species. There was a species difference in the amount of silver deposited (heavy deposits of silver in the rat and smaller amounts in the rabbit). Matuk et al. (1981) reported retardation of growth in rats given silver nitrate at 0.25% (15 mM) in drinking water for 10 wk. When continued for an additional 12 mo, all rats died. Examination of eyes showed deposits of silver particles in Bruch's membrane—the number and size of the particles increased with continued ingestion, but was found to be decreased in the group that was continued on silver-free water.

In a study involving biologic monitoring of workers (n = 37) in one of the silver smelting and refining industries in which the exposure is entirely by inhalation, silver was found in the blood (0.011 μg per milliliter [mL]), urine (<0.005 μg/mL), and feces (15 μg/g). Control subjects excreted about 1.5 μg/g in the feces (n = 35). The author suggests that human fecal excretion of silver at exposure levels equal to the Threshold Limit Value (TLV) (0.1 mg per cubic meter [m^3]) would be about 1 mg of silver per day (DiVincenzo et al. 1985).

Rungby (1986a) studied the anatomical distribution of silver in the peripheral nervous system of rats 4 d after intraperitoneal (silver lactate) or oral administration (silver lactate or silver nitrate). Silver was found to be distributed throughout the peripheral nervous system in dorsal root ganglia, peripheral nerve, adrenal medulla, and enteric ganglia. The localization of silver deposits in the orally treated animals was independent of the form of the salt. In all organs, large amounts were present in connective tissue fibers and basement membranes (Rungby 1986a).

In postmortem analyses for several metals in the tissues of 150 human adults who died instantaneously, silver was found to be present in all tissues (Tipton and Cook 1963) in the order of thyroid > skin > liver > adrenals > intestine > stomach and other tissues. East et al. (1980) did a detailed study on the uptake and disposition of silver in a 47-y-old woman who ingested antismoking lozenges containing 6 mg of silver acetate daily for 2.5 y and developed argyria in the process. Using radioactive tracer of silver acetate (4.5 mg; 4.43 μCi), they measured silver retention. At the end of first week, 18% of the ingested radioactivity was retained, and that remained constant up to 30 wk. The blood levels and the percent of excretion in urine over the first week were very low. Silver was detected at a high concentration in a skin biopsy sample (71.3 ± 3.7 μg/g). Uptake by the skin was substantial. In the Newton and Holmes (1966) study, whole-body monitoring of a 29-y-old man who accidentally inhaled an unknown amount of dust containing

^{110m}Ag from an experimental nuclear reactor showed that about 25% of the body burden of ^{110m}Ag (total radioactivity) was found near the liver.

Excretion

It was first demonstrated by Scott and Hamilton (1950) that when bile ducts were ligated in rats, the percent of silver excreted in the feces was much lower (by a factor of 10) than in control rats, although renal excretion increased, clearly demonstrating that silver is excreted primarily via the bile into feces. In a study on the mechanism of elimination of silver by the liver, Klaassen (1979) concluded that most of the silver excreted in feces was the result of elimination of silver through bile. When the disappearance of ^{110m}Ag from plasma and bile was measured 2 h after the intravenous administration at 0.01, 0.03, 0.1, and 0.3 mg/kg (^{110m}Ag was mixed with silver nitrate) in rats, the concentration of silver in bile was 20 times greater than that in plasma. Also, the concentration in liver was much higher than that in plasma, and 25-45% of the dose was excreted in the bile within 2 h (Gregus and Klaassen 1986). The disappearance of silver from the plasma and its excretion into the bile after intravenous administration of silver at 0.1 mg/kg in rats, rabbits, and dogs indicated marked species variations in the biliary excretion of silver. Rats excreted silver at the rate of 0.25 μg/min/kg, while rabbits and dogs excreted at rates of 0.05 μg/min/kg and 0.005 μg/min/kg, respectively. The species with the slowest excretion rate had the highest liver concentration. Dogs had the highest silver levels in the liver, and rats had the lowest, indicating that the transport from liver to bile is the governing factor (Klaassen 1979). This difference might also explain the rentention data obtained by Furchner et al. (1968) (see discussion below).

Scott and Hamilton (1950) studied the distribution of silver after an intramuscular administration of radioactive metallic silver alone as a tracer dose and then coadministered with two doses of nickel nitrate (0.4 mg/kg/d and 4.0 mg/kg/d). They reported that when excretion in the feces was decreased, a corresponding increase was noted in the deposition of silver in the pancreas, gastrointestinal tract, and thyroid. This increase suggested that the liver elimination pathway might be saturated.

Several studies indicate that the elimination of silver follows a 2- or 3-exponential profile, one with a short half-life and others with a half-life of several days. In the Newton and Holmes (1966) study cited above, calculation of the amount of silver excreted in the feces by a man who accidentally

inhaled an unknown amount of radioactive silver dust indicated that elimination from the body followed a biphasic exponential decay curve—the first phase had a half-life of 1 d, and the second terminal phase had a half-life of 43 d. Matuk (1983) reported similar results after an intraperitoneal injection of radioactive silver. There was an initial rapid loss of radioactivity from plasma, liver, and kidneys, which was followed by a slower rate of loss. The loss was somewhat linear and slower from forebrain and spleen. As shown above, silver is excreted predominantly in the feces and, to a minor extent, in the urine following an oral dose. The rate of excretion is rapid in the first week and then slows, showing biphasic elimination kinetics in humans given silver acetate orally (East et al. 1980).

Furchner et al. (1968) also reported that when radioactive silver nitrate was administered orally to mice, rats, dogs, and monkeys, 90-98% of the absorbed dose was eliminated in the feces (within 2-4 d) and only minor amounts were excreted in urine. They also reported interspecies differences in the clearance of silver. A 2-exponential component described the elimination data in mice and monkeys, and a 3-exponential component described the data from rats and dogs. Differences in the transit time through the gut has been offered as possible explanation (the transit time is 8 h in mice and rats and about 24 h in dogs and monkeys) (Furchner et al. 1968). It might also be attributed to the interspecies differences in biliary excretion rate reported by Kalaasen (1979).

Metabolism

Even though silver salts are not metabolized in the typical sense, silver salts that are transformed are reduced to metallic silver. It was suggested (ATSDR 1990) that the deposition of silver in tissues is the result of precipitation of insoluble silver chlorides and silver phosphates and that those silver salts are transformed to silver sulfides by forming complexes with amino or carboxyl groups in proteins or are reduced to metallic silver by reduction with ascorbic acid (Danscher 1981). Buckley et al. (1965) identified silver particles deposited in the dermis of a woman with argyria as silver sulfide. Similarly, Berry and Galle (1982) reported that deposits of silver in the internal organs of rats were identified as silver sulfide. Silver seems to interact with other metal salts, especially with selenium in the diet (Berry and Galle 1982, as cited in ATSDR 1990).

TOXICITY SUMMARY

One of the most commonly reported conditions in humans related to ingestion of silver is argyria, the blue-gray discoloring of skin resulting from the accumulation of silver in the dermis. It was mostly associated with frequent and long-term exposure, such as the use of silver amalgam, and occupational exposure to silver particles (in mines or in industries involving smelting, polishing, manufacture, and packaging of silver nitrate products). Argyria has been the result of exposure to metallic silver or silver compounds not only by the dermal route but also by oral and inhalation routes of exposure. Because of poor absorption of silver by all routes of exposures, chronic toxicities or physiologic effects at doses capable of causing argyria have not been documented. Gaul and Staud (1935) analyzed 70 cases of argyria where subjects had been exposed to silver either in a colloidal form or had it injected intravenously as a medication (e.g., silver arsphenamine for syphilis). Ten males and two females received a total of 31-100 intravenous injections of silver arsphenamine over a period of 2 to about 10 y. This amounted to a total exposure dose of 4-20 g of silver. No definite threshold could be identified for the incidence of argyria; some developed the condition after a total dose of 4 g of silver, while it appeared in others only after 20 g. Using a biospectrometric analysis of skin biopsies, the authors concluded that the skin discoloration was proportional to the amount of silver present. Based on the lowest level—4 g of silver arsphenamine—the EPA working group on silver (EPA 1992) calculated that argyria might occur at a total body burden approximately equivalent to 1 g or above. There is no functional impairment known to be associated with argyria. In clinical studies, 30 workers (of whom 6 had argyria and 20 had argyrosis [deposition of silver in the eye]) who were exposed to silver and silver oxide for more than 2 y had significant blood silver levels and abnormal clinical biochemistry (Rosenman et al. 1979). The exposed workers had complained of poor night vision, nausea, headache, nervousness, and tiredness. The authors reported that the presence of abdominal pain in 10 workers correlated with the level of silver in the blood. Rosenman et al. (1987) also reported possible nephrotoxic effects of silver in exposed workers in a precious-metal powder manufacturing plant. Workers with elevated concentrations of silver in the urine and in the blood had corneal deposits of silver and complained of poor night vision. They had significantly increased urinary levels of *N*-acetyl-beta-*d*-glucosaminidase (NAG) and a

decreased creatinine clearance (clinical markers of nephrotoxicity). Because of concurrent exposure to cadmium, also a well-known nephrotoxin, the effect of silver on nephrotoxicity could not be conclusively established in this study.

Acute Exposure (≤1 d)

Data on the toxicity of silver and its salts in humans come mainly from case reports and accidental exposures. A considerable amount of data is available from animal studies. Emphasis will be placed on oral bolus studies and drinking water studies with silver salts. Tamimi et al. (1998) determined the acute and subchronic toxicity in rats and rabbits after intraperitoneal injection and oral administration of an antismoking mouthwash containing silver nitrate at 0.5% (silver at 3.175 mg/mL) as an active ingredient. An oral LD_{50} (dose lethal to 50% of subjects) was reported at about 430 mg/kg for rats (males and females) and at about 1,300 mg/kg for rabbits (male and female). Postmortem and histopathologic examinations revealed congestion, edema, hemorrhage, and mucosal necrosis. It is not clear if other ingredients in the mouthwash might have been responsible for those effects.

Death in one human was reported in an accidental ingestion of a large amount of silver nitrate. Symptoms included abdominal pain, diarrhea, vomiting, corrosion of the gastrointestinal tract, shock, and convulsions. It was estimated that silver at 143 mg/kg might be a fatal single dose for humans (Hill and Pillsbury 1939; EPA 1992). LD_{50} studies indicate that in general, silver salts are acutely toxic to rodents. The toxicity and mortality also was dependent on the route of administration and the chemical nature (silver acetate, lactate, nitrate, or chloride) of the dose.

There are no known reports of hepatotoxicity, nephrotoxicity, or cardiotoxicity resulting from an acute exposure.

Short-Term Exposure (2-10 d)

Dequidt et al. (1974, as cited in ATSDR 1990) reported deaths in rats following oral ingestion of silver colloid at 1,680 mg/kg/d for 4 d. When the silver colloid was injected intraperitoneally at 420 mg/kg, rats died within 24-48 h. Dequidt et al. also reported that nitrate is 20 times more toxic than the colloidal form when given intraperitoneally. The actual cause of death was not reported in either of the above studies.

In mice given a single intraperitoneal injection of silver lactate at 20 mg/kg, lipid peroxidation significantly increased in the liver 3, 12, and 48 h after exposure as measured by levels of malondialdehyde (Rungby 1987). The levels were unaffected in the kidneys and the brain. The author concluded that silver interfered with free-radical scavenging mechanisms. A NOAEL (no-observed-adverse-effect level) for this effect could not be identified.

Twenty male and 10 female mice received two intraperitoneal injections of silver lactate on successive days totaling 1 mg. Ten days after the last injection, the animals were tested for open field behavior for 3 d (males) or 4 d (females), and all silver treated mice were hypoactive (Rungby and Danscher 1984). Hypoactivity was statistically significant in female mice 14 d after the injections and in male mice 11, 12, and 13 d after the injections. Female mice accumulated silver in their brainstem, cerebral cortex, basal ganglia, anterior olfactory nucleus, and in the red and cerebellar nuclei.

Subchronic Exposures (11-100 d)

Walker (1971) reported that three of 12 Sprague-Dawley rats receiving silver nitrate in drinking water at a concentration of 24 mM (311 mg/kg/d) died in 2 wk. No reports on short-term adverse effects on the gastrointestinal tract, liver, or kidneys were found.

In a 30-d subchronic study, Tamimi et al. (1998) swabbed the oral cavity of rats and rabbits with a new antismoking mouthwash containing silver nitrate at 0.5% at doses of 1.5, 15, and 150 mg/kg for 30 consecutive days. No differences were seen in body weight or hematologic parameters between control and treated groups.

Administration of 6 mM of silver nitrate (silver at 65 mg/kg/d) in the drinking water of mice resulted in the deposition of silver granules within the glomerular basement membrane after only 12 d of treatment (Day et al. 1976). When the silver ingestion was extended to 14 wk, larger aggregates were detected in the basement membrane. Even 21 wk after the termination of silver ingestion, the silver deposits were present. The authors reported that in their preliminary studies, when the concentration of silver was 12 mM (130 mg/kg/d), it was unacceptable to the mice, and there was a dramatic drop in water consumption. No further details were provided.

Wagner et al. (1975) did not find any growth depression or liver necrosis when Holtzman rats (10 per group) were given silver acetate in drinking water at 7.6 mg/kg/d for 52 d while ingesting a diet that had the recom-

mended concentrations of selenium and vitamin E. In another study by Diplock et al. (1967), no effects were seen in Holtzman rats given silver at 97 mg/kg/d as silver acetate in water for 50 d when the diet was complete. Liver necrosis was seen only in rats fed a vitamin-E deficient diet.

Van Vleet (1976) reported that four weanling swine fed a diet containing silver acetate at 0.5% for 4 wk (equivalent of 130 mg/kg/d) developed anorexia, diarrhea, and growth depression. Hepatic lesions were found in all four pigs at this dose. However, no lesions were found in pigs fed silver acetate at 0.2% (estimated dose of 52 mg/kg/d). Because the apparent NOAEL was only 2.5 times lower than the dose that killed three of four weanling swine and the dose-response was very steep, and considering the low number of subjects, the results were not considered for acceptable concentration (AC) calculations.

Silver has also been reported to inhibit glutathione (GSH) peroxidase, a seleno-enzyme. Administration of silver acetate at 751 parts per million (ppm) (silver at 484 mg/L or 73 mg/kg/d) in water for 15 wk to young Holtzman rats (fed a diet adequate in vitamin E and selenium) reduced liver GSH peroxidase activity to 5% of controls. In the erythrocytes and the kidneys, the enzyme activities were reduced to 37% of controls (Wagner et al. 1975). The same authors reported that when the rats were exposed to silver in drinking water at 76 mg/L (7.3 mg/kg/d) for 52 d, GSH peroxidase was only at 30% of the levels in control rats fed selenium at 0.5 ppm in the diet. These effects are probably due to the selenium deficiency caused by silver. A NOAEL could not be identified and hence could not be used to derive AC values.

Chronic Exposures (≥100 d)

In a 37-wk study of rats given silver nitrate in drinking water at a dose of 222.2 mg/kg/d, animals showed decreased weight gain and elevated mortality starting at 23 wk (Matuk et al. 1981). The authors also reported ocular argyria.

Neurotoxicity

Deposition of silver in the brains of animals and humans after short-term or long-term oral exposure through drinking water has been reported in several studies. In a 4-mo study (Rungby and Danscher 1984), administration of silver nitrate at 0.015% (estimated dose of 25 mg/kg) in drinking

water to 60-d-old NMRI-strain female mice (n = 20) resulted in mild hypoactivity as measured by the open field motor behavior test conducted for 4 d after the end of exposure. Even though the intake of water was less than in controls during the initial 3 d, it was not different for the remaining duration of the experiment. Body-weight gain among experimental subjects was comparable to the control group. The authors suggested that hypoactivity is consistent with the intraneuronal accumulation of silver in the lysosomes of the motor nuclei of the spinal cord and brain system that they reported earlier (Rungby and Danscher 1983) and was observed by other investigators (Landas et al. 1985). It also seems to be consistent with the ataxic disturbances in coordination and frontally located electroencephalgram (EEG) changes seen in argyric patients (cited by Rungby and Danscher 1984). This was the only study found on neurotoxicity determined by a test.

Argyria

Argyria is a consequence of chronic exposure to silver. The human data on argyric subjects came from occupational monitoring (e.g., Rosenman et al. 1979). Olcott (1947, 1948) conducted the only experimental long-term animal study on the progression of argyria. Rats were given silver nitrate in drinking water (63.5 mg/kg/d), and the rats' eyes were observed until the respective time of sacrifice. Tissue sections were autopsied at various sacrifice times, and the pigmentation of eyes was reported in various stages. The study continued up to 553 d (to a total estimated ingestion of 9.4 grams of silver as silver nitrate). The following stages were observed with respect to the intensity of pigmentation in the eyes: 218 d, slightly gray (stage 1); 373 d, more gray than pink (stage 2); 447 d, dark but translucent eyes (stage 3); and 553 d, opaque eyes (stage 4). At stage 4, the membranes were completely black. No other systemic toxicity was noted at autopsy.

In a study by Walker (1971), Sprague-Dawley rats were administered silver as silver nitrate at 1,290 mg/L (dose of 130 mg/kg/d) in drinking water for a total of 81 wk. Silver deposits were found in the glomerular basement membranes in the kidneys at 4, 6, 8, 10, 12, 25, and 60 wk. Silver deposits were also found in the colon, liver, thyroid, urinary bladder, and prostatic acini. During weeks 76 and 81, severe deterioration of clinical condition was found at this dose. However, in another group of rats given 65 mg/kg/d for 12 wk, there were no silver deposits noted and there was no reduction in water consumption. A LOAEL (lowest-observed-adverse-effect level) of 130 mg/kg/d and a NOAEL of 65 mg/kg/d were identified.

Cardiovascular Effects

No reports on the cardiovascular effects of silver ingestion in humans were available. Olcott (1950) studied the effect of ingestion of silver nitrate in rats (159 rats, males and females) administered silver nitrate at 0.1% in drinking water for 218 d. Few rats were also given silver chloride in sodium thiosulfate. Despite the fact that many rats had diseased lungs, the author concluded that there was a statistically significant increase in the incidence of ventricular hypertrophy (measured as a ratio of the weight of the left ventricle to body weight) in both male and female rats with normal lungs. The exposure corresponded to 89 mg/kg/d. Alhough autopsy revealed significant pigmentation in several organs, no direct relationship between the intensity of pigmentation in the heart and the ventricular hypertrophy could be established. The author did not successfully measure changes in the blood pressure in these rats. Thus, a cardiovascular effect was not clearly documented.

Factors That Influence Silver Toxicity

Diplock et al. (1967) reported that vitamin E and selenium in the diet could significantly influence the toxicity of silver. When weanling Norwegian hooded rats fed a basal vitamin-E deficient diet were provided drinking water containing silver at 970 mg/L (as silver acetate), all rats developed liver necrosis within 2-4 wk and died. In another group, when selenium was added at 1 ppm to the vitamin-E deficient diet, and the drinking water contained silver acetate, only four of nine rats died. In another group that was fed a diet containing vitamin E and was sacrificed after 50 d of silver exposure, no liver necrosis was found. Bunyan et al. (1968) reported similar observations in rats exposed to silver at 650 mg/L (as silver acetate) in drinking water. Liver necrosis was seen when the dietary selenium was reduced. Necrosis was induced at much lower doses of silver (80 mg/L). Vitamin E appeared to reverse that effect. Also, Grasso et al. (1969) reported that when silver (silver acetate) was fed either in the diet (at 130-1,000 ppm, or 4-33 mg/kg/d) or in drinking water (97.5 mg/kg/d) to vitamin-E deficient rats, fatal necrosis was noted. Alexander and Aaseth (1981) reported that depletion of liver GSH by diethyl maleate decreased biliary excretion of silver into the bile. Selenite also inhibited the biliary excretion of silver and increased its retention in the tissues. It was suggested that selenite formed an insoluble complex with silver that retarded biliary

excretion. It is not clear if that is in any way related to the effect of selenium-containing diets in reducing the GSH peroxidase (see Wagner et al. 1975, described above).

Reproductive Toxicity

No human data on the reproductive toxicity of silver are available. Male rats given water containing silver chloride or silver nitrate (89 mg/kg/d) over a 2-y period did not show any change in the appearance or production of spermatozoa or any accumulation of silver. There was a transient epididymal edema and deformation (Olcott 1948).

Developmental Toxicity

Only a few studies have reported developmental effects of silver or silver compounds. None of those pertains to ingestion. In a study of embryonic toxicity of 54 elements including silver in chicks (Ridgway and Karnofsky 1952), there were no effects of silver. Wistar rat pups injected subcutaneously with silver lactate at doses of 0.1, 0.2, or 0.35 mg/kg/d during weeks 1, 2, 3, or 4 had smaller pyramidal cells within the hippocampus, indicative of silver toxicity (Rungby et al. 1987), but the functional implications are not clear. Rungby and Danscher (1983) reported neuronal accumulation of silver in the brains of progeny from argyric rats. No effects related to developmental toxicity were found.

Genotoxicity and Mutagenicity

No genotoxic effects of silver in humans have been reported. Only data using in vitro bacterial and nonmammalian cell cultures were available. Using silver sulfadiazine, McCoy and Rosenkranz (1978) reported that silver was not mutagenic to different strains of *Salmonella typhimurium*. Using wild and recombinational repair-deficient strains of bacterium *Bacillus subtillis*, Strain H17 (Rec+) and M45 (Rec-), and exposing them to 50 microliters (μL) of silver chloride solution of 0.05 molar (M) for 24 h (only one concentration), Nishikova (1975) concluded that silver was not mutagenic to Rec+ and Rec- strains. When induction of streptomycin reverse mutations in *E.coli* was studied using six concentrations of silver

TABLE 9-4 Toxicity Summary[a]

Form	Concentration and Route	Duration	Species	Effect	Reference
Silver colloid	1,680 mg/kg/d, oral	4 d	Rat	Death at 4 d; no mortality with one bolus dose of 420 mg/kg	Dequidt et al. 1974
Silver nitrate	260 mg/kg/d, drinking water	2 wk	Rat (n = 12)	Reduced water consumption for the first 5 d; three of 12 died	Walker 1971
Silver nitrate	65 mg/kg/d and 130 mg/kg/d, in drinking water	12 d to 14 wk	Mouse (n = 18)	Silver deposits in kidney and reduced water consumption at 130 mg/kg/d; no effects at 65 mg/kg/d	Day et al. 1976
Silver acetate	130 mg/kg/d, diet	4 wk	Weanling swine (n = 4)	Anorexia, diarrhea, and growth depression; three of four died	Van Vleet (1976)
Silver acetate	11.5 mg/kg/d, drinking water	52 d	Rat (Holtzman) (n = 10)	No growth depression or liver necrosis	Wagner et al. 1975[b]
Silver acetate	97 mg/kg/d, drinking water	50 d	Rat (9-10 per group)	Liver necrosis in vitamin-E deficient animals only	Diplock et al. 1967
Silver acetate	114 mg/kg/d, drinking water	52 d	Rat (Holtzman) (n = 10)	Severely depressed growth in selenium deficient diet, but less pronounced in presence of selenium	Wagner et al. 1975[b]
Silver nitrate	65 mg/kg/d, drinking water	10 wk	Rat (n = 12)	Silver deposits in basement membrane	Walker 1971

Silver nitrate	25 mg/kg/d, drinking water	4 mo	Mouse, female (n = 20), NMRI strain	Hypoactive (open field behavior test)	Rungby and Danscher 1984
Silver nitrate	63.5 mg/kg/d, drinking water	182 d	Rat (n = 233)	Left ventricular hypertrophy	Olcott 1950
Silver nitrate	222 mg/kg/d, drinking water	37 wk (259 d)	Rat	Growth retardation; deposition of silver in the eyes; death	Matuk et al. 1981
Silver nitrate	63.5 mg/kg/d, drinking water	281 d	Rat (n = 98)	Mild argyria; silver deposits in the basement membrane in renal gomerulus	Olcott 1948
Silver nitrate	130 mg/kg/d, drinking water	60 wk (420 d)	Rat (n = 12)	Rapid clinical deterioration at weeks 76-81	Walker 1971
Silver nitrate	63.5 mg/kg/d, drinking water	373 d	Rat (n = 84)	Eyes more grey	Olcott 1947
Silver nitrate	63.5 mg/kg/d, drinking water	447 d	Rat (n = 37)	Eyes dark but translucent	Olcott 1947
Silver nitrate	63.5 mg/kg/d, drinking water	553 d	Rat (n = 5)	Eyes opaque	Olcott 1947

[a]Exposures by the oral route.
[b]Rats were fed a vitamin-E deficient diet.

nitrate over 3-25 h, the mutation rate was no greater than that of controls. But in a study on the enhancement of viral transformation for the evaluation of carcinogenic or mutagenic potential of inorganic salts on the transformation of Syrian hamster embryo cells by simian adenovirus SA7 (Casto et al. 1979), silver was listed as positive from 0.05 mM to 0.6 mM with out any noticeable cell killing. The exposure time was 72 h. Similarly, silver sulfide, added at 10 g/mL for 24 h to cultured Chinese hamster ovary (CHO) cells, induced DNA strand breaks (Robison et al. 1982). Rossman et al. (1986) reported that silver nitrate at 2.5E-07 M inhibited the growth of *E. coli* WP2s (lamda) but did not induce λ prophage in these bacteria. Silver iodide in acetone solution or as a suspension was mutagenic only at cytotoxic concentrations in the Ames/microsome tests in TA1935, TA102, TA97, and TA98 (e.g., 30 μg/mL in TA102 [-S9] and 150 μg/mL in TA97 [induced +S9]). Elipoulos and Mourelatos (1998) reported a lack of genotoxicity of silver iodide in the sister chromatid exchange (SCE) assay in vitro, in vivo, and in the Ames/ microsome test. Silver iodide did not induce SCEs in cultured human lymphocytes (1 μg/mL, 72 h incubation) or in P388 lympocytic leukemia cells cultured in the peritoneal cavity of 8- to 10-wk-old DBA mice injected intraperitoneally with 100 μg of silver iodide per gram. However, silver nitrate gave significant cytogenic effects from doses of 10 μg/g.

Carcinogenicity

The possibility of silver acting as a carcinogen in mammals when ingested or inhaled has not been studied directly. However, long-term toxicity tests examining other adverse effects in laboratory animals did not report the presence of any tumors. Long-term human occupational exposures, involving all three routes, have not been linked to carcinogenic effects. Schmachl and Steinhoff (1960) reported that subcutaneous injections of suspensions of colloidal silver (once a week for 10 mo) resulted in tumors in eight of 26 rats after 14 mo. Furst and Schlauder (1977) reported that 5 mg of silver powder suspended in an inert fluid and injected once a month intramuscularly to F-344 rats produced no fibrosarcomas at the site of injection. In another study, Furst (1981) observed local sarcomas after subcutaneous implantation of silver foils. In both cases, the tumors were observed at the site of implantation and are considered examples of silver foil acting as a nonspecific irritant rather than as a specific carcinogen. Therefore, those observations are not of particular relevance to human risk from ingestion of silver.

TABLE 9-5 Spacecraft Water Exposure Guidelines for Silver[a]

Duration	Concentration (mg/L)	Target Toxicity
1 d	5	Decreased water consumption
10 d	5	Decreased water consumption
100 d	0.6	Hypoactivity
1,000 d	0.4	Argyria[b]

[a]The 1-d and 10-d SWEGs were based on an AC derived from Day et al. (1976); the 100-d SWEG was based on an AC derived from Rungby and Danscher (1984); and the 1,000-d SWEG was based on an AC derived from Gual and Staud (1935) and Hill and Pillsbury (1939). WHO (1984) estimated that the upper bound level of silver intake from food was 80 μg/d, which is about 1.1 μg/kg/d for humans. That amount was not taken into consideration when deriving final ACs.
[b]Secondary SWEG. Argyria is not considered an adverse toxic effect. The 1,000-d value is similar to levels suggested by WHO (1984) for lifetime exposure.

Spaceflight Effects

Dehydration has been a serious concern during spaceflight, as seen in crews from Apollo, Skylab, and shuttle missions, resulting in reductions in total plasma volume and changes in RBC formation (Nicogossian et al. 1994). Silver in drinking water has been reported to cause a reduction in water consumption, which might exacerbate dehydration.

RATIONALE

The following paragraphs provide a rationale for proposing spacecraft water exposure guidelines (SWEGs) for silver in spacecraft drinking water for 1, 10, 100, and 1,000 d. The values (listed in Table 9-5, above) were set following the NRC guidance for developing of guideline levels for spacecraft water. (See Table 9-6 for standards set by other organizations.) In determining the ACs for silver, a spaceflight factor of 3 was used when the AC was determined on the basis of reduced water consumption. A factor of 10 was applied to account for differences in absorption between rodents and humans. The estimated absorption of silver (from retention data) in mice and rats was reported to be in the range of 1-2% (Furchner et al. 1968). Silver absorption was reported at about 20% in a study of one female human (Macintire et al. 1978; East et al. 1980). There were some limitations in the animal retention study and in the one human case study

TABLE 9-6 Drinking Water Silver Standards Set by Other Organizations

Organization	Standard	Concentration (mg/L)
EPA[a]	MCL	None
	MCLG	None
	SMCL (final)	0.1
	1-10 d HA (child)	0.2
	Long-term HA	0.2
	RfD	0.005
	DWEL	0.2
	Lifetime	0.1
	Cancer group	Group D
	Cancer risk	NA
ATSDR[b]	1-14 d MRL	None set
	15-365 d MRL	None set
	>365 MRL	None set

[a]There are no federally regulated standards for silver in drinking water. Only guideline levels are stated. The long-term HA refers to levels that will not cause any adverse noncarcinogenic effects up to 7 y (10% of lifetime). The RfD is an estimate of a daily exposure that is likely to be without appreciable risk of deleterious effect over a human lifetime. The DWEL assumes that water contributes to 100% of the exposure. Group D classification means not classifiable as a carcinogen to animals or humans (EPA 1996).
[b]ATSDR did not set MRLs because "sufficient data do not exist to identify a target organ or establish an MRL for acute duration or intermediate duration. General lack of quantitative information concerning this effect in humans or animals precludes the derivation of an MRL for chronic-duration exposure" (ATSDR 1990).
Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; DWEL, drinking water equivalent level; EPA, U.S. Environmental Protection Agency; HA, health advisory; MCL, maximum contaminant level; MCLG, maximum contaminant level goal; MRL, minimal risk level; RfD, reference dose; SMCL, secondary maximum contaminant level.

mentioned above. The doses in the animal study (Furchner et al. 1968) were extremely small and very different from each other. In the human study (Macintire et al. 1978) the subject already had significant body burden of silver, the test dose preparation had ammonium chloride as one of the components, and acetate was the salt form of silver. Due to these uncertainties,

it was decided to use a factor of 10 to account for the differences between rodents and humans. No additional factors were applied for differences in uptake of silver from water or from food due to lack of data. ACs were calculated assuming a total intake of 2.8 L of water per day. That includes an average of 0.8 L of water used for reconstitution of food and 2 L for drinking.

Ingestion for 1 d

A 1-d AC was not calculated because there are no data to support a 1-d value. Although a few animal studies in which rodents were exposed to silver (as salts) via drinking water showed decreases in water consumption for the first 3 d, water consumption returned to normal in the days following. The initial phenomenon may have been due to taste aversion. The 10-d value will be applied to protect against any 1-d effects.

Ingestion for 10 d

Water Consumption

Silver nitrate at 12 mM (dose of 130 mg/kg/d) in drinking water was unacceptable to mice, and water consumption dropped dramatically (Day et al. 1976). As early as 12 d, there was uniform deposition of silver within the glomerular membrane after exposure to 6 mM silver nitrate (calculated dose of 65 mg/kg/d), although there was no effect on water consumption. Hence, 65 mg/kg/d is considered a NOAEL for changes in water consumption. Factors of 10, 10, and 3 were applied for species extrapolation, differences in absorption between rodents and humans, and spaceflight effects, respectively. Thus, the 10-d AC for decreased water consumption was derived as

$$\text{10-d AC} = (65\ \text{mg/kg/d} \times 70\ \text{kg}) \div (10 \times 10 \times 2.8\ \text{L/d} \times 3);$$
$$\text{10-d AC} = 5.4\ \text{mg/L (rounded to 5 mg/L)}.$$

In Sprague-Dawley rats exposed to silver (as silver nitrate) in drinking water at 6, 12, or 24 mM (65, 130, or 260 mg/kg/d), the only effect observed was a decline in the amount of drinking water consumed in the 260 mg/kg group (Walker 1971). That was found as early as 1 wk. The middle-dose group exhibited only silver deposits in the kidneys without any

effects on the volume of ingested drinking water. Hence, for the 10-d AC, a dose of 130 mg/kg/d was used as the NOAEL for effects on water consumption. A factor of 10/7 was included for time extrapolation. The 10-d AC for decline in water consumption was derived as

10-d AC = (130 mg/kg/d × 70 kg) ÷ (10 × 10 × 2.8 L/d × 3 × 10/7);
10-d AC = 7.5 mg/L.

Nephrotoxicity

No human experimental data are available to establish an AC for this parameter. The industrial worker exposure survey reported by Rosenman et al. (1987), although strongly indicative of nephrotoxic effects of silver, is heavily masked by the presence of cadmium, a known nephrotoxin in the workplace. Hence, an AC for nephrotoxicity was not established.

Ingestion for 100 d

Cardiovascular Effects

There are no human data to indicate that silver causes any cardiovascular effects. In Olcott's (1950) study, rats administered silver nitrate in drinking water for 218 d at a dose of 89 mg/kg/d developed left ventricular hypertrophy. Thickening of the renal glomerular membrane was also noted. Although a large number of animals were used in that investigation, the way the effect was reported (as the weight of the left ventricle per 100 g body weight) was not reliable enough to derive a 100-d AC for cardiovascular effects.

Neurotoxicity

Rungby and Danscher (1984) reported that 60-d-old NMRI-strain female mice (n = 20) receiving silver nitrate at 0.015% in drinking water (silver at 0.095 mg/mL) for 125 d were hypoactive, as measured by open cage field behavior for 4 d after the end of exposure. The authors suggested that that effect might have been due to intraneuronal accumulation of silver in motor-control nuclei of the brain stem. An estimated dose of 25 mg/kg/d can be considered a LOAEL for that effect. There were no dose-response

or time-response data. A NOAEL was not identified. A factor of 10 was applied to calculate a NOAEL from the LOAEL; a factor of 10 was applied for species extrapolation; and a factor of 10 was applied for the differences in absorption between rodents and humans. No time factor was used because an AC derived from a 125-d study will be protective for a 100-d duration. A 100-d AC for neurotoxic effects can be calculated as

100-d AC = (25 mg/kg/d × 70 kg) ÷ (10 × 10 × 10 × 2.8L/d);
100-d AC = 0.6 mg/L (rounded).

Water Consumption

Day et al. (1976) reported that water consumption dramatically dropped in mice administered silver nitrate at 130 mg/kg/d in drinking water. But, in another batch of mice exposed to half that dose in drinking water and studied for 12 d to 14 wk, no reduction in water consumption was observed. Hence, 65 mg/kg/d appears to be a NOAEL for decreased water intake. Factors of 10, 10, 3, and 100/98 were applied for species extrapolation, differences in absorption between rodents and humans, spaceflight effects, and time extrapolation, respectively. A 100-d AC for decreased water consumption can be derived as

100-d AC = (65 mg/kg/d × 70 kg) ÷ (10 × 2.8 L/d × 100/98 × 3 × 10);
100-d AC = 5.0 mg/L (rounded).

Ingestion for 1,000 d

The deposition of silver in kidneys as a consequence of argyria has been reported to be associated with arteriosclerotic changes, and the deposition of silver in the eyes has been associated with poor night vision (NRC 1977). Deposition in various tissues, including basement membranes of kidneys, brain, and spinal cord, has been associated with changes in neuronal functions, such as loss of coordination and convulsions (Reinhardt 1971; Rosenman et al. 1979), EEG changes, and signs of cerebellar ataxia (Aaseth et al. 1981). Because these case report studies did not provide enough controlled data to derive an AC, and correlations are only suggestive, argyria was considered an aesthetic effect. To be conservative, an AC was derived for this end point using the following sets of data.

TABLE 9-7 Acceptable Concentrations (ACs)

Toxicity End Point	Exposure Data and Reference	Factors					ACs (mg/L)			
		To NOAEL	Species	Time	Spaceflight	Absorption	1 d	10 d	100 d	1,000 d
Decreased water consumption	NOAEL = 65 mg/kg/d, mice (Day et al. 1976)	1	10	1	3	10	5[a]	5	—	—
Decreased water consumption	NOAEL = 130 mg/kg/d, rats (Walker 1971)	1	10	10/7	3	10	—	7.5	—	—
Neurotoxicity (hypoactivity)	LOAEL = 25 mg/kg/d, female mice (Rungby and Danscher 1984)	10	10	1	1	10	—	—	0.6	—
Decreased water consumption	NOAEL = 65 mg/kg/d, mice (Day et al. 1976)	1	10	100/98	3	10	—	—	5	—
Skin and eye discoloration (argyria)	LOAEL = 0.39 mg/kg/d, human case reports (Gaul and Staud 1935; Hill and Pillsbury 1939)	10	1	1,000/365	1	1	—	—	—	0.4
Discoloration of eyes (argyria)	LOAEL = 63 mg/kg/d, rats (Olcott 1947)	10	10	1,000/218	1	10	—	—	—	0.4
SWEGs							5	5	0.6	0.4

[a]Because of a lack of data on which to base the 1-d AC, the 10-d AC was set as the 1-d value because it is also protective for 1 d.

There have been several reports of argyria in humans as a result of treatment using medications containing silver (Gaul and Staud 1935), after large doses of lozenges containing silver (East et al. 1980), and after ingesting capsules containing silver nitrate for 15 y (Blumberg and Carey 1934). Although these are human cases, there has been a lot of uncertainty about the exposure levels. In the latter two cases, there had been only one subject. The Gaul and Staud (1935) study is a report of 70 cases of generalized argyria in individuals who received several intravenous injections of silver arsphenamine for syphilis. The disadvantage of that study was that argyria developed at different total doses, indicating that some individuals were a lot more sensitive than others. A total dose of 4 g of silver arsphenamine (or 1 g of silver ions) over 1 y could cause argyria (Gaul and Staud 1935; Hill and Pillsbury 1939). Extrapolating intravenous exposure to an oral bolus, using a mean absorption in humans of 10%, that dose would be 10,000 mg over 365 d (accumulated dose over a year). One gram of silver by intravenous injection would correspond to10 g by oral dose (based on the assumed 10% absorption). That would correspond to 27.4 mg/d (10,000 mg/365 d), giving a dose of 0.39 mg/kg/d for a 70-kg person. Using that as a LOAEL for argyria, a 1,000-d AC was calculated. Factors of 10 and 1,000/356 were applied for deriving a NOAEL from the LOAEL and for time extrapolation, respectively. The AC was calculated as follows:

1,000-d AC = (0.39 mg/kg/d × 70 kg) ÷ (10 × 2.8 × 1,000/365);
1,000-d AC = 0.36 mg/L (rounded to 0.4 mg/L).

NASA also calculated a 1,000-d AC from the only long-term animal study found in the literature. Olcott (1947) described different levels of discoloration of eyes in 139 albino rats after administering a solution of silver nitrate (1:1,000) equivalent to 63 mg/kg/d. Slight gray color in the eyes was seen after 218 d of exposure to silver. The 63 mg/kg/d value was considered a LOAEL because the coloration was only slight (according to gradation of colors specified by the authors of the studies). Eye discoloration has been reported in studies in Wistar rats (Rungby 1986b) and in human cases involving use of eye drops or make-up containing silver (Greene and Su 1987). Workers exposed to silver for over 2 y had corneal deposits of silver, and some complained of poor night vision (Rosenman et al. 1979). Factors of 10, 10, 10, and 1,000/218 were applied for species extrapolation, differences in absorption between rodents and humans, deriv-

ing a NOAEL from a LOAEL, and time extrapolation, respectively. A 1,000-d secondary AC for this aesthetic effect was calculated as

1,000-d AC = (63 mg/kg/d × 70 kg) ÷ (10 × 10 × 10 × 2.8 L/d × 1,000/218);
1,000-d AC = 0.4 mg/L (rounded).

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