TOXICOLOGICAL PROFILE FOR
1,4-DIOXANE

April 2012

DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

UPDATE STATEMENT

A Toxicological Profile for 1,4-Dioxane, Draft for Public Comment was released in October 2007. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine/Applied Toxicology Branch
1600 Clifton Road NE
Mailstop F-62
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FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the toxic substances each profile describes. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The profiles focus on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. A health effects summary describes the adequacy of information to determine a substance's health effects. ATSDR identifies data needs that are significant to protection of public health.

Each profile:

(A) Examines, summarizes, and interprets available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) Determines whether adequate information on the health effects of each substance is available or being developed to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identifies toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are federal, state, and local health professionals; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other federal scientists also have reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the National Priorities List, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find this information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), route of exposure, and length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
NOTE: Not all health effects reported in this section are observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6 How Can (Chemical X) Affect Children?
Section 1.7 How Can Families Reduce the Risk of Exposure to (Chemical X)?
Section 3.7 Children's Susceptibility
Section 6.6 Exposures of Children

Other Sections of Interest:

Section 3.8 Biomarkers of Exposure and Effect
Section 3.11 Methods for Reducing Toxic Effects

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY) ***Fax:*** (770) 488-4178
E-mail: cdcinfo@cdc.gov ***Internet***: http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity*; and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Applied Toxicology Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

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PEER REVIEW

A peer review panel was assembled for 1,4-dioxane. The panel consisted of the following members:

1. Dr. George Alexeeff, Deputy Director for Scientific Affairs, Office of Environmental Health Hazard Assessment, CAL/EPA, Oakland, California;

2. Dr. Phillip Leber, Consultant in Toxicology, Akron, Ohio;

3. Dr. Raghubir Sharma, Emeritus Fred C. Davison Distinguished Chair in Toxicology, Department of Physiology and Pharmacology, University of Georgia College of Veterinary Medicine, Athens, Georgia; and

4. Dr. Alan Stern, Chief, Bureau for Risk Analysis, Division of Science and Research, Bureau for Risk Assessment, New Jersey Department of Environmental Protection, Trenton, New Jersey.

These experts collectively have knowledge of 1,4-dioxane's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about 1,4-dioxane and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. 1,4-Dioxane has been found in at least 31 of the 1,689 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which 1,4-dioxane is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure, and exposure to this substance may be harmful.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to 1,4-dioxane, many factors will determine whether you will be harmed. These factors include how much (the dose), how long (the duration), and how you come in contact with it. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

1.1 WHAT IS 1,4-DIOXANE?

Clear liquid with faint odor	1,4-Dioxane is a clear liquid with a faint pleasant odor. It mixes easily with water.
A solvent and laboratory reagent	It is used as a solvent in the manufacture of other chemicals and as a laboratory reagent.
A contaminant	1,4-Dioxane is a trace contaminant of some chemicals used in cosmetics, detergents, and shampoos. Manufacturers now reduce 1,4-dioxane from these chemicals to low levels before these chemicals are made into products used in the home.

1.2 WHAT HAPPENS TO 1,4-DIOXANE WHEN IT ENTERS THE ENVIRONMENT?

Found in air and water	1,4-Dioxane can be released into the air, water, and soil at places where it is produced or used as a solvent. In soil, 1,4-dioxane does not stick to soil particles, so it can move from soil into groundwater.
Break down	Compounds in the air can break down 1,4-dioxane into different compounds rapidly. In water, 1,4-Dioxane is stable and does not break down.

For more information on 1,4-dioxane in the environment, see Chapter 6.

1.3 HOW MIGHT I BE EXPOSED TO 1,4-DIOXANE?

Air	You can be exposed to 1,4-dioxane by breathing contaminated air. Current levels of 1,4-dioxane in air are not known. In the mid-1980s, average levels of 1,4-dioxane in air samples from the United States were about: • 0.4 micrograms per cubic meter ($\mu g/m^3$) for outdoor air • 4 $\mu g/m^3$ for indoor air
Water	You can be exposed to 1,4-dioxane in tap water. Current levels of 1,4-dioxane in water are not known. In the 1970s, the level of 1,4-dioxane in drinking water was 1 microgram per liter of water (1 µg/L).
Consumer products	Your skin may come into contact with 1,4-dioxane when you use cosmetics, detergents, and shampoos containing 1,4-dioxane. During 1992–1997, the average concentration of 1,4-dioxane in some cosmetic products reportedly ranged from 14 to 79 mg/kg. In a more recent survey reported by the Campaign for Safe Cosmetics, the levels of 1,4-dioxane in cosmetic products were found to be lower (1.5–12 ppm in baby and children's products and 2–23 ppm in adult products) than in the survey done by the FDA in the 1990s.

For more information on human exposure to 1,4-dioxane, see Chapter 6.

1.4 HOW CAN 1,4-DIOXANE ENTER AND LEAVE MY BODY?

Rapidly enters your body **Skin**	When you breathe air containing 1,4-dioxane, almost all of it will rapidly enter your body through your lungs. Almost all of the 1,4-dioxane in your drinking water will rapidly enter your body through the digestive tract. Studies have found that some 1,4-dioxane can pass through skin when applied with certain preparations such as lotions, but much of it will evaporate before it can be absorbed. .
Rapidly leaves your body	Once in your body, 1,4-dioxane is broken down into other chemicals. These other chemicals rapidly leave your body in the urine.

1.5 HOW CAN 1,4-DIOXANE AFFECT MY HEALTH?

This section looks at studies concerning potential health effects in animal and human studies.

The effects of 1,4-dioxane on human health depends on how much 1,4-dioxane you are exposed to and the length of exposure. The limited environmental monitoring data available suggest that the levels of 1,4-dioxane to which the general public might be exposed through contact or use of consumer products (including food), or that are normally found in environmental media, are generally significantly lower than those used in studies with experimental animals.

Short-term exposure effects	Eye and nose irritation was reported by people exposed to low levels of 1,4-dioxane for short periods of time. Exposure to very high levels may cause severe kidney and liver effects and possibly death.
Long-term exposure effects	Studies in animals have shown that breathing vapors of 1,4-dioxane affects mainly the nasal cavity and the liver and kidneys. Swallowing liquid 1,4-dioxane or contaminated drinking water, or having skin contact with liquid 1,4-dioxane also affects the liver and kidneys.

May cause cancer	Studies in workers did not indicate whether 1,4-dioxane causes cancer. Laboratory rats that breathed vapors of 1,4-dioxane during most of their lives developed cancer inside the nose and in the abdominal cavity; they also developed benign tumors in the liver. Laboratory rats and mice that drank water containing 1,4-dioxane during most of their lives developed liver cancer; the rats also developed cancer inside the nose. Some scientists believe that 1,4-dioxane may cause cancer by a nongenotoxic mechanism. Scientists are debating the degree to which the findings in rats and mice apply to exposure situations commonly encountered by people. The International Agency for Research on Cancer (IARC) has determined that 1,4-dioxane is possibly carcinogenic to humans. The U.S. Department of Health and Human Services (HHS) considers 1,4-dioxane as reasonably anticipated to be a human carcinogen. The EPA has established that 1,4-dioxane is likely to be carcinogenic to humans.

Further information on the health effects of 1,4-dioxane in humans and animals can be found in Chapters 2 and 3.

1.6 HOW CAN 1,4-DIOXANE AFFECT CHILDREN?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

Children are likely to have similar effects as adults	No data describe the effects of exposure to 1,4-dioxane on children or immature animals. It is likely that children would show the same health effects as adults. We do not know whether children differ from adults in their susceptibility to the effects of 1,4-dioxane.
Birth defects	We do not know whether 1,4-dioxane can harm an unborn child.

1.7 HOW CAN FAMILIES REDUCE THEIR EXPOSURE TO 1,4-DIOXANE?

Limit children's exposure to consumer products which may contain 1,4-dioxane	1,4-Dioxane may be a contaminant in certain ingredients used in cosmetics, detergents, shampoos, and some pharmaceuticals. 1,4-Dioxane is not intentionally added, but may occur as an unintentional byproduct in some ingredients, that may be listed on the product label, including: • PEG • polyethylene • polyethylene glycol • polyoxyethylene • -eth • -oxynol Many products on the market today (foods, pharmaceuticals, cosmetic products, detergents, etc.) contain 1,4-dioxane in very small amounts. However, some cosmetics, detergents, and shampoos may contain 1,4-dioxane at levels higher than recommended by the FDA for other products. Families wishing to avoid cosmetics containing the ingredients listed above may do so by reviewing the ingredient statement that is required to appear on the outer container label of cosmetics offered for retail sale.
Limit exposure to contaminated drinking water	1,4-Dioxane has been detected in some drinking water supplies. Bottled water may be less likely to be contaminated with 1,4-dioxane, and consumers should contact the bottler with specific questions on potential contaminants.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO 1,4-DIOXANE?

Can be measured in blood and urine	1,4-Dioxane and its breakdown products (metabolites) can be measured in blood and urine. The detection of 1,4-dioxane or these metabolites cannot be used to predict the kind of health effects that might develop from that exposure. The tests need to be conducted within days after exposure because 1,4-dioxane and its metabolites leave the body fairly rapidly.

For more information on the different substances formed by 1,4-dioxane breakdown and on tests to detect these substances in the body, see Chapters 3 and 7.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as "not-to-exceed" levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it.

Some regulations and recommendations for 1,4-dioxane include the following:

Levels in drinking water set by EPA	The EPA has determined that exposure to 1,4-dioxane in drinking water at concentrations of 4 mg/L for one day or 0.4 mg/L for 10 days is not expected to cause any adverse effects in a child.
Levels in workplace air set by OSHA	OSHA set a legal limit of 100 ppm 1,4-dioxane in air averaged over an 8-hour work day.
Levels set by NAS	The National Academy of Sciences (NAS) established a maximum specification of 10 ppm for 1,4-dioxane in the ingredient polysorbate, a food additive.

Levels set by FDA	FDA considered 10 ppm to be an acceptable limit for 1,4-dioxane during its evaluation of a spermicide, N-9, in a contraceptive sponge product. FDA also set a limit on 1,4-dioxane at 10 ppm in approving glycerides and polyglycerides for use as excipients in products such as dietary supplements. FDA keeps a record of raw materials and products contaminated with 1,4-dioxane.

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfiles™ CD-ROM by calling the toll-free information and technical assistance number at 1-800-CDCINFO (1-800-232-4636), by e-mail at cdcinfo@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine
1600 Clifton Road NE
Mailstop F-62
Atlanta, GA 30333
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Web site: http://www.ntis.gov/

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2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO 1,4-DIOXANE IN THE UNITED STATES

1,4-Dioxane is a stable, clear liquid at ambient temperatures and is miscible with water. It is used primarily as a solvent for chemical processing. It has also been used as a laboratory reagent; in plastic, rubber, insecticides, and herbicides; as a chemical intermediate; as part of a polymerization catalyst; and as an extraction medium of animal and vegetable oils. 1,4-Dioxane may also be found as a contaminant in ethoxylated surfactants, which are used in consumer cosmetics, detergents, and shampoos. Currently, manufacturers remove 1,4-dioxane from ethoxylated surfactants to low levels by vacuum stripping.

Current levels of 1,4-dioxane in ambient air, drinking water, and food samples are not available. In the mid 1980s, levels of 1,4-dioxane in ambient outdoor air ranged from 0.1 to 0.4 $\mu g/m^3$ (0.028–0.11 ppb). Mean concentrations of 1,4-dioxane in indoor air were a factor of 10 higher at 3.704 $\mu g/m^3$ (1.029 ppb). In the 1970s, municipal water supplies in the United States were reported to contain 1 µg/L (ppb) of 1,4-dioxane. 1,4-Dioxane has been detected in food volatiles which may indicate that 1,4-dioxane may be a natural constituent in some foods. Volatiles from chicken, meat, tomatoes, and small shrimp have been reported to contain 1,4-dioxane at unquantified levels. Dermal exposure to 1,4-dioxane may occur with the use of consumer cosmetics, detergents, and shampoos containing ethoxylated surfactants. Between the years 1992 and 1997, the average concentration of 1,4-dioxane in cosmetic finished products was reported to fluctuate from 14 to 79 ppm (mg/kg). In a more recent survey reported by the Campaign for Safe Cosmetics, the levels of 1,4-dioxane in cosmetic products that were tested were found to be lower (1.5–12 ppm in baby and children's products and 2–23 ppm in adult products) than in the survey done by the FDA in the 1990s.

2.2 SUMMARY OF HEALTH EFFECTS

Limited information exists regarding the health effects of 1,4-dioxane in humans. Yet, the available data are sufficient to clearly identify the liver and kidneys as the target organs for 1,4-dioxane toxicity following short-term exposure to relatively high amounts of 1,4-dioxane, regardless of the route of exposure. This has been corroborated in studies in animals. Workplace exposures to undetermined, but presumably high concentrations of 1,4-dioxane have resulted in death. Inhalation was the most likely route of exposure, although considerable dermal contact may also have taken place in one of these cases. Evaluation of the subjects prior to death did not provide a picture that could be considered unique to

1,4-dioxane. Subjects often complained of gastrointestinal pain, had high blood pressure, anuria, and leukocytosis, and exhibited signs of nervous system involvement. The deaths occurred 5–8 days after the initial symptoms of illness. Postmortem evaluation revealed extensive liver and kidney damage and in three out of five cases described in one study, kidney disease was considered to be the direct cause of death. Controlled exposures of volunteers to airborne 1,4-dioxane for periods ranging from a few minutes to 6 hours produced eye, nose, and throat irritation. The lowest exposure concentration that produced eye irritation was 50 ppm during a 6-hour exposure, but exposure in a much older study to 2,000 ppm for 3 minutes produced no complaints of eye or nasal discomfort. In a more recent study, exposure of volunteers to 20 ppm for 2 hours did not induce eye or respiratory irritation. Little is known about long-term exposure to lower concentrations of 1,4-dioxane. A study of workers exposed to 0.006–14.3 ppm 1,4-dioxane for an average of 25 years found no evidence of liver or kidney disease or any other clinical effects. An additional study that examined mortality rates among workers employed at a manufacturing and processing facility found no differences between observed and expected incidences of cancer. However, this study was limited in size and exposure duration. Although no information was available regarding reproductive, developmental, or immunological effects specific to 1,4-dioxane in humans, some occupational studies of workers exposed to 1,4-dioxane in combination with other solvents have reported elevated rates of spontaneous abortion, stillbirths, premature births, and low birth weights. These effects cannot be attributed either solely or in part to 1,4-dioxane.

Results from a recent 13-week study in rats and a 2-year study in rats indicate that the tissues in the nasal cavity are the most sensitive target for 1,4-dioxane following inhalation exposure. Adverse nasal effects were seen in rats exposed to ≥100 ppm in the 13-week study and in rats exposed to ≥50 ppm in the 2-year study. These exposure concentrations were the lowest tested. The liver and kidneys are also targets of 1,4-dioxane toxicity in animals following inhalation, oral and dermal exposure. There are no studies of the effects of 1,4-dioxane on reproductive function or immunocompetence in animals, and only one study in rats evaluated developmental end points following oral exposure during gestation. Slight fetotoxicity occurred at 1,033 mg/kg/day, a dose level that also affected the mothers. Chronic inhalation exposure of male rats to 1,4-dioxane induced benign tumors in the liver (1,250 ppm but not 250 ppm), squamous cell carcinoma in the nasal cavity (1,250 ppm but not 250 ppm), and mesothelioma in the peritoneum (≥250 ppm but not 50 ppm). Chronic administration of 1,4-dioxane in the drinking water produced liver cancer in rats (range, 398–1,015 mg/kg/day), mice (range, 77–380 mg/kg/day), and guinea pigs (1,014 mg/kg/day), and cancer of the nasal cavity in rats (range, 429–833 mg/kg/day). However, a 2-year inhalation study in rats exposed to 111 ppm 1,4-dioxane (equivalent to oral doses of approximately 105 mg/kg/day), provided no evidence of carcinogenicity or any other health effect. The mechanism of

carcinogenicity of 1,4-dioxane has not been elucidated, but the lack of or weak genotoxicity of 1,4-dioxane, its strong promotion properties, and the extensive cytotoxicity observed in some studies at dose levels that induce tumors suggest that 1,4-dioxane may be acting through a non-genetic mode of action.

Liver and Cancer Effects. Liver effects have occurred in humans and animals exposed to 1,4-dioxane, and the data in animals suggest that they occur regardless of the route of exposure. An occupational study and a case report provided a detailed description of the liver pathology in subjects following exposure to 1,4-dioxane that resulted in deaths within 1–2 weeks after the exposure. Upon postmortem examination, enlarged and pale liver and centrilobular necrosis were commonly observed. None of the subjects showed jaundice before death. Neither workers exposed to lower concentrations of 1,4-dioxane for many years nor volunteers exposed for a single 6-hour period to 50 ppm 1,4-dioxane showed indications of liver alterations.

One study provided detailed descriptions of liver pathology in several animal species exposed intermittently to 1,4-dioxane by inhalation for a period of up to 13 weeks and also exposed orally and by dermal contact. Both lethal and non-lethal concentrations (1,000–10,000 ppm) caused degrees of degeneration that varied from cloudy swelling to large areas of complete necrosis. Similar effects were seen following oral (1,428 mg/kg/day in rats) and dermal (143 mg/kg/day in guinea pigs; 57 mg/kg/day in rabbits) exposure. Hepatocyte vacuolation and swelling were reported in rats and mice dosed with 1,4-dioxane in the drinking water for 2 weeks (>2,500 mg/kg/day) or 13 weeks (≥126 mg/kg/day in rats; >550 mg/kg/day in mice). Evidence of hepatic degenerative changes was seen in Sherman rats that died after 2–4 months of receiving doses of 1,015 mg/kg/day 1,4-dioxane via the drinking water in a 2-year bioassay. Chronic inhalation exposure of male F344 rats to 1,250 ppm induced hepatic centrilobular necrosis and nuclear enlargement. Long-term oral studies in animals described hepatocellular degeneration and necrosis in Sherman rats at about 94 mg 1,4-dioxane/kg/day and increased cell foci in F344 rats at ≥55 mg/kg/day; hepatocytomegaly was observed in female Osborne-Mendel rats treated with approximately 350 mg/kg/day. The apparent different lesions and thresholds for the effects in the liver may reflect strain differences.

The mechanism by which 1,4-dioxane induces liver damage in unknown. Results from some studies suggest that toxicity occurs at high doses when the metabolism of 1,4-dioxane is saturated, which would suggest that the parent compound is the toxic form. This also is consistent with more recent observations that induction of hepatic CYP2B1/2 and CYP2E1 did not play a role in the toxicity of 1,4-dioxane, which

suggested that highly reactive and toxic intermediates do not play a major role in the liver toxicity of 1,4-dioxane, even under conditions of enhanced metabolism. Conversely, it has also been reported that the metabolite, 1,4-dioxane-2-one, was several-fold more toxic than 1,4-dioxane based on intraperitoneal LD_{50} determinations in rats.

All long-term studies in rats dosed with 1,4-dioxane via the drinking water reported an increased incidence of liver tumors, generally in the high-dose groups. In the better reported studies, tumor development occurred at doses that produced extensive liver toxicity, including hepatocellular hyperplasia and degeneration and evidence of hepatic regeneration, which has led some to suggest that cell damage and degeneration may be a necessary occurrence for the formation of liver tumors in rats. Oral exposure to 1,4-dioxane also induced tumors in the nasal cavity in rats and liver tumors in mice and guinea pigs. The relevance of the nasal tumors to humans following oral exposure to 1,4-dioxane has been questioned and some scientists suggested that the tumors resulted from inspiration of water containing 1,4-dioxane into the nasal cavity. A study reported that the addition of a fluorescent dye mixture to water containing 0.5% 1,4-dioxane and offered to rats as drinking water resulted in the fluorescent dye readily observed in numerous areas in the nasal cavity where bioassays have identified tumors. Little or no fluorescence associated with the dye mixture was found in a single rat that received the dye mixture by gavage. One study concluded that these results indicate that the rat nasal tissues are exposed by direct contact with drinking water under conditions of the bioassay. However, there is also evidence in support of the nasal alterations being caused, at least in part, by systemic delivery of either 1,4-dioxane or a metabolite (see Section 3.5.2). The lack of nasal cytotoxicity and nasal tumors in Wistar rats exposed intermittently to 111 ppm 1,4-dioxane in the air for 2 years suggests that the minimal effective dose may not have been reached, whether by direct contact alone or a combination of direct contact and internal exposure.

The mechanism of carcinogenicity of 1,4-dioxane has not been elucidated, but the results from several lines of investigation have led some to conclude that 1,4-dioxane has a non-genotoxic, yet unknown, mode of action. The EPA has developed cancer risk values for oral exposure to 1,4-dioxane, last revised in 2010, based on the increased incidence of hepatocellular adenoma and carcinomas in female Crj:BDF_1 mice in a 2-year drinking-water bioassay.

Liver toxicity has been proposed to be necessary for liver tumor formation in rats. Since this suggests to some scientists the existence of a threshold, they have suggested using approaches other than the Linearized Multistage Model for estimating human cancer risk due to exposure to 1,4-dioxane. Based on inadequate evidence in humans and sufficient evidence in experimental animals, the International Agency

for Research on Cancer (IARC) has determined that 1,4-dioxane is possibly carcinogenic to humans. The Department of Health and Human Services (DHHS) has stated that 1,4-dioxane is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. The EPA has established that 1,4-dioxane is likely to be carcinogenic to humans based on inadequate evidence of carcinogenicity in humans and sufficient evidence in animals.

Renal Effects. Kidney lesions appeared to be the cause of death of five workers who were exposed to unknown concentrations of 1,4-dioxane primarily by the inhalation route. Death occurred 1–2 weeks after episodes of elevated exposure started at work. All five cases experienced oliguria or anuria. Post mortem examination revealed swollen kidneys with hemorrhages and necrosis of the cortex. Similar findings were reported in a fatal case report. No renal alterations, as judged by urinalyses, were described in other reports of long-term occupational exposure to low levels of 1,4-dioxane or in a group of volunteers following a single 6-hour exposure to 50 ppm 1,4-dioxane. Very similar kidney lesions were observed in animals exposed to 1,4-dioxane by several routes of exposure. Rodents exposed to acutely lethal concentrations of 1,4-dioxane (≥5,000 ppm) showed severe kidney damage consisting of marked patchy cell degeneration of the cortical tubules and intense vascular congestion and hemorrhages both inter- and intra-tubular. Well-marked kidney lesions were present in animals that survived intermittent inhalation exposure to 1,000 ppm 1,4-dioxane for up to 12 weeks. Similar observations were made in intermediate-duration studies in rats and mice exposed orally (1,400–2,900 mg 1,4-dioxane/kg/day) and in guinea pigs (143 mg/kg) and rabbits (57 mg/kg) following dermal application of 1,4-dioxane. Evidence of renal degenerative changes was seen in Sherman rats that died after 2–4 months of treatment with 1,015 mg 1,4-dioxane/kg/day in a 2-year drinking water bioassay. Nuclear enlargement of the proximal tubule was reported in rats exposed to 657 mg 1,4-dioxane/kg/day in a 13-week study. Increased incidence of degeneration and necrosis of the tubular epithelium was seen in rats that received 94 mg/kg/day and survived until termination of the study, and similar findings were reported in Osborne-Mendel rats that received 240 mg/kg/day. Nuclear enlargement in the proximal convoluted tubule was reported in male F344 rats exposed to ≥250 ppm 1,4-dioxane vapors for 2 years. No compound-related neoplastic lesions were observed in the kidneys in other long-term studies conducted with 1,4-dioxane in rodents. The mechanism(s) by which 1,4-dioxane induces kidneys lesions is not known, and virtually no discussion about this topic was found in the reviews available. The findings in the case studies are consistent with an acute nephritic syndrome, which is characterized by oliguria and acute renal failure. It is not expected that exposure to concentrations commonly in the environment would cause adverse kidney effects in humans.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for 1,4-dioxane. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

- An MRL of 2 ppm has been derived for acute-duration inhalation exposure (14 days or less) to 1,4-dioxane.

The acute-duration inhalation MRL is based on a no-observed-adverse-effect level (NOAEL) of 20 ppm for eye and respiratory irritation and pulmonary function effects in humans (Ernstgård et al. 2006). In that study, six male and six female volunteers were exposed to 0 or 20 ppm 1,4-dioxane vapor for 2 hours under dynamic conditions. Each subject was exposed on two separate occasions to 0 or 20 ppm. End points monitored included self-rated symptoms on a visual analogue scale that measured discomfort of the eyes, nose and throat, breathing difficulty, solvent smell, headache, fatigue, nausea, dizziness and 'feeling of intoxication'. Rating was performed before, during (3, 60, and 118 minutes), and after exposure (20 and 180 minutes). Respiratory function was assessed by spirometry before exposure, immediately after and 3 hours after exposure ceased. The specific parameters measured included vital capacity, forced vital capacity, forced expiratory volume in 1 second, peak expiratory flow, and forced expiratory flow at 25, 50, and 75% of the forced vital capacity. Also assessed was nasal swelling before, immediately after,

and 3 hours after exposure. Eye blinking was monitored throughout the exposure period by electromyography. Also, two inflammatory markers, high sensitivity C-reactive protein and interleukin 6, were measured in blood before and 3 hours after exposure. Exposure to 1,4-dioxane under the conditions of the study did not significantly affect any of the end points monitored except the perception of smell of the chemical, which increased significantly after 3, 60, and 118 minutes of exposure. The NOAEL of 20 ppm was divided by an uncertainty factor of 10 (for human variability) to yield the MRL of 2 ppm.

Support for the acute-duration inhalation MRL of 2 ppm is provided by a study by Young et al. (1977) in which four healthy male volunteers were exposed to 50 ppm 1,4-dioxane for 6 hours under dynamic airflow conditions. Prior to the study, the subjects provided a complete history and underwent tests including chest x-ray, EKG, respiratory function tests, a conventional battery of 12 blood chemistry tests plus triglyceride and creatinine determinations, and complete hematological and urine analyses. Except for the chest x-ray, the tests were repeated 24 hours and 2 weeks after the exposure. The tests conducted 24 hours and 2 weeks after exposure did not reveal any exposure-related abnormalities, although no data were provided in the study. Eye irritation was a frequent and only complaint throughout the exposure. Perception of the odor of 1,4-dioxane diminished with time. Two of the subjects could not perceive the odor after 4 and 5 hours in the chamber. The 50 ppm exposure level constitutes a minimal LOAEL for eye irritation, although there was no control experiment, and possible low humidity in the exposure chamber (not addressed in the report) might have contributed to the eye irritation.

Other studies with volunteers also support the findings of Ernstgård et al. (2006) and Young et al. (1977). For example, Silverman et al. (1946) exposed 12 subjects to various concentrations of 1,4-dioxane for only 15 minutes and determined a no-observed-adverse-effect level (NOAEL) of 200 ppm for eye and nose irritation; the LOAEL was 300 ppm. Wirth and Klimmer (1936) reported that slight mucous membrane irritation started to take place in volunteers exposed to concentrations about 278 ppm for a few minutes (unspecified) and that at 1,390 ppm for several minutes, the subjects described prickling in the nose and scratchiness and dryness in the throat. Fairley et al. (1934) reported a NOAEL of 2,000 ppm (only level tested) for respiratory and ocular effects in six subjects exposed to 1,4-dioxane for only 3 minutes. Finally, Yant et al. (1930) described slight eye, nose, and throat irritation in a group of five subjects exposed to 1,600 ppm (only level tested) 1,4-dioxane for only 10 minutes. The available studies in animals used exposure concentrations that often caused death among the animals and were much higher than the concentrations tested by Ernstgård et al. (2006) and Young et al. (1977).

- An MRL of 0.2 ppm has been derived for intermediate-duration inhalation exposure (15–364 days) to 1,4-dioxane.

The intermediate-duration database for 1,4-dioxane consists of only two studies: an early study that exposed several animal species to high concentrations of 1,4-dioxane and monitored limited end points (Fairley et al. 1934); and a recent study that evaluated a comprehensive number of end points in rats exposed to several concentrations of 1,4-dioxane (Kasai et al. 2008). Fairley et al. (1934) exposed rats, mice, guinea pigs, and rabbits to airborne 1,4-dioxane 3 hours/day, 5 days/week for periods of up to 12 weeks. At termination, examination of the animals revealed moderate to severe liver and kidney toxicity occurring at all exposure levels in all of the species tested. The lowest exposure level was 1,000 ppm. In the recent study, groups of F344/DuCrj rats (10/sex/group) were exposed to target concentrations of 0 (clean air), 100, 200, 400, 800, 1,600, 3,200, or 6,400 ppm 1,4-dioxane vapors 6 hours/day, 5 days/week for 13 weeks (Kasai et al. 2008). End points evaluated included mortality, clinical signs (daily), body weight and food consumption (once per week), hematology, clinical chemistry and urinalysis at termination, and gross and microscopic pathology of all major organs and tissues. All rats in the 6,400 ppm group died during the first week of the study. Examination of these rats showed that death was primarily caused by renal failure, as judged by marked necrosis observed in the renal tubules. Lung congestion was also observed in males and females from this exposure group. At the remaining exposure concentrations, no abnormal clinical signs were observed during the study. Terminal body weight was reduced in all treated groups except the 100 ppm group; the final weight was reduced more than 10% relative to controls only in females exposed to 3,200 ppm. Data on food consumption were not provided. Significant changes in organ weight (>10% difference with controls) were limited to the liver, kidneys, and lungs and consisted of increases in relative organ weight, generally in the high dose groups of up to 15% relative to controls; data on absolute organ weights were not provided. Significant changes (although within normal values) in hematology and clinical chemistry parameters were limited to the 3,200 ppm groups and consisted of increases in mean corpuscular volume and serum alanine aminotransferase (ALT) in males, decrease in glucose and triglycerides in males, and increases in red blood cell count, hemoglobin, hematocrit, and aspartate aminotransferase (AST) and ALT serum activities in females. Histologically, exposure to 1,4-dioxane affected principally the respiratory tract, in particular the nasal cavity of males and females. Significant nuclear enlargement of the respiratory epithelium was seen in all exposed groups. The incidences in males were 0/10 in the control group and 7/10, 9/10, 7/10, 10/10, 10/10, and 10/10 in the exposed groups up to 3,200 ppm, respectively. The corresponding incidences in females were 0/10, 5/10, 9/10, 10/10, 10/10, 10/10, and 10/10. The severity of the lesion was concentration-related. Significant nuclear enlargement of the olfactory epithelium started at 200 ppm (5/10 in males and 6/10 in females). Similar lesions in the trachea and bronchus

appeared only in the high-exposure groups. The nuclear enlargement was characterized by the epithelial cells having a round to oval or elongated nucleus at least 4 times larger in diameter than normal. Significantly increased incidence of vacuolic change in the olfactory epithelium started in males at 400 ppm (0/10, 1/10, 3/10, 6/10, 10/10, 9/10) and in females at 800 ppm (0/10, 1/10, 2/10, 3/10, 7/10, 9/10, 10/10), while atrophy of the olfactory epithelium started in females at 800 ppm (0/10, 0/10, 2/10, 3/30, 5/10, 5/10, 4/10) and none was seen in males. Significant single cell necrosis and centrilobular swelling occurred in the liver of males exposed to 3,200 ppm 1,4-dioxane; females in this exposure group showed only centrilobular swelling. Significant kidney changes were seen only in females from the 3,200 ppm exposure group and consisted of hydropic changes in the proximal tubule. No treatment-related lesions were reported in any other tissue or organ examined.

Although nuclear enlargement of the respiratory and olfactory epithelium occurred at lower exposure levels than other nasal lesions, it was not selected as the critical effect for MRL derivation on the grounds that the toxicological significance of the lesion is uncertain. There is some evidence, although not conclusive at this time, suggesting that this alteration may represent a preneoplastic lesion. Indeed, preneoplastic and neoplastic lesions were observed in rats exposed chronically in the Kasai et al. (2009) study. Furthermore, as discussed by Kasai et al. (2008), nuclear enlargement occurred as an early histopathological change in the respiratory tract of rats simultaneously exposed to sulfur dioxide and treated intraperitoneally with several N-nitrosamines known to induce nasal tumors in rats (Fowlie et al. 1990). In addition, studies have shown a good correlation between *in vivo* carcinogenicity and the extent of nuclear enlargement in HeLa cells *in vitro* (Grant and Grasso 1978). Since MRLs are not based on a consideration of cancer effects, nuclear enlargement is not considered an appropriate basis for MRL derivation.

Incidence data for vacuolic change in the olfactory epithelium in male and female rats and for atrophy of the olfactory epithelium in female rats exposed to 1,4-dioxane vapors (Kasai et al. 2008) were analyzed using the benchmark dose/concentration (BMD/BMC) approach for MRL derivation (further details of the modeling are presented in Appendix A). A multistage (1-degree) model provided the best fit to the vacuolic change in both the male and female data, whereas a log-logistic model provided the best fit for the atrophy of the olfactory epithelium in female rats. From these models, the lowest predicted exposure concentrations associated with a 10% extra risk (BMC_{10}) was 40.39 ppm and corresponded to vacuolic changes in the respiratory epithelium of male rats; the corresponding lower 95% confidence limits on this concentration ($BMCL_{10}$) was 27.99 ppm.

The $BMCL_{10}$ of 27.99 ppm was converted to a human equivalent exposure concentration (HEC) ($BMCL_{10[HEC]}$) in consideration of EPA (1994) cross-species dosimetric methodology for inhaled gases. Although 1,4-dioxane produces portal-of-entry effects typical of a category 1 gas, it deviates from the strict definition of a category 1 gas in that: (1) it is not potently reactive, (2) it enters the systemic circulation via portal-of entry tissues, and (3) it induces systemic effects following inhalation exposure (i.e., liver and kidney effects). Both (1) and (3) are consistent with category 3 gases; however, atypical of a category 3 gas, 1,4-dioxane's critical effect from repeated inhalation exposure is a portal-of-entry effect (nasal lesions). 1,4-Dioxane may be more appropriately classified as a category 2 gas (moderately water-soluble and moderately to slowly reactive in respiratory tissue; EPA 1994); however, the EPA (1994) default dosimetric equations for respiratory effects from category 2 gases have not been widely used because they lead to human equivalent concentrations that are orders of magnitude lower than concentrations administered to rodents. Dosimetric equations for a category 1 gas (based on a ratio of the ratios of ventilation rate to extrathoracic surface area in rats to humans) indicate that effects observed in rodents at a specific air concentration will occur in humans at lower air concentrations (i.e., a $LOAEL_{human}$ will be about 0.25 x a $LOAEL_{rat}$, or the nasal dose experienced by humans at a specific air concentration will be higher than nasal doses experienced by rodents). In contrast, dosimetric equations for category 3 gases indicate that steady-state blood concentrations (and nasal doses) are determined principally by blood:air coefficients, and that, for chemicals like 1,4-dioxane with higher blood:air coefficients in rats (1,861; Sweeney et al. 2008) than in humans (1,666; Sweeney et al. 2008), effects observed in rats at a specific air concentration will occur in humans at equivalent or higher air concentrations (EPA 1994). This means that at a specific air concentration, nasal doses in humans would be equivalent to or less than nasal doses in rats. More sophisticated dosimetric cross-species extrapolation approaches have been developed for several other nasal toxicants linking computerized fluid dynamic (CFD) nasal airflow patterns and species-specific anatomical features with physiologically based pharmacokinetic (PBPK) models incorporating species-specific information about diffusion, metabolism, and kinetic distribution of the agents among tissues. Predictions from these models indicate that, at a specific air concentration, nasal doses would be nearly equivalent between humans and rats (e.g., vinyl acetate [Andersen et al. 2002]) or would be lower in humans than in rats (methyl acrylate [Andersen et al. 2002]; ethyl acrylate [Sweeney et al. 2004]; and formaldehyde [Conolly et al. 2004; Kimbell et al. 2001]). Because of these predictions, it is expected that the category 3 gas dosimetric equation is more appropriate for 1,4-dioxane than the category 1 gas dosimetric equation. Thus, the EPA (1994) category 3 gas dosimetric equation was used in deriving the MRL, in addition to a duration adjustment (6/24 hours x 5/7 days), which was considered appropriate in the absence of information regarding whether Haber's Law is applicable under the experimental conditions of the study.

The MRL is derived as follows:

$$BMCL_{10[HEC]} = BMCL_{10[ADJ]} \times (H_{b/g}A / H_{b/g}H)$$

where:
$BMCL_{10[ADJ]}$ = 27.99 ppm x 6/24 hours x 5/7 days = 4.998 ppm and
$H_{b/g}A$ = animal blood:air partition coefficient = 1,861 (Sweeney et al. 2008)
$H_{b/g}H$ = human blood:air partition coefficient = 1,666 (Sweeney et al. 2008)

$$(H_{b/g}A / H_{b/g}H) = 1{,}861/1{,}666 = 1.117$$

Because the ratio of the partition coefficients is higher than 1, a default value of 1 is used in accordance with EPA's RfC methodology (EPA 1994).

$$BMCL_{[HEC]} = 4.998 \text{ ppm} \times 1 = 4.998 \text{ ppm}$$

Applying an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) to the $BMCL_{[HEC]}$, rounded to the nearest single digit, yields an intermediate-duration inhalation MRL of 0.2 ppm for 1,4-dioxane.

- An MRL of 0.03 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to 1,4-dioxane.

The chronic inhalation database for 1,4-dioxane in humans and animals is limited. An occupational study by Thiess et al. (1976) provided no evidence of ill effects in a group of 74 German workers exposed to concentrations ranging from 0.006 to 14.3 ppm for an average of 25 years. In another epidemiological study, mortality rates were evaluated among workers exposed to 0.1–17 ppm 1,4-dioxane for up to 21 years (Buffler et al. 1978). No differences were found between observed and expected incidences of cancer. These studies were not considered for MRL derivation because they do not provide enough information to verify the conclusions of the authors.

Two chronic-duration inhalation studies in animals are available for 1,4-dioxane (Kasai et al. 2009; Torkelson et al. 1974). In a study conducted by Torkelson et al. (1974), groups of Wistar rats (288/sex) were exposed to 1,4-dioxane vapors at a concentration of 0.4 mg/L (111 ppm) 7 hours/day, 5 days/week for 2 years. Controls were exposed to filtered room air. End points examined included clinical signs, eye and nasal irritation, skin condition, respiratory distress, and tumor formation. Hematological parameters (hemoglobin, red blood cell count, total and differential leukocyte counts, corpuscular volume) were

determined after 16 and 23 months of exposure. Blood collected at termination was used also for determination of clinical chemistry parameters (serum ALT and AP activity, BUN, total protein). Liver, kidneys, and spleen were weighed and the major tissues and organs were processed for microscopic examination. Exposure to 1,4-dioxane vapors had no significant effect on mortality or body weight gain and induced no signs of eye or nasal irritation or respiratory distress. Slight but statistically significant changes in hematological and clinical chemistry parameters were within the normal physiological limits and were considered of no toxicological importance. Organ weights were not significantly affected. Microscopic examination of organs and tissues did not reveal treatment-related effects. It should be noted, however, that the tissues from the nasal cavity were not listed among the tissues that were subjected to microscopic examination by Torkelson et al. (1974), although in the discussion of the results, the investigators state that no nasal tumors were observed in any rats. Although there were no clinical signs, early mortality, nasal tumors, or any other indication that the health of the rats was compromised in the 2-year study, there is uncertainty regarding the possibility that the nuclear enlargement observed in the 13-week study might progress to cancer.

Kasai et al. (2009) whole-body exposed groups of male F344/DuCrj rats (50/group) to target concentrations of 0, 50, 250, or 1,250 ppm 1,4-dioxane vapors 6 hours/day, 5 days/week for 104 weeks; controls were exposed to clean air. End points evaluated included clinical signs and mortality (daily) and body weight and food consumption (once/week for the first 14 weeks, every 4 weeks thereafter). All rats were subjected to complete necropsy. Blood was collected at termination for clinical chemistry and hematology tests; urinary pH was measured in the last week of the study. All major organs were removed, weighed, and examined for macroscopic lesions. All major tissues and organs, including the entire respiratory tract, were examined microscopically. Survival rates in rats exposed to 250 ppm tended to decrease relative to controls, but the difference with controls was not statistically significant. Exposure to 1,250 ppm 1,4-dioxane significantly reduced ($p<0.05$) survival rate beginning on week 91. Terminal survival rate was 37/50, 37/50, 29/50, and 25/50 in the control, low-, mid-, and high-exposure groups, respectively. The decrease in survival rates was attributed to increased number of deaths due primarily to peritoneal mesotheliomas, although nasal tumors contributed to the causes of death. Terminal body weight was reduced 6.3% in the high-exposure group. Food consumption was not affected by exposure to 1,4-dioxane. Statistically significant increases in relative liver (27%) and lung (2%) weights were reported in the high-exposure group, but there was no clear dose-response relationship. Significant changes in hematology and clinical chemistry tests included reduced hemoglobin (13%), mean corpuscular volume (MCV, 6%), mean corpuscular hemoglobin (MCH, 8%), increased serum AST (46%), ALT (95%), AP (15%), and gamma-glutamyl transpeptidase (γ-GTP, 6–7-fold); urinary pH was

reduced 7%. All of these changes were restricted to the high-exposure group. Treatment-related pre- and nonneoplastic lesions occurred in the nasal cavity, liver, and kidney. All exposed groups had significant increases in nuclear enlargement of the respiratory epithelium (0/50, 50/50, 48/50, 38/50), nuclear enlargement of the olfactory epithelium (0/50, 48/50, 48/50, 45/50), atrophy of the olfactory epithelium (0/50, 40/50, 47/50, 48/50), and respiratory metaplasia of the olfactory epithelium (11/50, 34/50, 49/50, 48/50). Incidences of hydropic change and sclerosis of the lamina propia were significantly increased only in the mid- and high-exposure groups. Significant increases in liver lesions (centrilobular nuclear enlargement, acidophilic cell foci, basophilic cell foci, spongiosis hepatis, and centrilobular necrosis) occurred in the high-exposure group. Significant increases in nuclear enlargement of the proximal kidney tubule occurred in the mid- and high-exposure groups; significantly increased incidence of hydropic changes in the proximal tubule occurred in the high-exposure group. No significant changes occurred in other organs or tissues. The lowest exposure concentration tested, 50 ppm 1,4-dioxane, is a LOAEL for nasal lesions (atrophy of the olfactory epithelium); a NOAEL was not defined in this study.

Of the two available studies, the study by Kasai et al. (2009) was selected for MRL derivation because it conducted a complete examination of the respiratory tract, including nasal passages, plus Torkelson et al. (1974) established a free-standing NOAEL. The results of Kasai et al. (2009) clearly show that the nasal cavity was the most sensitive tissue following 2 years of exposure to 1,4-dioxane vapors. As discussed in the derivation of the intermediate-duration inhalation MRL for 1,4-dioxane, nuclear enlargement was not considered as the basis of an MRL because of evidence suggesting that the alteration may represent a pre-neoplastic lesion. Incidences of atrophy (0/50, 40/50, 47/50, and 48/50) and respiratory metaplasia (11/50, 34/50, 49/50, and 48/50) of the olfactory epithelium were also significantly elevated at all exposure levels tested. Of these two lesions, the atrophy of the olfactory epithelium was selected as the critical effect for MRL derivation because it showed a higher incidence rate at the LOAEL than respiratory metaplasia. Because the incidence of this lesion at the lowest exposure level (50 ppm) was close to the maximal response level (80% of 50-ppm animals showed this lesion), BMD analysis of the data was not conducted. This decision is in accordance with guidelines stating that studies in which responses are at or near the maximal response level are not considered adequate for BMD analysis (EPA 2000a).

The LOAEL of 50 ppm was converted to a HEC using the EPA cross-species dosimetric methodology (EPA 1994) for a category 3 gas as explained in the derivation of the intermediate-duration inhalation MRL. A duration adjustment (6/24 hours x 5/7 days) seemed appropriate in the absence of information

regarding whether Haber's Law is applicable under the experimental conditions of the study. The MRL is derived as follows:

$$LOAEL_{[HEC]} = LOAEL_{[ADJ]} \times (H_{b/g}A / H_{b/g}H)$$

where:
$LOAEL_{[ADJ]}$ = 50 ppm x 6/24 hours x 5/7 days = 8.9286 ppm and
$H_{b/g}A$ = animal blood:air partition coefficient = 1,861 (Sweeney et al. 2008)
$H_{b/g}H$ = human blood:air partition coefficient = 1,666 (Sweeney et al. 2008)

$$(H_{b/g}A / H_{b/g}H) = 1{,}861/1{,}666 = 1.117$$

Because the ratio of the partition coefficients is higher than 1, a default value of 1 is used in accordance with EPA's RfC methodology (EPA 1994).

$$LOAEL_{[HEC]} = 8.9286 \text{ ppm} \times 1 = 8.9286 \text{ ppm}$$

Applying an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, and 10 for human variability) to the $LOAEL_{[HEC]}$ yields a chronic-duration inhalation MRL of 0.03 ppm for 1,4-dioxane.

Oral MRLs

- An MRL of 5 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to 1,4-dioxane.

Only two acute-duration oral studies potentially useful for MRL derivation are available for 1,4-dioxane. JRBC (1998) conducted a 2-week drinking water study in F344 rats and $B6C3F_1$ mice and reported that the most sensitive effect was an increased incidence of nuclear enlargement of the olfactory epithelium in male and female rats receiving doses of approximately 1,010 and 1,040 mg 1,4-dioxane/kg/day, respectively; the corresponding NOAELs were 370 and 400 mg/kg/day. Liver and kidney alterations were observed at higher doses. Giavini et al. (1985) administered 1,4-dioxane by gavage in water to pregnant Sprague-Dawley rats on gestation days (Gd) 6 through 15 and reported slight but significant reductions in fetal weight and in the percent of ossified sternebrae in the group treated with the highest dose of 1,4-dioxane, 1,033 mg/kg/day; the NOAEL was 516 mg/kg/day. Most of the rest of the acute database consists of high-dose early studies aimed mainly at determining LD_{50} values and inadequate for risk assessment (de Navasquez 1935; Kesten et al. 1939; Laug et al. 1939; Pozzani et al. 1959; Smyth et al. 1941). The lowest dose that caused lethality was 327 mg 1,4-dioxane/kg/day in a study that tested

only three dogs (Schrenk and Yant 1936). This dose was provided in the drinking water and resulted in the death of one dog after 10 days of treatment. Doses of 375 mg/kg/day resulted in the death of another dog in 9 days. However, because the dogs were allowed to drink the 1,4-dioxane solution only twice daily and no other source of water was available, dehydration may have played a role in the death of the animals.

Of the two potentially useful studies for MRL derivation mentioned above, the developmental study conducted by Giavini et al. (1985) is preferred as basis for the acute-duration oral MRL for 1,4-dioxane mainly due to overall deficiencies in the JRBC (1998) study, namely, lack of statistical analysis of the results due to the fact that only 2 or 3 animals (out of 10/group) were examined, and the fact that end points such as hematology, clinical chemistry, clinical signs, and gross examinations were not conducted or reported. These limitations severely compromise the interpretation of the results.

In the study selected as basis for the acute oral MRL, groups of 17–20 pregnant Sprague-Dawley rats were treated with 0, 0.25, 0.5, or 1 mL 1,4-dioxane/kg/day (0, 258, 516, or 1,033 mg 1,4-dioxane/kg/day based on a specific gravity of 1.034) by gavage in water on Gd 6–15 (Giavini et al. 1985). Food consumption was determined daily and body weight was monitored every three days. Sacrifices were conducted on Gd 21 and the number of corpora lutea, implantations, resorptions and live fetuses was recorded. The fetuses were weighed and inspected for external malformations and half were examined for visceral abnormalities and the other half for skeletal malformations. Rats treated with 1,033 mg 1,4-dioxane/kg/day gained 18% less weight than controls during treatment days, although the difference was not statistically significant. Food consumption was slightly (5%) but significantly ($p<0.05$) reduced in these rats during treatment. The average fetal weight in the high-dose group was slightly but significantly ($p<0.01$) lower than in controls. Also, a slight but significant ($p<0.05$) reduction in sternum ossification was seen in high-dose fetuses. There were no significant effects on the number of implantations and live fetuses, post-implantation loss, or incidence of malformations. Based on the reduced maternal and fetal body weight and reduced sternum ossification, a maternal and developmental LOAEL of 1,033 mg 1,4-dioxane/kg/day can be defined; the maternal and developmental NOAEL is 516 mg/kg/day. Attempts made to apply dose-response models to the data were unsuccessful, as no adequate fits of EPA BMDS models to the data were obtained; therefore, the NOAEL/LOAEL approach was used for MRL derivation. An acute-duration oral MRL of 5 mg/kg/day was derived for 1,4-dioxane by dividing the NOAEL of 516 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

- An MRL of 0.5 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to 1,4-dioxane.

The intermediate-duration oral MRL is based on a NOAEL of 52 mg 1,4-dioxane/kg/day for liver effects in rats (Kano et al. 2008). In that study, groups of F344/DuCrj rats (10/sex/group) were administered 1,4-dioxane in the drinking water in concentrations of 0, 640, 1,600, 4,000, 10,000, or 25,000 ppm for 13 weeks (0, 52, 126, 274, 657, or 1,554 mg/kg/day in males; 0, 83, 185, 427, 756, or 1,614 mg/kg/day in females, estimated by the investigators). End points evaluated included clinical signs, food and water consumption, body weight, complete hematology and clinical chemistry tests, urinalysis, organ weights, gross necropsy, and histopathology. One female in the 1,614 mg/kg/day group died. Body weight gain was reduced at 756 mg/kg/day (12%) and 1,614 mg/kg/day (21%) in females and at 1,554 mg/kg/day (21%) in males. Food consumption was reduced 13% in females at 1,614 mg/kg/day. Water consumption was reduced in a dose-related manner in all male groups and in females at ≥126 mg/kg/day. Hematology tests showed significant increases in erythrocyte counts, hemoglobin, hematocrit, and neutrophils, and a decrease in lymphocytes in males at 1,554 mg/kg/day, and decreases in mean corpuscular volume and platelets in females at 1,614 mg/kg/day. Total protein and albumin were decreased in males at ≥274 mg/kg/day and in females at ≥427 mg/kg/day. Serum AST, ALT, alkaline phosphatase (AP), and leucine aminopeptidase (LAP) activities, and levels of cholesterol, triglycerides, sodium, and glucose were significantly elevated in high dose males and females. Urinary pH was decreased in males at ≥274 mg/kg/day and in females at ≥756 mg/kg/day. Absolute and relative kidney weights were increased in females at ≥231 mg/kg/day. Nuclear enlargement of the respiratory epithelium occurred in males at ≥126 mg/kg/day and in females at ≥185 mg/kg/day; nuclear enlargement of the olfactory and tracheal epithelium occurred in males at ≥274 mg/kg/day and in females at ≥427 mg/kg/day. Swelling of the central area of the liver was observed in males at ≥126 mg/kg/day and in females at ≥756 mg/kg/day, and vacuolar changes in the liver occurred in males at ≥657 mg/kg/day and in females at 1,614 mg/kg/day. The incidences of swelling of the central area of the liver in males were 0/10, 0/10, 9/10, 10/10, 10/10, and 10/10 in the control, 52, 126, 274, 657, and 1,554 mg/kg/day dose groups, respectively. Nuclear enlargement of the proximal tubule of the kidneys was seen in males at ≥657 mg/kg/day and in females at ≥756 mg/kg/day. Hydropic changes in the proximal tubule of the kidneys and vacuolar changes in the brain occurred in high-dose males and females (1,554 and 1,614 mg/kg/day, respectively). The study LOAEL was 126 mg/kg/day for liver effects in male rats. Limitations of the study include the lack of reporting on clinical signs and gross necropsy. To derive the MRL, the NOAEL of 52 mg/kg/day for liver effects in males was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability), yielding an intermediate-

duration oral MRL of 0.5 mg/kg/day. The steepness of the dose-response relationship for liver lesions rendered the data set inadequate for BMD analysis.

A promotion study by Lundberg et al. (1987) supports the liver findings of Kano et al. (2008). The study used male Sprague-Dawley rats (8–11/group) that were treated with 100 or 1,000 mg 1,4-dioxane/kg by gavage in saline 5 days/week for 7 weeks. One week after the last treatment, the rats were sacrificed, and the livers were processed for microscopic examination. The livers of high-dose rats showed enlarged foamy hepatocytes mainly in midzonal regions. The foamy appearance was due to vacuoles shown to contain fat. No treatment-related histopathological alterations were observed in the liver at the 100 mg/kg/day dose level. Because of the limited scope of the study by Lundberg et al. (1987) (only the liver was examined) and because the NOAEL of 100 mg/kg/day is practically the same as the LOAEL of 126 mg/kg/day identified by Kano et al. (2008), the latter study was preferred for MRL derivation. Also supporting the findings from Kano et al. (2008) is a report by Stott et al. (1981) who found that repeated dosing of rats with 1,000 mg 1,4-dioxane/kg/day for 7 or 11 weeks produced hepatocyte swelling and histopathology. Similar findings were reported in an earlier study in which rats were treated with doses of approximately 1,428 mg 1,4-dioxane/kg/day in the drinking water for 34 days (Fairley et al. 1934).

Although available rat and mouse PBPK models (Leung and Paustenbach 1990; Reitz et al. 1990; Sweeney et al. 2008) provide adequate fits of high-dose observations, they do not perform well against low-dose data; thus, they were not used for MRL derivation. The human model could not replicate the limited human experimental inhalation data available (Sweeney et al. 2008). Further, it assumes equivalency with mice in eliminating β-hydroxyethoxyacetic acid (HEAA), and has no value derived for oral absorption. Based on these significant limitations, the Sweeney et al. (2008) model for 1,4-dioxane in rats, mice, and humans, which is a more refined version of the earlier models, is not adequate for MRL derivation. This applies also to the use of PBPK models for derivation of the chronic-duration oral MRL for 1,4-dioxane described below.

- An MRL of 0.1 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to 1,4-dioxane.

The chronic-duration oral MRL is based on a NOAEL of 9.6 mg 1,4-dioxane/kg/day for liver effects in male rats in a study by Kociba et al. (1974). In that study, groups of Sherman rats (60/sex/dose level) were treated with 1,4-dioxane in the drinking water at levels of 0 (controls), 0.01, 0.1, or 1% for 716 days. Based on body weight and water consumption data, the investigators estimated that the water provided doses of 1,4-dioxane of 0, 9.6, 94, and 1,015 mg/kg/day for males and 0, 19, 148, and 1,599 mg/kg/day

for females. Blood samples were collected from controls and high-dose rats during the 4th, 6th, 12th, and 18th months of the study and at termination. Additional end points evaluated included clinical signs, body weight, organ weights, and gross and microscopic examination of major tissues and organs. Treatment with 1,4-dioxane significantly increased mortality in males dosed with 1,015 mg/kg/day and in females dosed with 1,599 mg/kg/day beginning at about 2–4 months of treatment. These rats showed degenerative changes in both the liver and kidneys. Rats in these groups also showed significantly reduced water consumption during the first year of the study and body weight gain was significantly reduced from the beginning of the study. Microscopic lesions in other groups were restricted to the liver and kidneys in males treated with ≥94 mg/kg/day and females dosed with ≥148 mg/kg/day. The liver lesions consisted of various degrees of hepatocellular degeneration and necrosis and evidence of hepatic regeneration as indicated by hepatocellular hyperplastic nodule formation. The NOAEL for liver effects was 9.6 mg/kg/day in males and 19 mg/kg/day in females. The LOAELs were 94 mg/kg/day in males and 148 mg/kg/day in females. The kidneys showed tubular epithelial degeneration and necrosis, and there was evidence of renal tubular regeneration as indicated by increased tubular epithelial regenerative activity (≥94 mg/kg/day in males and ≥148 mg/kg/day in females). There were no compound-related alterations in hematological parameters at any time point. The MRL of 0.1 mg/kg/day was calculated by dividing the male rat NOAEL of 9.6 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability). The lack of quantitative information regarding incidences of non-neoplastic lesions precludes the use of BMD methodology for MRL derivation.

The NOAEL and LOAEL for liver effects from Kociba et al. (1974) are supported by the results of Kano et al. (2009). In that study, groups of F344/DuCrj rats (50/sex/dose level) received 1,4-dioxane in the drinking water for 104 weeks. 1,4-Dioxane was administered at levels of 0, 200, 1,000, and 5,000 ppm for 2 years (0, 11, 55, and 274 mg/kg/day for males; 0, 18, 83, and 429 mg/kg/day for females). End points evaluated included clinical signs, food and water consumption, body and organ weights, and gross and microscopic examination of major organs and tissues. Terminal body weight was reduced 9% in high-dose males (274 mg/kg/day) and 20% in high-dose females (429 mg/kg/day). In males, relative liver weight was significantly increased at 55 mg/kg/day (14%) and 274 mg/kg/day (72%). A significant increase incidence of mixed cell foci was observed in the liver from male rats dosed with ≥55 mg 1,4-dioxane/kg/day. Increased incidence of acidophilic and mixed cell foci were reported in the liver from high-dose females (429 mg/kg/day). In addition, both high-dose male (274 mg/kg/day) and female (429 mg/kg/day) rats had significantly increased incidence of nuclear enlargement and squamous cell metaplasia of the respiratory epithelium; females dosed with ≥83 mg 1,4-dioxane/kg/day also showed a significantly increased incidence of nuclear enlargement of the nasal olfactory epithelium.

The NCI (1978) bioassay in Osborne-Mendel rats used somewhat higher dose levels than Kociba et al. (1974) and Kano et al. (2009), but did not observe liver lesions in male rats dosed with 240 mg 1,4-dioxane/kg/day. However, a dose level of 55 mg/kg/day induced cell foci in the liver from male F344 rats, and 94 mg/kg/day caused hepatocyte degeneration in Sherman rats. Since the dosing method was the same in all three studies (via the drinking water) the different results may reflect differences in strain sensitivity.

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3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 1,4-dioxane. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of 1,4-dioxane are indicated in Table 3-2 and Figure 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for 1,4-dioxane. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes when suitable data are available. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

3.2.1.1 Death

Several cases of death in humans have been documented after exposure to high concentrations of 1,4-dioxane. Barber (1934) described five deaths that occurred within a period of 2 weeks among factory workers engaged in a process that involved primarily exposure to 1,4-dioxane vapors, although minimal dermal exposure could not have been avoided. Three of the subjects suffered from abdominal pain and vomiting before death occurred. Post-mortem examination of the subjects showed extensive gross and microscopic lesions to the liver and kidneys. Based on his observations, Barber (1934) suggested that the effects on the kidneys may have been responsible for the fatal outcome and that liver necrosis, although widespread, was compatible with recovery. No exposure levels were available in these case reports. Johnstone (1959) described an additional fatal case of a worker exposed to 1,4-dioxane for only 1 week and whose post-mortem examination showed kidney and liver alterations similar to those described by Barber (1934). In the Johnstone case, the room in which the patient had worked had no exhaust ventilation and the worker was not provided a respirator. The minimum concentration of 1,4-dioxane in the room was 208 ppm and the maximum was in excess of 650 ppm; the average concentration was 470 ppm. In addition, dermal exposure may have been considerable in this case.

Studies in animals, mostly early studies, provide information on lethality of relatively high concentrations of 1,4-dioxane in several species and also indicate that the kidneys and liver, and in some cases, the lungs, are the main targets of high airborne concentrations of 1,4-dioxane. Short-term exposure to 5,000 ppm 1,4-dioxane was lethal to rats, mice, and rabbits, whereas 10,000 ppm was lethal to guinea pigs (Fairley et al. 1934). A 4-hour LC_{50} of 14,261 ppm was calculated for rats (Pozzani et al. 1959). An additional study in guinea pigs reported that the minimum period of exposure that caused the death of the majority of a group of six animals was 180 minutes to 30,000 ppm; no deaths occurred in groups exposed to up to 10,000 ppm for up to 480 minutes (Yant et al. 1930). One out of four rabbits exposed to 2,000 ppm 1,4-dioxane 3 hours/day, 5 days/week died on week 4 of exposure, and the cause of death was attributed to renal and hepatic lesions (Fairley et al. 1934). In a more recent 13-week intermittent exposure study, all rats (10/sex) exposed to 6,400 ppm 1,4-dioxane vapors 6 hours/day, 5 days/week died during the first week of the study (Kasai et al. 2008). The primary cause of death was reported to be renal failure, as judged by marked necrosis of the renal tubules. Similar exposure of male rats to 1,250 ppm 1,4-dioxane for 2 years significantly decreased survival rate relative to controls beginning on week 91 of the study (Kasai et al. 2009). This was attributed to increased number of deaths due primarily to peritoneal

mesotheliomas, although nasal tumors also contributed. Exposure to 250 ppm 1,4-dioxane did not significantly decrease survival rates.

The LOAEL values for death in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal, endocrine, dermal, or body weight effects in humans after inhalation exposure to 1,4-dioxane.

The highest NOAEL and all reliable LOAEL values from each study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. In a group of six individuals exposed to 2,000 ppm 1,4-dioxane vapors for 3 minutes in a 10 m^3 chamber, there were no complaints of nasal discomfort, but one out of four subjects exposed to 1,000 ppm for 5 minutes complained of constriction of the throat (Fairley et al. 1934); however, the exposure concentrations were not verified. Exposure of five subjects to about 278 ppm for a few minutes (unspecified) produced slight mucous membrane irritation, and 1,390 ppm caused a slight prickling in the nose and scratchiness and dryness in the throat (Wirth and Klimmer 1936). Exposure to 300 ppm 1,4-dioxane for 15 minutes produced nose and throat irritation among a group of 12 volunteers (Silverman et al. 1946). At 200 ppm, the report does not indicate the presence or absence of symptoms, but considers the exposure acceptable. A 10-minute exposure to 1,600 ppm 1,4-dioxane produced slight nose and throat irritation that persisted throughout the test in a group of five individuals (Yant et al. 1930). In another experiment by the same investigators, exposure of the same five persons to 5,500 ppm 1,4-dioxane for 1 minute resulted in a burning sensation to the nose and throat (Yant et al. 1930). Exposure of four men to 50 ppm for 6 hours reportedly caused no adverse respiratory signs or alterations in respiratory function, assessed 24 hours and 2 weeks after exposure (Young et al. 1977); however, no data were provided in the study. Exposure of 12 volunteers (6 males and 6 females) to 20 ppm 1,4-dioxane for 2 hours caused no significant respiratory effects during exposure or up to 3 hours after exposure (Ernstgård et al. 2006). Subjective symptoms were assessed with a questionnaire and respiratory function was assessed by spirometry. Also assessed was nasal swelling. The study by Ernstgård et al. (2006) was used to derive an acute-duration inhalation MRL for 1,4-dioxane.

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form	Comments
ACUTE EXPOSURE									
Death									
1	Rat (NS)	1 wk 5 d/wk 3 hr/d				5000	(3/3 deaths before a total of 16 hours of exposure)	Fairley et al. 1934	
2	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d				6400	(all rats died during first week of exposure)	Kasai et al. 2008	
3	Rat (Wistar)	4 hr				14261 F	(4-hour LC50)	Pozzani et al. 1959	
4	Mouse (NS)	1 wk 5 d/wk 3 hr/d				5000	(1/3 deaths after 3 hours of exposure)	Fairley et al. 1934	
5	Gn Pig (NS)	1 wk 5 d/wk 3 hr/d				10000	(6/6 deaths before 7.5 hours of exposure)	Fairley et al. 1934	
6	Gn Pig (NS)	10-540 min				30000	(death of majority of animals in 180 minutes)	Yant et al. 1930	
7	Rabbit (NS)	1 wk 5 d/wk 3 hr/d				5000	(1/4 death after 16.5 hours of exposure)	Fairley et al. 1934	

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Systemic								
8	Human	2 hr	Resp	20[b]			Ernstgard et al. 2006	NOAELS are for sensory irritation and pulmonary function.
			Ocular	20				
9	Human	5 min	Resp		1000	(1/4 throat constriction)	Fairley et al. 1934	
10	Human	3 min	Resp	2000			Fairley et al. 1934	
			Ocular	2000				
11	Human	15 min	Resp	200	300	(nose and throat irritation)	Silverman et al. 1946	
			Ocular	200	300	(eye irritation)		
12	Human	10 min	Resp		1600	(slight nose and throat irritation)	Yant et al. 1930	
			Ocular		1600	(slight eye irritation and lacrimation)		

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form	Comments
13	Human	6 hr	Resp	50 M					Young et al. 1977	Tests done included chest X-ray, EKG, respiratory function, clinical chemistry and hematology, and urinalysis.
			Cardio	50 M						
			Hemato	50 M						
			Hepatic	50 M						
			Renal	50 M						
			Ocular		50 M	(eye irritation)				
14	Rat (CD)	4 hr	Hepatic		1000 M	(increased serum transaminases indicative of liver injury)			Drew et al. 1978	
15	Gn Pig (NS)	10-540 min	Resp		1000	(immediate nasal irritation)	30000	(dyspnea, gasping, shallow breathing, hyperemia, congestion)	Yant et al. 1930	
			Ocular	1000	2000	(eye irritation)				
Neurological										
16	Rat (Wistar)	2 wk 5 d/wk 4 hr/d		1500 F	3000 F	(depressed avoidance response)			Goldberg et al. 1964	

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE									
Death									
17	Rabbit (NS)	3-12 wk 5 d/wk 3 hr/d				2000	(1/4 death on week 4 of exposure)	Fairley et al. 1934	
Systemic									
18	Rat (NS)	3-12 wk 5 d/wk 3 hr/d	Resp	1000				Fairley et al. 1934	No gross or microscopic lesions in the lungs.
			Hepatic			1000	(hepatocyte degeneration)		
			Renal			1000	(renal cortex degeneration)		

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
19	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d	Resp	200 M	400 M[c] (vacuolic change in olfactory epithelium)		Kasai et al. 2008	NOAELs are for tissue or organ histopathology.
			Cardio	3200				
			Gastro	3200				
			Hemato	3200				
			Musc/skel	3200				
			Hepatic	1600 M	3200 M (cell necrosis, centrilobular swelling)			
			Renal	1600 F	3200 F (hydropic change in proximal tubule)			
			Endocr	3200				
			Dermal	3200				
			Ocular	3200				
			Bd Wt	1600 F	3200 F (final body weight reduced 10.2%)			

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form	Comments
20	Mouse (NS)	3-12 wk 5 d/wk 3 hr/d	Resp	1000				Fairley et al. 1934	No gross or microscopic lesions in the lungs.
			Hepatic			1000	(hepatocyte degeneration)		
			Renal			1000	(renal cortex degeneration)		
21	Gn Pig (NS)	3-12 wk 5 d/wk 3 hr/d	Resp	5000				Fairley et al. 1934	No lesions were seen in the lungs.
			Hepatic			1000	(hepatocyte degeneration)		
			Renal			1000	(cortical cell degeneration)		
22	Rabbit (NS)	3-12 wk 5 d/wk 3 hr/d	Resp	1000				Fairley et al. 1934	No lesions were seen in the lungs.
			Hepatic			1000	(hepatocyte degeneration)		
			Renal			1000	(cortical cell degeneration)		

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
23	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d		3200			Kasai et al. 2008	NOAEL is for histopathology of lymphoreticular tissues.
Neurological								
24	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d		3200			Kasai et al. 2008	NOAEL is for histopathology of central and peripheral nervous tissues.
Reproductive								
25	Rat F344/DuCrj	13 wk 5 d/wk 6 hr/d		3200			Kasai et al. 2008	NOAEL is for histopathology of reproductive organs.
CHRONIC EXPOSURE								
Death								
26	Rat F344/DuCrj	104 wk 5 d/wk 6 hr/day				1250 M (decreased survival rate)	Kasai et al. 2009	

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Systemic								
27	Rat F344/DuCrj	104 wk 5 d/wk 6 hr/day	Resp		50 M[d] (atrophy of olfactory epithelium)		Kasai et al. 2009	NOAELs are for histopathology of organs and tissues examined.
			Cardio	1250 M				
			Gastro	1250 M				
			Hemato	250 M	1250 M (decreased hemoglobin, MCV and MCH)			
			Musc/skel	1250 M				
			Hepatic	250 M	1250 M (centrilobular nuclear enlargement and necrosis; increased serum transaminases)			
			Renal	50 M	250 M (nuclear enlargement in proximal tubule)			
			Endocr	1250 M				
			Dermal	1250 M				
			Ocular	1250 M				
			Bd Wt	1250 M				

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
28	Rat (Wistar)	2 yr 5 d/wk 7 hr/d	Resp	111			Torkelson et al. 1974	Only one exposure level tested. End points evaluated included clinical signs, hematology and clinical chemistry, and histopathology.
			Cardio	111				
			Gastro	111				
			Hemato	111				
			Hepatic	111				
			Renal	111				
			Endocr	111				
			Dermal	111				
			Ocular	111				
			Bd Wt	111				
Immuno/ Lymphoret								
29	Rat F344/DuCrj	104 wk 5 d/wk 6 hr/day		1250 M			Kasai et al. 2009	NOAEL is for histopathology of lymphoreticular tissues.
30	Rat (Wistar)	2 yr 5 d/wk 7 hr/d		111			Torkelson et al. 1974	No gross or microscopic alterations in spleen or lymph nodes.

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Neurological								
31	Rat F344/DuCrj	104 wk 5 d/wk 6 hr/day		1250 M			Kasai et al. 2009	NOAEL is for histopathology of central and peripheral nervous tissues.
32	Rat (Wistar)	2 yr 5 d/wk 7 hr/d		111			Torkelson et al. 1974	No signs of altered behavior. No gross or microscopic alterations in the brain.
Reproductive								
33	Rat F344/DuCrj	104 wk 5 d/wk 6 hr/day		1250 M			Kasai et al. 2009	NOAEL is for histopathology of primary or secondary sex organs.
34	Rat (Wistar)	2 yr 5 d/wk 7 hr/d		111			Torkelson et al. 1974	No gross or microscopic alterations in testes, ovaries, uterus, and vagina.

Table 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Cancer								
35	Rat F344/DuCrj	104 wk 5 d/wk 6 hr/day				250 M (CEL:peritoneum mesothelioma)	Kasai et al. 2009	NOAELs are for histopathology of organs and tissues examined.

a The number corresponds to entries in Figure 3-1.

b Used to derive an acute-duration inhalation minimal risk level (MRL) of 2 ppm for 1,4-dioxane; the MRL was derived by dividing the NOAEL by an uncertainty factor of 10 for human variability.

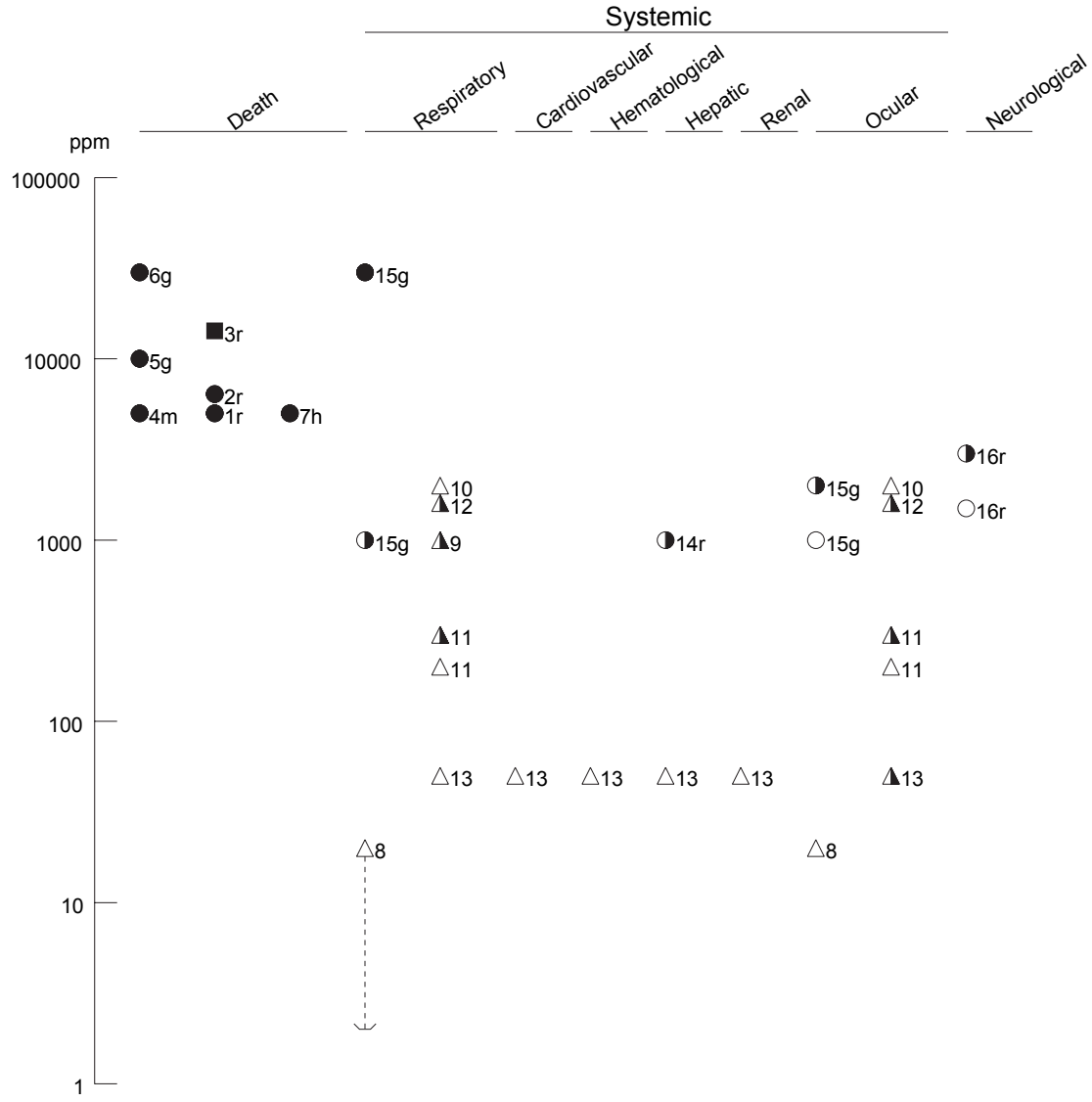
c Used to derive an intermediate-duration inhalation MRL of 0.2 ppm for 1,4-dioxane; the MRL was derived by dividing the BMCL10HEC by an uncertainty factor of 30 (3 for using dosimetric adjustments and 10 for human variability).

d Used to derive a chronic-duration inhalation MRL of 0.03 ppm for 1,4-dioxane; the MRL was derived by dividing the LOAELHEC by an uncertainty factor of 300 (3 for using dosimetric adjustments, 10 for using a LOAEL, and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); EKG = electrocardiogram; F = Female; Gastro = gastrointestinal; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); NOAEL = no-observed-adverse-effect level; NS = not specified; ppm = parts per million; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation

Acute (≤14 days)



c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◮ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation *(Continued)*

Intermediate (15-364 days)

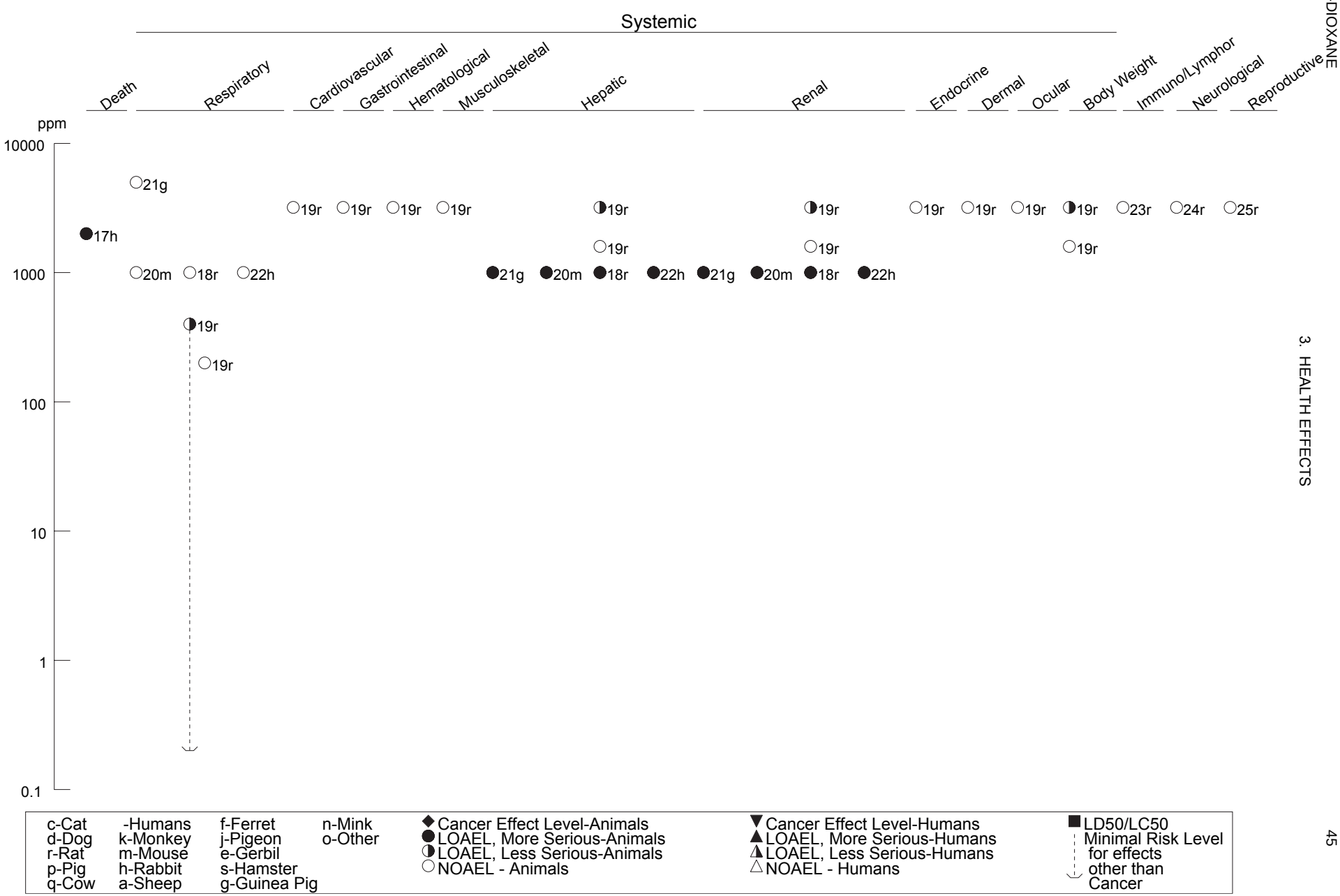
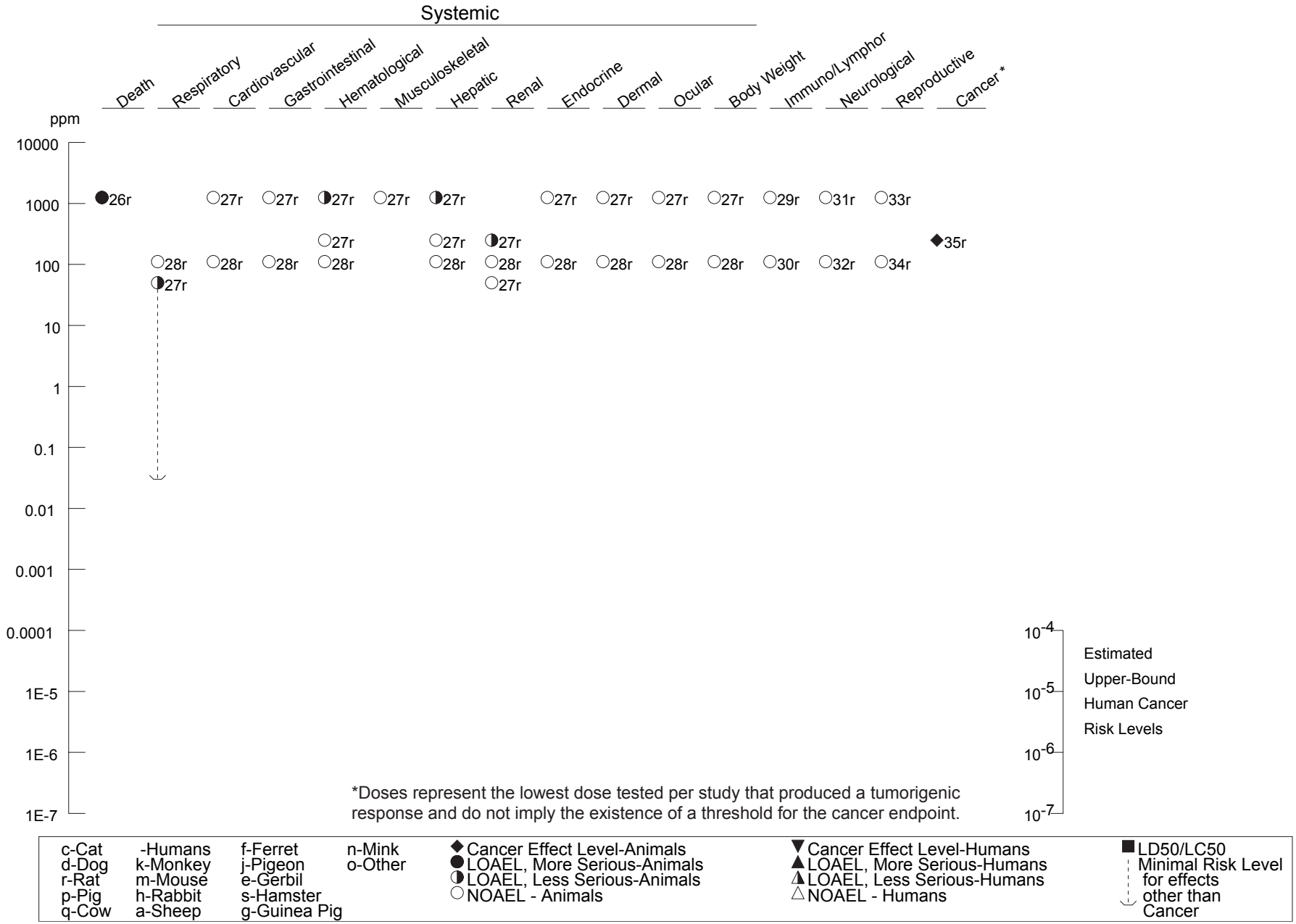


Figure 3-1 Levels of Significant Exposure to 1,4-Dioxane - Inhalation *(Continued)*

Chronic (≥365 days)



In a study in guinea pigs exposed to 1,000–30,000 ppm 1,4-dioxane for periods ranging from 10 to 540 minutes, nasal irritation was evident almost immediately at all exposure levels (Yant et al. 1930). No functional respiratory changes were noticed with concentrations of up to 10,000 ppm for 480 minutes, but dyspnea and gasping occurred at 30,000 ppm in 45–116 minutes. The 30,000 ppm level caused death in the animals in about 180 minutes. Gross necropsy revealed exposure-duration-related hyperemia in the lungs. Surviving guinea pigs autopsied 8–10 days after exposure showed no gross pathological changes except for a few cases of hyperemic areas of congestion in the lungs. Exposure of rats and guinea pigs to acute lethal concentrations of 1,4-dioxane produced vascular congestion of the lungs (Fairley et al. 1934). No lung lesions were seen in rats, mice, or rabbits exposed to 1,000 ppm 1,4-dioxane for 3–12 weeks or in guinea pigs exposed to 5,000 ppm for the same duration (Fairley et al. 1934). However, male and female F344 rats exposed intermittently to ≥100 ppm 1,4-dioxane vapors for 13 weeks showed an increased incidence of nuclear enlargement of the respiratory epithelium of the nasal cavity (Kasai et al. 2008). Nuclear enlargement of the olfactory epithelium was reported at ≥200 ppm and vacuolar changes and atrophy in the olfactory epithelium were noted at ≥400 ppm. Similar changes were reported in the tracheal and bronchial epithelium, but only in rats exposed to ≥1,600 ppm 1,4-dioxane. The study by Kasai et al. (2008) was used to derive an intermediate-duration inhalation MRL for 1,4-dioxane. In the 2-year inhalation study in rats by Torkelson et al. (1974), intermittent exposure to 111 ppm 1,4-dioxane caused no signs of nasal irritation, respiratory distress, or histopathologic alterations in the lungs and trachea of the animals. However, it appears that the rats' nasal cavity may have not been examined. More recently, a 2-year study in male F344 rats reported that intermittent exposure to ≥50 ppm 1,4-dioxane vapors (the lowest exposure concentration tested) significantly increased the incidence of nuclear enlargement in the respiratory and olfactory epithelia and also induced atrophy and respiratory metaplasia in the olfactory epithelium (Kasai et al. 2009). This study was used to derive a chronic-duration inhalation MRL for 1,4-dioxane.

Cardiovascular Effects. A study of four men exposed to 50 ppm 1,4-dioxane for 6 hours reported no abnormalities in the electrocardiograms (EKG) taken 24 hours and 2 weeks after exposure compared to EKGs taken prior to the study (Young et al. 1977); however, no data were provided in the study. High blood pressure was reported in subjects who eventually died following exposure to high amounts of 1,4-dioxane (Barber 1934; Johnstone 1959), but this may have been a non-specific response to a stressful condition or due to acute renal failure.

The only available information in animals is that no gross or histopathological alterations were observed in the heart from rats exposed to up to 3,200 ppm 1,4-dioxane 6 hours/day, 5 days/week for 13 weeks

(Kasai et al. 2008), to 111 ppm 1,4-dioxane for 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974), or to up to 1,250 ppm 1,4-dioxane 6 hours/day, 5 days/week for 2 years (Kasai et al. 2009).

Gastrointestinal Effects. Abdominal pain and vomiting were common features among subjects who eventually died after exposure to high concentrations of 1,4-dioxane (Barber 1934; Johnstone 1959). Barber (1934) suggested that the abdominal pain may have been due to stretching of the capsule of the liver and kidneys. No gross or histologic alterations were observed in the gastrointestinal tract from rats exposed to up to 3,200 ppm 1,4-dioxane 6 hours/day, 5 days/week for 13 weeks (Kasai et al. 2008), to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974), or to up to 1,250 ppm 1,4-dioxane 6 hours/day, 5 days/week for 2 years (Kasai et al. 2009).

Hematological Effects. A study of four male volunteers exposed to 50 ppm 1,4-dioxane for 6 hours reportedly did not show any significant effect of exposure on hematology parameters (Young et al. 1977); however, no data were provided in the study. Blood was collected prior to exposure and 24 hours and 2 weeks after exposure and subjected to a complete hematological analysis. Leukocytosis and eosinophilia were described in subjects who survived exposure to high concentrations of 1,4-dioxane described by Barber (1934). A cross-sectional study of 74 workers exposed to concentrations of 1,4-dioxane between 0.006 and 14.3 ppm for an average length of exposure of almost 25 years found no significant alterations in hemoglobin concentration, erythrocyte counts, and total and differential leukocyte counts among the subjects (Thiess et al. 1976).

Female rats exposed intermittently to 3,200 ppm 1,4-dioxane for 13 weeks showed statistically significant elevations in red blood cell counts, hemoglobin, and hematocrit, whereas males showed only an increase in mean corpuscular volume (Kasai et al. 2008). The values were elevated <5% relative to controls and were within normal limits. No statistically significant changes were reported at ≤1,600 ppm 1,4-dioxane. In the 2-year inhalation study in rats by Torkelson et al. (1974), hematological parameters were measured in blood collected after 16 and 23 months of exposure. In this study, the rats were exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week. The specific hematological parameters measured were packed corpuscular volume, erythrocyte counts, hemoglobin concentration, and total and differential leukocyte counts. No toxicologically significant deviations from normal limits were found. Intermittent exposure to 1,250 ppm 1,4-dioxane for 2 years induced significant decreases in hemoglobin (13%), MCV (6%), and MCH (8%) (Kasai et al. 2009). No significant effects were seen in rats exposed to ≤250 ppm 1,4-dioxane.

Musculoskeletal Effects. Intermittent exposure of rats to up to 3,200 ppm 1,4-dioxane for 13 weeks did not result in gross or microscopic alterations in bone (sternum, femur, and joint) or skeletal muscle (thigh) (Kasai et al. 2008). No gross or microscopic alterations were reported in skeletal muscle or bone from male rats exposed intermittently to up to 1,250 ppm 1,4-dioxane for 2 years (Kasai et al. 2009). No further information was located.

Hepatic Effects. Short-term exposure of humans to concentrations that eventually caused death produced serious liver damage. Barber (1934) described five lethal cases in which postmortem examination of the patients revealed an enlarged liver and centrilobular necrosis of the liver cells. Similar lesions were observed in a lethal case described by Johnstone (1959). A group of four men were exposed to 50 ppm 1,4-dioxane for 6 hours and were given 12 "standard clinical chemistry tests" at 24 hours and 2 weeks after exposure (Young et al. 1977). Although the nature of clinical chemistry tests was not specified, there were no effects related to exposure. A cross sectional study of 74 workers exposed to concentrations of 1,4-dioxane between 0.006 and 14.3 ppm for an average length of exposure of almost 25 years found no conclusive evidence of serious liver damage (Thiess et al. 1976). Although 6 out of 24 current workers had elevated serum transaminase levels, all 6 were known as habitual alcohol drinkers.

Guinea pigs exposed to acute lethal concentrations of 1,4-dioxane had liver lesions ranging from cloudy swelling to areas of complete necrosis (Fairley et al. 1934). The effect of 1,4-dioxane on the levels of serum ALT, AST, ornithine carbamyl transferase (OCT), and glucose-6-phosphatase was studied in groups of male rats exposed to 0, 1,000, or 2,000 ppm 1,4-dioxane for 4 hours (Drew et al. 1978). The enzyme levels were used as indication of liver damage. Exposure to 1,4-dioxane markedly increased the activities (concentration-related) of AST, ALT, and OCT, particularly 48 hours after exposure. The activity of glucose-6-phosphatase was slightly increased 48 hours after exposure.

A study in which rats, mice, guinea pigs, and rabbits were exposed to 1,000 ppm (the lowest concentration tested) 3 hours/day, 5 days/week for 3–12 weeks reported hepatocyte degeneration of varying severity in all of the species tested (Fairley et al. 1934). Increased incidence of single cell necrosis and centrilobular swelling was described in the liver of male rats exposed intermittently to 3,200 ppm 1,4-dioxane for 13 weeks; no such effects were observed at ≤1,600 ppm (Kasai et al. 2008). Females exposed to 3,200 ppm only showed centrilobular swelling. In the 2-year inhalation bioassay in rats exposed intermittently to 111 ppm 1,4-dioxane, there was no evidence of any exposure-related gross or microscopic liver alterations or alterations in serum AST and AP activities (Torkelson et al. 1974). Another study reported significant increases in the incidences of centrilobular nuclear enlargement,

acidophilic and basophilic cell foci, spongiosis hepatis, and centrilobular necrosis in male rats following exposure to 1,250 ppm 1,4-dioxane for 2 years (Kasai et al. 2009). No such alterations were observed in the liver of rats exposed to ≤250 ppm 1,4-dioxane, which is consistent with the findings of Torkelson et al. (1974). Serum transaminases were also significantly elevated following exposure to 1,250 ppm 1,4-dioxane.

Renal Effects. Swollen kidneys with hemorrhage was seen in subjects who died following exposure to unknown amounts of 1,4-dioxane in the air described by Barber (1934). These subjects showed oliguria and/or anuria, and in one case there was bloody urine. Microscopic examination showed hemorrhage around the glomeruli with some necrosis. Barber (1934) stated that in at least three of the five cases he described, kidney disease was the direct cause of death. In a fatal case of a patient described by Johnstone (1959), postmortem examination revealed necrosis in the kidney cortex, with extensive interstitial hemorrhage and oliguria. A group of four men were exposed to 50 ppm 1,4-dioxane for 6 hours and were given urinalysis tests at 24 hours and 2 weeks after exposure (Young et al. 1977). There were no effects related to exposure. No evidence of kidney damage was found in a cross-sectional study of 74 workers exposed to concentrations of 1,4-dioxane between 0.006 and 14.3 ppm for an average length of exposure of almost 25 years (Thiess et al. 1976).

Kidney lesions were commonly observed in rodents exposed to acute lethal concentrations of 1,4-dioxane (Fairley et al. 1934). Examination of rats, mice, guinea pigs, and rabbits exposed to 1,000 ppm 1,4-dioxane (the lowest concentration tested) 3 hours/day, 5 days/week for 3–12 weeks, showed varying degrees of kidney damage ranging from vascular congestion to renal cortex degeneration (Fairley et al. 1934). In general, exposure to higher concentrations increased the severity of the effects. In a 13-week intermittent exposure study, female rats exposed to 3,200 ppm 1,4-dioxane showed a significant increased incidence of hydropic change in the proximal tubule; however, no renal alterations were seen in males exposed to that concentration or in females exposed to ≤1,600 ppm 1,4-dioxane (Kasai et al. 2008). In a 2-year inhalation study in rats exposed intermittently to 111 ppm 1,4-dioxane, there were no treatment-related gross or microscopic alterations in the kidneys or significant alterations in blood-urea nitrogen and total protein concentration (Torkelson et al. 1974). In other 2-year study, intermittent exposure of male rats to ≥250 ppm 1,4-dioxane significantly increased the incidence of nuclear enlargement in the proximal tubule and (Kasai et al. 2009); exposure to 1,250 ppm also increased the incidence of hydropic change in the proximal tubule.

Endocrine Effects. No gross or microscopic alterations were observed in the pituitary, adrenal, thyroid, or parathyroid glands from rats exposed intermittently to up to 3,200 ppm 1,4-dioxane for 13 weeks (Kasai et al. 2008), in the thyroid and pituitary glands from rats exposed intermittently to 111 ppm for 2 years (Torkelson et al. 1974), or in the thyroid, parathyroid, adrenal, and pituitary glands from male rats exposed intermittently to up to 1,250 ppm 1,4-dioxane for 2 years (Kasai et al. 2009).

Dermal Effects. No gross or microscopic alterations were reported in the skin from rats exposed intermittently to up to 3,200 ppm 1,4-dioxane for 13 weeks (Kasai et al. 2008). In the 2-year study in rats by Torkelson et al. (1974), the investigators indicated that intermittent exposure to a concentration of 111 ppm 1,4-dioxane in the air had no significant effect on skin condition; no microscopic examination of the skin was conducted. However, Kasai et al. (2009) conducted microscopic examinations of the skin of male rats exposed to up to 1,250 ppm 1,4-dioxane for years and did not report significant alterations. Had skin condition been affected in these studies, it would have been most likely due to direct contact with the chemical rather than due to inhaled 1,4-dioxane.

Ocular Effects. In a group of six individuals exposed to 2,000 ppm 1,4-dioxane vapors for 3 minutes in a 10-m^3 chamber, there were no complaints of ocular discomfort (Fairley et al. 1934). Exposure to 300 ppm 1,4-dioxane for 15 minutes produced eye irritation among a group of 12 volunteers (Silverman et al. 1946). A 10-minute exposure to 1,600 ppm 1,4-dioxane produced slight eye irritation and lacrimation that persisted throughout the test in a group of five individuals (Yant et al. 1930). In another experiment by the same investigators, exposure of the same five persons to 5,500 ppm 1,4-dioxane for 1 minute resulted in irritation of the eyes (Yant et al. 1930). Eye irritation throughout exposure was a frequent and the only complaint among four men exposed to 50 ppm for 6 hours in a study by Young et al. (1977). It is assumed that, in these cases, the irritation was caused by direct contact of the vapor with the eyes. In addition, there was no control experiment, and possible low humidity in the exposure chamber might contribute to the eye irritation. A study of six men and six women exposed to 20 ppm 1,4-dioxane for 2 hours reported no discomfort in the eyes (burning, irritation, or running eyes) among the subjects during or after exposure (Ernstgård et al. 2006). Also unaffected was the eye blinking frequency, monitored by electromyography.

In a study in guinea pigs exposed to 1,000–30,000 ppm 1,4-dioxane for 10–540 minutes, eye irritation was observed at 2,000 ppm in a 5-minutes exposure and 3,000 ppm for 8 minutes of exposure, but not at 1,000 ppm for 480 minutes (Yant et al. 1930). No exposure-related gross or microscopic alterations were reported in the eyes (retina, optic nerve, and eyelids) of rats exposed to up to 3,200 ppm 1,4-dioxane for

13 weeks (Kasai et al. 2008). No evidence of eye irritation was observed in rats exposed intermittently to 111 ppm 1,4-dioxane for 2 years, but no histological examination of the eyes was performed (Torkelson et al. 1974). Microscopic examination of the eyes of male rats exposed to up to 1,250 ppm 1,4-dioxane for 2 years did not reveal exposure related alterations (Kasai et al. 2009).

Body Weight Effects. Intermittent exposure of rats to 200–3,200 ppm 1,4-dioxane for 13 weeks resulted in <10% reduction in final body weight relative to controls, except in females exposed to 3,200 ppm, whose final body weight was reduced by 10.2% (Kasai et al. 2008). Food consumption data were not provided in that study. No significant effect on body weight gain was observed in rats exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974). Intermittent exposure of male rats to up to 1,250 ppm 1,4-dioxane for 2 years resulted in a terminal body weight 6.3% lower than control rats (Kasai et al. 2009); food consumption was not affected.

3.2.1.3 Immunological and Lymphoreticular Effects

Exposure of 12 volunteers to 20 ppm 1,4-dioxane for 2 hours did not result in inflammatory changes, as measured by the levels of high sensitivity C-reactive protein and interleukin 6 in blood collected before and 3 hours after exposure (Ernstgård et al. 2006). No further information was located regarding immunological and lymphoreticular effects in humans following inhalation exposure to 1,4-dioxane.

No gross or microscopic alterations were observed in the spleen, thymus, or lymph nodes of rats exposed intermittently to up to 3,200 ppm 1,4-dioxane for 13 weeks (Kasai et al. 2008), in the lymph nodes or the spleen from rats exposed to intermittently to 111 ppm 1,4-dioxane for 2 years (Torkelson et al. 1974), or in the spleen, thymus, and lymph nodes from male rats exposed to up to 1,250 ppm 1,4-dioxane for 2 years (Kasai et al. 2009). These values are presented as NOAELs for lymphoreticular effects in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

In a study of volunteers (six males and six females) exposed to 20 ppm 1,4-dioxane for 2 hours, self-reported ratings of headache, fatigue, nausea, and ‘feeling of intoxication’ during and after exposure were no different than before exposure (Ernstgård et al. 2006). Edema of the brain was observed in three of the five fatal cases described by Barber (1934). However, as suggested by NIOSH (1977), these changes were probably terminal, rather than specific toxic effects of 1,4-dioxane. Also, brain damage, possibly secondary to anoxia and cerebral edema, was observed in a worker who died following combined

inhalation and dermal exposure to a high amount of 1,4-dioxane (Johnstone 1959). Postmortem examination showed moderate perivascular widening of the brain and demyelination and partial loss of nerve fibers in small areas of the basal nuclei.

Exposure of rats to ≥3,000 ppm 1,4-dioxane 4 hours/day 5 days/week for 2 weeks resulted in depression of an avoidance response (Goldberg et al. 1964). The maximal effect was obtained after the second day of exposure. All of the effects on behavior were reversible. Intermittent exposure of rats to up to 3,200 ppm 1,4-dioxane for 13 weeks did not affect the gross or microscopic appearance of the brain, spinal cord, or peripheral nerves (Kasai et al. 2008). Exposure of rats to 111 ppm 1,4-dioxane for 2 years caused no significant gross or microscopic alterations in the brain (Torkelson et al. 1974). Similar exposure of male rats to up to 1,250 ppm 1,4-dioxane for 2 years also did not cause gross or microscopic alterations in the brain, spinal cord, or peripheral nerves (Kasai et al. 2009). Although no neurological testing was conducted in these studies, the rats were observed throughout the studies for signs of toxicity, including activity and demeanor; therefore, the values of 3,200 ppm, 111 ppm, and 1,250 ppm are presented as NOAELs for neurological effects in Table 3-1 and are plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

Elevated rates of spontaneous abortion and stillbirths were associated with occupational exposure to a combination of chemicals (1,4-dioxane among them) used in a silk screening process (NIOSH 1988). Increased incidences of miscarriages, premature births, and low birth weights were also reported in women occupationally exposed to a combination of chemicals that included 1,4-dioxane in the electronics industry in Russia, as noted by the Australia National Industrial Chemicals Notification and Assessment Scheme (NICNAS 1998). These effects cannot be attributed solely to 1,4-dioxane.

No alterations were observed in the primary and secondary reproductive organs from male and female rats exposed intermittently to up to 3,200 ppm 1,4-dioxane for 13 weeks (Kasai et al. 2008) or similarly to 111 ppm 1,4-dioxane for 2 years (Torkelson et al. 1974). Exposure of male rats to up to 1,250 ppm 1,4-dioxane for 2 years also did not induce gross or microscopic alterations in the reproductive organs (Kasai et al. 2009). These NOAELs are presented in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or in animals following inhalation exposure to 1,4-dioxane.

3.2.1.7 Cancer

Limited information exists regarding exposure to 1,4-dioxane and cancer in humans. Thiess et al. (1976) conducted a cross-sectional study of 74 workers exposed to concentrations of 1,4-dioxane between 0.006 and 14.3 ppm for an average length of exposure of almost 25 years. Twelve deaths had been reported and two were attributed to cancer, but the overall death rate and the cancer death rate were not significantly different than expected rates. Buffler et al. (1978) also found no evidence 1,4-dioxane-induced cancer in an occupational study of 165 workers exposed intermittently to mean concentrations of 1,4-dioxane between 0.1 and 17 ppm (the maximums ranged between 1.5 and 3.2 ppm) at least 1 month during a 21-year period. However, the study was limited in power to detect an effect due to the small size of the cohort, low levels of exposure, and the relatively short exposures.

No evidence of carcinogenicity due to 1,4-dioxane was found in a study in Wistar rats (288/sex) in which the animals were exposed to 111 ppm 1,4-dioxane 7 hours/day, 5 days/week for 2 years (Torkelson et al. 1974). A group of 192 rats of each sex served as controls, and the evaluation included all major tissues and organs, but there was no direct indication that the nasal cavity was examined. In the 2-year study conducted by Kasai et al. (2009), male F344/DuCrj rats were exposed intermittently to 0, 50, 250, or 1,250 ppm 1,4-dioxane. Significantly increased incidences of peritoneum mesothelioma occurred in the 250 and 1,250 ppm groups compared to controls (2/50, 4/50, 14/50, and 41/50 in control, low-, mid-, and high-exposure groups, respectively) ($p<0.01$ in both the mid- and high-exposure groups). Exposure to 1,250 ppm 1,4-dioxane also significantly increased the incidence of squamous cell carcinoma in the nasal cavity (0/50, 0/50, 1/50, 6/50) ($p<0.05$). High-exposure rats also showed a significant increase in the incidence of hepatocellular adenoma (1/50, 2/50, 3/50, 21/50) ($p<0.01$).

The EPA is in the process of deriving a cancer risk assessment for inhalation exposure to 1,4-dioxane.

3.2.2 Oral Exposure

3.2.2.1 Death

No reports of death in humans following oral exposure to 1,4-dioxane were found in the literature reviewed. Studies in animals have reported lethal doses in various species. Reported single dose LD_{50} values in rats include 5,346 mg/kg (Laug et al. 1939), 6,369 mg/kg (Pozzani et al. 1959), and 7,120 mg/kg (Smyth et al. 1941). Laug et al. (1939) also reported an LD_{50} of 5,852 mg/kg in mice and 4,033 mg/kg in

guinea pigs. Two of 10 female rats dosed with 2,750 mg 1,4-dioxane/kg/day for 2 weeks in the drinking water died before the end of the study (JBRC 1998). Smyth et al. (1941) calculated an LD_{50} of 3,150 mg/kg in guinea pigs, whereas de Navasquez (1935) reported 100% lethality in a group of five rabbits within 6 days of administration of a single dose of 2,068 mg of 1,4-dioxane/kg by gavage in water; a lower dose of 1,034 mg/kg was not lethal, but produced narcolepsy, and doses of 207 mg/kg repeated at weekly intervals did not appear to affect the animals. All 10 female mice dosed with 3,230 mg 1,4-dioxane/kg/day in the drinking water died, and 9 of 10 males dosed with 3,630 mg/kg/day also died (JBRC 1998). In a study using three dogs, consumption of approximately 327 mg 1,4-dioxane/kg/day via the drinking water killed one dog in 10 days, and consumption of approximately 375 mg/kg/day was lethal to an additional dog in 9 days (Schrenk and Yant 1936). Upon necropsy, common features in these animals were severe kidney and liver lesions consisting of cellular degeneration of the renal cortex, hemorrhages and vascular congestion in the kidneys, and cellular degeneration in the liver. Because the dogs were allowed to drink the 1,4-dioxane solution only twice daily and no other source of water was available, dehydration may have played a role in their death.

In an intermediate-duration study, five of six rats dosed through drinking water that provided approximately 1,000 mg 1,4-dioxane/kg/day died between the 14th and 35th day of the study (Fairley et al. 1934). Necropsy of these animals revealed kidney and liver lesions. In a 2-year cancer bioassay in Sherman rats, significant early mortality beginning at about 2–4 months in the study was observed in males and females treated with 1,015 and 1,599 mg 1,4-dioxane/kg/day, respectively, in the drinking water (Kociba et al. 1974). Although the specific cause of death was not discussed, the investigators indicated that rats dying early showed degenerative changes in the liver and kidneys. Early mortality also was reported in other long-term studies in rats given ≥240 mg 1,4-dioxane/kg/day in the drinking water for 104–110 weeks (NCI 1978), or ≥274 mg/kg/day for 2 years (Kano et al. 2009) and in mice treated similarly with ≥380 mg/kg/day for 90–104 weeks (NCI 1978) or ≥278 mg/kg/day for 2 years (Kano et al. 2009). In the Kano et al. (2009) study, early death in male rats was attributed to nasal cavity tumors and peritoneal mesothelioma; deaths in female rats were attributed to nasal and hepatic tumors. Death in mice was attributed to liver tumors.

The LOAEL values for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Fischer- 344)	2 wk ad lib (W)				2750 F (2/10 deaths)	JBRC 1998a	
2	Rat (NS)	12 d (W)				1034 (8/10 deaths within 12 days)	Kesten et al. 1939	
3	Rat (NS)	once (G)				5346 (LD50)	Laug et al. 1939	
4	Rat (Wistar)	once (G)				6369 F (LD50)	Pozzani et al. 1959	
5	Rat (Wistar)	once (GW)				7120 (LD50)	Smyth et al. 1941	
6	Mouse (B6C3F1)	2 wk ad lib (W)				3230 F (10/10 deaths)	JBRC 1998a	
7	Mouse (NS)	once (G)				5852 (LD50)	Laug et al. 1939	
8	Gn Pig (NS)	once (G)				4033 (LD50)	Laug et al. 1939	
9	Gn Pig (NS)	once (GW)				3150 (LD50)	Smyth et al. 1941	

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
10	Rabbit (NS)	once (GW)				2068 (5/5 deaths in 2-6 days)	De Navasquez 1935	
Systemic								
11	Rat (Fischer- 344)	2 wk ad lib (W)	Resp	370 M	1010 M (nuclear enlargement of olfactory epithelium)		JBRC 1998a	
			Hepatic	1040 F	2750 F (hepatocyte swelling and vacuolation)			
			Renal	1040 F	2750 F (hydropic change in proximal tubule)			
			Bd Wt	1040 F		2750 F (24% reduced body weight gain)		
12	Rat (NS)	12 d (W)	Hepatic		1034 (unspecified liver abnormalities)		Kesten et al. 1939	
			Renal			1034 (kidney degeneration)		
13	Rat (Sprague-Dawley)	once (GW)	Hepatic	1000 M			Stott et al. 1981	No histopathological alterations in the liver.
14	Mouse (B6C3F1)	2 wk ad lib (W)	Hepatic	1380 M	2550 M (swelling of central area)		JBRC 1998a	
			Bd Wt	1380 M	2550 M (swelling of central area)			

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Neurological								
15	Rat (Fischer- 344)	2 wk ad lib (W)		1040 F		2750 F (vacuolar changes in the brain)	JBRC 1998a	
16	Rabbit (NS)	once (GW)		207		1034 (narcolepsy, slow gate, ataxia)	De Navasquez 1935	
17	Rabbit (NS)	once (G)		1760		4400 (staggering)	Knoefel 1935	
Developmental								
18	Rat (Sprague-Dawley)	9 d Gd 6-15 (GW)		516[b]	1033 (decreased fetal weight; reduced sternum ossification)		Giavini et al. 1985	
INTERMEDIATE EXPOSURE								
Death								
19	Rat (NS)	34 d ad lib (W)				1000 (5/6 deaths before the 35th day)	Fairley et al. 1934	
20	Rat (Sherman)	2-4 mo ad lib (W)				1015 M (significant early mortality beginning at 2 months in the study)	Kociba et al. 1974	

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form	Comments
Systemic								
21	Rat (NS)	34 d ad lib (W)	Gastro		1428 (gastroenteritis)		Fairley et al. 1934	
			Hepatic			1428 (hepatocyte degeneration)		
			Renal			1428 (renal cortex degeneration)		

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
22	Rat (Fischer- 344)	13 wk ad lib (W)	Resp	52 M	126 M (nuclear enlargement of respiratory epithelium)		Kano et al. 2008	NOAELs are for histopathology of organs and tissues.
			Cardio	1614 F				
			Gastro	1614 F				
			Hemato	657 M	1554 M (increased red blood cell, hemoglobin, hematocrit, neutrophils)			
			Musc/skel	1614 F				
			Hepatic	52[c] M	126 M (swelling in central area)			
			Renal	274 M 83[d] F	657 M (nuclear enlargement of proximal tubule) 185[d] F (increased kidney weight)			
			Endocr	1614 F				
			Dermal	1614 F				
			Ocular	1614 F				
			Bd Wt	657 M	756 F (12% reduction in weight gain)	1614 F (21% reduction in body weight gain)		
23	Rat (Sprague-Dawley)	7 wk 5 d/wk (GW)	Hepatic	100 M	1000 M (fatty vacuoles in cytoplasm of hepatocytes)		Lundberg et al. 1987	

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
24	Rat (Sprague-Dawley)	11 wk ad lib (W)	Hepatic	10 M	1000 M (minimal hepatocellular swelling)		Stott et al. 1981	
			Bd Wt	1000 M				
25	Mouse (NS)	67 d ad lib (W)	Hepatic			2916 (hepatocyte degeneration)	Fairley et al. 1934	Only one dose level was tested.
			Renal			2916 (cell degeneration in renal cortex)		

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
26	Mouse (B6C3F1)	13 wk ad lib (W)	Resp	170 F	387 F (nuclear enlargement of bronchial epithelium)		Kano et al. 2008	NOAELs are for microscopic examination of organs and tissues.
			Cardio	2669 F				
			Gastro	2669 F				
			Hemato	882 M	1570 M (increase red blood cell, hemoglobin, hematocrit, corpuscular volume)			
			Musc/skel	2669 F				
			Hepatic	231 M	585 M (single cell necrosis and swelling of central area)			
			Renal	1620 F	2669 F (increased relative kidney weight)			
			Endocr	2669 F				
			Dermal	2669 F				
			Ocular	2669 F				
			Bd Wt	882[d] M		1570 M (29% reduced body weight gain)		
				2669 F				
Immuno/ Lymphoret								
27	Rat (Fischer- 344)	13 wk ad lib (W)		1614 F			Kano et al. 2008	No histological effects in lymph nodes, spleen, or thymus.

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
28	Mouse (B6C3F1)	13 wk ad lib (W)		2669 F			Kano et al. 2008	No histological effects in lymph nodes, spleen, or thymus.
Neurological								
29	Rat (Fischer- 344)	13 wk ad lib (W)		657 M		1554 M (vacuolar changes in the brain)	Kano et al. 2008	
30	Mouse (B6C3F1)	13 wk ad libi (W)		2669 F			Kano et al. 2008	No histological effects in brain, spinal cord, or sciatic nerve.
Reproductive								
31	Rat (Fischer- 344)	13 wk ad lib (W)		1554[d] M 1614 F			Kano et al. 2008	No histological effects in reproductive organs.
32	Mouse (B6C3F1)	13 wk ad lib (W)		1570[d] M 2669 F			Kano et al. 2008	No histological effects on reproductive organs.
CHRONIC EXPOSURE								
Death								
33	Rat F344/DuCrj	2 yr ad lib (W)				274 M (increased early mortality)	Kano et al. 2009	

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
34	Rat (Osborne-Mendel)	110 wk ad lib (W)				240 M (early mortality)	NCI 1978	
35	Mouse Crj:BDF1	2 yr ad lib (W)				278 F (increased early mortality)	Kano et al. 2009	
36	Mouse (B6C3F1)	90 wk ad lib (W)				380 F (early mortality)	NCI 1978	
Systemic								
37	Rat (Wistar)	452 d ad lib (W)	Renal			584 M (glomerulonephritis)	Argus et al. 1965	

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
38	Rat F344/DuCrj	2 yr ad lib (W)	Resp	18 F	83 F (nuclear enlargement of olfactory epithelium)		Kano et al. 2009	NOAELs are for no histopathological effects in organs and tissues.
			Cardio	429 F				
			Gastro	429 F				
			Musc/skel	429 F				
			Hepatic	11 M	55 M (mixed cell foci)			
			Renal	429 F				
			Endocr	429 F				
			Dermal	429 F				
			Ocular	429 F				
			Bd Wt	83 F		429 F (20% reduced body weight gain)		

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
39	Rat (Sherman)	716 d ad lib (W)	Resp	1599 F			Kociba et al. 1974	NOAELs are for no histopathological effects in organs and tissues.
			Cardio	1599 F				
			Gastro	1599 F				
			Hemato	1599 F				
			Hepatic	9.6[e] M		94 M (hepatocellular degeneration and necrosis)		
			Renal	9.6 M		94 M (degeneration and necrosis of tubular epithelium)		
			Endocr	1599 F				
			Bd Wt	94 M	1015 M (>10% reduced weight gain)			

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
40	Rat (Osborne-Mendel)	110 wk ad lib (W)	Resp		240 M (increased incidence of pneumonia)		NCI 1978	NOAELs are for no histopathological effects in organs and tissues.
			Cardio	640 F				
			Gastro		240 M (stomach ulcers)			
			Musc/skel	640 F				
			Hepatic	240 M	350 F (hepatocytomegaly)			
			Renal			240 M (cortical tubular degeneration)		
			Endocr	640 F				
			Dermal	640 F				
			Bd Wt	240 M	530 M (reduced body weight gain, unquantified)			

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
41	Mouse Crj:BDF1	2 yr ad lib (W)	Resp	49 M	191 M (nuclear enlargement of olfactory epithelium in nasal cavity)		Kano et al. 2009	NOAELs are for no histopathological effects in organs and tissues.
			Cardio	964 F				
			Gastro	964 F				
			Musc/skel	964 F				
			Hepatic	49 M	191 M (increased relative liver weight)			
			Renal	964 F				
			Endocr	964 F				
			Dermal	964 F				
			Ocular	964 F				
			Bd Wt	191 M 66 F[d]	278 F (16% reduced terminal body weight)	677 M (45% reduced terminal body weight)		

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
42	Mouse (B6C3F1)	90 wk ad lib (W)	Resp		380 F (increased incidence of pneumonia)		NCI 1978	NOAELs are for no histopathological effects in organs and tissues.
			Cardio	860 F				
			Gastro	860 F				
			Musc/skel	860 F				
			Hepatic	860 F				
			Renal	860 F				
			Endocr	860 F				
			Dermal	860 F				
			Bd Wt	830 M 380 F[d]	860 F (decreased body weight gain, unquantified)			
Immuno/ Lymphoret								
43	Rat F344/Du/DuCr	2 yr ad lib (W)		429 F			Kano et al. 2009	No histopathological effects in spleen, lymph nodes, or thymus.
44	Rat (Sherman)	716 d ad lib (W)		1599 F			Kociba et al. 1974	No histopathological effects in spleen or mesenteric lymph nodes.
45	Rat (Osborne-Mendel)	110 wk ad lib (W)		640 F			NCI 1978	No histopathological effects in spleen, lymph nodes, or thymus.

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
46	Mouse Crj:BDF1	2 yr ad lib (W)		964 F			Kano et al. 2009	No histopathological effects in spleen, lymph nodes, or thymus.
47	Mouse (B6C3F1)	90 wk ad lib (W)		860 F			NCI 1978	No histopathological effects in spleen, lymph nodes, or thymus.
Neurological								
48	Rat F344/DuCrj	2 yr ad lib (W)		429 F			Kano et al. 2009	No histopathological effects in brain, spinal cord, or sciatic nerve.
49	Rat (Sherman)	716 d ad lib (W)		1599 F			Kociba et al. 1974	No histopathological alterations in the brain or spinal cord.
50	Rat (Osborne-Mendel)	110 wk ad lib (W)		640 F			NCI 1978	No histopathological effects in the brain, spinal cord, or sciatic nerve.
51	Mouse Crj:BDF1	2 yr ad lib (W)		964 F			Kano et al. 2009	No histopathological effects in the brain, scpinal cord, or sciatic nerve

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
52	Mouse (B6C3F1)	90 wk ad lib (W)		860 F			NCI 1978	No histopathological alterations in the brain, spinal cord, or sciatic nerve.
Reproductive								
53	Rat F344/DuCrj	2 yr ad lib (W)		429 F			Kano et al. 2009	No histopathological effects in reproductive organs.
54	Rat (Sherman)	716 d ad lib (W)		1599 F			Kociba et al. 1974	No histopathological effects in reproductive organs.
55	Rat (Osborne-Mendel)	110 wk ad lib (W)		640 F			NCI 1978	No histopathological effects in reproductive organs.
56	Mouse Crj:BDF1	2 yr ad lib (W)		964 F			Kano et al. 2009	No histopathological effects in reproductive organs.
57	Mouse (B6C3F1)	90 wk ad lib (W)		860 F			NCI 1978	No histopathological effects in reproductive organs.

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Cancer								
58	Rat (Wistar)	452 d ad lib (W)				584 M (CEL: liver tumors)	Argus et al. 1965	
59	Rat F344/DuCrj	2 yr ad lib (W)				274 M (CEL: hepatocellular adenoma or carcinoma; mesothelioma of the peritoneum)	Kano et al. 2009	
60	Rat (Sherman)	716 d ad lib (W)				1015 M (CEL: hepatocellular carcinomas)	Kociba et al. 1974	
61	Rat (Osborne-Mendel)	110 wk ad lib (W)				350 F (CEL: hepatocellular carcinomas)	NCI 1978	
						240 (CEL: nasal carcinomas in both sexes)		
62	Mouse Crj:BDF1	2 yr ad lib (W)				66 F (CEL: hepatocellular adenomas or carcinomas)	Kano et al. 2009	

Table 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
63	Mouse (B6C3F1)	90 wk ad lib (W)				380 F (CEL: increased incidence of hepatocellular carcinomas and adenomas)	NCI 1978	
64	Gn Pig (NS)	23 mo ad lib (W)				1014 M (CEL: increased incidence of hepatomas)	Hoch-Ligeti and Argus 1970	

a The number corresponds to entries in Figure 3-2.

b Used to derive an acute-duration oral minimal risk level (MRL) of 5.0 mg/kg/day for 1,4-dioxane; the MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 animal to human extrapolation and 10 to protect sensitive populations).

c Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.5 mg/kg/day for 1,4-dioxane; the MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 animal to human extrapolation and 10 to protect sensitive populations).

d Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

e Used to derive a chronic-duration oral minimal risk level (MRL) of 0.1 mg/kg/day for 1,4-dioxane; the MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 animal to human extrapolation and 10 to protect sensitive populations).

ad lib = ad libitum; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; Gn pig = guinea pig; (GW) = gavage in water; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; (W) = drinking water; wk = week(s)

Figure 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral

Acute (≤14 days)

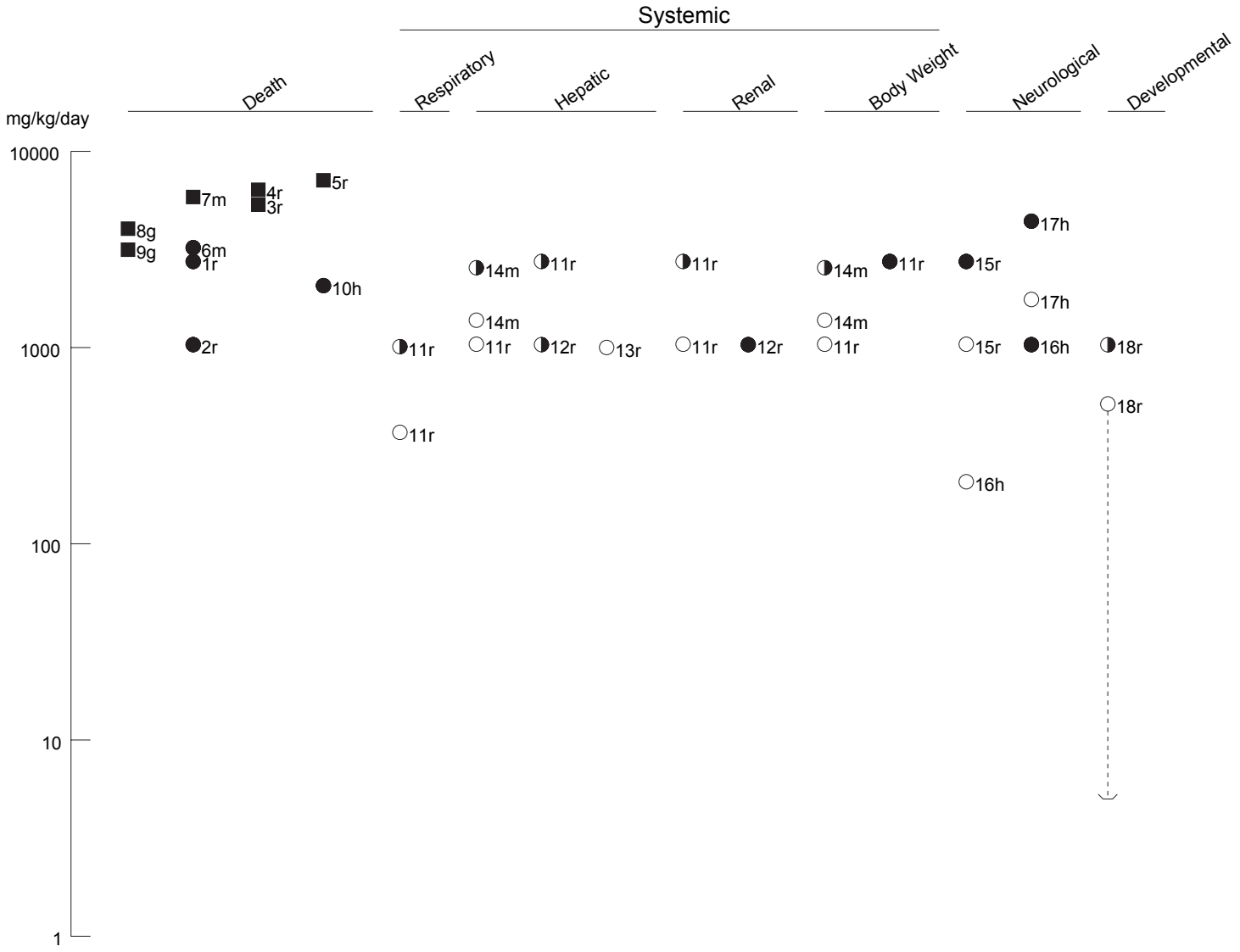


Figure 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral *(Continued)*

Intermediate (15-364 days)

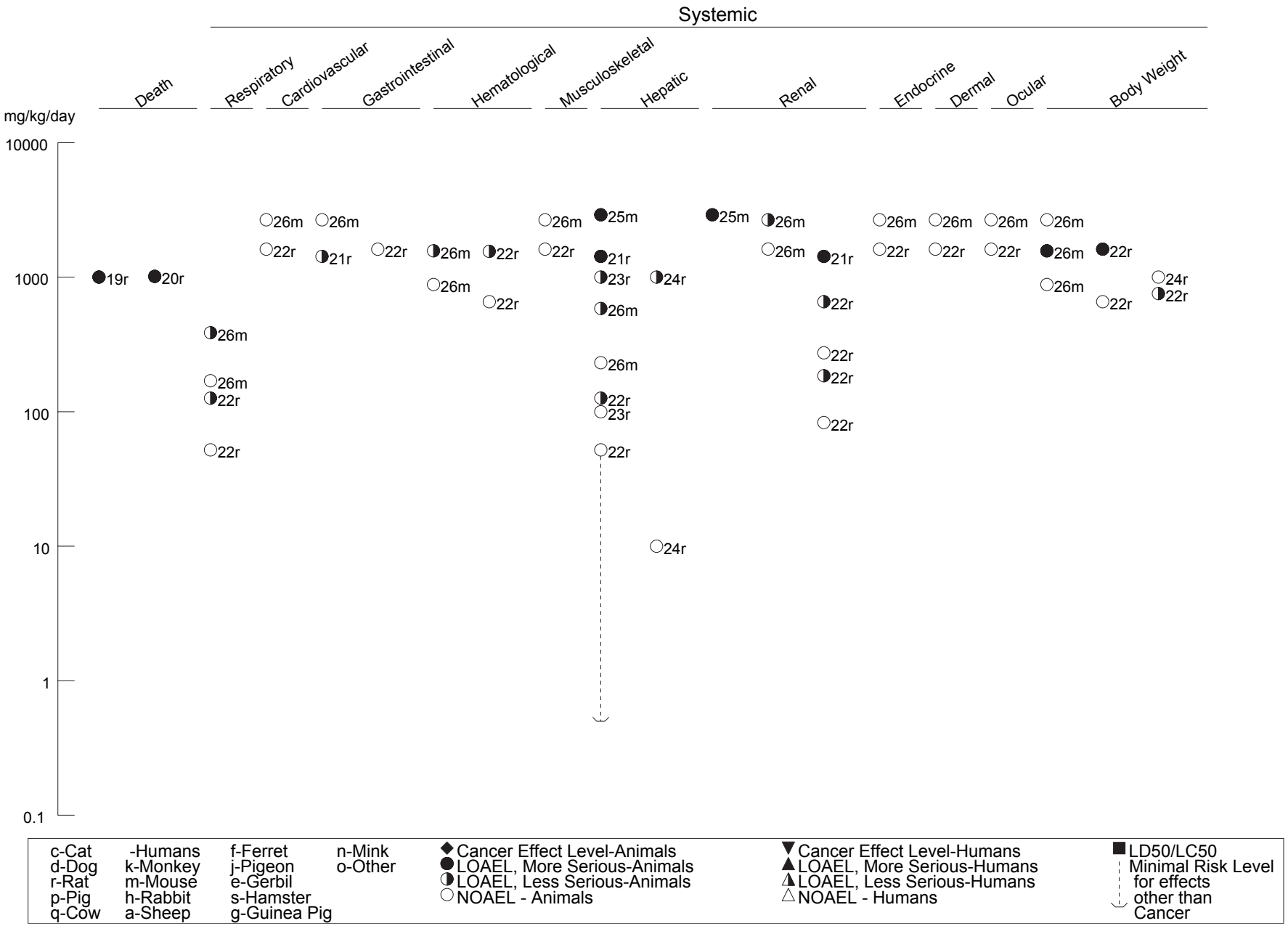
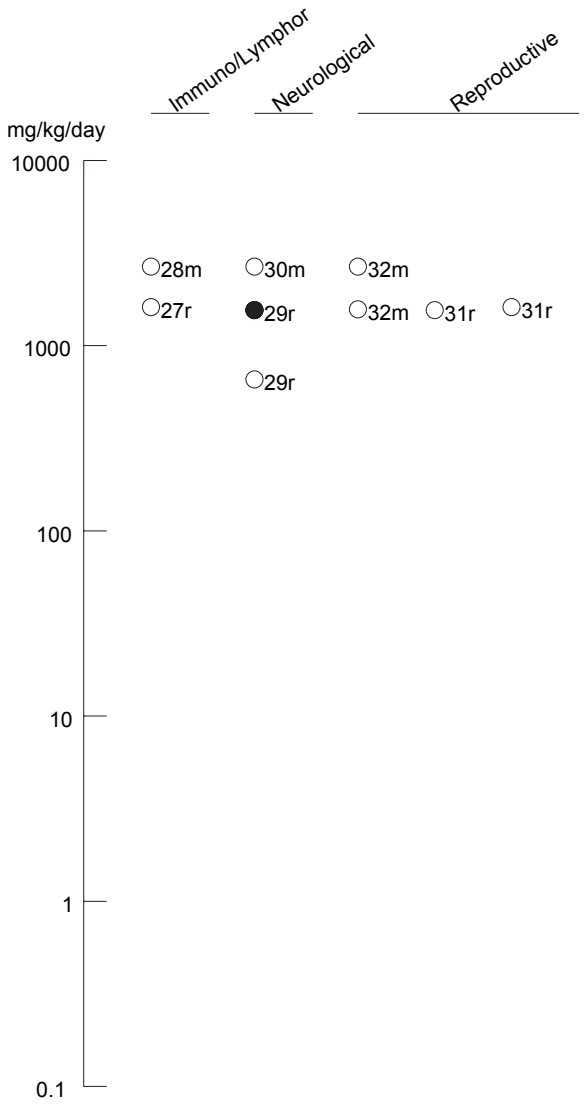


Figure 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral *(Continued)*

Intermediate (15-364 days)



c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◮ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral *(Continued)*

Chronic (≥365 days)

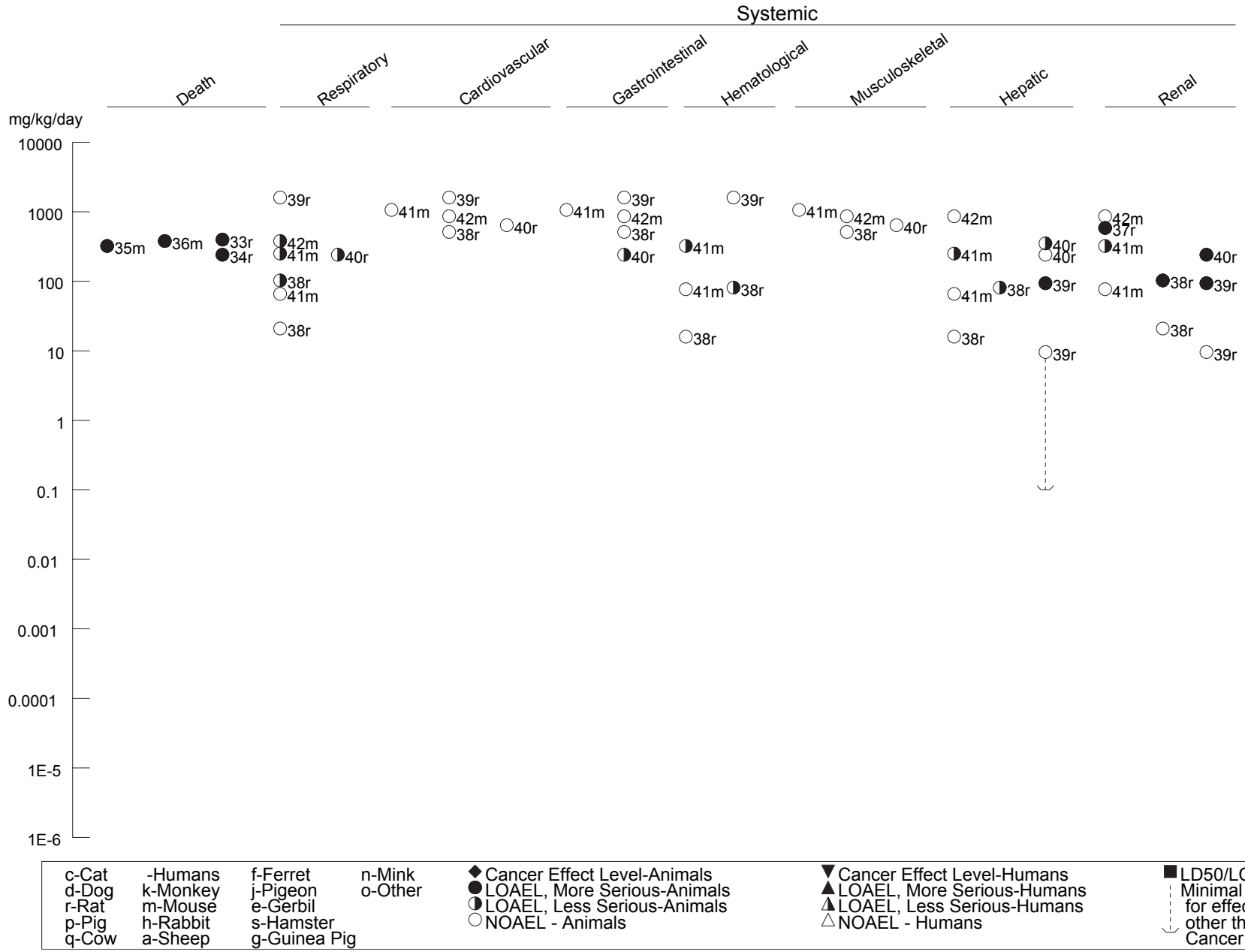
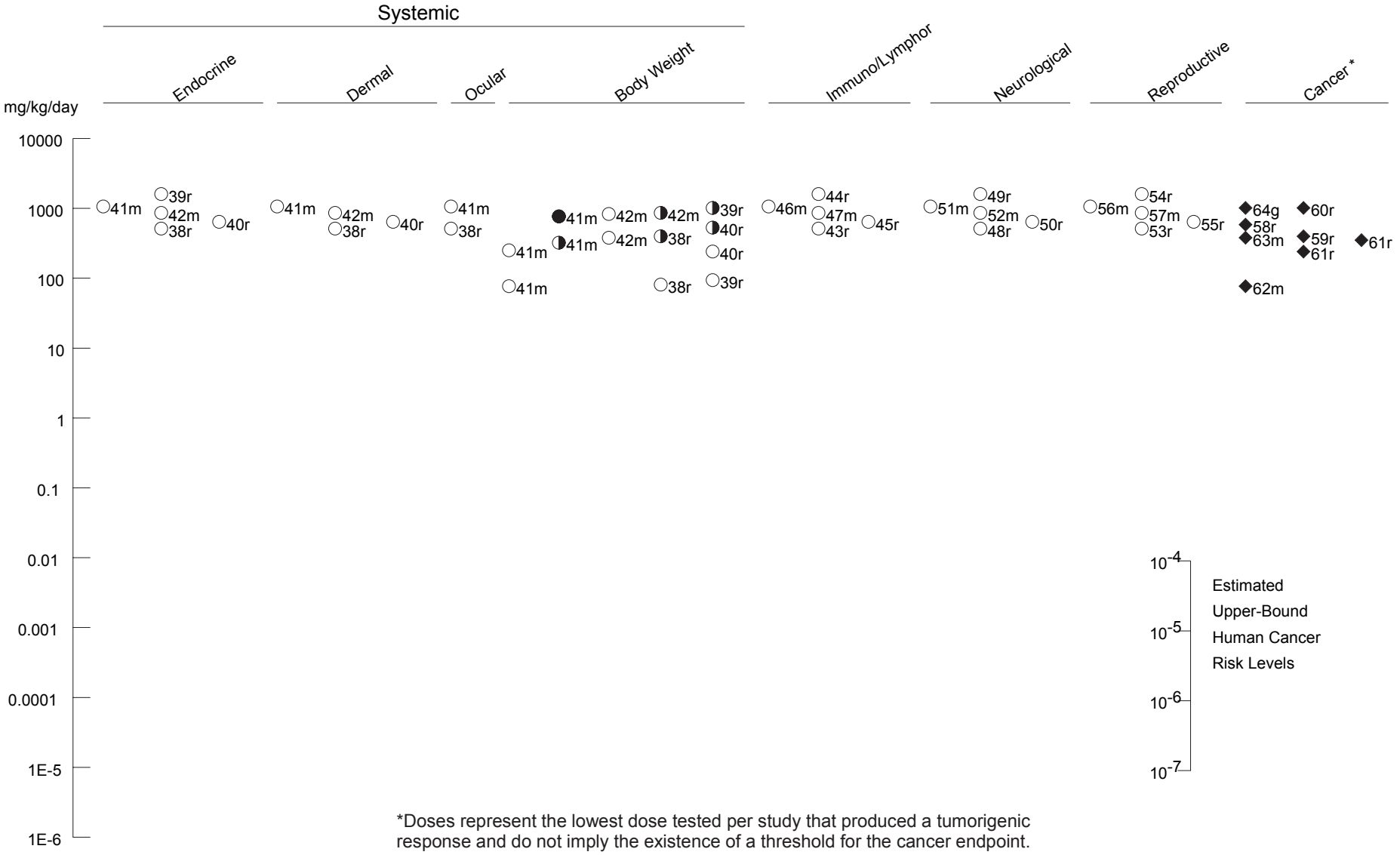


Figure 3-2 Levels of Significant Exposure to 1,4-Dioxane - Oral *(Continued)*

Chronic (≥365 days)



3.2.2.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, and body weight effects in humans after oral exposure to 1,4-dioxane.

The highest NOAEL and all reliable LOAEL values from each study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. Information exists regarding nasal and respiratory effects in animals after oral exposure to 1,4-dioxane. Nuclear enlargement of the olfactory epithelium was observed in male and female F344/DuCrj rats dosed with 1,010 and 1,040 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 2 weeks (JBRC 1998); the respective NOAELs were 370 and 400 mg/kg/day. The same type of lesions were observed in male and female rats treated with 126 and 185 mg 1,4-dioxane/kg/day, respectively, for 13 weeks; the respective NOAELs were 52 and 83 mg/kg/day (Kano et al. 2008). It should be noted, however, that a recent study suggested that the nasal lesions in rats exposed to 1,4-dioxane in drinking water may be caused by direct contact of the chemical with the nasal tissue as the rats drink the water (Sweeney et al. 2008). Male and female Crj:BDF_1 mice dosed with 585 and 387 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks showed nuclear enlargement of the bronchial epithelium; higher doses also involved the nasal cavity, trachea, and lungs (Kano et al. 2008); the respective NOAELs were 231 and 170 mg/kg/day. No histopathologic changes were observed in the lungs and nasal turbinates from Sherman rats dosed with up to 1,599 mg 1,4-dioxane/kg/day in the drinking water for 716 days (Kociba et al. 1974). No significant non-neoplastic lesions were seen in the lungs and trachea from Osborne-Mendel rats dosed with up to 640 mg 1,4-dioxane/kg/day via drinking water for 110 weeks (NCI 1978). However, an increased incidence of pneumonia was seen in treated males and females, although the incidence was not dose-related. The investigators speculated that the development of nasal carcinomas might have been a contributing factor (NCI 1978). Female F344 rats dosed with ≥83 mg 1,4-dioxane/kg/day, also in the drinking water, for 104 weeks showed increased incidence of nuclear enlargement of the olfactory epithelium in the nasal cavity; the NOAEL was 18 mg/kg/day (Kano et al. 2009). In males, nasal cavity lesions were observed at 274 mg/kg/day, and the NOAEL in males was 55 mg/kg/day.

In $B6C3F_1$ mice treated with 1,4-dioxane in the drinking water for 90 weeks, there was a dose-related increase in the incidence of pneumonia in males and females and of rhinitis in females (NCI 1978); males were dosed with 720 or 830 mg/kg/day and females were dosed with 380 or 860 mg/kg/day. Examination of the lungs and trachea did not reveal any other treatment-related non-neoplastic alterations. Male $Crj:BDF_1$ mice dosed with ≥191 mg 1,4-dioxane/kg/day in the drinking water for 2 years showed nuclear enlargement of olfactory epithelium in the nasal cavity; the NOAEL was 49 mg/kg/day (Kano et al. 2009). This lesion was significantly increased in females dosed with ≥278 mg/kg/day, but not 66 mg/kg/day. Doses of 677 mg/kg/day in males and 964 mg/kg/day in females significantly increased the incidence of nuclear enlargement of the respiratory epithelium in the nasal cavity.

Cardiovascular Effects. No gross or histological alterations were observed in the heart from rats and mice dosed with up to 1,614 and 2,669 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (Kano et al. 2008). Also, no gross or histological alterations were reported in the heart from rats dosed with up to 1,599 mg 1,4-dioxane/kg/day in the drinking water for 2 years (Kano et al. 2009; Kociba et al. 1974; NCI 1978) or in mice dosed with up to 860 mg/kg/day for 90 weeks (NCI 1978) or 964 mg/kg/day for 2 years (JBRC 1998b).

Gastrointestinal Effects. Hemorrhage of the stomach was reported in rats, mice, guinea pigs, and dogs administered acute lethal doses of 1,4-dioxane by gavage (Laug et al. 1939; Schrenk and Yant 1936; Smyth et al. 1941). Gastroenteritis was also reported in rats that died after drinking water that provided approximately 1,428 mg 1,4-dioxane/kg/day for up to 34 days (Fairley et al. 1934). No gross or histological alterations were observed in the gastrointestinal tract from rats and mice dosed with up to 1,614 and 2,669 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (Kano et al. 2008). In chronic-duration studies, no gastrointestinal alterations were reported in Sherman rats dosed with up to 1,599 mg 1,4-dioxane/kg/day (Kociba et al. 1974), in F344/DuCrj rats dosed with up to 429 mg/kg/day (Kano et al. 2009), in $B6C3F_1$ mice treated with up to 860 mg/kg/day (NCI 1978), or in $Crj:BDF_1$ mice dosed with up to 964 mg/kg/day (Kano et al. 2009). However, male Osborne-Mendel rats treated with ≥240 mg/kg/day for 110 weeks developed stomach ulcers; no such lesions were seen in control males or in female rats (NCI 1978).

Hematological Effects. Hematological changes consisting of increased red blood cell counts, hemoglobin, and hematocrit were reported in F344/DuCrj male rats dosed with 1,554 mg 1,4-dioxane /kg/day in the drinking water for 13 weeks; no significant changes occurred at 657 mg/kg/day (Kano et al. 2008). In females, there was a decrease in mean corpuscular volume and platelet concentration at

1,614 mg/kg/day. Sherman rats showed no significant deviations from normality in hematological parameters in a 2-year study (Kociba et al. 1974). In that study, the rats received doses of up to 1,599 mg 1,4-dioxane/kg/day in the drinking water; blood samples were collected during the 4th, 6th, 12th, and 18th month and at termination, and analyzed for packed cell volume, total erythrocyte counts, hemoglobin concentration, and total and differential white blood cell counts.

Musculoskeletal Effects. No gross or histological alterations were observed in bone and skeletal muscle (neither bone or muscle were specified) from rats and mice dosed with up to 1,614 and 2,669 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (Kano et al. 2008), or in rats and mice dosed with up to 429 and 964 mg 1,4-dioxane/kg/day, respectively, for 2 years (Kano et al. 2009). Similarly, no histopathologic alterations were observed in skeletal muscle from rats dosed with up to 640 mg 1,4-dioxane/kg/day for 110 weeks or in mice treated with up to 860 mg/kg/day for 90 weeks (NCI 1978).

Hepatic Effects. Acute oral doses of 1,4-dioxane that caused lethality in rats, mice, rabbits, guinea pigs, and dogs (see Section 3.2.2.1) induced varying degrees of liver damage, including liver congestion and degeneration (de Navasquez 1935; Kesten et al. 1939; Laug et al. 1939; Schrenk and Yant 1936; Smyth et al. 1941). In general, single doses that caused death were higher than 2,000 mg/kg. A single dose of 1,000 mg/kg administered to rats, and that did not cause death, produced no histopathologic alterations in the liver (Stott et al. 1981). Hepatocyte swelling and vacuolation of the central area were reported in the liver from F344/DuCrj rats dosed with 2,750–2,960 mg 1,4-dioxane/kg/day for 2 weeks in the drinking water (JBRC 1998), but no significant liver alterations were seen at 1,010–1,040 mg/kg/day. Crj:BDF_1 mice treated in the same manner with 2,550–3,230 mg 1,4-dioxane/kg/day showed single cell necrosis and swelling of the central area; no significant alterations were reported at 1,380–1,780 mg/kg/day (JBRC 1998).

Repeated administration of doses of approximately 1,428 mg 1,4-dioxane/kg/day in the drinking water for 34 days was lethal to rats, and examination of the animals showed liver congestion and hepatocyte degeneration (Fairley et al. 1934). The same types of liver lesions were seen in mice treated in the same manner with approximately 2,916 mg 1,4-dioxane/kg/day; in this experiment, the mice survived up to day 67, at which time they were sacrificed (Fairley et al. 1934). Repeated dosing of rats with 1,000 mg 1,4-dioxane/kg/day for 7 or 11 weeks produced hepatocyte swelling and histopathology (Stott et al. 1981) and fatty vacuoles in the hepatocytes (Lundberg et al. 1987). Male F344/DuCrj rats dosed with ≥126 mg 1,4-dioxane/kg/day for 13 weeks in the drinking water showed swelling of the central area (Kano et al.

2008); the NOAEL was 52 mg/kg/day. Higher doses also produced vacuolar changes and granulation, and changes in clinical chemistry parameters indicative of liver toxicity. In female rats, granulation was evident at 427 mg/kg/day and hepatocyte swelling at 756 mg/kg/day. The findings from the Kano et al. (2008) study in rats were used to derive an intermediate-duration oral MRL of 0.5 mg/kg/day for 1,4-dioxane based on a NOAEL of 52 mg/kg/day for male rats. In Crj:BDF_1 mice treated in the same manner, doses of 585–898 mg 1,4-dioxane/kg/day caused single cell necrosis and swelling in the central area, but doses ≤410 mg/kg/day were without significant effect (Kano et al. 2008). Changes in clinical chemistry parameters suggestive of liver damage were reported also in mice dosed with ≥585 mg 1,4-dioxane/kg/day (Kano et al. 2008).

In the 2-year bioassay by Kociba et al. (1974) in Sherman rats, significant early deaths occurred with doses between 1,015 and 1,599 mg/kg/day beginning at about 2–4 months in the study, and the authors indicated that these rats exhibited degenerative changes in the liver, although it was not made clear whether these changes along with renal lesions were the cause of death. Rats treated chronically with 1,4-dioxane in the drinking water (≥94 mg/kg/day for males, ≥148 mg/kg/day for females) had liver lesions consisting of various degrees of hepatocellular degeneration and necrosis and evidence of hepatic regeneration, as indicated by hepatocellular hyperplastic nodule formation (Kociba et al. 1974). No significant effects were seen in males at 9.6 mg/kg/day and in females at 19 mg/kg/day. The findings from Kociba et al. (1974) were used to derive a chronic-duration oral MRL of 0.1 mg/kg/day for 1,4-dioxane. An elevated incidence of hepatocytomegaly was observed in female rats treated with ≥350 mg 1,4-dioxane/kg/day (the lowest dose tested in females) and in males dosed with 530 mg/kg/day of the chemical in the drinking water for 2-years (NCI 1978); the NOAEL in males was 240 mg/kg/day. In another 2-year drinking water study in F344/DuCrj rats, males dosed with ≥55 mg/kg/day showed increased mixed cell foci; the NOAEL was 11 mg/kg/day (Kano et al. 2009). Females treated with 429 mg/kg/day showed increased mixed cell foci, but no such lesions were observed at 83 mg/kg/day.

Mice dosed with up to 860 mg 1,4-dioxane/kg/day via the drinking water for 90 weeks showed no treatment-related non-neoplastic liver lesions (NCI 1978). Although the investigators stated that hepatocytomegaly was commonly found in treated mice, the incidences were not significantly different than in controls, and no trend was apparent. Male Crj:BDF_1 mice dosed with ≥191 mg 1,4-dioxane for 2 years had significantly increased relative liver weight (Kano et al. 2009); no significant increase was reported at 49 mg/kg/day.

Renal Effects. Acute lethal doses of 1,4-dioxane in rodents caused kidney lesions ranging from kidney enlargement to extensive kidney degeneration (de Navasquez 1935; Kesten et al. 1939; Laug et al. 1939; Smyth et al. 1941). Severe kidney damage was seen also in an acute study in dogs (Schrenk and Yant 1936). Hydropic changes of the proximal renal tubule were reported in male and female F344/DuCrj rats dosed with 2,960 and 2,750 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 2 weeks (JBRC 1998); the corresponding NOAELs were 1,010 and 1,040 mg/kg/day. Treatment for 13 weeks resulted in nuclear enlargement of the proximal renal tubules at 657 and 756 mg 1,4-dioxane/kg/day in male and female rats, respectively (Kano et al. 2008); higher doses also induced hydropic changes in the proximal tubules. The NOAELs for morphological alterations in the kidneys were 274 and 427 mg/kg/day in males and females, respectively. Other significant findings in this study were a decrease in urinary pH in males at ≥274 mg/kg/day and an increase in absolute and relative kidney weight in females at ≥185 mg/kg/day. In another study, rats dosed for up to 34 days with 1,428 mg 1,4-dioxane/kg/day, a dose that caused deaths, had vascular congestion in the kidneys and cell degeneration in the cortical epithelium (Fairley et al. 1934). Similar lesions were observed in mice treated in the same fashion with approximately 2,916 mg/kg for up to 67 days (Fairley et al. 1934). Changes resembling glomerulonephritis were reported in male Wistar rats exposed to approximately 584 mg 1,4-dioxane/kg/day in the drinking water for about 452 days (Argus et al. 1965). Treatment of female Crj:BDF_1 mice with 1,620 mg 1,4-dioxane/kg/day in the drinking water for 13 weeks increased absolute kidney weight and decreased urinary pH; urinary pH was decreased in males at 882 mg/kg/day. Doses of 585 and 898 mg/kg/day in males and females, respectively, did not cause any significant renal effects (Kano et al. 2008).

Kociba et al. (1974) observed degenerative changes in the kidneys from Sherman rats that died after 2–4 months of drinking water that provided approximately 1,015 mg 1,4-dioxane to males and 1,599 mg/kg/day to females. At termination of this 2-year study, the kidneys of both males (≥94 mg/kg/day) and females (≥148 mg/kg/day) showed tubular epithelial degeneration and necrosis, and there was evidence of renal tubular regeneration as indicated by increased tubular epithelial regenerative activity. The NOAELs in males and females were 9.6 and 19 mg/kg/day, respectively, which were also the NOAELs for liver effects in the study (Kociba et al. 1974). In the NCI (1978) bioassay in Osborne-Mendel rats, kidney lesions consisting of vacuolar degeneration and/or focal tubular epithelial regeneration in the proximal cortical tubules and occasionally hyaline casts were seen with significantly higher incidence in treated males (≥240 mg/kg/day, dose-related) and in high-dose females (640 mg/kg/day). No significant histological alterations were reported in the kidneys from F344/DuCrj rats or Crj:BDF_1 mice dosed with up to 429 or 964 mg 1,4-dioxane/kg/day, respectively, in the drinking

water for 2 years (Kano et al. 2009). No treatment-related kidney lesions were observed in B6C3F$_1$ mice treated via the drinking water with up to 860 mg 1,4-dioxane/kg/day for 90 weeks (NCI 1978).

Endocrine Effects. No gross or histological alterations were observed in the thyroid, parathyroid, adrenal, pituitary, pancreas, and salivary glands from rats and mice dosed with up to 1,614 and 2,669 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (Kano et al. 2008), or in rats and mice dosed with up to 429 and 964 mg 1,4-dioxane/kg/day, respectively, for 2 years (Kano et al. 2009). No gross or microscopic alterations were seen in the pituitary, adrenal, thyroid, and parathyroid glands from rats dosed with up to 1,599 mg 1,4-dioxane/kg/day in the drinking water for 2 years (Kociba et al. 1974; NCI 1978) or in the same organs from mice dosed in the same manner with up to 860 mg 1,4-dioxane/kg/day (NCI 1978). No further information was located on effects of 1,4-dioxane on endocrine parameters.

Dermal Effects. No gross or histological alterations were observed in the skin from rats and mice dosed with up to 1,614 and 2,669 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (Kano et al. 2008), or in rats and mice dosed with up to 429 and 964 mg 1,4-dioxane/kg/day, respectively, for 2 years (Kano et al. 2009). Treatment of rats with up to 640 mg 1,4-dioxane/kg/day in the drinking water for 2 years or mice with up to 860 mg 1,4-dioxane/kg/day had no significant effect on the gross or microscopic appearance of the skin (NCI 1978).

Ocular Effects. No gross or histological alterations were observed in the eyes from rats and mice dosed with up to 1,614 and 2,669 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (Kano et al. 2008), or in rats and mice dosed with up to 429 and 964 mg 1,4-dioxane/kg/day, respectively, for 2 years (Kano et al. 2009). No other relevant information was located.

Body Weight Effects. Administration of a single dose of 10 mg 1,4-dioxane/kg by gavage in water to rats reduced body weight gain by approximately 32% relative to controls in a 7-day period (Stott et al. 1981). According to the investigators, this was likely due to a reduction in food consumption, consistent with the histological observation that hepatocytes were depleted of glycogen. However, treatment with 10 mg/kg/day by gavage in water for 11 weeks had no significant effect on weight gain, and doses of 1,000 mg/kg/day for 11 weeks decreased body weight only about 9% relative to controls (Stott et al. 1981). In 2-week drinking water studies, doses of approximately 2,750–2,960 mg 1,4-dioxane/kg/day reduced body weight gain in F344/DuCrj rats (JBRC 1998). In Crj:BDF$_1$ mice, a significant reduction in body weight gain occurred in males at 2,550 mg/kg/day, but not at 1,380 mg/kg/day (JBRC 1998).

Reduced body weight gain was also reported in female F344/DuCrj rats treated for 13 weeks with ≥756 mg 1,4-dioxane/kg/day and in male and female Crj:BDF_1 mice treated for the same duration with ≥1,570 mg/kg/day (Kano et al. 2008). In the JBRC (1998) and Kano et al. (2008) studies, reduction in weight gain was usually associated with reduced food consumption and/or reduced water consumption. Sherman rats treated with 1,015–1,599 mg 1,4-dioxane/kg/day for 2 years gained approximately 10% less weight throughout the study (estimated from graphic data in the paper) than controls or rats dosed with 94–148 mg/kg/day (Kociba et al. 1974). In the NCI (1978) bioassay, body weight of male rats in the high-dose group (530 mg/kg/day) and female mice (860 mg/kg/day) were lower than controls during the second year of the study. No data on food consumption were provided in these two chronic-duration studies. In another chronic study, treatment of male F344/DuCrj rats with up to 274 mg 1,4-dioxane/kg/day did not significantly affect body weight, but females dosed with 429 mg/kg/day had a terminal weight 20% lower than controls; the NOAEL in females was 83 mg/kg/day (Kano et al. 2009). Neither food nor water consumption was significantly altered in this case. Male Crj:BDF_1 mice dosed with 677 mg 1,4-dioxane/kg/day for 2 years had a terminal weight 45% lower than controls, and females dosed with 278 and 964 mg/kg/day had a final weight 16% and 45% lower than controls, respectively (Kano et al. 2009). The NOAELs for males and females were 191 and 66 mg/kg/day, respectively. In mice, the reductions in weight gain were accompanied by significant reductions in water and food consumption.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after oral exposure to 1,4-dioxane. No gross or histological alterations were observed in the lymph nodes, spleen, and thymus from rats and mice dosed with up to 1,614 and 2,669 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (Kano et al. 2008), or in rats and mice dosed with up to 429 and 964 mg 1,4-dioxane/kg/day, respectively, for 2 years (Kano et al. 2009). No histopathologic alterations were observed in the spleen, thymus, and lymph nodes from rats dosed via drinking water with up to 1,599 mg 1,4-dioxane/kg/day for 2 years or from mice dosed similarly with up to 860 mg/kg/day (Kociba et al. 1974; NCI 1978). These NOAEL values for lymphoreticular effects are listed in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to 1,4-dioxane. In an acute study in rabbits, a single gavage dose of 4,400 mg 1,4-dioxane/kg induced staggering in one of three rabbits, and 6,600 mg/kg produced narcosis in one of three rabbits and was lethal to two other

rabbits (Knoefel 1935). No further details were provided in this early study. Male and female F344/DuCrj rats dosed with 2,960 and 2,750 mg 1,4-dioxane/kg/day, respectively, for 2 weeks showed vacuolar changes in the brain (JBRC 1998); the respective NOAELs were 1,010 and 1,040 mg/kg/day. Similar effects were reported in male and female F344/DuCrj rats dosed with 1,554 and 1,614 mg 1,4-dioxane/kg/day, respectively, for 13 weeks in the drinking water (Kano et al. 2008); the respective NOAELs were 657 and 756 mg/kg/day. However, no significant alterations were seen in the spinal cord or sciatic nerve. In the same study, no histopathological alterations were observed in the brain, spinal cord, and sciatic nerve from Crj:BDF_1 mice dosed with up to 2,669 mg 1,4-dioxane/kg/day. No histopathologic alterations were observed in the brain, spinal cord, and sciatic nerve from rats dosed via the drinking water with up to 1,599 mg 1,4-dioxane/kg/day for 2 years or from mice dosed similarly with up to 964 mg/kg/day (Kano et al. 2009; Kociba et al. 1974; NCI 1978). The NOAEL and LOAEL values for neurological effects are listed in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to 1,4-dioxane.

Standard reproductive toxicity studies on 1,4-dioxane in animals were not located. Only ancillary information is available. No gross or histological alterations were observed in the reproductive organs (testes, prostate, seminal vesicles, epididymis, uterus, ovaries, vagina) from rats and mice dosed with up to 1,614 and 2,669 mg 1,4-dioxane/kg/day, respectively, in the drinking water for 13 weeks (Kano et al. 2008), or in rats and mice dosed with up to 429 and 964 mg 1,4-dioxane/kg/day, respectively, for 2 years (Kano et al. 2009). No evidence of gross or microscopic alterations was found in the reproductive organs from rats exposed through the drinking water to up to 1,599 mg 1,4-dioxane/kg/day for up to 2 years (Kociba et al. 1974; NCI 1978) or from mice exposed to up to 860 mg 1,4-dioxane/kg/day for up to 90 weeks (NCI 1978). These values are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to 1,4-dioxane. Only one relevant animal study was located. In that study, groups of pregnant Sprague-Dawley rats were administered 0, 258, 516, or 1,033 mg 1,4-dioxane by gavage on gestation days 6–15 and sacrifices were conducted on gestation day 21 (Giavini et al.1985). Dams in the high-dose group gained less weight than controls, and fetal weight in this group was reduced by 5.3% relative to controls. In addition, a slightly but significantly higher incidence of reduced sternum ossification was noticed in the high-dose group. No

other significant differences between treated and control groups were observed, including number of implantations and of live fetuses, post-implantation loss, and incidence of malformations. This study was used to derive an acute-duration oral MRL for 1,4-dioxane. The NOAEL and LOAEL values are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located regarding oral exposure of humans to 1,4-dioxane and cancer, but numerous studies have examined the carcinogenicity of 1,4-dioxane in animals exposed orally; in all of them the test material has been administered in the drinking water. In general, the studies in animals can be divided into a group in which numerous limitations are apparent, including small number of animals, low tumor incidences, lack of statistical analyses, and only one dose level tested, and another small group of standard bioassays. To the former category belong Argus et al. (1965, 1973), Hoch-Ligeti and Argus (1970), and Hoch-Ligeti et al. (1970), whereas the standard bioassays include JBRC (1998b), Kociba et al. (1974), and NCI (1978).

Argus et al. (1965) exposed a group of 26 male Wistar rats to 1,4-dioxane in the drinking water at a concentration of 1% for 452 days. Nine rats served as controls. The maximal dose per rat was 132 g, which divided by an average exposure time of 452 days yields a daily dose of 584 mg/kg/day, assuming a reference body weight of 0.5 kg for mature male Wistar rats. End points examined included gross necropsy and histopathologic examination of tissues, but the range of tissues examined was not specified. Six of the 26 rats treated with 584 mg 1,4-dioxane/kg/day developed liver tumors that ranged in appearance from small neoplastic nodules to multifocal hepatocellular carcinomas. One treated rat had a transitional cell carcinoma of the kidney and one rat that received a total dose of 116 g for 387 days (599 mg/kg/day) developed leukemia. One control rat developed a lymphosarcoma.

In a subsequent study by the same group of investigators, groups of male Sprague-Dawley rats (28–32/group) were treated with 1,4-dioxane in the drinking water for 13 months at levels of 0 (controls), 0.75, 1.0, 1.4, or 1.8% (Hoch-Ligeti et al. 1970). At termination, complete necropsy and histopathological examinations were conducted. Doses were estimated by ATSDR to be approximately 444, 607, 833, and 1,004 mg/kg/day assuming 13 months equals 390 days, a body weight of 0.6 kg for the rats, and the total dose provided in the study was 104–256 g. Six treated rats developed tumors of the nasal cavity. All of the tumors consisted of squamous cell carcinomas with marked keratinization. The incidences were as follows: one at 0.75%, one at 1%, two at 1.4%, and two at 1.8%. Hepatocellular carcinomas

were also observed in the rats that had nasal carcinomas in the 1.4 and 1.8% groups (Argus et al. 1973). In the latter study, in addition to incidences of hepatomas and hepatocellular carcinomas, the authors reported the incidences of "incipient" hepatomas. Two types of incipient hepatomas were observed, one consisting of large cells, apparently filled and distended with fat, and the other of finger-like strands of rather smaller hepatocytes with large hyperchromic nuclei and dense cytoplasm. According to Argus et al. (1973), these nodules appeared as histologically characteristic of fully developed hepatomas. The following tumor incidences were reported: 4 incipient tumors at 0.75%, 9 incipient tumors at 1%, 13 incipient tumors and 3 hepatomas at 1.4%, and 11 incipient tumors and 12 hepatomas at 1.8% 1,4-dioxane. No tumors were found in the lungs. The authors stated that the effective tumor dose (TD5), the 50% tumor dose (TD50), and the maximum effective dose (TD95) were 72, 149, and 260 g, respectively, evaluated from the probit plot of the dose-response (Argus et al. 1973).

In the Kociba et al. (1974) study, groups of Sherman rats (60/sex/dose level) were treated with 1,4-dioxane in the drinking water at levels of 0 (controls), 0.01, 0.1, or 1% for 716 days. This corresponded to doses of 0, 9.6, 94, and 1,015 mg/kg/day in males and 0, 19, 148, and 1,599 mg/kg/day in females based on body weight and water consumption data. Treatment with 1,4-dioxane significantly increased mortality in high-dose males and females beginning at about 2–4 months of treatment. These rats showed degenerative changes in both the liver and kidneys. Body weight gain was significantly reduced in high-dose animals from the beginning of the study. Carcinogenic effects were limited to the liver and nasal turbinates. The investigators combined the incidence of tumors in males and females and expressed them as the effective incidences in the number of rats that survived for 12 months. The incidence of all hepatic tumors was 2/106 (1.9%), 0/110 (0%), 1/106 (0.9%), and 12/66 (18.2%, p=0.0022) in controls, low-, mid-, and high-dose rats, respectively. The corresponding incidences of hepatocellular carcinomas were 1/106 (0.9%), 0/110 (0%), 1/106 (0.9%), and 10/66 (15.2%, p=0.00033). Only three high-dose rats (one male and two females) had nasal carcinomas (p=0.05491) that were considered treatment-related by the investigators.

In the NCI (1978) bioassay, groups of Osborne-Mendel rats (35/sex/dose level) were administered 1,4-dioxane in the drinking water for 110 weeks. The estimated doses were 0 (controls), 240, and 530 mg/kg/day in males and 0, 350, and 640 mg/kg/day in females. Neoplasms associated with the administration of 1,4-dioxane occurred in the nasal cavity from males and females, liver from females, and testis/epididymis in males. The incidences of squamous cell carcinomas in the nasal turbinates were 0/33, 12/33 (36%), and 16/34 (47%) in control, low-, and high-dose males, respectively; the corresponding incidences in females were 0/34, 10/35 (29%), and 8/35 (23%). The first tumors were seen

at week 52 in males and week 66 in females. Statistical analyses of these incidences revealed a significant dose-related trend and significant differences between treated groups and controls. The incidences of hepatocellular carcinomas in females were 0/31, 10/33 (30%), and 11/32 (34%) in controls, low-, and high-dose groups, respectively. A higher incidence of mesotheliomas of the vaginal tunics of the testis/epididymis was seen in treated males than in controls (2/33, 4/33, and 5/34 in controls, low-, and high-dose, respectively). The incidences of other neoplasms were not related to treatment with the test material by type, site, test group, or sex. Under the conditions of the study, NCI (1978) concluded that 1,4-dioxane induced hepatocellular carcinomas in female rats and squamous cell carcinoma of the nasal turbinates in male and female rats.

In the Kano et al. (2009) study, groups of F344/DuCrj rats (50/sex/dose level) received 1,4-dioxane in the drinking water at levels of 200, 1,000, and 5,000 ppm for 2 years (0, 11, 55, and 274 mg/kg/day for males; 0, 18, 83, and 964 mg/kg/day for females). Survival was significantly decreased in the high-dose groups due to nasal and peritoneal mesothelioma in males and nasal and hepatic tumors in females. Twenty-two of 50 high-dose male rats survived compared to 40/50 in controls; 24/50 of high-dose females survived compared to 38/50 in controls. In high-dose males (278 mg/kg/day), the incidence of combined nasal cavity tumors was 7/50 ($p<0.01$) compared to none in the other groups; in high-dose females (429 mg/kg/day), the combined incidence was 8/50 ($p<0.01$) compared to none in the other groups. The nasal tumors included squamous cell carcinomas and esthesioneuroepithelioma, and the incidence of squamous cell carcinoma was significant by itself (7/50 $p<0.05$). The incidence of combined hepatocellular adenoma or carcinoma in males was 3/50, 4/50, 7/50, and 39/50 ($p<0.01$) in the control, low-, mid-, and high-dose male rats, respectively; the corresponding incidences in females were 3/50, 1/50, 6/50, and 48/50 ($p<0.01$). High-dose males also had an increased incidence of mesothelioma of the peritoneum (28/50 compared to 2/50 in controls, $p<0.01$). High-dose females had an increased incidence of mammary gland adenomas (16/50 compared to 6/50 in controls, $p<0.05$).

Two studies have been conducted in mice. Groups of $B6C3F_1$ mice (50/sex/dose level) were administered 1,4-dioxane in the drinking water for 90 weeks (NCI 1978). Based on body weight and water consumption data, the investigators estimated that the water provided doses of 0 (controls), 720, and 830 mg/kg/day in males, and 0, 380, and 860 mg/kg/day in females. Mortality was significantly increased (dose-related) in female mice. In female mice, 28/50, (56%) in the high-dose group, 39/50 (78%) in the mid-dose group, and 45/50 (90%) in the control group were still alive on week 91 of the study. In males, at least 90% of the mice in each group were still alive at week 91. Treatment with 1,4-dioxane significantly increased the incidence of liver tumors. The incidences of hepatocellular

carcinoma were 2/49 (4%), 18/50 (36%), and 24/47 (51%), in controls, low-, and high-dose males, respectively; the corresponding incidences in females were 0/50, 12/48 (25%), and 29/37 (78%). The incidences of hepatocellular carcinomas or adenomas in males were 8/49 (16%), 19/50 (38%), and 28/47 (60%); the incidences in females were 0/50, 21/48 (44%), and 35/37 (95%) for the respective control, low-, and high-dose groups. Statistical analysis showed significance for dose-related trend and for direct comparisons with controls. No other neoplasm, benign or malignant, was found to be associated to treatment with 1,4-dioxane.

In another study (Kano et al. (2009), groups of Crj:BDF_1 mice (50/sex/dose level) received 1,4-dioxane in the drinking water at levels of 500, 2,000, and 8,000 ppm for 2 years (0, 49, 191, 677 mg/kg/day for males; 0, 66, 278, and 964 mg/kg/day for females). Early mortality occurred in female mice, and this was attributed to liver tumors. Survival rates at 104 weeks in females were 29/50, 29/50, 17/50, and 5/50 in control, low-, mid-, and high-dose groups, respectively. A significant and dose-related increase in the incidence of liver adenomas or carcinomas was found in female mice. The incidences of combined adenomas or carcinomas in control, low-, mid-, and high-dose females were 5/50, 35/50, 41/50, and 46/50 ($p<0.01$ for all treated groups). High-dose males (677 mg/kg/day) also showed a significant increased incidence of hepatocellular carcinomas; the combined incidences of adenomas or carcinomas, as the dose increased, were 23/50 (controls), 31/50, 37/50 ($p<0.05$), and 40/50 ($p<0.01$). There were no nasal cavity tumors in male or female mice.

In the single study in guinea pigs, a group of 22 male guinea pigs was administered 1,4-dioxane in the drinking water at concentrations of 0.5–2% for 23 months (Hoch-Ligeti and Argus 1970). Ten guinea pigs served as controls. The investigators stated that the total intake of 1,4-dioxane during the 23 months of the experiment was 588–625 g. Assuming a reference body weight of 0.84 kg and converting 23 months into 690 days (30 days/month), the intake of 1,4-dioxane was approximately 1,014–1,075 mg/kg/day. All of the animals were sacrificed within 28 months. Very little additional data were presented in this brief note. Examination of the lungs revealed peri- or intrabronchial epithelial hyperplasia and nodular mononuclear infiltration in nine of the treated guinea pigs. In addition, two guinea pigs had carcinoma of the gall bladder, three had early hepatomas, and one had adenoma of the kidney. No tumors were found in the controls.

1,4-Dioxane was tested also as a cancer initiator in mice (Bull et al. 1986) and promoter in rats (Lundberg et al. 1987). Female Sencar mice received doses of 1,000 mg 1,4-dioxane/kg by gavage before receiving topical applications of 1 μg of 12-O-tetradecanoylphorbol-13-acetate (TPA) 3 times/week for 20 weeks.

A control group was initiated with acetone before the TPA application. Administration of 1,4-dioxane did not increase the formation of papillomas compared to mice initiated with the solvent (unspecified whether emulphor, saline, or water) and promoted with TPA, indicating a lack of initiating activity under the conditions of the study. The tumor promotion activity of 1,4-dioxane was also studied in groups of male Sprague-Dawley rats (8–11/group) (Lundberg et al. 1987). All rats underwent a 2/3 hepatectomy before receiving a single intraperitoneal injection of 30 mg/kg of diethylnitrosamine (DENA). Five days later, treatment by gavage with 100 or 1,000 mg/kg of 1,4-dioxane in saline started once daily, 5 days/week for 7 weeks. One week after the last treatment, the rats were killed, the liver was removed and stained for gamma-glutamyl-transpeptidase (GGT), and the number and total volume of GGT-positive foci was studied. 1,4-Dioxane alone had no significant effect on the end points evaluated. In DENA initiated rats, the high-dose of 1,4-dioxane induced a significant increase in the number of foci and total volume of foci relative to rats treated with DENA alone. The livers of high-dose rats showed enlarged foamy hepatocytes mainly in midzonal regions. The foamy appearance was due to vacuoles shown to contain fat. Thus, 1,4-dioxane promoted the carcinogenic potential of DENA.

The data available indicate that 1,4-dioxane produced liver and nasal cancer in rats and liver tumors in mice. The EPA has derived an oral cancer potency factor of 0.1 $(mg/kg/day)^{-1}$ for 1,4-dioxane using the Log-Logistic Model (IRIS 2011). This factor was calculated from oral exposure data reported by Kano et al. (2009) regarding incidence of hepatocellular adenoma or carcinoma in female BDF_1 mice exposed to 1,4-dioxane in the drinking water for 2 years. The lifetime average doses that would result in risk of $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, and $1x10^{-7}$ are $1x10^{-3}$, $1x10^{-4}$, $1x10^{-5}$, and $1x10^{-6}$ mg/kg/day, respectively, as indicated in Figure 3-2.

3.2.3 Dermal Exposure

3.2.3.1 Death

As mentioned in Section 3.2.1.1, Johnstone (1959) described a fatal case of a worker exposed to 1,4-dioxane for only 1 week and whose post-mortem examination showed kidney and liver alterations. The room in which the patient had worked had no exhaust ventilation, and the worker was not provided a respirator. Dermal exposure in this case may have been considerable, since the worker used liquid 1,4-dioxane to keep his hands free of glue. A dermal LD_{50} of 7,600 mL/kg was reported for rabbits (RTECS 2004); this value is presented in Table 3-3.

Table 3-3 Levels of Significant Exposure to 1,4-Dioxane - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL				Reference Chemical Form	Comments
				Less Serious		Serious			
ACUTE EXPOSURE									
Death									
Rabbit (NS)	once					7600 ml/kg	(LD50)	RTECS 2004	
Systemic									
Human	3 min	Ocular	2000 ppm					Fairley et al. 1934	
Human	15 min	Ocular	200 B ppm	300 B ppm	(eye irritation)			Silverman et al. 1946	
Human	10 min	Ocular		1600 ppm	(slight eye irritation)			Yant et al. 1930	
Human	6 hr	Ocular		50 M ppm	(eye irritation)			Young et al. 1977	
Rat (Wistar)	once	Dermal	8300 M mg/kg					Clark et al. 1984	No signs of skin irritation.

Table 3-3 Levels of Significant Exposure to 1,4-Dioxane - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE							
Systemic							
Gn Pig (NS)	49-101 d 5 d/wk 2 x/d	Hepatic			143 mg/kg (cloudy swelling and patchy cell degeneration)	Fairley et al. 1934	No signs of skin irritation.
		Renal			143 mg/kg (degeneration and necrosis of cortical tubules)		
		Dermal	143 mg/kg				
Rabbit (NS)	49-101 d 5 d/wk 2 x/d	Hepatic			57 mg/kg (patchy cell degeneration)	Fairley et al. 1934	No signs of skin irritation.
		Renal			57 mg/kg (tubular cell degeneration)		
		Dermal	57 mg/kg				

B = both male and female; d = day(s); Gn pig = guinea pig; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); NOAEL = no-observed-adverse-effect level; NS = not specified; ppm = parts per million; wk = week(s); x = time(s)

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, endocrine, or body weight effects in humans or in animals after dermal exposure to 1,4-dioxane. No studies were located regarding hepatic and renal effects in humans following dermal exposure to 1,4-dioxane.

The highest NOAEL and all reliable LOAEL values from each study for systemic effects in each species and duration category are recorded in Table 3-3.

Hepatic Effects. In a study with four guinea pigs, approximately 143 mg 1,4-dioxane/kg was applied to a clipped area of the nape 5 days/week for 49–101 days (Fairley et al. 1934). Upon sacrifice on days 49, 66, 77, and 101, no gross alterations of the liver were observed, but there were indications of patchy cell degeneration. The same protocol conducted in four rabbits applied doses of approximately 57 mg/kg showed vascular congestion of the liver and patchy cell degeneration in two of the rabbits (Fairley et al. 1934).

Renal Effects. Application of approximately 143 mg 1,4-dioxane/kg to a clipped area of the nape of guinea pigs 5 days/week for 49–101 days resulted in renal cortical cell degeneration and hemorrhages. The same experiment conducted in rabbits applied approximately 57 mg 1,4-dioxane/kg resulted in the same type of kidney lesions (Fairley et al. 1934).

Dermal Effects. Application of a single dose of up to 8,300 mg 1,4-dioxane/kg to an uncovered area of the skin of rats produced no signs of skin irritation within the period of observation of 14 days (Clark et al. 1984). Application of approximately 143 mg 1,4-dioxane/kg 5 days/week for 40–101 days to a clipped area of the nape from guinea pigs did not produce skin irritation (Fairley et al. 1934). Similar results were obtained in rabbits applied approximately 57 mg 1,4-dioxane/kg using the same protocol (Fairley et al. 1934).

Ocular Effects. The ocular effects observed in humans and in animals described in Section 3.2.1.2 and listed in Table 3-1 are assumed to have occurred by direct contact of vapors of 1,4-dioxane with eyes and are also listed in Table 3-3.

No studies were located regarding the following health effects in humans or animals after dermal exposure to 1,4-dioxane:

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

The carcinogenicity and initiator and promoter properties of 1,4-dioxane have been evaluated. To test whether 1,4-dioxane is a complete carcinogen, 0.2 mL of a solution of 1,4-dioxane in acetone (unspecified concentration) were applied 3 times/week to the shaved back from Swiss-Webster mice for 60 weeks (King et al. 1973). Examination of the skin at week 60 revealed only one skin sarcoma and one lymphoma, suggesting that under the conditions of the study, 1,4-dioxane was not a complete carcinogen. King et al. (1973) also tested whether 1,4-dioxane is a promoter by applying 50 µg of dimethyl-benzanthracene (DMBA) to groups of Swiss-Webster followed 1 week later by the application of 0.2 mL of a solution of 1,4-dioxane (unspecified concentration) to the shaved back for 60 weeks. At week 60, only 4 males and 5 females were still alive (out of 30/sex). Treatment with 1,4-dioxane in mice initiated with DMBA resulted in an increased number of tumors in the skin, lungs, and kidneys. The activity of 1,4-dioxane in promoting skin tumors was similar to that observed with croton oil as a promoter. However, croton oil led to a much higher multiplicity of skin tumors per mouse than 1,4-dioxane. Bull et al. (1986) tested 1,4-dioxane as an initiator. In that study, female Sencar mice were applied topical doses of 1,000 mg 1,4-dioxane/kg before receiving topical applications of 1 µg 12-O-tetradecanoylphorbol-13-acetate (TPA) 3 times/week for 20 weeks. A control group received an application of acetone before the TPA application. 1,4-Dioxane did not increase the formation of papillomas compared to mice initiated with acetone and promoted with TPA.

3.3 GENOTOXICITY

Studies of the *in vitro* and *in vivo* genotoxicity of 1,4-dioxane are summarized in Tables 3-4 and 3-5, respectively. 1,4-Dioxane was not genotoxic in standard *in vitro* tests of gene mutation in bacteria in the presence or absence of metabolic activation (Haworth et al. 1983; Khudoley et al. 1987; Morita and Hayashi 1998; Nestmann et al. 1984; Stott et al. 1981). Kwan et al. (1990) tested 1,4-dioxane in a strain of *Photobacterium phosphoreum*, which is sensitive to chemicals that are DNA-damaging agents,

Table 3-4. Genotoxicity of 1,4-Dioxane *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Salmonella typhimurium (TA100, TA98, TA1535, TA1537)	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	–	–	Stott et al. 1981
S. typhimurium (TA100, TA1535)	Gene mutation	–	–	Nestmann et al. 1984
S. typhimurium (TA98, TA100, TA1530, TA1535, TA1537)	Gene mutation	–	–	Khudoley et al. 1987
Photobacterium phosphoreum	DNA damage	NT	–	Kwan et al. 1990
Escherichia coli K-12 *uvrB*/*recA*	DNA damage	–	–	Hellmer and Bolcsfoldi 1992
S. typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Morita and Hayashi 1998
E. coli (WP2, WP2 *uvrA*)	Gene mutation	–	–	Morita and Hayashi 1998
Saccharomyces cerevisiae (D61M)	Chromosomal malsegregation	NT	–	Zimmermann et al. 1985
Mouse lymphoma cells	Gene mutation	–	–	Morita and Hayashi 1998
CHO cells	Chromosomal aberrations	–	–	McElroy et al. 2003
CHO-K1 cells	Chromosomal aberrations	–	–	Morita and Hayashi 1998
CHO-K1 cells	Sister chromatid exchange	–	–	Morita and Hayashi 1998
CHO-K1 cells	Micronuclei	–	–	Morita and Hayashi 1998
Rat hepatocytes	DNA repair	–	–	Goldsworthy et al. 1991
CHO-W-B1 cells	Chromosomal aberrations	–	–	Galloway et al. 1987
CHO-W-B1 cells	Sister chromatid exchange	–	±	Galloway et al. 1987
Mouse lymphoma cells	Gene mutation	–	–	McGregor et al. 1991
BALB/3T3 cells	Cell transformation	NT	+	Sheu et al. 1988

– = negative result; + = positive result; ± = weak positive result; CHO = Chinese hamster ovary; NT = not tested

Table 3-5. Genotoxicity of 1,4-Dioxane *In Vivo*

Species (test system)	End point	Results	Reference
Human peripheral lymphocytes	Chromosomal aberrations	–	Thiess et al. 1976
Rat hepatocytes	DNA repair	–	Goldsworthy et al. 1991
Rat nasal epithelial cells	DNA repair	–	Goldsworthy et al. 1991
Mouse hepatocytes	Micronuclei	+	Morita and Hayashi 1998
Mouse hepatocytes	Micronuclei	+	Roy et al. 2005
Mouse peripheral blood	Micronuclei	–	Morita and Hayashi 1998
Rat hepatocytes	DNA alkylation or repair	–	Stott et al. 1981
Rat hepatocytes	DNA damage	+	Kitchin and Brown 1990, 1994
Mouse bone marrow	Micronuclei	–	Tinwell and Ashby 1994
Mouse bone marrow	Micronuclei	+	Roy et al. 2005
Mouse bone marrow (C57BL6)	Micronuclei	+	Mirkova 1994
Mouse bone marrow (BALB/c)	Micronuclei	–	Mirkova 1994
Mouse bone marrow	Micronuclei	inc	McFee et al. 1994
Drosophila (food)	Dominant lethal	–	Yoon et al. 1985
Drosophila (food)	Meiotic non-disjunction	+	Muñoz and Barnett 2002

– = negative result; + = positive result; ± = weak positive result; inc = inconclusive

DNA-intercalating agents, DNA-synthesis inhibitors, and direct mutagens. 1,4-Dioxane showed no activity in the absence of metabolic activation, but was not tested with metabolic activation. No evidence of DNA damage was seen in *Escherichia coli* K-12 *uvrB/recA* incubated with 1,4-dioxane with or without metabolic activation (Helmér and Bolcsfoldi 1992). A study in the yeast *Saccharomyces cerevisiae* strain D61M also gave negative results for chromosomal aneuploidy without activation (Zimmermann et al. 1985), but was not tested in the presence of metabolic activation. Studies with isolated mammalian cells exposed to 1,4-dioxane have also yielded negative results. For example, assays for induction of micronuclei, sister chromatid exchanges, and chromosomal aberrations in Chinese hamster ovary cells (CHO) were negative with and without metabolic activation (McElroy et al. 2003; Morita and Hayashi 1998). A similar study by Galloway et al. (1987) also found no increase in chromosomal aberrations in CHO cells, but did observe a slight increase in the incidence of sister chromatid exchanges in the absence of activation. Morita and Hayashi (1998) and McGregor et al. (1991) found no increase in gene mutations in mouse lymphoma cells incubated with 1,4-dioxane. 1,4-Dioxane did not induce DNA damage in rat hepatocytes (Goldsworthy et al. 1991), but increased cell transformations in BALB/3t3 cells at cytotoxic concentrations (Sheu et al. 1988). A test for DNA single strand breaks in rat hepatocytes incubated with 1,4-dioxane yielded positive results only at cytotoxic concentrations of 1,4-dioxane (Sina et al. 1983).

Studies of *in vivo* exposure of organisms to 1,4-dioxane also have been mostly negative, although some positive results have been reported. The only information in humans is that no increases in chromosomal aberrations were observed in peripheral lymphocytes from a groups of six workers employed in 1,4-dioxane production, relative to observations made in six control subjects (Thiess et al. 1976).

Several studies have reported results regarding micronuclei formation. An assay in bone marrow cells from C57BL6 mice after single gavage doses of up to 3,600 mg 1,4-dioxane/kg found a dose-related increase in the incidence of micronuclei, but the results in BALB/c mice were negative (Mirkova 1994). A similar study by Tinwell and Ashby (1994) found that 1,4-dioxane did not induce micronuclei in bone marrow cells from CBA mice treated with a single oral dose of 1,800 mg/kg or from C57BL6 mice dosed with 3,600 mg/kg. Studies reported by McFee et al. (1994) of several trials conducted by two different laboratories yielded equivocal results for micronuclei formation in mouse bone marrow. More recent data by Morita and Hayashi (1998) in CD-1 mice treated with a single gavage dose of up to 3,000 mg 1,4-dioxane/kg showed an increase in micronuclei in hepatocytes, but not in peripheral blood reticulocytes. Roy et al. (2005) also reported an increased incidence of micronuclei in hepatocytes and bone marrow from male CD-1 mice treated for 5 days with ≥2,500 and ≥1,500 mg 1,4-dioxane/kg,

respectively. Their results indicate that at high doses, 1,4-dioxane can induce chromosome breakage resulting in micronuclei.

Hepatocytes from Sprague-Dawley rats dosed with a single dose of 1,000 mg 1,4-dioxane/kg by gavage showed no evidence of DNA alkylation or DNA repair activity (Stott et al. 1981). This dose level administered via the drinking water to the rats for 11 weeks induced minimal hepatocellular swelling, which was accompanied by increased DNA synthesis (Stott et al. 1981). In male F344 rats administered single doses of up to 2,000 mg 1,4-dioxane/kg by gavage, 1,4-dioxane did not induce replicative DNA synthesis in hepatocytes (Uno et al. 1994), but it did in a subsequent study by the same group of investigators (Miyagawa et al. 1999). In liver tissue from Sprague-Dawley rats given two doses of 2,550 or 4,200 mg 1,4-dioxane/kg, there was a dose-related increase in DNA damage (assessed by alkaline elution) and cytochrome P-450 content; no significant effect was seen at ≤840 mg/kg (Kitchin and Brown 1990). Administration of a single oral dose of 1,000 mg 1,4-dioxane/kg to F344 rats produced no evidence of hepatocyte DNA repair, and the same negative response was obtained in rats dosed for a week via drinking water containing up to 2% 1,4-dioxane (Goldsworthy et al. 1991). No DNA repair activity was also observed in nasal epithelial cells from rats given 1% 1,4-dioxane in the drinking water for 8 days followed by a single gavage dose of 1,000 mg/kg (Goldsworthy et al. 1991). 1,4-Dioxane did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* in one study (Yoon et al. 1985), but was positive for meiotic non-disjunction in another study in *D. melanogaster* (Muñoz and Barnett 2002).

The information available indicates that 1,4-dioxane is not genotoxic in *in vitro* tests in eukaryotic and prokaryotic cells. Tests *in vivo* have been mostly negative, but a few tests yielded positive results in animals treated with 1,4-dioxane in high doses, many times higher than environmental exposures.

3.4 TOXICOKINETICS

Data in volunteers acutely exposed to vapors of 1,4-dioxane suggest that the chemical is readily and almost completely absorbed through the lungs. Studies in animals also show that 1,4-dioxane is readily absorbed after inhalation and oral exposure, but much less through the skin. No information is available regarding distribution of 1,4-dioxane or metabolites in humans. In animals injected with radiolabelled 1,4-dioxane, 1,4-dioxane-derived radioactivity distributed widely throughout the body, and no organ seemed to preferentially accumulate radiolabel. In humans and animals, 1,4-dioxane is metabolized to HEAA by mixed-function oxidase enzymes; HEAA can be converted to 1,4-dioxane-2-one under acidic

conditions. Both of these products are rapidly and extensively eliminated in the urine. Unchanged 1,4-dioxane can also be excreted in the urine and in exhaled air, but mainly after high-dose exposure. Studies have shown that the metabolism of 1,4-dioxane in rats is saturable at high doses. There is virtually no information regarding the toxicokinetics of 1,4-dioxane in humans following oral or dermal exposure. There is no indication that 1,4-dioxane or HEAA accumulates in the body.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Young et al. (1977) exposed a group of four healthy male volunteers to 50 ppm of 1,4-dioxane vapor for 6 hours. Plasma concentrations of 1,4-dioxane climbed rapidly during the first 2 hours of exposure, indicating an initial rapid absorption. This was followed by a gradual slow down in the rate of absorption until a plateau was reached at approximately 3 hours. Thus, steady state was reached during exposure. In contrast, the concentration of HEAA in plasma peaked approximately one hour after exposure ceased. Based on the presence of 1,4-dioxane and its main metabolite (HEAA) in the urine, the investigators calculated that the subjects absorbed a total mean dose of 5.4 mg 1,4-dioxane/kg at a mean rate of 76.1 mg/hour.

Experiments conducted in four male Sprague-Dawley rats exposed head-only to 50 ppm 1,4-dioxane vapors revealed that during a 6-hour exposure period, the rats absorbed a mean total dose of 71.9 mg 1,4-dioxane/kg; this figure is based on the amounts of parent compound and HEAA measured in the urine over a 48-hour period (Young et al. 1978a, 1978b). At the end of the exposure period, the concentration of 1,4-dioxane in the plasma was 7.3 µg/mL. It is worth noting that the value for total absorbed dose in this study, on a per body weight basis, is considerably greater than that calculated from volunteers exposed to the same airborne concentration of 1,4-dioxane for the same length of time (Young et al. 1977).

3.4.1.2 Oral Exposure

Data on the absorption of 1,4-dioxane following oral exposure in humans are not available.

Young et al. (1978a, 1978b) administered single doses of 10, 100, or 1,000 mg/kg of uniformly labeled ^{14}C-1,4-dioxane exposed to groups of male Sprague-Dawley rats by gavage for 17 days, and reported that <2% of the label was found in the feces in the first 24 hours (10 mg/kg dose) or 72 hours (100 or

1,000 mg/kg doses), indicating rapid and nearly-complete absorption of the compound from the gastrointestinal tract. In another experimental series reported in the same manuscripts (Young et al. 1978a, 1978b), groups of male Sprague-Dawley rats were given 10, 100, or 1,000 mg/kg of uniformly labeled ^{14}C-1,4-dioxane by gavage daily for 17 days. Less than 2% of the total administered label was recovered in the feces in 480 hours post-exposure, indicating that at least 98% absorption had occurred.

3.4.1.3 Dermal Exposure

Data on the absorption of 1,4-dioxane in humans following dermal exposure are not available, but a study with excised human skin reported that 10 times more 1,4-dioxane penetrates the skin under occluded conditions than under unoccluded conditions (3.2% of the applied dose vs. 0.30%, values obtained 205 minutes after application) (Bronaugh 1982). In the experiments, ^{14}C-1,4-dioxane was dissolved in a popular lotion and applied to the skin in occluded and unoccluded diffusion cells. The author explained that rapid evaporation was easily observed in the experiment. The rate of penetration of 1,4-dioxane in water (0.36±0.03 µg cm^{-2}hr^{-1}) was similar to that in a popular lotion (0.23±0.03 µg cm^{-2}hour^{-1}) and about 3 times slower than in a lipoidal vehicle, isopropyl myristate (0.94±0.10 µg cm^{-2}hour^{-1}) (Bronaugh 1982). A lethal case of intoxication with 1,4-dioxane in which the patient had extensive dermal contact with 1,4-dioxane in addition to inhalation of vapors suggests that dermal absorption is possible (Johnstone 1959).

Data in animals are limited to a study by Marzulli et al. (1981) in which uniformly labeled ^{14}C-1,4-dioxane, dissolved in either methanol or skin lotion, was applied to the unoccluded, clipped skin of Rhesus monkeys (4 µg/cm^2 over 3–12 cm^2) for 24 hours. Assuming a body weight of approximately 10 kg for an adult Rhesus monkey, the applied dose of 1,4-dioxane ranged from 1.2 to 4.8 mg/kg. The ability of the compound to penetrate the skin was assessed by analysis of radiolabel in the urine. The skin penetration of 1,4-dioxane was <4% in all cases; however, because the skin was unoccluded, evaporation may have influenced the study results. The differences between the results in the Bronaugh (1982) absorption data and those of Marzulli et al. (1981) could be due to differences in experimental conditions, that is, excised human skin in diffusion cells versus *in vivo* exposure of monkey skin.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Data on the distribution of 1,4-dioxane following inhalation exposure in humans or animals are not available.

3.4.2.2 Oral Exposure

Data on the distribution of 1,4-dioxane following oral exposure in humans or animals are not available.

3.4.2.3 Dermal Exposure

Data on the distribution of 1,4-dioxane following dermal exposure in humans or animals are not available.

3.4.2.4 Other Routes of Exposure

The only relevant information regarding distribution of 1,4-dioxane is that reported in studies involving intraperitoneal exposure of animals. In a study by Woo et al. (1977b), the distribution of ^{3}H-1,4-dioxane-derived radioactivity was followed in tissues from male Sprague-Dawley rats administered a single dose of 6.97 mg/kg intraperitoneally. Levels of radioactivity were measured in whole blood, liver, kidney, spleen, lung, colon, and skeletal muscle at 1, 2, 6, and 16 hours after dosing. The radioactivity was found to be widely distributed among the tissues examined and, for the most part, tissues had comparable levels of specific activity (nmol/g wet tissue). For example, the concentrations of dioxane-derived radioactivity at 1 and 16 hours decreased from 93.4 to 41.4 nmol/mL in the blood, from 59.1 to 24.2 nmol/g in the liver, from 116.1 to 31.9 nmol/g in the kidney, from 49.6 to 30 nmol/g in the spleen, from 52.2 to 23.2 nmol/g in the lung, from 56.1 to 27.7 nmol/g in the colon, and from 45.3 to 28.1 nmol/g in skeletal muscle. It should be noted that the tissue samples were not perfused or corrected for levels of blood in the tissue, so there might have been some influence of the blood-borne activity on the reported tissue values. Within the tissues, the percent covalent binding at 16 hours was universally <20%, with the highest levels in the colon (17.3% bound), spleen (16.4% bound), and liver (13.7% bound), followed by the lung (11.2% bound), kidney (9.5% bound), whole blood (3.1% bound), and skeletal muscle (2.7% bound). Within the cells, the highest activity levels were found in the cytosol (~68% at 6 hours post-exposure), with lesser amounts in the microsomal (~15% at 6 hours post-exposure), mitochondrial (~14% at 6 hours post-exposure), and nuclear (<3% at 6 hours post-exposure) fractions. Interestingly, percent covalent binding was entirely opposite in proportion to total activity levels, with the greatest percent

binding found in the nuclear fraction (~65%), followed by the mitochondrial (~46%), microsomal (~34%), and cytosolic (~5%) fractions.

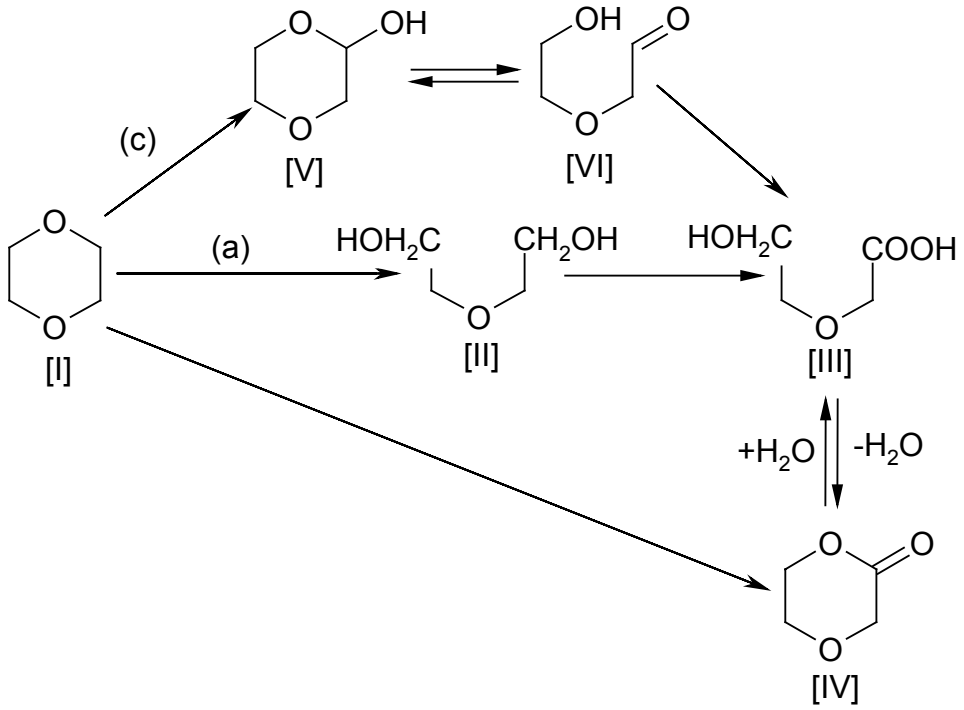
Mikheev et al. (1990) exposed rats to ^{14}C-1,4-dioxane by intraperitoneal injection and evaluated the levels of radioactivity in the blood, brain, testes, liver, and kidney at 5, 15, and 30 minutes and at 1, 3, and 6 hours post-injection in order to determine the tissue:blood concentration ratios. For all evaluated tissues at all time points, the tissue:blood ratio was between 0.5 and 1.5, indicating that 1,4-dioxane is distributed evenly and does not appreciably accumulate in any of the evaluated tissues. The maximum accumulation time (T_{max}) was 5 minutes for liver and kidney, and 15 minutes for the blood, brain, and testes.

3.4.3 Metabolism

A proposed metabolism scheme for 1,4-dioxane is diagrammed in Figure 3-3.

The exact metabolic pathways of 1,4-dioxane are not known. However, numerous studies have reported that 1,4-dioxane is metabolized to a single urinary metabolite, believed to be HEAA. There is some question as to whether HEAA or 1,4-dioxane-2-one is the ultimate metabolite (Braun and Young 1977; Woo et al. 1977a, 1977b, 1977c; Young et al. 1977). This arises from the fact that under acid conditions, such as are often used in analytical assays, HEAA can be converted to 1,4-dioxane-2-one, and under alkaline conditions, the reverse reaction occurs. It is of note that HEAA is not volatile, and as a result, is often catalyzed to 1,4-dioxane-2-one in order to facilitate analysis, which may explain why Woo et al. (1977a, 1977d) reported 1,4-dioxane-2-one, rather than HEAA. As mentioned above, acid conditions, such as were employed by the assays of Woo et al. (1977a, 1977d) result in the formation of 1,4-dioxane-2-one from HEAA. Additional evidence for HEAA as the primary metabolite, rather than 1,4-dioxane-2-one, comes from structure-activity relationship analyses of the genotoxicity of the two putative 1,4-dioxane metabolites (Blake 1995; Gombar 1995). 1,4-Dioxane-2-one is predicted to be strongly mutagenic, based on its structure, while HEAA would be only weakly genotoxic; the observed results of tests of genotoxicity for 1,4-dioxane correlate much closer with the predicted results from HEAA than from those of 1,4-dioxane-2-one. Further support for HEAA as the main metabolite of 1,4-dioxane was provided by the results of U.S. Army (2010), which showed no 1,4-dioxane-2-one in the urine of rats during an 8-hour period following a single gavage dose of 10 or 1,000 mg 1,4-dioxane/kg. Furthermore, incubating a urine sample from untreated rats with 1,4-dioxane-2-one showed rapid break down of the compound, presumably to HEAA, with a half-life of approximately 0.4 hours. Similar incubation with HEAA showed no conversion to 1,4-dioxane-2-one. An experiment conducted in

Figure 3-3. Suggested Metabolic Pathways of 1,4-Dioxane in the Rat



I = 1,4-dioxane; II = diethylene glycol; III = β-hydroxyethoxy acetic acid (HEAA); IV = 1,4-dioxane-2-one; V = 1,4-dioxane-2-ol; VI = β-hydroxyethoxy acetaldehyde

Source: adapted from Woo et al. (1977c)

nonbiological media that did not involve extraction, separation, or derivatization of the two metabolites showed a primary equilibrium constant of 0.016 ± 0.001 between the two compounds, indicating that thermodynamically HEAA is very strongly favored in the equilibrium. Mixed-function oxidase enzymes, and cytochrome P-450 in particular, are critical to the metabolism of 1,4-dioxane, as induction of these enzymes increases the rate of HEAA formation, and inhibition decreases HEAA formation (Woo et al. 1977c, 1978). The initial step in metabolism is likely a P-450-catalyzed oxidative step; however, the specific oxidation that occurs has not yet been determined. One possibility is diagrammed in pathway (a) of Figure 3-3. Cytochrome P-450 could act on one of the oxane oxygens, resulting in decyclization and the formation of diethylene glycol. Evidence supporting this pathway comes from the fact that in animals injected with diethylene glycol, HEAA was found as the major metabolite (Woo et al. 1977a). Diethylene glycol could then be further metabolized to HEAA through an additional oxidative metabolic step. Alternately, cytochrome P-450 enzymes could act on one of the carbons in 1,4-dioxane to add a single oxygen atom, resulting in the direct formation of 1,4-dioxane-2-one as diagrammed in pathway (b) of Figure 3-3; however, no evidence is presently available to support this possible pathway. Another possibility is that rather than a single oxygen, a hydroxyl group could be added to a carbon atom, resulting in 1,4-dioxane-2-ol, as shown in pathway (c) of Figure 3-3. Additional oxidation to HEAA, resulting in a breaking of the ring structure and further hydrolysis to HEAA could follow. As with pathway (b), there is no direct evidence supporting pathway (c) as the pathway for 1,4-dioxane metabolism.

1,4-Dioxane is extensively metabolized to HEAA in humans. Young et al. (1977) reported that over 99% of the urinary elimination of 1,4-dioxane after a 4-hour exposure of volunteers to 50 ppm occurred as HEAA rather than the parent compound. In an earlier study, the ratio of HEAA to dioxane in the urine of humans following a 7.5-hour exposure to 1.6 ppm dioxane was 118:1, indicating nearly complete metabolism at this exposure concentration (Young et al. 1976).

Recently, Sweeney et al. (2008) reported that rate constants and the metabolic profile from isolated human hepatocytes were very similar to those from isolated hepatocytes from rats and mice. The V_{max} for the production of HEAA from 1,4-dioxane in human hepatocytes ranged from 2.4 to 8.7 μg/h/10^6 cells, and the K_m ranged from 3.8 to 17.6 mg/mL. The respective mean values in rats and mice were 1.92 and 3.74 μg/h/10^6 cells and 2.51 and 2.63 mg/mL. Additional studies with the metabolism marker substrates coumarin and dextromethorphan suggested the involvement of the combined activities of P-450s 2A and 2D in the metabolism of 1,4-dioxane.

The metabolism of 1,4-dioxane to HEAA in animals is nearly complete, as evidenced by studies examining the urine of exposed animals. Following inhalation exposure of rats to 50 ppm of 1,4-dioxane for 6 hours, the ratio of HEAA to dioxane in the urine over the 48-hour observation period was >3,000, indicating that for urinary elimination, nearly all of the compound was eliminated as the metabolite, rather than as the parent compound (Young et al. 1978a, 1978b).

The available animal data indicate that the metabolism of 1,4-dioxane is saturable. Young et al. (1978a, 1978b) reported that with an increasing oral dose level, a greater percentage of the total dose was eliminated as expired 1,4-dioxane, suggesting that the normally-rapid metabolism of 1,4-dioxane had reached a maximum, allowing the free compound to circulate in the blood and be eliminated by expiration; no dose-related differences were seen in elimination as CO_2 or in the feces that could otherwise account for this difference. A similar pattern was seen following 17 repeated doses of 10 or 1,000 mg/kg of ^{14}C-1,4-dioxane by gavage, with a greater elimination of label, primarily as the metabolite, in the urine at the lower dose, with the higher dose resulting in a greater elimination, as both ^{14}C-1,4-dioxane and $^{14}CO_2$, in the expired air (Young et al. 1978a, 1978b). In an intravenous study reported in the same manuscript, the metabolic clearance of 1,4-dioxane decreased from 2.82 mL/minute following a single injection of 10 mg/kg to 0.17 mL/minute following an injection of 1,000 mg/kg, indicating that the metabolic capacity to metabolize 1,4-dioxane to HEAA had been saturated.

Woo et al. (1977a) reported that in the urine of rats orally exposed to 1–4 g/kg, only one metabolite was detected by gas chromatography. This metabolite was identified as 1,4-dioxane-2-one using nuclear magnetic resonance (NMR), infrared, and gas chromatograph-mass spectroscopy. Administration of diethylene glycol to rats resulted in the formation of the same metabolite, leading the study authors to hypothesize that diethylene glycol may represent an intermediate metabolite in the formation of 1,4-dioxane-2-one. In a later study by the same authors (Woo et al. 1977b), urine samples were collected, with glacial acetic acid as a preservative, from rats for 2 days following intraperitoneal injection of 50–400 mg/kg 1,4-dioxane. Gas chromatography identified a single metabolite, which was confirmed to be 1,4-dioxane-2-one by NMR, infrared, and mass spectroscopy.

Braun and Young et al. (1977) exposed groups of rats to radiolabeled 1,4-dioxane and characterized the major radiolabeled metabolite in the urine. The metabolite behaved identically to standards of both HEAA and 1,4-dioxane-2-one when evaluated using gas chromatography coupled with mass spectroscopy, preventing determination of the identity of the metabolite by this method. Using thin-layer chromatography, the metabolite's R_f value (the ratio of spot distance traveled to distance of the solvent

front) of 0.60 correlated with that of HEAA (0.61) rather than that of 1,4-dioxane-2-one (1.00). The study authors therefore concluded that the identity of the urinary metabolite in rats was HEAA, rather than 1,4-dioxane-2-one, but noted the tendency for HEAA to cyclize under acidic conditions, forming 1,4-dioxane-2-one.

Woo et al. (1977c, 1978) pretreated groups of rats with phenobarbital (PB), Arochlor 1254 (PCB), or 3-methylcholanthrene (MC) and examined the effects on the metabolism of an intraperitoneal dose of 1,4-dioxane. Pretreatment with PB resulted in a much more rapid metabolism of 1,4-dioxane, with the majority of the dose eliminated in the urine as HEAA within 32 hours, compared to 40 hours for the controls. The addition of 2,4-dichloro-6-phenylphenoxy ethylamine (DPEA), an inhibitor of cytochrome P-450, resulted in a reversal of this effect. Pretreatment with PCB resulted in similar effects as PB, while pretreatment with MC had no effect. Pretreatment with cobaltous chloride, to suppress P-450 synthesis, resulted in decreased metabolite elimination, further implicating cytochrome P-450 enzymes in the metabolism of 1,4-dioxane.

A study in male Sprague-Dawley rats showed that 1,4-dioxane induces several isomers of cytochrome P-450 in various tissues from male Sprague-Dawley rats and that the induction is tissue-specific (Nannelli et al. 2005). For example, administration of 2,000 mg 1,4-dioxane/kg/day by gavage for 2 days or ingestion of 1.5% 1,4-dioxane in the drinking water (approximately 2,200 mg 1,4-dioxane/kg/day) resulted in significant increases in the activities of CYP2B1/2, CYP2C11, and CYP2E1 in hepatic microsomes, but only CYP2E1 activity was increased in the kidney and nasal mucosa, and no alterations of P-450 activities were recorded in the lungs. Administration of the chemical by gavage also resulted in a significant increase in CYP3A activity in the liver. In addition, CYP4A1 activity was not enhanced by any treatment with 1,4-dioxane. According to Nannelli et al. (2005), the increase in liver CYP2C11 activity (2α-testosterone hydroxylase), which is normally under hormonal control and is suppressed in the presence of CYP2B1/2 and CYP2E1, may have been due to 1,4-dioxane altering plasma growth hormone levels. It was also noted that the increases in CYP2E1 activities in the kidney and renal mucosa were accompanied by increases in 2E1 mRNA, which suggested that 2E1 induction in these tissues is controlled by transcriptional activation. In contrast, the lack of an increase in 2E1 mRNA in the liver suggested that induction of CYP2E1 in hepatocytes is regulated via a post-transcriptional mechanism. In a different experiment, Nannelli et al. (2005) showed that induction of CYPB1/2 and CYP2E1 with phenobarbital or fasting did not increase the toxicity of 1,4-dioxane as measured by hepatic glutathione content or serum activity of ALT. This led the authors to suggest that reactive intermediates do not play a major role in the liver toxicity of 1,4-dioxane. The authors also suggested that a sustained induction of

CYP2E1 may lead to production of reactive oxygen species that contribute to target organ toxicity and regenerative cell proliferation, but no data were provided to support this hypothesis.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

1,4-Dioxane and its metabolite, HEAA, were found in the urine of workers exposed to a time-weighted average concentration of 1.6 ppm of 1,4-dioxane for 7.5 hours (Young et al. 1976). The concentration of HEAA was 414 µmol/L, and that of unchanged 1,4-dioxane was only 3.5 µmol/L, suggesting rapid and extensive metabolism. In four volunteers exposed to 50 ppm of 1,4-dioxane for 6 hours, over 99% of the urinary elimination of the compound occurred as its metabolite HEAA (Young et al. 1977) during the exposure period or within 18 hours post-exposure; the remainder of the urinary elimination occurred as the parent compound. The half-life of elimination of 1,4-dioxane from plasma was 59 minutes, of dioxane in urine was 48 minutes, and of HEAA in the urine was 2.7 hours. The urinary elimination data suggested that elimination kinetics of 1,4-dioxane and HEAA are best described with first-order, one-compartment kinetic models. Elimination by other pathways (e.g., feces, expired air) was not evaluated in this study.

Following inhalation exposure in animals, the primary route of elimination is believed to be the urine. Young et al. (1978a, 1978b) reported that following inhalation exposure in rats, urinary elimination of 1,4-dioxane was primarily as HEAA, rather than as the parent compound.

3.4.4.2 Oral Exposure

Data on the elimination of 1,4-dioxane in humans following oral exposure are not available.

The administered dose of 1,4-dioxane has an effect on elimination of the compound. While urinary elimination is the predominant pathway regardless of dose, at large doses, elimination in the expired air plays a greater role, possibly due to the saturable pathways of 1,4-dioxane metabolism. After single oral doses of ^{14}C-1,4-dioxane in rats, 99% of the label was recovered in the urine and <1% was recovered in the expired air at 10 mg/kg; 86% of the label was recovered in the urine and 4.7% in the expired air at 100 mg/kg; and 76% of the label was found in the urine and 25% in the expired air at 1,000 mg/kg (Young et al. 1978a, 1978b). Similar results were seen following 17 daily gavage doses of ^{14}C-1,4-dioxane in rats, with 99 and 83% of the label found in the expired air, 1.3 and 8.9% of the label

found as expired dioxane, and 4.1 and 7% found as expired CO_2 in animals receiving 10 and 1,000 mg/kg, respectively. Elimination of 1,4-dioxane in both the expired air and in the urine appear to be first-order kinetic processes (Young et al. 1978a, 1978b).

3.4.4.3 Dermal Exposure

Data on the elimination of 1,4-dioxane following dermal exposure in humans and animals are not available.

3.4.4.4 Other Routes of Exposure

After a single intravenous dose of 10 mg/kg of 1,4-dioxane in rats, 4% of the dioxane was eliminated in the urine as dioxane, 92% as HEAA, and 1% was eliminated in the expired air (Young et al. 1978a, 1978b). Following a 1000 mg/kg dose, 11% was eliminated in the urine as dioxane, 60% as HEAA, and 27% in the expired air, indicating a dose-related shift in the elimination of the compound, possibly due to metabolic saturation. At low intravenous doses, 1,4-dioxane is eliminated from the plasma with apparently linear kinetics, while higher doses are eliminated progressively more slowly, achieving linear kinetics only after a non-linear phase. This indicates the involvement of a saturable process, very likely metabolic saturation, in elimination of the compound. Elimination of 1,4-dioxane in both the expired air and in the urine following intravenous exposure appear to be first-order kinetic processes (Young et al. 1978a, 1978b). Pretreatment of rats with phenobarbital resulted in a 2.7-fold greater elimination of HEAA in the urine than in rats that were not pretreated (Woo et al. 1977c, 1978). The addition of DPEA partly reduced this effect, with the PB + DPEA animals eliminating 1.8-fold the HEAA of controls.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

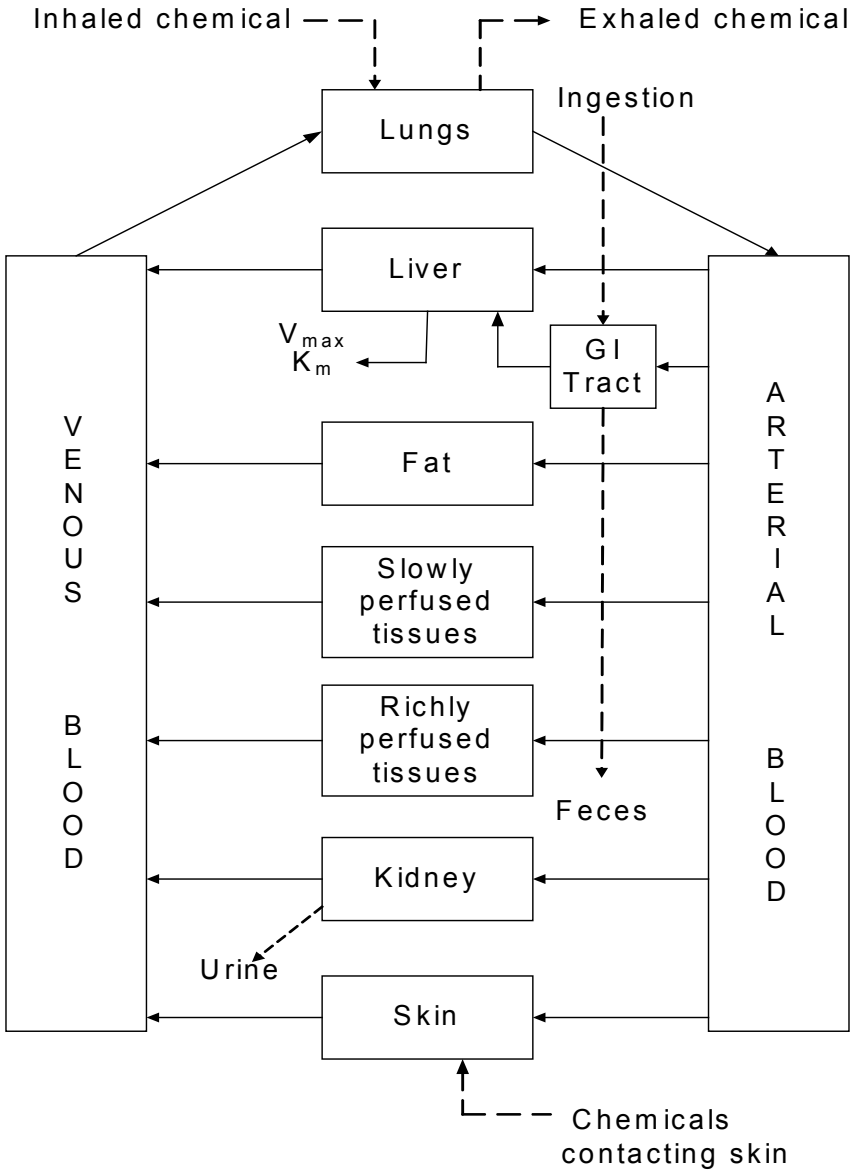
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, as well as species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites), based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan and Andersen 1994

If PBPK models for 1,4-dioxane exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Leung and Paustenbach (1990) Model.

Leung and Paustenbach (1990) developed a PBPK model for 1,4-dioxane for rats and humans, based on the styrene model of Ramsey and Andersen (1984). The model simulates concentrations of 1,4-dioxane in the four modeled tissue compartments, as well as in arterial and venous blood, and the amount of metabolites formed.

Description of the Model. The model consists of compartments for liver, fat, slowly perfused tissues, and richly perfused tissues. Model parameters are presented in Table 3-6. Model inputs included inhalation, where 1,4-dioxane input was assumed to occur at a rate equal to the cardiac output, and drinking water, where 1,4-dioxane absorption was considered to be a zero-order process depositing 1,4-dioxane directly into the liver compartment. Tissue/air partition coefficients for rat blood, liver, fat, and muscle were determined by vial equilibration, and tissue/blood values were calculated by dividing the tissue/air coefficient by the blood/air coefficient. The richly perfused coefficient was set equal to that of the liver. Human coefficients were assumed to be identical to the rat. For the human model, alveolar ventilation rate and cardiac output were estimated using a $(\text{body weight})^{0.74}$ scalar. The metabolic constants were obtained by optimization of the model with experimental data from Young et al. (1977, 1978a, 1978b); these studies were also used to calibrate the model, using data on blood 1,4-dioxane levels, 1,4-dioxane in expired air, and urinary excretion of 1,4-dioxane and HEAA to compare with model predictions.

Validation of the Model. The model parameters were optimized against the rat and human data of Young et al. (1977, 1978a, 1978b). Comparisons of model simulations against data from studies other than those used in model development were not presented.

Risk Assessment. The model attempts to estimate concentrations of 1,4-dioxane in the blood and in the tissue compartments, as well as the levels of metabolites formed, following an inhalation or oral exposure to 1,4-dioxane. These internal dose surrogates could be used in the assessment of health risks from exposure to 1,4-dioxane. The study authors used liver tumor data from a rat study (Kociba et al. 1974) to estimate risk-specific doses for tumor formation following oral exposure, fitting the rat data to a

Table 3-6. Parameters Used in the PBPK Model for 1,4-Dioxane

	Rat	Human
Weights		
Body (kg)	0.25	84.1
Liver (percent)	4	4
Fat (percent)	7	20
Richly perfused (percent)	5	5
Slowly perfused (percent)	75	62
Blood Flow		
Cardiac output (L/hour)	5.4	399
Liver (percent)	25	25
Fat (percent)	5	5
Richly perfused (percent)	51	51
Slowly perfused (percent)	19	19
Air flow		
Alveolar ventilation (L/hour)	5.4	399
Partition coefficients		
Liver/blood	0.85	0.85
Fat/blood	0.4	0.4
Richly perfused/blood	0.85	0.85
Slowly perfused/blood	0.54	0.2[a]
Metabolic constants		
V_{max}	1.9[a]	300[a]
K_m	7.5[a]	15[a]

[a]Values obtained by model optimization.

Source: Adapted from Leung and Paustenbach (1990)

multistage model and using liver dioxane concentrations as the dose metric for conversion from rat to human values. Human risk levels calculated by dividing the rat value by 5.5 (a body surface area scaling factor) were compared with values calculated using the model to calculate a human equivalent concentration; the model values resulted in a 7–8-fold greater value for maximum likelihood exposure (MLE) risk values than did division of the rat values by 5.5. It is important to note that the application of a PBPK model only addresses differences in pharmacokinetic behavior between species, and that differences in pharmacodynamic behavior must be discussed separately.

Target Tissues. The model simulates concentrations of 1,4-dioxane in the four modeled tissue compartments, as well as in arterial and venous blood, and the amount of metabolites formed. While two of the compartments represent actual body tissues (liver, fat), it is not known whether the model's estimates of tissue concentrations of 1,4-dioxane in these tissues is representative of the actual concentration in the tissues. However, the model's predictions of metabolite formation have been calibrated with actual data, providing evidence that the estimate of internal dose to the liver (where all metabolism is assumed to occur) is accurate.

Species Extrapolation. The Leung and Paustenbach (1990) PBPK model for 1,4-dioxane was developed in rats and humans, and human data on the pharmacokinetics of 1,4-dioxane was used in the optimization of model parameters. As such, interspecies extrapolation using the two models should be possible, although it has not yet been presented.

Interroute Extrapolation. The model includes inputs for both inhalation and oral exposures, and as such, should provide a means to estimate an internal dose to a target tissue compartment or other dose metric regardless of which of these two exposure routes is used. The use of the model for interroute extrapolation is therefore feasible, although it has not yet been performed.

Reitz et al. (1990) Model

Reitz et al. (1990) have also published a PBPK model for 1,4-dioxane in rats, mice, and humans, again building on the basic structure of the Ramsey and Andersen (1984) model for styrene. The model estimates concentrations of 1,4-dioxane in the modeled tissue compartments, as well as in arterial and venous blood, and the amount of metabolites formed.

Description of the Model. The model consisted of six compartments: lung, fat, liver, venous blood, slowly perfused tissues, and richly perfused tissues. The model was designed to simulate inhalation, intravenous, and oral exposures. Oral exposures could be by gavage or in the drinking water, and were assumed to pass through the liver before entering the systemic circulation. Intravenous injection was simulated by direct addition to the venous blood compartment, while inhalation deposited directly into arterial blood at a rate dependent on ventilation, cardiac output, and the blood/air partition coefficient for 1,4-dioxane. Tissue/air partition coefficients for 1,4-dioxane in human blood, rat blood, rat fat, rat muscle, and rat liver were determined by vial equilibration. Organ volumes, blood flows, and air flows were similar to those employed by Andersen et al. (1987), except that ventilation and cardiac output rates in humans were increased to provide a better simulation of the human blood level data. Metabolic rate constants were determined from data presented by Young et al. (1977, 1978a, 1978b) during optimization of the model. Metabolism was assumed to occur only in the liver, and metabolites were assumed to be removed from the system. Elimination of parent compound was modeled in the expired air and in the urine. The model parameters are presented in Table 3-7. After optimization using data from Young et al. (1977, 1978a, 1978b) the results of model runs and the corresponding experimental data were presented for venous blood concentrations in rats and humans following inhalation exposure, and venous blood concentrations in rats following intravenous exposure.

Validation of the Model. The model parameters were optimized against the rat and human data of Young et al. (1977, 1978a, 1978b). Comparisons of model simulations against data from studies other than those used in model development were not presented.

Risk Assessment. The model attempts to estimate concentrations of 1,4-dioxane in the blood and in the tissue compartments, as well as the levels of metabolites formed, following an inhalation, oral, or intravenous exposure to 1,4-dioxane. These internal dose surrogates could be used in the assessment of health risks from exposure to 1,4-dioxane. The study authors used the model to estimate "Human Virtually Safe Doses" (VSDs) based on tumor data from oral and inhalation studies in rats and mice (Kociba et al. 1974; NCI 1978; Torkelson et al. 1974). The VSDs were calculated by converting the rat no observable effect level (NOEL) for tumor formation to a human equivalent dose, and then dividing by a safety factor 100. The authors calculated a risk-specific water concentration of 20,000 µg/L for upper bound lifetime cancer risk of 1 in 100,000, calculated to represent the lower 95% confidence limit on administered dose producing a lifetime increase in risk of developing liver cancer, using the weighted average of area under the liver concentration/time curve and area under the metabolite concentration/time curve as the dose surrogate for conversion between species. Assuming a daily water intake of 2 L and a

Table 3-7. Parameters Used in the Reitz et al. (1990) PBPK Model for 1,4-Dioxane

	Mice	Rats	Humans
Body weight (kg)	0.035	0.400	70.0
Percentage of body weight			
Liver	4.0	4.0	3.1
Fat	4.0	7.0	23.1
Rapidly perfused	5.0	5.0	3.7
Slowly perfused	73.0	70.0	56.1
Blood	5.0	5.0	5.0
Flows (L/hour)			
Alveolar ventilation	2.34	7.61	696
Cardiac output	2.34	7.61	696
Percent of cardiac output			
Liver	25.0	25.0	25.0
Fat	5.0	5.0	5.0
Rapidly perfused	52.0	52.0	52.0
Slowly perfused	18.0	18.0	18.0
Partition coefficients			
Blood/air	2,750	1,850	3,650
Liver/air	1,557	1,557	1,557
Fat/air	851	851	851
Rapidly perfused/air	1,557	1,557	1,557
Slowly perfused/air	1,557	1,557	1,557
Saline/air	2,066	2,066	2,066
Metabolic constants (allometric)			
$V_{max}c$ (mg/hour)	10.0	13.7	6.35
K_m (mg/L)	16.2	29.4	3.00
Miscellaneous constants			
Ka ($hour^{-1}$)	5.0	5.0	5.0
K_{me} ($hour^{-1}$)	0.42	0.28	0.56
Water consumption (mL/day)	9.8	54	2,000

reference body weight of 70 kg, 20,000 µg/L of 1,4-dioxane in drinking water corresponds to a dose of approximately 0.6 mg/kg/day. For the purpose of comparison, the intake estimated by EPA for the same upper bound lifetime cancer risk shown in Figure 3-2 is 0.0001 mg/kg/day. It is important to note, however, that the application of a PBPK model only addresses differences in pharmacokinetic behavior between species, and that differences in pharmacodynamic behavior must be discussed separately.

Target Tissues. The model simulates concentrations of 1,4-dioxane in the four modeled tissue compartments, as well as in arterial and venous blood, and the amount of metabolites formed. While three of the compartments represent actual body tissues (liver, fat, venous blood), experimental data been compared to model simulations for only venous blood levels. It is not known whether the model's estimates of tissue concentrations of 1,4-dioxane in the other tissues is representative of the actual concentration in the tissues. However, the model's predictions of metabolite formation have been calibrated with actual data, providing evidence that the estimate of internal dose to the liver (where all metabolism is assumed to occur) is accurate.

Species Extrapolation. The Reitz et al. (1990) PBPK model for 1,4-dioxane was developed for rats, mice, and humans. Human and rat data on the pharmacokinetics of 1,4-dioxane were used in the optimization of model parameters; mouse parameters were generally assumed to be equivalent to those in the rat. As such, interspecies extrapolation using the models for the different species should be possible.

Interroute Extrapolation. The model includes inputs for both inhalation, oral, and intravenous exposures, and as such, should be able to estimate an internal dose to a target tissue compartment or other dose metric regardless of which of these exposure routes is used. The use of the model for interroute extrapolation is therefore feasible, although it has not yet been performed.

Sweeney et al. (2008) Model

Sweeney et al. (2008) developed a PBPK model for 1,4-dioxane for mice, rats, and humans. The model simulates concentrations of 1,4-dioxane in the four tissue compartments and venous blood. The amount and total body concentration of unspecified metabolite (presumed to be HEAA) is also simulated.

Description of the Model. The model consists of compartments for liver, fat, slowly perfused tissues (i.e., muscle and skin), well perfused tissues (excluding the liver), and venous blood. The model parameter values are presented in Table 3-8. Values for tissue volumes and fractional blood flow rates

Table 3-8. Parameters Used in the Sweeney et al. (2008) PBPK Model for 1,4-Dioxane

	Rats	Mice	Humans
Body weight (kg)	0.25	0.025	70.0
Percentage of body weight			
Liver	3.4	5.5	3.3
Fat	7.0	7.0	21.4
Rapidly perfused	1 – (sum of other tissue volumes)		
Slowly perfused	59.4	54.9	43.7
Blood	7.4	4.9	7.9
Unperfused tissue	5.0	5.4	7.1
Flows (L/hour/kg$^{0.74}$)			
Alveolar ventilation	13	20	13
Cardiac output	13	20	13
Percent of cardiac output			
Liver	18.3	16.1	22.7
Fat	7.0	7.0	5.2
Rapidly perfused	1 – (flow to liver, fat, and slowly perfused)		
Slowly perfused	33.6	21.7	24.9
Partition coefficients			
Blood/air	1,861	2,002	1,666
Liver/air	1,862	1,143	1,500
Fat/air	851	879	865
Rapidly perfused/air	560	560	560
Slowly perfused/air	1,341	1,705	1,503
Saline/air	2,446	2,446	2,446
Metabolic constants (allometric)			
$V_{max}c$ (mg/hour/kg$^{0.74}$)[a]	7.5 or 12.7	39 or 46	54, 75, or 192
K_m (mg/L) [a]	21	21	29, 32, or 147
Miscellaneous constants			
K_a (hour^{-1})[a]	0.8	0.8	Not derived
K_{me} (hour^{-1})[a]	0.48	0.35	0.35
Metabolite volume of distribution (L/kg)	1.0	0.83	0.83

[a]Values obtained by model optimization.

Source: Adapted from Sweeney et al. (2008)

were taken from the compendium of physiological parameters by Brown et al. (1997). Gastrointestinal absorption is described as first-order transport of the oral dose from the stomach to the liver. The first-order oral absorption rate constant (K_a) of 0.8 h^{-1} was optimized in mice using 2,000 mg/kg gavage data generated by the model authors. This value was assumed for rats. No value for K_a was derived for humans. Inhalation assumes rapid equilibration of the 1,4-dioxane concentration between inhaled air and blood described by an experimentally measured blood:air partition coefficient.

1,4-Dioxane is distributed throughout the body via venous blood flow to the tissue compartments with transfer from blood to tissues governed by tissue:blood partition coefficients, which were derived by dividing the measured tissue:air by blood:air partition coefficients. Blood:air partition coefficients were measured by the model authors for all three species. The authors also measured tissue:air partition coefficients for liver, fat, kidney, and muscle in mice, and liver and muscle in rats. Mouse values for kidney:air and muscle:air were used as surrogates for rapidly and slowly perfused tissue:air. These values, as well as mouse fat:air and kidney:air, were used for rats. The average values for rat and mouse tissue:air partition coefficients were used for humans. Elimination of 1,4-dioxane occurs by exhalation or following hepatic metabolism.

Metabolism of 1,4-dioxane is described as a saturable Michaelis-Menten process. Two parameter values for maximum metabolic velocity ($V_{max}c$ in mg/hour/kg body weight$^{0.7}$) and metabolic affinity (K_m in mg/L) were fitted for rats. Because the intravenous data of Young et al. (1978a, 1978b) suggest that higher doses result in rapid induction of metabolic activity, the 1,000 mg/kg intravenous data were used to optimize $V_{max}c$ values in the "induced " state, while data for intravenous doses of 3, 10, 30, or 100 mg/kg were used to optimize $V_{max}c$ in the "uninduced" state. The K_m for rats was fitted to all of the Young et al. (1978a, 1978b) intravenous data. The K_m for mice was chosen to be equivalent to rats because the *in vitro* values for rat and mouse K_m were very similar. Mouse $V_{max}c$ was optimized against the gavage data from 200 and 2,000 mg/kg doses generated by the model authors. The human $V_{max}c$ and K_m values were derived by multiplying the *in vitro* value from human hepatocytes, measured by the study authors, by the ratio of rat optimized $V_{max}c$ and scaled $V_{max}c$ (from rat hepatocytes) (known as the parallelogram approach).

The metabolite is eliminated via a first-order elimination rate constant (K_{me}). For the rat, K_{me} was optimized against the urinary HEAA data following intravenous doses of 100 or 1,000 mg/kg 1,4-dioxane and gavage doses of 10, 100, or 1,000 mg/kg (Young et al. 1978a, 1978b). For mice, K_{me} and the volume of distribution (VDMC) for total 1,4-dioxane metabolite was derived by optimization of data for HEAA

in blood following gavage doses of 200 or 2,000 mg/kg 1,4-dioxane. The value for human K_{me} was assumed to be equivalent to that of mice.

Validation of the Model. Following optimization of parameter values for $V_{max}c$, K_m, K_a, K_{me}, and VDMC (for mice), additional data from intravenous, gavage, and inhalation exposures were used to evaluate model performance. For the rat model, simulated exhaled 1,4-dioxane levels were very similar to observations from intravenous or gavage doses of 1,000 mg/kg, but were ~3–5-fold higher than observations for 10 mg/kg doses. For 6-hour 50 ppm exposures to 1,4-dioxane, rat simulations of blood levels were similar to observations, but HEAA urinary excretion was 3-fold lower than observations. For mice, simulations of 20 mg/kg gavage doses predicted no rise of blood 1,4-dioxane levels above the observed background concentration of 1.6 mg/L, but predicted levels of HEAA were not in agreement with observations. Human predictions of blood 1,4-dioxane levels and urinary HEAA excretion during and following a 6-hour 50 ppm inhalation exposure were not in good agreement with observations. The model authors attempted to model the 1,4-dioxane body burden in workers based on cumulative 1,4-dioxane and HEAA in urine samples following a single 7.5-hour inhalation exposure to an average of 1.6 ppm 1,4-dioxane (Young et al. 1976). Although the predicted and observed body burdens were similar, the results are uncertain because the scheme used to calculate body burden was unclear. The human urinary production rate was assumed to be 1 mL/hour, and human urinary HEAA elimination was determined using elimination parameter values for mice.

Risk Assessment. The mouse, rat, and human models have not been used previously in risk assessment. Although the rat and mouse models provide adequate fits of high-dose observations, they do not perform well against low-dose data. The human model could not replicate the limited human experimental inhalation data available. Further, it assumes equivalency with mice in eliminating HEAA, and has no value derived for oral absorption. Based on these significant limitations, the Sweeney et al. (2008) model for 1,4-dioxane in rats, mice, and humans is not adequate for MRL derivation.

Target Tissues. The model simulates concentrations of 1,4-dioxane in liver, fat, and lumped rapidly and slowly perfused tissues. However, performance of the model to predict tissue levels of 1,4-dioxane or metabolites cannot be evaluated, as experimental data for tissues are not available for rodents or humans.

Species Extrapolation. The Sweeney et al. (2008) model for 1,4-dioxane was developed for mice, rats, and humans. However, the inability of the model to replicate low-dose 1,4-dioxane levels in rats or humans precludes its use for species extrapolation.

Interroute Extrapolation. The model includes inputs for intravenous, oral, and inhalation exposures, providing a direct means for interroute extrapolation.

Takano et al. (2010) Model

Takano et al. (2010) developed a simplified two-compartment PBPK model for 1,4-dioxane for rats and humans.

Description of the Model. The Takano et al. (2010) model simulates 1,4-dioxane in two compartments: the liver compartment and the central compartment. The model used data from (1) *in vivo* studies in rats (repeated oral exposure to 500 mg/kg/day for 14 days and using intraperitoneal administration of 500 mg/kg) to establish absorption elimination kinetics and cytochrome P450 parameters, (2) *in vitro* studies using rat and human liver microsomes, and (3) *in silico* estimation of the fraction of unbound plasma 1,4-dioxane and octanol-water partition coefficient. Other parameters, such as hepatic volumes and blood flow rates, were taken from the literature. The model parameter values are presented in Table 3-9. The rat model simulated 1,4-dioxane levels in the blood were similar to measured blood levels. The PBPK model showed little accumulation of 1,4-dioxane in the rat 24 hours after daily treatment with 1,4-dioxane. Comparisons of simulated blood 1,4-dioxane levels after single or multiple exposures to 500 mg/kg in humans showed an accumulation of 1,4-dioxane.

Validation of the Model. Comparisons of model simulations against data from studies other than those used in model development were not presented.

Risk Assessment. The model attempts to estimate concentrations of 1,4-dioxane in the blood following oral exposure to 1,4-dioxane. Although the model provided adequate fit of rat blood 1,4-dioxane data, the model has not been validated using different dose levels or using data from other studies. Based on these significant limitations, this model is not adequate for MRL derivation.

Table 3-9. Parameters Used in the Takano et al. (2010) PBPK Model for 1,4-Dioxane

	Rat	Human
Octanol-water partition coefficient	-0.31	-0.31
Hepatic intrinsic clearance (L/hour)	0.0244	1.76
Liver-plasma concentration ratio	0.692	0.692
Renal clearance (L/hour)	0.000290	0.0873
Plasma unbound fraction	0.806	0.806
Ratio of blood to plasma concentration	1.00	1.00
Volume of systemic circulation (L)	0.0810	23.7
Hepatic volume (L)	0.00850	1.50
Hepatic blood flow rate of systemic circulation to tissue compartment (L/hour)	0.853	96.6
Absorption rate constant ($hour^{-1}$)	0.280	0.208
Fraction absorbed x intestinal availability	1.00	1.00
Dose (mg)	125	3,500

Target Tissues. The model simulates concentrations of 1,4-dioxane in blood. However, performance of the model to predict tissue levels of 1,4-dioxane or metabolites cannot be evaluated, as experimental data for tissues, as experimental data for tissues are not available for rats or humans.

Species Extrapolation. The Takano et al. (2010) PBPK model for 1,4-dioxane was developed for rats and humans. Human and rat data on the pharmacokinetics of 1,4-dioxane were used in the optimization of model parameters. As such, interspecies extrapolation using the models for the different species should be possible.

Interroute Extrapolation. The model is limited to oral exposure and does not provide a means for interroute extrapolation.

Fisher et al. (1997) Model

Fisher et al. (1997) have published a general PBPK model for volatile organic chemicals, which incorporates a compartment for elimination of the chemical in the breast milk. Model simulations predicted a high degree (18%) of lactational transfer of 1,4-dioxane. While the study authors note that the model is applicable to 1,4-dioxane, simulations using the model have not been compared to data from humans or animals exposed to 1,4-dioxane.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. The absorption of 1,4-dioxane following inhalation or oral exposure is rapid and essentially complete; absorption following dermal exposure is very low (Marzulli et al. 1981; Young et al. 1977, 1978a, 1978b). The mechanisms involved in the absorption of 1,4-dioxane have not been evaluated, but given the speed of the absorption and the chemical similarity of 1,4-dioxane to other low-molecular-weight compounds, absorption is generally assumed to occur through passive diffusion.

Distribution. The mechanisms of distribution of 1,4-dioxane have not been evaluated. Data on the distribution of 1,4-dioxane are limited to studies following injection of the compound (Mikheev et al. 1990; Woo et al. 1977b). In those studies, distribution of 1,4-dioxane was rapid (5–15 minutes to T_{max}).

1,4-Dioxane has been detected in all tissues that have been evaluated, but has not been shown to appreciably accumulate in tissues, possibly due to its high water solubility.

Metabolism. Studies on the metabolism of 1,4-dioxane have clearly identified the primary metabolite as HEAA/1,4-dioxane-2-one, but have not confirmed a clear pathway for the formation of metabolites from 1,4-dioxane. Cytochrome P-450 enzymes are clearly involved, as evidenced by the studies of Woo et al. (1977c, 1978) and Nannelli et al. (2005). It has been suggested that P-450-mediated metabolism may result in the formation of diethylene glycol, since injection of diethylene glycol in rats also results in the formation of HEAA (Woo et al. 1977a); however, additional data supporting this pathway have not been presented.

The issue of whether metabolism of 1,4-dioxane represents a detoxifying event or a process that generates toxic intermediates has not been resolved. Data from Kociba et al. (1974, 1975) and Young et al. (1978a, 1978b) indicate that toxicity occurs at high doses when the metabolism of 1,4-dioxane is saturated, which would suggest that the parent compound is the toxic form. This also is consistent with more recent data from Nannelli et al. (2005) who observed that induction of hepatic CYP2B1/2 and CYP2E1 did not play a role in the toxicity of 1,4-dioxane, which suggested that highly reactive and toxic intermediates do not play a major role in the liver toxicity of 1,4-dioxane, even under conditions of enhanced metabolism. On the other hand, Woo et al. (1978) reported that the metabolite, 1,4-dioxane-2-one, was several-fold more toxic than 1,4-dioxane based on intraperitoneal LD_{50} determinations in rats.

Excretion. The elimination of 1,4-dioxane occurs primarily (>95%) in the urine, as the primary metabolite, at low doses. At higher doses, metabolism becomes saturated, and a greater fraction is eliminated in the expired air; however, urinary elimination remains the primary method of elimination. Elimination of 1,4-dioxane in both the expired air and the urine following intravenous exposure appear to be first-order kinetic processes (Young et al. 1978a, 1978b). Evidence for active secretion or uptake of 1,4-dioxane from the kidney has not been reported.

3.5.2 Mechanisms of Toxicity

The mechanism of carcinogenicity of 1,4-dioxane has not been elucidated, but the results from several lines of investigation suggest that 1,4-dioxane has a non-genotoxic mode of action (Goldsworthy et al. 1991; Leung and Paustenbach 1990; Stott et al. 1981). Briefly, the collective evidence from evaluations of genetic toxicity suggests that 1,4-dioxane is unlikely to be genotoxic (see Section 3.3, Genotoxicity).

1,4-Dioxane was not mutagenic in bacterial assays with or without metabolic activation (Haworth et al. 1983; Khudoley et al. 1987; Morita and Hayashi 1998; Nestmann et al. 1984; Stott et al. 1981), did not induce chromosomal aneuploidy in yeast (Zimmermann et al. 1985), mutations in mouse lymphoma cells (Morita and Hayashi 1998; McGregor et al. 1991), or sex-linked recessive lethal mutations in *D. melanogaster* (Yoon et al. 1995). Moreover, in an occupational study there was no evidence of an increased incidence of chromosomal aberrations among workers chronically exposed to relatively low levels of 1,4-dioxane compared to controls (Thiess et al. 1976). No significant increase in chromosomal aberrations was observed in Chinese hamster ovary cells incubated with 1,4-dioxane with or without metabolic activation, but there was a weak increase in sister chromatid exchanges when the cells were incubated without metabolic activation (Galloway et al. 1987). Also, incubation of BALB/3t3 cells with 1,4-dioxane increased the incidence of transformations leading to the formation of foci, but at concentrations of 1,4-dioxane that were cytotoxic to over 50% of the cells (Sheu et al. 1998).

Several structure-activity analyses have been conducted to study the mechanism of carcinogenicity of 1,4-dioxane. For example, a computerized structure relationship analysis using TOPKAT (version 3.0) in male rat and female mouse models showed that the -O-CH_2- fragment of the molecule increased the carcinogenic potential in both models; however, the male rat model indicated that the symmetry of the 1,4-dioxane molecule is more important in the carcinogenicity of 1,4-dioxane than the -O-CH_2- fragment (Blake 1995). Additional modeling efforts of the potential role of 1,4-dioxane's metabolites, HEAA and 1,4-dioxane-2-one, in the carcinogenicity and genotoxicity of 1,4-dioxane predicted that HEAA would be noncarcinogenic and nonmutagenic, whereas 1,4-dioxane-2-one was predicted to have a strong positive influence in the female mouse carcinogenicity model and the Ames mutagenicity model (Gombar 1995). It should be noted, as mentioned in Section 2.4.3, Metabolism, experiments conducted by U.S. Army (2010) showed that the chemical instability of 1,4-dioxane-2-one suggested that it is unlikely to be involved in the mode of action for carcinogenesis of 1,4-dioxane. A structure-activity relationship analysis using the Computer-Automated Structure Evaluation (CASE) methodology found the fragment -O-CH_2- to be associated with a high probability of induction of micronuclei in mice bone marrow cells (Rosenkranz and Klopman 1992). According to unpublished results cited by Rosenkranz and Klopman (1992), the concordance between experimental results and CASE predictions of the induction of micronuclei is in excess of 83%. However, Rosenkranz and Klopman (1992) indicated that because the -O-CH_2- fragment does not seem to have intrinsic electrophilicity, they could not envision a possible DNA-reactive metabolite; however, the -O-CH_2- fragment might contribute to a non-genotoxic effect of 1,4-dioxane resulting in the induction of micronuclei. Since the experimental results of micronuclei-induction studies in mice have been mixed (McFee et al. 1994; Mirkova 1994; Morita and Hayashi 1998;

Tinwell and Ashby 1994), Ashby (1994) suggested that it is not always possible to categorize a chemical as either unequivocally positive or negative in genotoxic activity.

Numerous studies have examined the possible role of DNA damage, DNA synthesis, cell proliferation, or peroxisome proliferation in the carcinogenic effects of 1,4-dioxane. For instance, a test for DNA single strand breaks in rat hepatocytes incubated with 1,4-dioxane gave positive results, although only at cytotoxic concentrations (Sina et al. 1983). Stott et al. (1981) reported that hepatocytes from Sprague-Dawley rats dosed with 1,4-dioxane for 11 weeks showed no evidence of DNA alkylation or DNA repair activity, but showed increased levels of DNA synthesis. Based on these results, Stott et al. (1981) suggested that 1,4-dioxane induces tumors by a non-genetic mechanism.

The role of cell proliferation in the carcinogenicity of 1,4-dioxane was further evaluated in two studies that yielded inconclusive results. Administration of single doses of up to 2,000 mg 1,4-dioxane/kg by gavage to male F344 failed to induce replicative DNA synthesis in hepatocytes (Uno et al. 1994), which led the authors to suggest that 1,4-dioxane may induce liver cancer only in initiated cells. However, in a subsequent study by the same group of investigators, and using the same experimental protocol, 1,4-dioxane did increase hepatocyte cell proliferation (Miyagawa et al. 1999); the reason for the discrepancy in the results between the two studies is not apparent.

In liver tissue from Sprague-Dawley rats given single doses of 1,4-dioxane, there was a small but statistically significant amount of DNA damage (assessed by alkaline elution) at dose levels that did not induce cytotoxicity (Kitchin and Brown 1990, 1994). Liver toxicity was assessed by light microscopy and measurements of serum levels of ALT (no significant increase was observed). The DNA damage was accompanied by an increase in cytochrome P-450 content and by large increases in the activity of hepatic ornithine decarboxylase, suggesting strong promoter activity for 1,4-dioxane.

Another study of the potential mechanisms of carcinogenicity of 1,4-dioxane showed that neither 1,4-dioxane nor the metabolite 1,4-dioxane-2-one induced DNA repair activity in primary rat hepatocytes following incubation *in vitro* with the chemicals (Goldsworthy et al. 1991). Administration of a single oral dose of 1,4-dioxane to F344 rats produced no evidence of hepatocyte DNA repair, and did not increase DNA synthesis, relative liver weight, or the activity of palmitoyl CoA oxidase (an indicator of peroxisome proliferation) (Goldsworthy et al. 1991). Furthermore, administration of a single dose of 1,000 mg/kg of 1,4-dioxane did not increase the hepatocyte labeling index 24 or 48 hours after dosing, but exposure to 1% 1,4-dioxane in the drinking water for 2 weeks resulted in a 2-fold increase in hepatic

labeling index (Goldsworthy et al. 1991); the latter suggested that cell proliferation may play a role in the induction of liver carcinoma. In addition, no DNA repair activity or evidence of cells proliferation was observed in nasal epithelial cells from rats administered 1% 1,4-dioxane in the drinking water for 2 weeks (Goldsworthy et al. 1991). However, it must be mentioned that Goldsworthy et al. (1991) acknowledged that a 2-week period of exposure might have been too short for detecting a proliferative response. Goldsworthy et al. (1991) concluded that the mechanism of carcinogenicity of 1,4-dioxane remains obscure and may involve a novel mechanism. In support of some of Goldsworthy's findings, Nannelli et al. (2005) also provided evidence that excluded peroxisome proliferation as a way to explain the toxicity of 1,4-dioxane. These investigators found that administration of approximately 2,200 mg 1,4-dioxane/kg/day in the drinking water for 10 days to rats also failed to induce palmitoyl CoA oxidase activity.

Gold et al. (1993) analyzed 351 chemicals in the Carcinogenic Potency Database (CPDB) and pointed out that, relative to non-mutagenic chemicals, mutagens are more likely to be carcinogenic, more likely to induce tumors at multiple target sites and, more likely to be carcinogenic in two species. Since 1,4-dioxane was carcinogenic in more than one species and induced tumors at multiple sites, one would expect that 1,4-dioxane would behave like a mutagen, but the empirical data suggest otherwise. Gold (1993) pointed out that among the CPDB chemicals tested for mutagenicity, 75% are mutagens, compared to 45% for non-mutagens. This suggested that administration of large doses in cancer bioassays result in mitogenesis, which in turn increases the rate of mutagenesis and thus carcinogenesis.

The lack of significant genotoxicity along with the cytotoxicity observed at dose levels that induce tumors supports the view that 1,4-dioxane acts via an unknown non-genotoxic mechanism.

The mechanism(s) by which 1,4-dioxane induces kidneys lesions is not known, and virtually no discussion about this topic was found in the available reviews. The findings in the cases described by Barber (1934) and Johnstone (1959) are consistent with an acute nephritic syndrome, which is characterized by acute renal failure and oliguria. The impaired glomerular filtration rate causes extracellular fluid volume expansion, edema, and hypertension. A study of controlled exposures in volunteers showed that 1,4-dioxane has poor renal clearance, 0.34 mL/minute (Young et al. 1977), which probably contributes to accumulation of the chemical in the kidneys as biotransformation to the metabolite HEAA becomes saturated under conditions of high exposure.

The issue of the nasal effects observed in rats exposed to 1,4-dioxane via the drinking water studies is controversial. As indicated in Section 2.2, Sweeney et al. (2008) presented evidence suggesting that nasal

effects may result from the splashing of drinking water into the nasal cavity. However, in the 13-week drinking water study by Kano et al. (2008), the enlarged nuclei of the respiratory and olfactory epithelial cells were distributed over the entire respiratory and olfactory region, respectively. Similarly, Kano et al. (2009) also found nasal tumors distributed over the entire region of the nasal cavity from rats exposed to 1,4-dioxane in the drinking water for 2 years. These findings support a systemic delivery of a chemical. The mechanism by which oral exposure to 1,4-dioxane can lead to effects on the respiratory epithelium has not been elucidated. However, Kasai et al. (2009) suggested that induction of P-450 isozymes in the olfactory epithelium, as shown by Nannelli et al. (2005), may lead to the formation of toxic metabolites not only from the inhaled and circulating 1,4-dioxane, but also from the 1,4-dioxane exhaled from the alveoli. A role for unchanged 1,4-dioxane cannot be ruled out. Further research on this issue is necessary.

3.5.3 Animal-to-Human Extrapolations

Exposure to high concentrations of 1,4-dioxane induces liver and kidneys effects in humans and in animals, regardless of the route of exposure. Based solely on the similarity of target organs, it would appear that results from animal studies could be used as valid models to predict health effects in humans resulting from high-dose exposure to 1,4-dioxane.

Long-term oral exposure of rodents to 1,4-dioxane has induced liver tumors in rats and mice as well as tumors in the nasal cavity of rats (JBRC 1998b; Kociba et al. 1974; NCI 1978). Therefore, the issue is whether these long-term, relatively high-exposure studies in animals are relevant to the low environmental exposures to 1,4-dioxane experienced by the general population. Studies of humans exposed chronically to relatively low concentrations of 1,4-dioxane in the air in occupational settings have provided no evidence of ill effects, including cancer, associated with 1,4-dioxane among the workers (Buffler et al. 1978; Thiess et al. 1976). However, it is unclear if the effects reported in humans are consistent with the potency estimated in rodents.

Some studies have shown that the liver tumors in rats are accompanied by extensive toxicity, as evidenced by hepatocyte hyperplasia, accumulation of fat in the cytoplasm, and degenerative changes (JBRC 1998b; Kociba et al. 1974; NCI 1978), which has led some to suggest that cell damage and degeneration may be a necessary occurrence for the formation of liver tumors in rats. Since liver toxicity in rats seems to occur only at dose levels at which plasma clearance and excretion of HEAA are reduced and plasma concentrations of unchanged 1,4-dioxane are increased, it may be appropriate to consider the

differences in metabolic disposition when extrapolating from effects that occur only with high doses to low-dose events (Kociba et al. 1975).

The relevance to humans of the nasal lesions and nasal tumors consistently seen in rats following exposure to 1,4-dioxane through the drinking water in many studies has been questioned (Stickney et al. 2003). Some have suggested that the tumors resulted from inspiration of water containing 1,4-dioxane into the nasal cavity (Goldsworthy et al. 199; Reitz et al. 1990; Sweeney et al. 2008). However, as mentioned above, there are studies that support a systemic delivery of 1,4-dioxane or a metabolite (Kano et al. 2008, 2009). The lack of nasal tumors in mice in chronic drinking water studies could be due to differences in tissue sensitivity and/or repair mechanisms, or to differences in anatomical features. However, species differences are difficult to establish, since 1,4-dioxane acts via an unknown mechanism to produce tumors in liver, nasal cavity, and other sites.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system, because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated that EPA develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial,

scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones responsible for maintaining homeostasis, reproduction, development, and/or behavior in humans (EPA 1997b). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Based on the available information, there is no evidence that 1,4-dioxane is an endocrine disruptor in humans or in animals, but appropriate tests have not been conducted. The only relevant information that was located is that 1,4-dioxane tested negative for estrogenic activity in a reporter gene expression assay using yeast cells (Nishihara et al. 2000). A substance was considered positive when its activity was more than 10% of the activity of 10^{-7} M 17β-estradiol.

Long-term oral studies have found no histopathologic (non-neoplastic) alterations in endocrine glands and reproductive organs from rats and mice (Kociba et al. 1974; NCI 1978), and the same was found in a chronic-duration inhalation study in rats (Torkelson et al. 1974). However, neoplasms associated with the administration of 1,4-dioxane occurred in the testis/epididymis in male rats administered ≥240 mg 1,4-dioxane/kg/day in the drinking water for 2 years (NCI 1978). Another 2-year bioassay reported an increased incidence of mammary gland adenomas in rats treated with 514 mg 1,4-dioxane/kg/day in the drinking water (JBRC 1998b).

Standard reproductive toxicity studies on 1,4-dioxane were not located, and only one study that examined the developmental effects of 1,4-dioxane was available (Giavini et al. 1985). The latter study reported slight fetotoxicity occurring at a dose level that also affected the mothers.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their

alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

There are no studies that specifically address the health effects of exposure to 1,4-dioxane in children or in immature animals; therefore, it is unknown whether children differ from adults in their susceptibility to health effects from 1,4-dioxane. Data in adults were derived from occupational studies (Barber 1934; Buffler et al. 1978; Thiess et al. 1976) and studies in volunteers (Fairley et al. 1934; Silverman et al. 1946; Yant et al. 1930; Young et al. 1977). The former showed that exposure to high concentrations of 1,4-dioxane in the air (and also dermally) can severely damage the liver and kidneys and can be lethal. The studies of controlled exposure with volunteers showed that exposure to 1,4-dioxane in the air can produce eye, nose, and throat irritation. It is reasonable to assume that the same types of effects would be seen in children accidentally exposed to high amounts of 1,4-dioxane.

There is no information regarding possible adverse developmental effects in humans exposed to 1,4-dioxane. A study in rats exposed orally to 1,4-dioxane during gestation found slight fetotoxicity, but at a dose level that also affected the mothers (Giavini et al. 1985). There is evidence that 1,4-dioxane is at most a weak genotoxic compound. Therefore, it is unlikely that parental exposure would result in adverse childhood development or cancer development as a result of 1,4-dioxane metabolite exposures to parental germ cells.

There is no information regarding pharmacokinetics of 1,4-dioxane in children. Analysis of urine samples from humans exposed to 1,4-dioxane suggests the involvement mainly of phase I metabolic enzymes in the biotransformation and elimination of 1,4-dioxane. A recent study showed that 1,4-dioxane can induce several P-450 isozymes in the liver of rats (Nannelli et al. 2005), and one of them was CYP2E1, which has been shown to be developmentally-regulated (Vieira et al. 1996). However, the question of whether metabolism of 1,4-dioxane represents a detoxifying mechanism or a process generating toxic intermediates is still unclear. It is not known whether 1,4-dioxane can cross the placenta, and there are no reports on levels of 1,4-dioxane in maternal milk.

There are no biomarkers of exposure or effect for 1,4-dioxane that have been validated in children or in adults exposed as children. No relevant studies were located regarding interactions of 1,4-dioxane with other chemicals in children or adults.

No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to 1,4-dioxane, reducing body burden, or interfering with the mechanisms of action for toxic effects.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to 1,4-dioxane are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction, such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by 1,4-dioxane are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, "Populations That Are Unusually Susceptible."

3.8.1 Biomarkers Used to Identify or Quantify Exposure to 1,4-Dioxane

1,4-Dioxane and its metabolite, HEAA, were found in the urine of workers exposed to a time-weighted average air concentration of 1.6 ppm of 1,4-dioxane for 7.5 hours (Young et al. 1976). The concentration of HEAA was 414 μmol/L and that of unchanged 1,4-dioxane was only 3.5 μmol/L, suggesting rapid and extensive metabolism. 1,4-Dioxane in the urine is a specific biomarker for exposure to 1,4-dioxane, but HEAA can also be produced by exposure to 1,4-dioxane-2-one and diethylene glycol. In a controlled-exposure study with volunteers exposed to 50 ppm 1,4-dioxane vapors for 6 hours, the half-life for elimination of 1,4-dioxane from plasma was 59 minutes (Young et al. 1977). The plasma concentration of HEAA reached a peak at about 1 hour after exposure ceased and decreased linearly thereafter. Of all the 1,4-dioxane detected in the urine within a 48-hour period, 90% was excreted during the exposure period and none could be detected 6 hours after termination of the exposure. The half-life for elimination of 1,4-dioxane in the urine was 48 minutes, and that of HEAA was 2.7 hours. Almost all the 1,4-dioxane was excreted in the urine as HEAA. About half of the total HEAA excreted was excreted during the exposure period, and the excretion was complete 18 hours after the exposure ceased. A simulation of repeated exposures to 50 ppm 1,4-dioxane for 8 hours/day showed that 1,4-dioxane will reach a peak in plasma at the end of each exposure day and will not accumulate; neither will HEAA. Collectively, these results imply that 1,4-dioxane and HEAA in plasma and urine can be used as biomarkers of recent isolated exposure or multiple daily exposures, but that could not differentiate between the two types of exposure (providing the exposure concentrations are below about 50 ppm). In addition, because these substances are rapidly eliminated, they cannot be used as biomarkers of past exposure to 1,4-dioxane. Given the low levels of 1,4-dioxane reported in the environment, it is not unlikely that the levels of 1,4-dioxane and HEAA in members from the general population fall under the detection levels of the available analytical methods.

Some chemicals bind to macromolecules (i.e., DNA, hemoglobin, etc.) to form compounds that can be used as specific biomarkers of exposure. That is not the case for 1,4-dioxane. In liver preparations from rats administered a single intraperitoneal dose of radioactive 1,4-dioxane, most of the radioactivity was

bound non-covalently in the cytosol (Woo et al. 1977b). Covalent binding to macromolecules was highest in nuclear fraction followed by mitochondrial, microsomal, whole homogenate, and cytosol fractions. The binding was nonspecific and not associated with DNA. Pretreatment of rats with microsomal enzyme inducers had no significant effect on the covalent binding to macromolecules. There was no microsomally-mediated binding of radioactivity to DNA.

3.8.2 Biomarkers Used to Characterize Effects Caused by 1,4-Dioxane

The liver and kidneys are targets for 1,4-dioxane toxicity, but lesions to these organs cannot be considered specific biomarkers for 1,4-dioxane because, exposure to many different chemicals or health conditions unrelated to chemical exposures can produce similar effects.

3.9 INTERACTIONS WITH OTHER CHEMICALS

The only information located that is relevant to environmental or occupational exposures is from a study by Buffler et al. (1978) in workers, even though it provides only suggestive evidence that interactions may have played a role in the outcome. In a cohort of 165 workers exposed intermittently to concentrations of 1,4-dioxane between 0.1 and 17 ppm, seven deaths were identified among those working in the manufacturing area and five among those involved in the processing area (Buffler et al. 1978). The exposure histories of the seven subjects indicated that all were exposed to other chemicals of possible significance at earlier times and for longer intervals than their exposure to 1,4-dioxane. In addition, the five deaths that occurred among the processing area were exposed to vinyl chloride simultaneously with their exposure to 1,4-dioxane. No firm conclusions can be drawn from this study regarding interactions of 1,4-dioxane with other chemicals.

If cytochrome CYP2E1 is involved in the metabolism of 1,4-dioxane (the cytochrome P-450 system is known to be involved in 1,4-dioxane metabolism), then ethanol could alter the hepatic effects of 1,4-dioxane if one assumes that the toxic entity is a metabolite of 1,4-dioxane. In the Thiess et al. (1976) study, some workers exposed to 1,4-dioxane who consumed alcohol frequently had elevated serum levels of transaminases; however, the values became normal after the workers reduced their alcohol consumption, suggesting that the elevated transaminase values were purely or largely primarily due to exposure to ethanol and not to the combination of 1,4-dioxane and ethanol, at least at the relative low level of exposure experienced by the workers in this occupational study (maximum 14.3 ppm).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to 1,4-dioxane than will most persons exposed to the same level of 1,4-dioxane in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of 1,4-dioxane, or compromised function of organs affected by 1,4-dioxane. Populations who are at greater risk due to their unusually high exposure to 1,4-dioxane are discussed in Section 6.7, Populations with Potentially High Exposures.

Because 1,4-dioxane is a liver and kidney toxicant at high concentrations, people with compromised liver or kidney function may be more susceptible to the effects of exposure to 1,4-dioxane than healthy individuals. Among those unusually susceptible would be, for example, individuals who drink excessive amounts of alcohol, those on medications known to affect the liver or the kidneys, or those with genetic or other diseases of the kidney.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to 1,4-dioxane. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to 1,4-dioxane. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

No information was found that provided specific information about treatment following exposure to 1,4-dioxane.

3.11.1 Reducing Peak Absorption Following Exposure

The only relevant information that was located is that the skin and eyes should be immediately flushed with water for at least 15 minutes following skin and eye contact (NIOSH 1977). If 1,4-dioxane is swallowed, vomiting should be induced immediately if the patient is conscious (NIOSH 1977).

3.11.2 Reducing Body Burden

No information was located regarding reducing body burden following exposure to 1,4-dioxane. As mentioned in Section 3.4, Toxicokinetics, 1,4-dioxane and its main metabolites do not accumulate and are rapidly eliminated from the body in the urine.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The liver and kidneys are targets for 1,4-dioxane toxicity in humans and animals. Lesions have been found in humans acutely exposed to relatively high concentrations of 1,4-dioxane and in animals following inhalation, oral, and dermal exposure (see Section 3.2). Also, 1,4-dioxane has induced liver cancer in rats and mice and nasal cancer in rats. The mechanism(s) of toxic action of 1,4-dioxane has not been elucidated, but there is increasing evidence that the liver lesions seen in animals evolve into neoplasms induced by 1,4-dioxane through a non-genotoxic mechanism of action. Any attempt to discuss possible mechanisms to interfere with this action would be pure speculation at this time.

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 1,4-dioxane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 1,4-dioxane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of 1,4-Dioxane

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 1,4-dioxane are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing

information concerning the health effects of 1,4-dioxane. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 3-5, there is limited information on the effects of 1,4-dioxane in humans. The available information is derived from occupational studies in which exposure was assumed to have been primarily by inhalation of vapors, but that may have also involved dermal exposure. These studies provided information on acute systemic effects and lethality and also effects due to long-term exposure. A few studies of controlled inhalation exposures with volunteers are also available and these studies provided data on acute systemic effects. No information was located regarding oral exposure of humans to 1,4-dioxane.

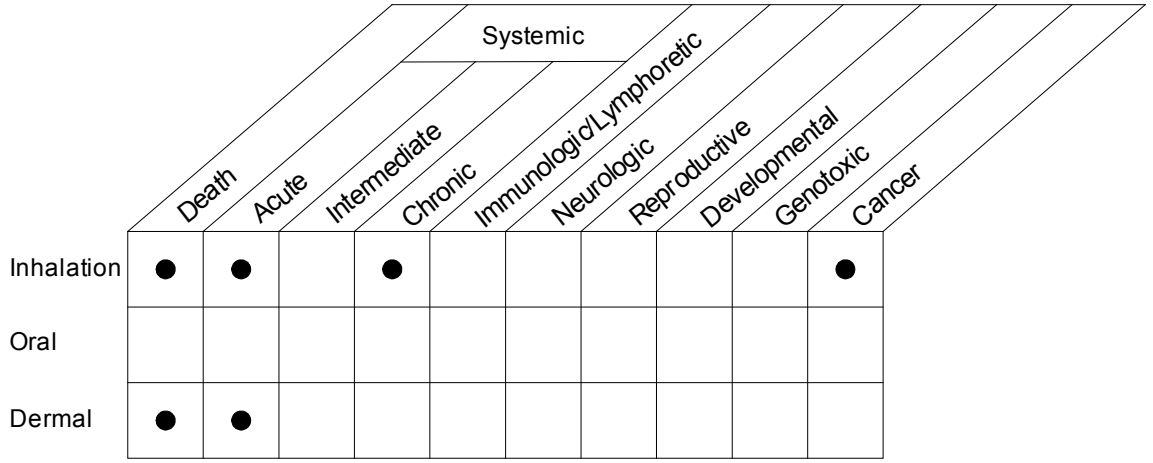
In animals, the studies available for review provided information on lethality and on systemic, neurological, and cancer effects following inhalation exposure to 1,4-dioxane. For oral exposure, there are studies that evaluated systemic, neurological, developmental, genotoxic, and cancer effects. No studies were available regarding chronic systemic effects, or immunological, neurological, reproductive, developmental, or genotoxic effects after dermal exposure to 1,4-dioxane.

The information available from human and animals studies suggests that the effects of 1,4-dioxane are not route-dependent. In addition, the limited environmental monitoring data available suggests that the levels of 1,4-dioxane to which the general population might be exposed through contact or use of consumer products (including food), or that are normally found in environmental media, are generally orders of magnitude lower than those used in studies with experimental animals.

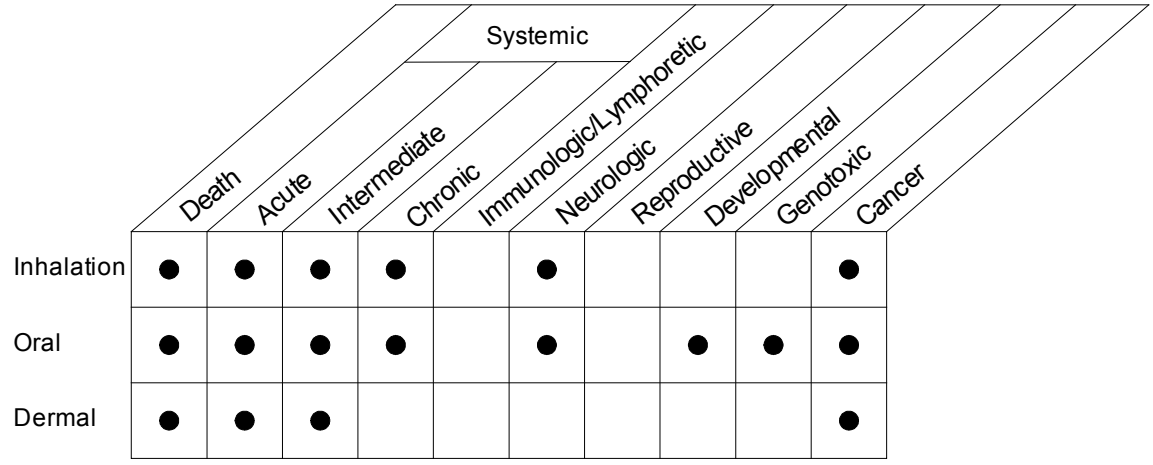
3.12.2 Identification of Data Needs

Acute-Duration Exposure. Two occupational studies provided acute inhalation data for 1,4-dioxane, Barber (1934) and Johnstone (1959). Barber (1934) described five lethal cases among factory workers exposed to 1,4-dioxane, whereas Johnstone (1959) described one additional lethal,

Figure 3-5. Existing Information on Health Effects of 1,4-Dioxane



Human



Animal

● Existing Studies

occupational case in which dermal exposure also occurred. Exposure to unknown, but lethal concentrations of 1,4-dioxane produced serious liver and kidney effects. A few additional studies in volunteers evaluated mostly clinical signs, such as eye and nose irritation, during exposures varying from 3 minutes to 6 hours (Ernstgård et al. 2006; Fairley et al. 1934; Silverman et al. 1946; Yant et al. 1930; Young et al. 1977). Ernstgård et al. (2006) also evaluated pulmonary function by spirometry immediately after and 3 hours after exposure of volunteers to 20 ppm 1,4-dioxane for 2 hours and reported no alterations relative to measurements before exposure. The lowest concentration that produced an effect in the studies mentioned above was 50 ppm during a 6-hour exposure, which caused eye irritation (Young et al. 1977). Data from the studies by Young et al. (1977) and Ernstgård et al. (2006) were used to derive an acute-duration inhalation MRL for 1,4-dioxane.

The animal database consists mainly of early studies in rodents exposed to lethal or near lethal concentration of 1,4-dioxane that indicated that the liver and kidneys are the main targets of 1,4-dioxane toxicity in animals (Fairley et al. 1934; Yant et al. 1930). Additional acute inhalation studies conducted according to current guidelines would be helpful to establish dose-response relationships for liver and kidney effects at low levels of exposure. No data were located regarding acute oral exposure of humans to 1,4-dioxane. Most studies in animals provided lethal dose levels and also showed that the liver and kidneys are the organs most severely affected by high oral doses of 1,4-dioxane (de Navasquez 1935; Kesten et al. 1939; Laug et al. 1939; Schrenk and Yant 1936; Smyth et al. 1941). A more recent 2-week drinking water study in rats, although with limitations, provided information on systemic end points but had limitations that precluded its use for derivation of an acute-duration oral MRL for 1,4-dioxane (JBRC 1998). Instead, a developmental study in rats was used as the basis for deriving an acute-duration oral MRL for 1,4-dioxane (Giavini et al. 1985).

Additional acute oral studies conducted according to current guidelines could provide information on thresholds for liver and kidney effects. Also, exposures to low or moderate single oral doses followed by long observation periods would provide information on reversibility of the effects. Limited acute dermal data were found. In the lethal occupational case described by Johnstone (1959), considerable dermal exposure occurred, since the subject used to wipe his hands with 1,4-dioxane to clean them; this probably contributed to the liver and kidney toxicity observed. In the studies with volunteers mentioned above, eye irritation was most likely due to direct contact of the eye with the vapors of 1,4-dioxane and not due to inhaled 1,4-dioxane. A study in rats applied a dose of 8,300 mg/kg of 1,4-dioxane to a shaved area of the skin found no signs of skin irritation during a 14-day observation period (Clark et al. 1984). Additional

acute dermal studies may be tied to studies of the pharmacokinetics of 1,4-dioxane by this route of exposure, which has not been well characterized.

Intermediate-Duration Exposure. No intermediate-duration studies in humans were available. An early study by Fairley et al. (1934) in several animal species provided enough information to determine that the liver and kidneys are targets for 1,4-dioxane toxicity. The lowest concentration of 1,4-dioxane to which rats, mice, and guinea pigs were exposed intermittently for 3–12 weeks was 1,000 ppm, which caused moderate to severe kidney toxicity. A 13-week inhalation study in rats (Kasai et al. 2008) reported nasal lesions in males and females exposed to ≥100 ppm and served as the basis for derivation of an intermediate-duration inhalation MRL for 1,4-dioxane. Several oral studies in animals provided information on lethal doses (Fairley et al. 1934; Kociba et al.1974) and on systemic effects, mostly hepatic and renal (Fairley et al. 1934; Lundberg et al. 1987; Stott et al. 1981). A more recent 90-day drinking water study in rats provided sufficient information on multiple end points and was used as the basis (liver effects) for an intermediate-duration oral MRL for 1,4-dioxane (Kano et al. 2008). Kano et al. (2008) also reported nasal lesions in rats. Further research to elucidate the mechanism by which oral exposure to 1,4-dioxane can cause nasal lesions is warranted. Information by the dermal route of exposure was limited to a study of intermittent application of 1,4-dioxane to the skin of rabbits and guinea pigs for up to 101 days (Fairley et al. 1934). There were no dermal effects in either species at dose levels that induced liver and kidney lesions, which appeared to be more severe in rabbits than in guinea pigs. Although the study by Kasai et al. (2008) showed that the most sensitive target for 1,4-dioxane in rats following inhalation exposure is the nasal cavity, additional inhalation studies with lower concentrations of 1,4-dioxane and following current testing standards are needed to better define the threshold region. Information on effects on other organs is limited in intermediate-duration oral studies.

Chronic-Duration Exposure and Cancer. An occupational study of workers exposed to 1,4-dioxane provides information regarding long-term exposure to this chemical. Thiess et al. (1976) found no adverse effects in workers exposed to 0.006–14.3 ppm 1,4-dioxane for an average of 25 years. Two chronic-duration studies by the inhalation route are available (Kasai et al. 2009; Torkelson et al. 1974). The study by Torkelson et al. (1974) provided information on multiple organs and tissues and hematology parameters in rats exposed to 111 ppm 1,4-dioxane; no adverse effects were found. Since only one exposure concentration was tested, the NOAEL may be higher. In addition, the study did not explicitly indicate whether the nasal cavity was examined. The nasal cavity was a target for 1,4-dioxane in a 13-week inhalation study in rats (Kasai et al. 2008). The chronic-duration study by Kasai et al. (2009) also provided information on multiple organs and tissues, including the nasal cavity. The latter

was the most sensitive target and the increased incidence of vacuolic changes in the olfactory epithelium of the nasal cavity in male rats was used to derive a chronic-duration inhalation MRL for 1,4-dioxane. Additional studies in animals do not seem necessary at this time. Several chronic-duration oral studies in rats and mice are available (Kano et al. 2009; Kociba et al. 1974; NCI 1978). These studies provided information on clinical signs, changes in body weight, hematology, blood chemistry, urinalysis, and gross and microscopic appearance of major organs and tissues. The liver and kidneys were the main targets for 1,4-dioxane toxicity. A NOAEL of 9.6 mg/kg/day for liver effects in male Sherman rats was used to derive a chronic-duration oral MRL for 1,4-dioxane (Kociba et al. 1974). Additional chronic oral studies do not seem necessary at this point. No chronic dermal studies were located, but it is not apparent what new key information such studies could provide.

Very limited information was found regarding human exposure to 1,4-dioxane and cancer. A study of 165 workers exposed intermittently to 0.1–17 ppm 1,4-dioxane for up to 21 years found no significant increases in the incidences of deaths due to cancer (Buffler et al. 1978). 1,4-Dioxane was not carcinogenic in rats in the only single inhalation bioassay (Torkelson et al. 1974). However, only one exposure level was used; therefore, a dose-response relationship for cancer could not be estimated. In addition, the maximum tolerated dose (MTD) may have not been achieved. Increased incidences of squamous cell carcinoma in the nasal cavity and hepatocellular adenomas were reported in male rats exposed intermittently to 1,250 ppm 1,4-dioxane for 2 years (Kasai et al. 2009); exposure concentrations ≥250 ppm significantly increased the incidence of peritoneum mesothelioma. Long-term oral administration of 1,4-dioxane induced liver cancer in rats and mice, peritoneum mesothelioma in male rats, and also nasal tumors in rats (Argus et al. 1965, 1973; Hoch-Ligeti et al. 1970; Kano et al. 2009; Kociba et al. 1974; NCI 1978). 1,4-Dioxane was not a complete carcinogen in a 60-week dermal exposure study in mice (King et al. 1973), but showed promoter activity in oral (Lundberg et al. 1987) and dermal studies (King et al. 1973). 1,4-Dioxane was not an initiator in a dermal assay in mice (Bull et al. 1986). Since the mechanism of carcinogenicity of 1,4-dioxane is yet unknown, continued research on this topic and on the role of metabolism in carcinogenicity is necessary, particularly regarding a mechanism by which oral exposure to 1,4-dioxane can induce nasal tumors. Some have suggested that liver toxicity and subsequent tumor development in rats only occurs when metabolism of 1,4-dioxane is saturated. Under current EPA guidelines for assessing cancer risk (EPA 2005a), it might be more appropriate to apply a nonlinear model to cancer risk assessment. The EPA (IRIS 2011) derived an oral slope factor of $1x10^{-1}$ per mg/kg/day for 1,4-dioxane based on increased incidences of hepatocellular adenoma and carcinoma in female BDF_1 mice in a drinking water study (Kano et al. 2009).

Genotoxicity. The genotoxic effects of 1,4-dioxane have been well characterized in studies in microorganisms *in vitro* (Haworth et al. 1983; Hellmer and Bolcsfoldi 1992; Khudoley et al. 1987; Kwan et al. 1990; Morita and Hayashi 1998; Nestmann et al. 1984; Stott et al. 1981; Zimmermann et al. 1985) and in mammalian cells (Galloway et al. 1987; Goldsworthy et al. 1991; McGregor et al. 1991; Morita and Hayashi 1998; Sheu et al. 1988). Most of these studies were conducted both in the presence and absence of metabolic activation systems, which would suggest that metabolites of 1,4-dioxane are also not mutagenic. The results from *in vivo* studies also provided mostly negative evidence of genotoxicity (Goldsworthy et al. 1991; Kitchin and Brown 1990, 1994; McFee et al. 1994; Mirkova 1994; Morita and Hayashi 1998; Muñoz and Barnett 2002; Stott et al. 1981; Tinwell and Ashby 1994; Yoon et al. 1985). The total weight of evidence suggests that 1,4-dioxane is either weakly genotoxic or not genotoxic, and it is unlikely that further studies will provide new information.

Reproductive Toxicity. No reliable information was located regarding reproductive effects of 1,4-dioxane in humans. There are studies that examined the gross and microscopic appearance of the reproductive organs from rats following intermediate (Kasai et al. 2008) chronic inhalation exposure (Torkelson et al. 1974) and from rats and mice following intermediate oral exposure (Kano et al. 2008) and chronic oral exposure to 1,4-dioxane (JBRC 1998b; Kociba et al. 1974; NCI 1978), but no assessments of reproductive function or examinations of sperm characteristics have been made. The lack of effects on reproductive organs observed in these studies diminishes the need to conduct a 2-generation reproductive study. In addition, only one study was located that tested the estrogenic properties of 1,4-dioxane in an assay *in vitro* (Nishihara et al. 2000), with negative results. Additional standard *in vivo* and *in vitro* studies to assess whether 1,4-dioxane has endocrine disruptor properties would be useful.

Developmental Toxicity. There is no information on developmental effects in humans exposed to 1,4-dioxane. If populations were identified that are exposed to high levels of 1,4-dioxane, it would be useful to determine whether 1,4-dioxane or metabolites are found in breast milk. This can also be done in surveys monitoring chemicals in the general population at the national level. Only one study was located that evaluated developmental parameters in rats exposed orally by gavage during gestation (Giavini et al. 1985). Slight fetotoxicity was seen at a dose level that affected the mothers. The study by Giavini et al. (1985) was used as the basis for derivation of an acute-duration oral MRL for 1,4-dioxane. Additional studies are necessary to determine whether adverse developmental effects can occur without maternal toxicity. In addition, a developmental neurotoxicity study in rats in which pups are tested at various ages after being exposed *in utero* and/or via maternal milk would fill a data gap.

Immunotoxicity. Virtually no information was located regarding immunotoxic effects in humans following exposure to 1,4-dioxane. Ernstgård et al. (2006) reported that exposure of volunteers to 20 ppm 1,4-dioxane did not cause inflammatory changes as monitored by measurements of high sensitivity C reactive protein and interleukin 6 in blood. This information is clearly insufficient to determine whether exposure to 1,4-dioxane affects the immune system in humans. The information from animal studies is restricted to gross and microscopic examination of the spleen, thymus, and lymph nodes from rats exposed intermittently to up to 3,200 ppm 1,4-dioxane for 13 weeks (Kasai et al. 2008), of the lymph nodes and spleen from rats similarly exposed to 111 ppm 1,4-dioxane vapors for 2 years (Torkelson et al. 1974), and of the lymph nodes, spleen, and thymus from rats and mice dosed with up to 2,669 mg 1,4-dioxane/kg/day in the drinking water for 13 weeks (Kano et al. 2008), or in rats and mice dosed with up to 1,599 mg 1,4-dioxane/kg/day in the drinking water for up to 2 years (JBRC 1998b; Kociba et al. 1974; NCI 1978). No treatment-related effects were observed. Although there was no indication that immunocompetence was compromised in these studies, a study performing a complete Tier I battery of tests may be warranted to evaluate the possibility that exposure to 1,4-dioxane might cause subtle alterations in immune parameters.

Neurotoxicity. Edema of the brain was observed in lethal cases of intoxication with 1,4-dioxane vapors (Barber 1934; Johnstone 1959). Occupational studies of long-term exposure to lower concentrations of 1,4-dioxane did not report signs or symptoms that would indicate neurological damage, but sensitive tests were not conducted (Buffler et al. 1978; Thiess et al. 1976). Exposure of mice and rats for 4 hours to 1,800–2,400 ppm 1,4-dioxane had a narcotic effect (Frantik et al. 1994), and exposure to 3,000 ppm intermittently for 2 weeks affected an avoidance response in rats (Goldberg et al. 1964), which also could have been due to narcosis. High oral doses also induced narcosis in rabbits (Knoefel 1935). Vacuolar changes were observed in the brain from rats exposed to 2,750–2,960 mg 1,4-dioxane/kg/day for 2 weeks (JBRC 1998) and from rats exposed to 1,554–1,614 mg/kg/day for 13 weeks (Kano et al. 2008). Long-term inhalation (Kasai et al. 2008; Torkelson et al. 1974) and oral studies (JBRC 1998b; Kano et al. 2008; Kociba et al. 1974; NCI 1978) in rats and mice have provided no indication of adverse clinical signs in the animals, and examination of the brain, spinal cord, and sciatic nerve was unremarkable. The overall information suggests that 1,4-dioxane may have narcotic properties at high concentrations, but it would be useful to determine whether possible subtle behavioral effects can be detected with more sensitive tests at exposure concentrations that do not induce narcosis.

Epidemiological and Human Dosimetry Studies. Information on the health effects of 1,4-dioxane in humans is derived from cases of accidental exposure at work to relatively high

concentrations of 1,4-dioxane, which caused death (Barber 1934; Johnstone 1959), and studies of long-term exposure, also at work, to lower concentrations of 1,4-dioxane (Buffler et al. 1978; Thiess et al. 1976). Follow-up evaluations of individuals who may have been occupationally exposed would provide valuable information. No specific group from the general population that may have been subjected to unusually high amounts of 1,4-dioxane was identified. If such a situation arises, for example due to an accidental spill or leak from a waste site resulting in contaminated water or soil, individuals potentially exposed to 1,4-dioxane should be monitored for liver and kidney effects with standard function tests, since the liver and the kidneys have been identified as targets for 1,4-dioxane toxicity.

Biomarkers of Exposure and Effect.

Exposure. 1,4-Dioxane and its main metabolite, HEAA, have been identified in the blood and urine from workers exposed to 1,4-dioxane vapors (Young et al. 1976) and from volunteers exposed to controlled amounts 1,4-dioxane vapors (Young et al. 1977). Under condition of low to moderate exposure, the transformation of 1,4-dioxane to HEAA is rapid and extensive, and HEAA is rapidly eliminated in the urine (Young et al. 1977). The development of models that would support quantitative estimates of exposure to 1,4-dioxane based on urine levels of HEAA may be valuable in cases of high exposure, but given the very low levels of 1,4-dioxane to which the general population is exposed, the development of analytical methods capable to detect and quantify HEAA in the general population may be more useful.

Effect. There are no biomarkers of effect specific for 1,4-dioxane. Exposure to high amounts of 1,4-dioxane affects the liver and kidneys, but no 1,4-dioxane-induced health effects have been reported in populations exposed to low amounts of 1,4-dioxane (Buffler et al. 1978; Thiess et al. 1976). Research to identify reliable biomarkers for exposure to 1,4-dioxane in humans would be useful in order to evaluate the prevalence and magnitude of exposure in an at-risk population.

Absorption, Distribution, Metabolism, and Excretion. Among the areas of absorption, distribution, metabolism, and excretion, the greatest data need lies in metabolism, specifically, the determination of the metabolic pathways involved in the metabolism of 1,4-dioxane to its primary metabolite, HEAA or 1,4-dioxane-one (Braun and Young 1977; Woo et al. 1977a, 1977b, 1977c; Young et al. 1977). While the identity of the metabolite has been determined and the involvement of cytochrome P-450 enzymes has been demonstrated (Nannelli et al. 2005; Woo et al. 1977c, 1978), the formation of intermediate metabolites, and their identities, has not been demonstrated. Additional information regarding this pathway may be useful in the refinement of PBPK models and in the development of

biomarkers of exposure and/or effect. Data are lacking on the absorption of 1,4-dioxane in humans following oral exposure and dermal exposure *in vivo*, but this information would likely do little to further our understanding of the pharmacokinetic processes of 1,4-dioxane.

Comparative Toxicokinetics. Studies directly comparing the toxicokinetics of 1,4-dioxane across species are not available. Some limited data on 1,4-dioxane absorption following inhalation exposure suggest large differences in the absorbed dose, expressed on a per body weight basis, between rats and humans (Young et al. 1977, 1978a, 1978b). However, these studies did not measure absorption efficiencies. Studies examining absorption efficiency in humans and rats following inhalation and oral exposures would provide valuable data for evaluating possible species differences. The available data on metabolism and elimination of 1,4-dioxane in humans and rats indicate that the compound behaves similarly in the two species (Woo et al. 1977a, 1977b, 1977c, 1978; Young et al. 1976, 1977, 1978a, 1978b). Studies of the toxicokinetic behavior of 1,4-dioxane in animal species other than the rat would provide additional insight into potential interspecies differences, while studies directly comparing the toxicokinetic behavior of 1,4-dioxane in multiple species would add to our understanding of the comparative toxicokinetic behavior of 1,4-dioxane. Limitations of the available PBPK models (Leung and Pastenbauch 1990; Reitz et al. 1990; Sweeney et al. 2008) precluded their use for MRL derivation; further refinement of these models is necessary.

Methods for Reducing Toxic Effects. No specific methods for the mitigation of effects of acute exposure to 1,4-dioxane were located other than measures to support vital functions. No information was located concerning mitigation of effects of lower-level or longer-term exposure to 1,4-dioxane. This, in part, may reflect the fact that no population has been identified as having been subjected or currently undergoing exposure to excessive amounts of 1,4-dioxane. Attempts to propose studies of specific methods to reduce possible adverse effects do not appear warranted at this time.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures and developmental effects expressed either prenatally or during childhood are discussed in detail in the Developmental Toxicity subsection above.

There are no studies that specifically addressed exposure to 1,4-dioxane in children. Workers exposed to high amounts of 1,4-dioxane vapors experienced liver and kidney effects and some died (Barber 1934; Johnstone 1959). Volunteers exposed to low concentrations of 1,4-dioxane in the air experienced eye and nose irritation (Silverman et al. 1946; Yant et al. 1930; Young et al. 1977). It is reasonable to assume that

children exposed in similar manners will experience similar effects. There is no information on whether the developmental process is altered in humans exposed to 1,4-dioxane. Very limited evidence with 1,4-dioxane in rats suggests that fetotoxicity may occur only at maternally toxic levels (Giavini et al. 1985), but further studies are necessary on this issue. The possibility that 1,4-dioxane may have endocrine-disrupting ability in mammals has not been systematically studied.

There are no data to evaluate whether pharmacokinetics of 1,4-dioxane in children are different from adults. There is no information on whether 1,4-dioxane can cross the placenta and there are no studies on whether 1,4-dioxane can be transferred from mother to offspring through maternal milk. Cross-fostering studies can provide important information regarding the role of *in utero* vs. lactation exposure to 1,4-dioxane in normal development. There are no data to permit an evaluation of whether metabolism of 1,4-dioxane is different in children from adults.

Research into the development of sensitive and specific biomarkers of exposures and effects for 1,4-dioxane would be valuable for both adults and children. There are no data on the interactions of 1,4-dioxane with other chemicals in children. There are no pediatric-specific methods to reduce peak absorption 1,4-dioxane, to reduce body burdens, or to interfere with the mechanisms of action. Based on the information available, it is reasonable to assume that the supportive methods recommended for maintaining vital functions in adults will also be applicable to children.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

No ongoing studies pertaining to 1,4-dioxane were identified in the Federal Research in Progress (FEDRIP 2009) database.

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4. CHEMICAL AND PHYSICAL INFORMATION

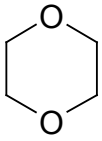
4.1 CHEMICAL IDENTITY

1,4-Dioxane or para-dioxane is also commonly referred to as simply ‘dioxane’. However, 1,4-dioxane should not be confused with dioxin (or dioxins), which are a different class of chemical compounds. Information regarding the chemical identity of 1,4-dioxane is located in Table 4-1.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

1,4-Dioxane is a colorless liquid. 1,4-Dioxane is also completely miscible in water and organic solvents. The technical-grade product is >99.9% pure, but may contain bis(2-chloroethyl) ether as an impurity (DeRosa et al. 1996). Information regarding the physical and chemical properties of 1,4-dioxane is located in Table 4-2.

Table 4-1. Chemical Identity of 1,4-Dioxane

Characteristic	Information
Chemical name	1,4-Dioxane
Synonym(s)	1,4-diethylenedioxide; 1,4-dioxacyclohexane; 1,4-dioxanne (French); di(ethylene oxide); diethylene dioxide; diethylene ether; dioksan (Polish); diossano-1,4 (Italian); dioxaan-1,4 (Dutch); dioxan; dioxan-1,4 (German); dioxane; dioxane-1,4; dioxanne (French); dioxyethylene ether; glycol ethylene ether; para-dioxane; *p*-dioxan (Czech); *p*-dioxane; *p*-dioxin, tetrahydro-; tetrahydro-1,4-dioxin; tetrahydro-para-dioxin; tetrahydro-*p*-dioxin
Registered trade name(s)	No data
Chemical formula	$C_4H_8O_2$
Chemical structure	
Identification numbers:	
CAS Registry	123-91-1
NIOSH RTECS	JG8225000
EPA Hazardous Waste	U108; A toxic waste when a discarded commercial chemical product or manufacturing chemical intermediate or an off-specification commercial chemical product or a manufacturing chemical intermediate
OHM/TADS	No data
DOT/UN/NA/IMDG	UN 1165; IMDG 3.2
HSDB	81
NCI	No data

CAS = Chemical Abstracts Services; CIS = Chemical Information System; DOT/UN/NA/IMDG = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substance Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Physical and Chemical Properties of 1,4-Dioxane

Property	
Molecular weight (g/mol)	88.11[a]
Color	Clear[b]
Physical state	Liquid[a]
Melting point	11.8 °C[a]
Boiling point	101.1 °C[a]
Density	1.0329[a]
Odor	Faint pleasant odor[a]
Odor threshold:	
Water	230 ppm w/v[b]
Air	24 ppm v/v[b]
Taste	No data
Solubility:	
Water	Miscible[c]
Other solvents	Soluble in organic solvents[a]
Partition coefficients:	
Log K_{ow}	-0.27[d]
Log K_{oc}	1.23[b]
Vapor pressure at 25 °C	38.1 mm Hg[e]
OH radical rate constant	1.09×10^{-11} cm^3/molecule-sec[f]
Henry's law constant at 25 °C	4.80×10^{-6} atm-cm^3/mole[g]
Autoignition temperature	356 °F (180 °C)[h]
Flashpoint	5–18 °C[a]
Flammability limits at 25 °C	Lower: 2.0%; Upper: 22%[b]
Incompatibilities	Strong oxidizers, decaborane, triethynyl aluminum[h]
Conversion factors (25 °C and 1 atm)	1 ppm = 3.6 mg/m^3; 1 mg/m^3 = 0.278 ppm[b]
Explosive limits	Vapor forms explosive mixtures with air over wide range[i]

[a]O'Neil et al. 2001.
[b]EC 2002.
[c]Riddick et al. 1986.
[d]Hansch et al. 1995.
[e]Daubert and Danner 1985.
[f]Atkinson 1989.
[g]Park et al. 1987.
[h]NIOSH 2001.
[i]ScienceLab 2005.

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5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

1,4-Dioxane is manufactured in a closed system by acid catalyzed conversion of diethylene glycol via dehydration and ring closure. The use of mono-, tri-, and polyethylene glycol and their ethers as raw materials has also been reported. Concentrated sulfuric acid (ca. 5%) is used as the acid catalyst, although phosphoric acid, *p*-toluenesulfonic acid, strongly acidic ion-exchange resins, and zeolites are alternatives. Operating conditions vary; temperatures range from 130 to 200 °C, and pressures range from a partial vacuum to slight pressure (i.e., 188–825 mm Hg). The ideal temperature is reported to be 160 °C. The reaction process is continuous and carried out in a heat vessel. The raw 1,4-dioxane product forms an azeotrope with water, which is then vaporized from the reaction vessel by distillation. 1,4-Dioxane vapors are passed through an acid trap and two distillation columns to remove water and purify the product. Yields of ca. 90% are achievable. 2-Methyl-1,3-dioxolane, 2-ethyl-1,3-dioxolane, and acetaldehyde are the main by-products. To a lesser extent, crotonaldehyde and polyglycol are also formed during the production. The crude 1,4-dioxane is further cleaned by heating with acids, distillation (to remove glycol and acetaldehyde), salting out with NaCl, $CaCl_2$, or NaOH, and fine subsequent distillation (EC 2002; Surprenant 2002).

While the latter production process is the most important industrially, two other processes are especially useful for the production of substituted dioxanes. 1,4-Dioxane can be prepared by ring closure of 2-chloro-2'-hydroxydiethyl ether (formed from ethylene glycol reacting with 1,2-dibromoethane) through heating with 20% sodium hydroxide, and by catalysed cyclo-dimerisation of ethylene oxide either over $NaHSO_4$, SiF_4, or BF_3, or at an elevated temperature with an acidic cation-exchange resin (EC 2002; Surprenant 2002).

Commercial production of 1,4-dioxane in the United States was first reported in 1951, but semi-commercial quantities were available in 1929 (NCI 1985). Currently, 1,4-dioxane is produced in the United States by two manufacturers: Dow Chemical (production site, Freeport, Texas) and Ferro Corporation (production site, Baton Rouge, Louisiana) (SRI 2003). Outside of the United States, 1,4-dioxane is produced by BASF AG in Lugwigshafen, Germany, Osaka Yuki and Toho Chem, Japan; and also in other countries around the world (EC 2002).

Recent information was not available on the production volumes of 1,4-dioxane in the United States. The total production of 1,4-dioxane for 1982 was estimated at 15 million pounds (6,800 metric tons), up from

12 million pounds (5,400 metric tons) reported in 1977 (HSDB 2010). Between 1 and 10 million pounds of 1,4-dioxane were produced in the United States in 2002 (HSDB 2010). The worldwide production capacity in 1985 was estimated to be 11,000–14,000 metric tons/year. In 1995, the production capacity of known producers and the worldwide production volume were estimated at 8,000 and 10,000 metric tons/year, respectively. In Europe, the production volume in 1997 was estimated to be 2,000–2,500 metric tons (EC 2002). However, current production levels of 1,4-dioxane are expected to be significantly less due to changing use patterns.

Table 5-1 lists the facilities in each state that manufacture or process 1,4-dioxane, the intended use, and the range of maximum amounts of 1,4-dioxane that are stored on-site. There are 381 facilities that produce or process 1,4-dioxane in the United States. The data from the Toxics Release Inventory (TRI) listed in Table 5-1 should be used with caution, however, since only certain types of facilities were required to report (EPA 1995). This is not an exhaustive list (TRI07 2009).

5.2 IMPORT/EXPORT

No information was located on the current import/export levels of 1,4-dioxane for the United States. In 1977, at least $9.1x10^4$ kg of 1,4-dioxane were imported into the United States (HSDB 2010). However, current import levels of 1,4-dioxane are expected to be significantly less due to changing use patterns.

5.3 USE

Because of its broad range of solvent properties, 1,4-dioxane has found a variety of applications. 1,4-Dioxane is used as a solvent for chemical processing. 1,4-Dioxane has also been used as a laboratory reagent (e.g., mobile phase in chromatography); in plastic, rubber, insecticide, and herbicides; as a chemical intermediate; as part of a polymerization catalyst; and as an extraction medium of animal and vegetable oils. Other minor uses are in the manufacture of membrane filters, for measuring optical activity, and for cryoscopic determination. 1,4-Dioxane has been reported to be used in the production processes of the following product categories: pharmaceuticals/pesticides, magnetic tape, and adhesives. In the past, 1,4-dioxane was used primarily as a stabilizer in chlorinated solvents, particularly 1,1,1-trichloroethane. Approximately 90% of former production of 1,4-dioxane was used in this application. 1,4-Dioxane was typically used at a concentration of about 3.5% in chlorinated solvents. However, at the end of 1995, the use of 1,1,1-trichloroethane was limited under the Montreal Protocol due to the ozone depletion potential of 1,1,1-trichloroethane. Thus, current use of 1,4-dioxane as a stabilizer of 1,1,1-trichloroethane will not be significant (EC 2002; Hartung 1989; HSDB 2010; NICNAS 1998).

Table 5-1. Facilities that Produce, Process, or Use 1,4-Dioxane

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AL	6	1,000	999,999	1, 5, 6, 7, 10, 11
AR	6	1,000	999,999	1, 5, 7, 10, 12
AZ	1	1,000	9,999	12
CA	34	0	99,999	2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13
CO	1	1,000	9,999	10
CT	5	100	99,999	1, 2, 3, 5, 6, 8, 10
GA	3	0	9,999	1, 5, 7
IA	4	0	99,999	8, 12
IL	14	100	9,999,999	1, 2, 4, 5, 7, 9, 10, 11, 12
IN	12	0	99,999	2, 7, 10, 11, 12
KS	2	0	999	1, 2, 3, 7, 11
KY	2	100	999	7, 12
LA	23	0	49,999,999	1, 4, 5, 7, 9, 11, 12, 13
MA	5	1,000	99,999	7, 10, 11, 12
MD	1	1,000	9,999	7
ME	2	100	9,999	11, 12
MI	10	0	99,999	7, 9, 11, 12
MN	9	0	99,999	1, 2, 3, 4, 5, 7, 10, 11, 12, 13
MO	15	0	999,999	1, 3, 5, 6, 7, 8, 9, 10, 12, 13
MS	12	0	999,999	1, 2, 5, 6, 7, 9, 11, 12
NC	21	0	9,999,999	1, 2, 5, 6, 7, 9, 12, 13, 14
NE	2	10,000	99,999	12
NH	2	100	99,999	11
NJ	6	0	99,999	1, 2, 4, 5, 7, 9, 10, 12, 14
NY	17	100	99,999	1, 5, 7, 9, 10, 11, 12, 13
OH	21	0	999,999	1, 5, 7, 9, 10, 11, 12
OK	2	100	9,999	7, 8
OR	2	1,000	99,999	10
PA	9	100	99,999	7, 9, 10, 11, 12
PR	4	0	999,999	10, 12
SC	23	0	9,999,999	1, 5, 6, 7, 9, 11, 12, 13, 14
TN	16	0	99,999	1, 5, 10, 12, 13, 14
TX	41	0	9,999,999	1, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14
UT	10	0	999,999	7, 10, 11, 12
VA	7	0	99,999	1, 2, 5, 10, 12
WA	1	1,000	9,999	11
WI	9	0	999,999	1, 5, 7, 9, 10, 11, 12, 13

Table 5-1. Facilities that Produce, Process, or Use 1,4-Dioxane

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
WV	20	0	999,999	1, 3, 5, 6, 7, 8, 12, 13, 14
WY	1	0	99	12

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

Source: TRI07 2009 (Data are from 2007)

1,4-Dioxane has been found as an impurity in cosmetics, household and industrial detergents, and pharmaceuticals due to its occurrence as a by-product in ethoxylated emulsifiers (Hartung 1989). Currently, most manufacturers utilize vacuum stripping to remove 1,4-dioxane before formulation of ethoxylated surfactants in consumer cosmetics and household products (EC 2002).

5.4 DISPOSAL

The primary method of disposal of 1,4-dioxane is by incineration. Small amounts of 1,4-dioxane can be diluted with large amounts of water and subsequently discharged to waste water treatment plants (United Nations 1985). However, since 1,4-dioxane does not undergo significant biodegradation in waste water treatment plants, much of the 1,4-dioxane disposed by this method will end up in the environment.

In contrast to biological or physical methods, chemical treatment has been found to be highly effective for the removal of 1,4-dioxane from water. 1,4-Dioxane is rapidly degraded by hydrogen peroxide in combination with a ferrous salt. Chlorination has also been found to be highly effective for the removal of 1,4-dioxane from water. For example, chlorine and hypochlorous acid are capable of oxidizing 1,4-dioxane (Dow Chemical Co. 1989). However, the extent to which 1,4-dioxane is removed from waste streams by these methods is unknown.

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6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

1,4-Dioxane has been identified in at least 31 of the 1,689 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2007). However, the number of sites evaluated for 1,4-dioxane is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, all are located within the United States.

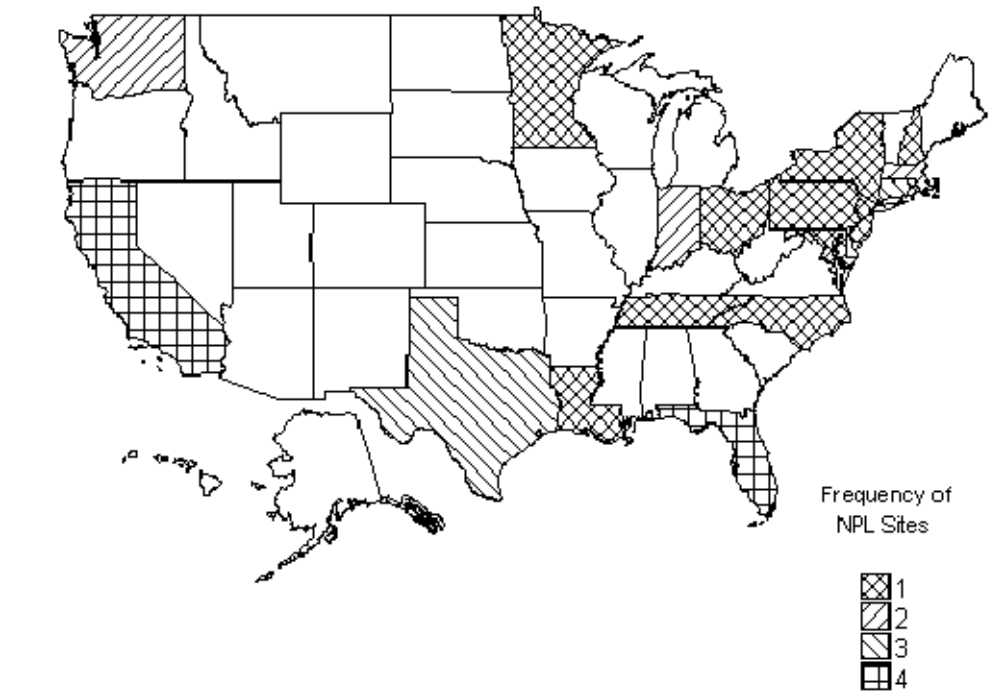
1,4-Dioxane is released into the environment during its production, the processing of other chemicals, its use, and with its unintentional formation during the manufacture of ethoxylated surfactants (EC 2002). In the past, 1,4-dioxane was released into the environment with its use as a stabilizer for 1,1,1-trichloro-ethane (TCA). Since the use of TCA has been discontinued, current releases from this source are expected to be very low.

Given its vapor pressure and Henry's Law constant, the rate of volatilization of 1,4-dioxane from water and soil surfaces is expected to be moderate. In air, it is subject to photooxidation with an estimated half-life of 1–3 days. 1,4-Dioxane biodegrades very slowly in water and soils and is considered recalcitrant. It adsorbs weakly to soil and will move quickly into groundwater. Bioconcentration, bioaccumulation, and biomagnification are not considered important environmental fate processes for 1,4-dioxane.

Current levels of 1,4-dioxane in the environment are unavailable. Historical data (i.e., 1980s or earlier) suggest that ambient levels were 0.1–0.4 μg/m^3 in air and 1 μg/L in water. Higher concentrations of 1,4-dioxane have been observed primarily in groundwaters.

The general population is exposed to negligible levels of 1,4-dioxane. The primary routes of human exposure to 1,4-dioxane are inhalation of 1,4-dioxane in air, ingestion of contaminated food and drinking water containing 1,4-dioxane, and dermal contact with contaminated consumer products (e.g., products containing ethoxylated surfactants). Because 1,4-dioxane may be found in tap water, human exposure to 1,4-dioxane may also occur during activities such as showering, bathing, and laundering. Occupational exposure occurs during the production, processing, and use of 1,4-dioxane, which results in inhalation or dermal exposure.

Figure 6-1. Frequency of NPL Sites with 1,4-Dioxane Contamination



Derived from HazDat 2007

6.2 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005b). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ 10 or more full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005b).

1,4-Dioxane is released into the environment during its production, processing, use, and with its unintentional formation during the manufacture of ethoxylated surfactants (EC 2002). In the past, 1,4-dioxane was released into the environment with its use as a stabilizer for TCA. Since the use of TCA has been discontinued, current releases from this source are expected to be very low.

1,4-Dioxane is unintentionally formed as an impurity during the manufacture of alkyl ether sulphates (AES) and other ethoxylated substances. However, much of the 1,4-dioxane impurity in these chemicals is removed through a stripping process during their manufacture. The stripper condensates from the manufacturing processes are discharged through normal plant effluents, where they are diluted by other waste streams and discharged as industrial wastes (NICNAS 1998). 1,4-Dioxane remaining as a by-product in end-use products (a large percentage of which may be used in domestic detergents and personal care products) will be released to publicly owned treatment works (POTWs) along with the surfactants, although this release will be far more diffuse.

According to the TRI, a total of 182,338 pounds (82,693 kg) of 1,4-dioxane were released to the environment in 2007 from facilities required to report to the TRI (TRI07 2009). In addition, an estimated 2,794 pounds (361 kg) were transferred off-site, including to POTWs (TRI07 2009). The TRI data should

be used with caution, because only certain types of facilities are required to report. This is not an exhaustive list. Since 1988, total on-site releases of 1,4-dioxane appear to be decreasing from a high of 1,234,968 pounds (560,172 kg) in 1993 to a low of 182,338 (82, 707) pounds in 2007.

6.2.1 Air

Estimated releases of 125,341 pounds (~57 metric tons) of 1,4-dioxane to the atmosphere from 45 domestic manufacturing and processing facilities in 2007 accounted for about 69% of the estimated total on-site environmental releases from facilities required to report to the TRI (TRI07 2009). These releases are summarized in Table 6-1.

1,4-Dioxane may be released to air during its production, the processing of other chemicals (e.g., pharmaceuticals/pesticides), and its use (EC 2002). The total emissions of 1,4-dioxane from stationary sources in California are estimated to be at least 210,000 pounds per year, based on data reported under the Air Toxics "Hot Spots" Program (California ARB 1997).

No further information was located on the emissions of 1,4-dioxane to air.

1,4-Dioxane has been identified in air samples collected at 6 of the 1,689 NPL hazardous waste sites, where it was detected in some environmental media (air, groundwater, surface water, soil, and sediment) (HazDat 2007).

6.2.2 Water

Estimated releases of 56,996 pounds (~26 metric tons) of 1,4-dioxane to surface water from 45 domestic manufacturing and processing facilities in 2006 accounted for about 31% of the estimated total on-site environmental releases from facilities required to report to the TRI (TRI07 2009). An additional 2,794 pounds (~1 metric ton) were transferred off-site, which included releases to POTWs (TRI07 2009). These releases are summarized in Table 6-1.

1,4-Dioxane may be released to surface water and groundwater during its production, the processing of other chemicals, its use, and with its unintentional formation during the manufacture of ethoxylated surfactants (EC 2002).

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use 1,4-Dioxane[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AL	1	1,038	0	0	0	0	1,038	0	1,038
AR	2	7,383	49	0	0	490	7,432	490	7,922
CA	2	2,554	0	0	0	0	2,554	0	2,554
CO	1	1,000	0	0	0	0	1,000	0	1,000
CT	1	0	No data	0	0	0	0	0	0
IL	1	6,209	0	0	2	0	6,211	0	6,211
LA	4	3,702	13,711	0	3	840	17,413	843	18,256
MI	1	0	No data	0	0	0	0	0	0
MN	1	1,950	No data	0	0	870	1,950	870	2,820
MO	2	20	1	0	0	0	21	0	21
MS	1	1,508	0	0	2	0	1,508	2	1,511
NC	4	20,230	4,926	0	12	0	25,156	12	25,168
OH	1	250	0	0	500	0	250	500	750
OR	1	908	0	0	24	0	908	24	932
PA	1	1,660	No data	0	0	0	1,660	0	1,660
PR	1	156	0	0	34	0	156	34	190
SC	8	38,818	13,876	0	18	0	52,694	18	52,713
TN	2	7,840	23,167	0	0	0	31,007	0	31,007
TX	5	30,088	1,266	0	0	0	31,354	0	31,354
WI	2	10	0	0	0	0	10	0	10

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use 1,4-Dioxane[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
WV	2	16	0	0	0	0	16	0	16
Total	44	125,341	56,996	0	596	2,200	182,338	2,794	185,132

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, waste water treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other on-site landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI07 2009 (Data are from 2007)

1,4-Dioxane was detected at 1 µg/L in effluents from the North Side and Calumet sewage treatment plants on the Lake Michigan Basin (Konasewich et al. 1978). 1,4-Dioxane has been detected in discharges into Lake Michigan near Chicago in 1977 (Konasewich et al. 1978) and in the Haw River in North Carolina (Dietrich et al. 1988). However, no information about the concentration of 1,4-dioxane or detection limit was provided in these sources.

The U.S. Geological Survey (USGS) sampled groundwater and surface water in the vicinity of a former waste-oil refinery near Westville, Indiana from 1997 to 2000 (USGS 2002). The site was operational from the mid 1930s until 1987, and included numerous storage tanks, a filter press, several cracking towers, a cannery, and waste oil storage lagoons. 1,4-Dioxane concentrations in the groundwater plume ranged from 3 to 31,000 µg/L and eventually discharged to a ditch approximately half a mile away from the refinery. 1,4-Dioxane levels ranged from 8 to 140 µg/L in the surface water collected from the network of ditches surrounding the site.

Effluent of a sewage treatment plant discharging into the River Lee (United Kingdom) contained <1 ng/L in 8-hour mixed samples (EC 2002). Effluent of a sewage treatment plant from a polyethylene terephthalate (PET) manufacturing process contained 100 mg/L of 1,4-dioxane in 1995 (EC 2002).

In Kanagawa prefecture, Japan, Abe (1999) reported that 1,4-dioxane concentrations in effluents from chemical plants that used the compound as a solvent ranged from 0.4 to 4,020 µg/L the combined collection treatments of apartment houses and river basin sewage systems were 0.8–46 and 1.0–97 µg/L, respectively. No further data were located for emissions of 1,4-dioxane to water.

1,4-Dioxane has been identified in surface water and groundwater samples, collected at 5 and 18 sites, respectively, of the 1,689 NPL hazardous waste sites, where it was detected in some environmental media (HazDat 2007).

6.2.3 Soil

Estimated releases of 596 pounds (<1 metric ton) of 1,4-dioxane to soils from 45 domestic manufacturing and processing facilities in 2007 accounted for <1% of the estimated total on-site environmental releases from facilities required to report to the TRI (TRI07 2009). These releases are summarized in Table 6-1.

1,4-Dioxane may be released to soil during its production, the processing of other chemicals, its use, and with its unintentional formation during the manufacture of ethoxylated surfactants (EC 2002).

Between 1976 and 1985, Pall Life Sciences' (PLS) predecessor, Gelman Sciences in Ann Arbor, Michigan disposed of large quantities of waste water containing 1,4-dioxane on soil in a holding pond and through a waste injection well. 1,4-Dioxane was used as a solvent for cellulose acetate, a component of micro-porous filters. This chemical contaminated soil and rock layers and seeped into the groundwater. Disposal of this chemical in this way was stopped in 1986 (City of Ann Arbor 2003; MSU 2001). No further data were located for emissions of 1,4-dioxane to soil.

1,4-Dioxane has been identified in soil samples collected at 6 of the 1,689 NPL hazardous waste sites, where it was detected in some environmental media (HazDat 2007).

6.3 ENVIRONMENTAL FATE

1,4-Dioxane is expected to volatilize at a moderate rate from water and soil surfaces. In air, it is subject to photooxidation with an estimated half-life of 1–3 days. 1,4-Dioxane is relatively resistant to biodegradation in water and soils. It binds weakly to soils and will therefore move readily into groundwater. Bioconcentration, bioaccumulation, and biomagnification are not significant for 1,4-dioxane.

6.3.1 Transport and Partitioning

The Henry's law constant for 1,4-dioxane is 4.8×10^{-6} atm m^3/mole, which indicates that 1,4-dioxane is expected to volatilize from water surfaces (Park et al. 1987; Thomas 1990). Based on this Henry's law constant, the volatilization half-life from a model river (1 m deep, flowing 1 m/second, wind velocity of 3 m/second) is estimated as 7 days. The volatilization half-life from a model lake (1 m deep, flowing 0.05 m/second, wind velocity of 0.5 m/sec) is estimated as 56 days (EPA 2000b). The Henry's law constant for 1,4-dioxane also indicates that volatilization from moist soil surfaces may occur. The potential for volatilization of 1,4-dioxane from dry soil surfaces may exist based upon a vapor pressure of 38.1 mm Hg (Daubert and Danner 1985). Even though volatilization of 1,4-dioxane may occur, risks to human health from inhalation exposures are expected to be low under environmental conditions.

According to a classification scheme, an estimated K_{oc} value of 17 suggests that 1,4-dioxane is expected to have very high mobility in soil (Swann et al. 1983). This estimated K_{oc} value was calculated using a

log K_{ow} of -0.27 and a regression-derived equation (Hansch et al. 1995; Thomas 1990). In the absence of significant degradation processes (see Section 6.3.2), 1,4-dioxane is susceptible to leaching from soil into groundwater. In clay soils, 1,4-dioxane will not be adsorbed as a result of any specific interaction with the surface of clay minerals. However, 1,4-dioxane can get trapped in the interfacial region of clay soils due to its strong interaction with water molecules. This may result in a lower than expected mobility for 1,4-dioxane in clay soils (Zhang et al. 1990). Groundwater retardation factors (R_f) for 1,4-dioxane range from 1.0 to 1.6. These values indicate that 1,4-dioxane is expected to be a mobile compound (e.g., R_f for chloride=1.0, which is indicative of no retardation) in groundwater (Priddle and Jackson 1991).

According to a classification scheme, a bioconcentration factor (BCF) value of 3 for 1,4-dioxane suggests that the potential for bioconcentration in aquatic organisms is low (Franke et al. 1994). This estimated BCF (the BCF is the concentration of the chemical in fish tissues over concentration of chemical in water) was calculated using a log K_{ow} of -0.27 and a regression-derived equation (Hansch et al. 1995; Meylan et al. 1999). The results of an experimental bioconcentration study also reported very low BCF values (e.g., 0.2–0.7) for 1,4-dioxane (EC 2002). Therefore, bioconcentration, bioaccumulation, and biomagnification are unlikely to be significant for 1,4-dioxane.

6.3.2 Transformation and Degradation

6.3.2.1 Air

The primary loss mechanism for 1,4-dioxane in the atmosphere is photooxidation with OH radicals, while photolysis, reaction with ozone molecules, and reaction with nitrate radicals are insignificant in comparison (Grosjean 1990). The second-order rate constant for OH radical photooxidation of 1,4-dioxane is 1.09×10^{-11} cm^3/molecule-sec (Atkinson 1989). Using OH radical concentrations between 0.5×10^6 and 1.5×10^6 OH radicals/cm^3 and a 12-hour day, the atmospheric half-lives for 1,4-dioxane are 2.9 and 1.0 days, respectively. A reaction product from OH radical photooxidation is 2-oxodioxane (or c-$C_4H_7O_2$). The lifetime of this alkyl radical, 2-oxodioxane in air at 1 atm is 0.02 microseconds with respect to the addition of O_2 to give the corresponding peroxy radical (c-$C_4H_7O_2$)O_2. These radicals react rapidly ($t_{½}$=6 minutes based on NO concentration of 2.5×10^8 molecules/cm^3) with NO to produce NO_2 and by inference (c-$C_4H_7O_2$)O alkoxy radicals. The sole atmospheric fate of this alkoxy radical is decomposition *via* C-C bond scission, leading to the formation of ethylene glycol diformate (Platz et al. 1997). There are no known reactions for the *in situ* formation of 1,4-dioxane in the atmosphere (Grosjean 1990).

6.3.2.2 Water

Since 1,4-dioxane does not have functional groups that are susceptible to hydrolysis (Wolfe and Jeffers 2000), hydrolysis of 1,4-dioxane is not expected to occur in the environment. Since 1,4-dioxane does not adsorb light in the environmental spectrum (i.e., >290 nm), 1,4-dioxane is not expected to undergo direct photolysis in aqueous media. 1,4-Dioxane may undergo indirect photolysis by aqueous hydroxyl radicals near the water surface. The half-life for this reaction is 336 days at pH 7 (Anbar and Neta 1967). However, the extent of this reaction of OH radicals with 1,4-dioxane in the environment is unknown.

1,4-Dioxane has been found to be resistant to biodegradation (Alexander 1973; Dow Chemical Co. 1989; Fincher and Payne 1962; Heukelekian and Rand 1955; Mills and Stack 1954). Results of a biological oxygen demand (BOD) test for 1,4-dioxane indicate that negligible oxygen was consumed over a 20-day test period (Swope and Kenna 1950). Mills and Stack (1954) noted that degradation of 1,4-dioxane was not observed in cultures of sewage microorganisms exposed for 1 year to waste water treatment plant effluents adjusted to contain 1,4-dioxane at concentrations ranging from 100 to 900 mg/L. In a different study, microorganisms present in either municipal or industrial activated sludge were unable to degrade 1,4-dioxane during 2 days of continuous exposure to concentrations ranging from 10 to 100 mg/L (Dow Chemical Co. 1989). Accordingly, it appears that 1,4-dioxane will not undergo significant degradation in conventional biological treatment systems. Thus, 1,4-dioxane has been classified as not readily biodegradable, and it is not expected to rapidly biodegrade in the environment (Kawasaki 1980; Lyman et al. 1982).

Acclimated microbial cultures may be capable of degrading 1,4-dioxane under certain conditions. Roy et al. (1994) investigated the biodegradability of 1,4-dioxane in industrial wastes using microorganisms obtained from acclimated industrial waste. These authors found that pure 1,4-dioxane and industrial wastes containing 1,4-dioxane are biodegradable. Following a 10-day lag period, complete degradation of 150 mg/L of 1,4-dioxane was observed after 32 days of treatment using a electrolytic respirometer cell. However, partial degradation of 1,4-dioxane was observed at higher concentrations, which may have resulted from the build up of intermediates inhibitory to the biodegradation process (Roy et al. 1994, 1995). Zenker et al. (2000) reported that a mixed microbial culture enriched from a 1,4-dioxane contaminated aquifer was capable of aerobically degrading 1,4-dioxane in the presence of tetrahydrofuran (THF). No biodegradation of 1,4-dioxane was observed in the absence of THF, and the measured cell yield was similar during degradation of 1,4-dioxane with THF or with THF alone. This suggests that

1,4-dioxane was biodegraded via a co-metabolic process (i.e., transformation of a non-growth substance [1,4-dioxane] in the presence of a growth substrate [THF] or another transformable compound).

Zenker et al. (1999) reported that a mixed microbial culture enriched from a 1,4-dioxane contaminated soil was capable of aerobically degrading 1,4-dioxane in the presence of THF. 1,4-Dioxane and THF were added to the soil microcosm at a concentration of 200 mg/L under enhanced conditions, which included incubation at 35 °C and the addition of nitrogen, phosphorus, and trace minerals. Both 1,4-dioxane and THF were completely degraded within 100 days, while 1,4-dioxane alone degraded completely after 300 days of incubation. Microcosms incubated under ambient conditions exhibited no biodegradation of 1,4-dioxane or THF (Zenker et al. 1999).

6.3.2.3 Sediment and Soil

Limited information was located on the transformation and degradation of 1,4-dioxane in soils and sediment. Kelley et al. (2001) investigated the potential to enhance 1,4-dioxane biodegradation in both planted and unplanted soil, by adding the 1,4-dioxane-degrading actinomycete, *Amycolata* sp. CB1190. 1,4-Dioxane was not removed within 120 days in sterile controls or in viable microcosms not amended with CB1190. Popular root extract (40 mg/L as chemical oxygen demand [COD]) stimulated 1,4-dioxane degradation in bioaugmented soil, and 100 mg/L of 1,4-dioxane was removed within 45 days. Other co-substrates that enhanced 1,4-dioxane degradation by CB1190 included THF and 1-butanol, while glucose and soil extract did not affect 1,4-dioxane degradation (Kelley et al. 2001). While long-term enrichments eventually yield cultures of CB1190 that are capable of growth on 1,4-dioxane alone, THF appears to be the preferred growth substrate for CB1190 (Parales et al. 1994).

6.3.2.4 Other Media

Pure 1,4-dioxane is known to react with molecular oxygen at ambient temperatures to form peroxides and hydroperoxides in the course of long-term storage and handling (Howard and Ingold 1969). Peroxides are formed primarily with exposure to air and UV light. Formate esters are formed from subsequent transformations of peroxides and hydroperoxides by way of free-radical mechanisms (Jewett and Lawless 1980).

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to 1,4-dioxane depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of 1,4-dioxane in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on 1,4-dioxane levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable. The analytical methods available for monitoring 1,4-dioxane in a variety of environmental media are detailed in Chapter 7.

Recent information on the levels of 1,4-dioxane in the ambient environment are unavailable. Historical data (i.e., 1980s or earlier) suggest that ambient levels were 0.1–0.4 μg/m^3 in air and 1 μg/L in water. Higher concentrations of 1,4-dioxane in groundwaters have been observed in aquifers contaminated with TCA.

6.4.1 Air

Recent information on the ambient levels of 1,4-dioxane in air is unavailable. Because the use of 1,4-dioxane has declined in recent years, current levels of 1,4-dioxane in the ambient air are likely to be less than levels reported in the 1980s or in earlier periods. In 1984, the concentration of 1,4-dioxane ranged from 0.1–0.4 μg/m^3 in ambient air sampled from the United States. No information was provided in this source on the locations where the air sampling occurred (EC 2002). In the early to mid 1980s, the mean ambient levels of 1,4-dioxane in outdoor air was measured as part of the VOC National Ambient Database in the United States (Shah and Singh 1988). The mean concentration of 1,4-dioxane was 0.107 ppbv or 0.385 μg/m^3 (n=617; median, 0.000 ppbv). 1,4-Dioxane was detected in outdoor air samples from the United States between 1981 and 1984 (detection limit unspecified). In the winter of 1984, 1,4-dioxane was detected in 67% of outdoor air samples from Los Angeles communities (n=25) at a median concentration of 0.27 μg/m^3. In the summer of 1984, 1,4-dioxane was detected in 22% of outdoor air samples from Los Angeles communities (n=23) at a median concentration of 0.02 μg/m^3. In the summer of 1984, 1,4-dioxane was detected in 20% of outdoor air samples from Antioch/West Pittsburg, California (n=10) at a median concentration of 0.03 μg/m^3 (Pellizzari et al. 1986). Between 1979 and 1984, the mean concentration of 1,4-dioxane in ambient air was 0.44 μg/m^3 (range, 0–30 μg/m^3; detected in 187 of 533 samples) in samples collected from 12 unspecified urban/suburban locations in the United States (EPA 1993).

In the summer of 1981 (July 6–August 16), the geometric mean concentrations of 1,4-dioxane in air near three industrialized urban areas (i.e., Newark, Elizabeth, and Camden, New Jersey) of the United States were 0.01 (21 of 38 samples positive), 0.02 (15 of 38 samples positive), and 0.005 μg/m^3 (21 of 35 samples positive), respectively (Harkov et al. 1983). The three same sites were also sampled from January 18–February 26, 1982. The geometric means of these samples ranged from 0 to 0.01 μg/m^3; 20% of samples were positive, with a maximum value of 5.31 μg/m^3 (Harkov et al. 1984; EC 2002). Two ambient air samples taken in New Jersey were reported to contain 1,4-dioxane (Harkov et al. 1985). In 1983, near the Kramer Landfill in New Jersey, sampled ambient air contained 1,4-dioxane at a geometric mean concentration of 0.01 ppbv or 0.4 μg/m^3 (maximum, 0.09 ppbv or 0.3 μg/m^3). In 1982, at an urban/industrial site in Newark, New Jersey, ambient air contained 1,4-dioxane at a geometric mean concentration of 0.01 ppbv, or 0.4 μg/m^3 (n=26; maximum, 1.45 ppbv or 5.22 μg/m^3). At various landfills in the United States, the concentration of 1,4-dioxane in landfill gas was reported to be 0.62 μg/m^3 and 0.33 g/m^3 (EC 2002).

In the early to mid 1980s, the mean ambient levels of 1,4-dioxane in indoor air was measured as part of the VOC National Ambient Database in the United States (Shah and Singh 1988). The mean concentration of 1,4-dioxane in indoor air was 1.029 ppbv, or 3.704 μg/m^3 (n=585; median, 0.000 ppbv). 1,4-Dioxane was detected in indoor air samples from the United States between 1981 and 1984 (detection limit unspecified). In the winter of 1984, 1,4-dioxane was detected in 64% of indoor air samples from Los Angeles communities (n=25) at a median concentration of 0.26 μg/m^3. In the summer of 1984, 1,4-dioxane was detected in 17% of indoor air samples from Los Angeles communities (n=23) at a median concentration of 0.02 μg/m^3. In the summer of 1984, 1,4-dioxane was detected in 10% of indoor air samples from Antioch/West Pittsburg, California (n=10) at a median concentration of 0.07 μg/m^3 (Pellizzari et al. 1986). In a multi-national survey taken between 1978 and 1990, mean 1,4-dioxane levels were 11 μg/m^3 in indoor air samples taken from buildings (i.e., schools and offices) with reported unspecified complaints among the occupants (Brown et al. 1994). In June of 1990, 125 households in Woodland, California were monitored for a variety of toxic air contaminants. Approximately 21% of the indoor samples collected contained measurable amounts of 1,4-dioxane. The average concentration of 1,4-dioxane was below the quantifiable limit of 0.11 μg/m^3, and the measurements ranged from below the quantifiable limit to 140 μg/m^3 (California ARB 1997).

6.4.2 Water

Recent information on the concentration levels of 1,4-dioxane in groundwater, surface water, and drinking water is limited. However, because the use of 1,4-dioxane has declined in recent years, current levels of 1,4-dioxane in aqueous media are likely to be less than levels reported in the 1980s or in earlier periods.

In the 1970s, municipal water supplies in the United States were reported to contain 1 μg/L of 1,4-dioxane (Kraybill 1978); however, the frequency of this level was not provided. In a drinking water well in Massachusetts, a concentration of 2,100 μg/L was reported (Burmaster 1982). However, this well appeared to be contaminated. In six drinking water wells (37% of samples) near a solid waste landfill located 60 miles southwest of Wilmington, Delaware, two wells were found to contain 0.1 and 0.5 μg/L 1,4-dioxane, but no 1,4-dioxane was detectable in the finished drinking water in the municipality using that well field (DeWalle and Chian 1981). Concentrations in private wells ranged from 0.001–1 to 200 mg/L (from 1–1,000 to 200,000 μg/L). The concentration of 1,4-dioxane in five wells near Circleville, Ohio ranged from <1 to 360 μg/L after contamination of groundwater following treatment of industrial waste water (Hartung 1989). Drinking water from the Netherlands contained 1,4-dioxane at a concentration of 0.5 μg/L (EC 2002). Drinking water samples from homes near the Durham Meadows Superfund site in Durham, Connecticut had maximum 1,4-dioxane concentrations of 26 μg/L in untreated water in one residential well and 12 μg/L in treated water from another residential well (EPA 2004g).

1,4-Dioxane was determined at 1.1–109 μg/L in contaminated groundwater in California (Draper et al. 2000). Extensive groundwater contamination (<0.01–220 mg/L or <10–220,000 μg/L) with more limited surface water contamination (<0.01–0.29 mg/L or <10–290 μg/L) resulted from treatment of industrial waste water in an unlined oxidation lagoon in Ann Arbor, Michigan (DeRosa et al. 1996). Current levels of 1,4-dioxane were about 1 μg/L in eight groundwater wells located in the vicinity of this site. However, the number of non-detects was not provided in this source (Michigan DEQ 2004). 1,4-Dioxane was discovered in groundwater at more than 250 ppm (mg/L) at a San Jose, California solvent recycling facility in 1998. In a survey of TCA release sites in California, it was found that 1,4-dioxane was present in a majority of these sites (concentrations unspecified) (Mohr 2004). At the Stanford Linear Accelerator Center (SLAC) in Menlo Park, California, the occurrence of 1,4-dioxane in groundwater is closely associated with TCA and its abiotic degradation product, 1,1-dichloroethane. It was found at this location at a maximum concentration of 7,300 ppb (Mohr 2004). Leachates from wells located near low level radioactive waste disposal sites contained 1,4-dioxane, but no quantitative data were presented (Francis et

al. 1980). Between 1983 and 1986, 1,4-dioxane was detected in groundwater near three landfills in Canada at concentrations <1 µg/L (EC 2002). In groundwater beneath a landfill, the concentration of 1,4-dioxane was 500 µg/L at a site in Canada sampled in 1982 (EC 2002). Groundwater samples obtained from an abandoned waste-oil refinery near Westville, Indiana contained 1,4-dioxane at levels ranging from approximately 3 to 31,000 µg/L (USGS 2002).

In 1982, 1,4-dioxane was detected in samples of river water from the Haw River in North Carolina, which flows through an industrialized section of the North Carolina Piedmont (Dietrich et al. 1988). However, no information on the levels of 1,4-dioxane in these samples were reported by the authors. 1,4-Dioxane at 1 µg/L was detected in the Chicago Sanitary and Ship Channel in the Lake Michigan basin (Konasewich et al. 1978). Surface water from the provincial area of Drenthe in the Netherlands contained 1,4-dioxane at concentrations ranging from 1 to 10 µg/L (EC 2002). At five different locations near the banks of the Rhine River in Germany, surface water contained <10 µg/L 1,4-dioxane in 1996 (EC 2002). River water collected from an unspecified river in the United Kingdom contained 1,4-dioxane, but no quantitative data were presented (Gelman Sciences 1989c). Dioxane concentrations in river water ranged from <0.024 to 0.69 µg/L in Kitakyushu, Japan (Kawata et al. 2003) and from 0.1 to 16 µg/L in Kanagawa, Japan (Abe 1999). The Japanese Ministry of the Environment reported that 1,4-dioxane in river and coastal waters ranged from <0.08 to 46 µg/L at 34–35 sites in Japan during fiscal years 1997–1999 (Kawata et al. 2003). 1,4-Dioxane was detected in surface water samples from 11 of 19 sites from Niigata, Japan at concentrations ranging from <0.03 to 0.39 µg/L (Kawata et al. 2003).

During the period of 1988–1991, the maximum concentration of 1,4-dioxane detected at Superfund sites in 21 states was 10 µg/L (Canter and Sabatinti 1994). 1,4-Dioxane ranged in concentration from 1.1 to 109 µg/L in leachate from hazardous waste disposal sites in Japan (Yasuhara et al. 1997). In 2000–2001, the concentration of 1,4-dioxane in leachate from a closed hazardous waste landfill in Japan ranged from 0.16 to 0.50 µg/L. At an open hazardous waste landfill, the concentration of 1,4-dioxane in leachate ranged from 0.91 to 10.6 µg/L (Yasuhara et al. 2003). At these landfills, waste plastics were disposed after either incineration or crushing and pressing under heat. The heating process appears to have resulted in the formation of 1,4-dioxane, although a mechanism for this process was not provided (Yasuhara et al. 2003). The concentration of 1,4-dioxane in landfill leachates from eight hazardous disposal sites in Japan ranged from 1.100 to 109 µg/L (median, 3.900 µg/L) (Yasuhara et al. 1997). In May 1988, the concentration of 1,4-dioxane in an outwash aquifer near the Gloucester Hazardous Waste Landfill (Ottawa, Canada) ranged from ~300 to 2,000 µg/L, with a 13% frequency of detection (detection

limit=150 µg/L) (Lesage et al. 1990). The concentrations of 1,4-dioxane were reported to be 11, 8, and 36 µg/L in landfill leachates sampled from three municipal landfills in Göteburg, Sweden (Paxéus 2000).

In an industrial/urban area of Japan (i.e., Kanagawa prefecture), the concentration of 1,4-dioxane in river water ranged from 0.3 to 0.9 µg/L during the period of 1996–1998; the concentration in groundwater ranged from 0.2 to 0.4 µg/L during the period of 1995–1997 in the same area (Abe 1999). High concentrations of 1,4-dioxane in polluted groundwater from this area ranged from <0.1 (not detected) to 52 µg/L and were correlated with TCA contamination of groundwater (Abe 1999). Romero et al. (1998) measured the concentration of 1,4-dioxane in industrial waste waters from producers of polyester resins in Barcelona, Spain. The polymer resins were polymerized using different glycols in acid catalyzed condensation reactions. In these waste water samples, 1,4-dioxane was detected at a mean concentration of 6,400 µg/L (range, <100–31,400 µg/L) and a frequency of 48.6% (Romero et al. 1998).

6.4.3 Sediment and Soil

No quantitative data were located on the concentrations of 1,4-dioxane in sediments or soil.

6.4.4 Other Environmental Media

There have been no systematic studies designed to determine the levels of 1,4-dioxane in foods. However, 1,4-dioxane has been detected in some foods, which may indicate that 1,4-dioxane may be a natural constituent. 1,4-Dioxane was identified, but not quantified, in chicken flavor and meat volatiles (Shahidi et al. 1986). 1,4-Dioxane was also identified in volatile flavor compounds from fried chicken (Tang et al. 1983); however, no concentration levels were reported. Chung et al. (1983) identified 1,4-dioxane in the volatile components of tomato juices and tomato juice products by mass spectrometry, although levels of 1,4-dioxane were not quantified. 1,4-Dioxane was formed in trilinolein (a component of fat oil used in deep-frying foods) after deep-fry heating (Chang et al. 1978). However, the concentration of 1,4-dioxane was not specified. Odor from cooked small shrimp was reported to contain 1,4-dioxane at unquantified levels (Choi et al. 1983). Sanceda et al. (1984) detected 1,4-dioxane in patis, a Philippine fermented fish sauce, which is a commonly used food condiment in the diet of Southeast Asian people. Patis also may be readily available in some gourmet food stores in the United States. The concentration of 1,4-dioxane in patis was not specified. A study of the Japanese diet found that samples of a representative basket of foods contained 1,4-dioxane in levels from undetected to 13 ppm (mg/kg) (Nishimura et al. 2004). 1,4-Dioxane was identified, but not quantified, in adipose tissue of pasture-

raised or concentrate-fed lambs (Sivadier et al. 2008). No further information on the detection of 1,4-dioxane in foods was located.

Food additives have been reported to contain 1,4-dioxane, although current levels were unavailable. For example, polysorbate 60 and polysorbate 80, which are used as food additives, have historically been found to contain 1,4-dioxane (Birkel et al. 1979). Polysorbate 60 and polysorbate 80 are produced from the polymerization of polyoxyethylene. Levels of 1,4-dioxane in these compounds have been reported to range from 4.8 to 6.0 ppm (mg/L) and from 5.3 to 5.8 ppm (mg/L), respectively. No further information on the levels of 1,4-dioxane in food additives was located.

In the FDA Cosmetic Handbook, it was reported that "cosmetics containing as ingredients ethoxylated surface active agents, i.e., detergents, foaming agents, emulsifiers, and certain solvents identifiable by the prefix, word or 'PEG,' 'Polyethylene,' 'Polyethylene glycol,' 'Polyoxyethylene,' '-eth-,' or '-oxynol-,' may be contaminated with 1,4-dioxane." It is also reported that "it (1,4-dioxane) may be removed from ethoxylated compounds by means of vacuum stripping at the end of the polymerizations process without unreasonable increase in raw material cost" (FDA 1992).

Although manufacturers are able to remove 1,4-dioxane from ethoxylated raw materials by vacuum stripping, studies by FDA indicate that some ethoxylated raw materials may still contain 1,4-dioxane at significant levels. Since 1979, FDA has conducted periodic surveys of levels of 1,4-dioxane in ethoxylated raw materials used in cosmetic products and finished cosmetic products (Black et al. 2001). In 1997, the average concentration of 1,4-dioxane in ethoxylated raw materials used in cosmetic products was 348 ppm (range, 45–1,102 ppm). In previous years, the average concentrations of 1,4-dioxane were 49 ppm (1979), 207 ppm (1980), 71 ppm (1993), and 180 ppm (1996). The average concentration of 1,4-dioxane in ethoxylated alkyl sulfate surfactants was reported to be 229 ppm (range, 71–580 ppm), 226 ppm (range, 6–1,410 ppm), 80 ppm (range, 16–243 ppm), 188 ppm (range, 20–653 ppm), and 348 ppm (range, 45–1,102 ppm) in the years 1979, 1980, 1983, 1993, 1996, and 1997, respectively (Black et al. 2001).

Although industry has taken steps to reduce 1,4-dioxane in ethoxylated surfactants, some cosmetic and household products may contain 1,4-dioxane at levels >10 ppm. For example, EPA (1992) examined 1,159 household products for chemical contaminants such as 1,4-dioxane. In one of six samples of laundry presoak spray analyzed, 1,4-dioxane was reported at a concentration of 15.0 w/w% In an FDA survey of cosmetic finished products in the United States, the average concentrations of 1,4-dioxane were

reported to be 50 ppm (range, 2–279 ppm), 19 ppm (range, 2–36 ppm), and 2 ppm (range, 1–8 ppm) for the years 1981, 1982, and 1983, respectively (Black et al. 2001). After a 10-year break, FDA resumed its surveys of cosmetic finished products in 1992. The number of products analyzed for 1,4-dioxane between 1992 and 1997 totaled 99. Since 1994, the focus was on children's shampoos and bubble baths, which are typically formulated with ethoxylated raw materials. FDA observed that the previous downward trend in the levels of 1,4-dioxane in products in the late 1980s was no longer evident in the 1990s. The average concentrations of 1,4-dioxane in cosmetic finished products were reported to be 41 ppm (range, 5–141 ppm), 79 ppm (range, 50–112 ppm), 45 ppm (range, 20–107 ppm), 74 ppm (range, 42–90 ppm), 14 ppm (range, 6–34 ppm), and 19 ppm (range, 6–34 ppm) in the years 1992, 1993, 1994, 1995, 1996, and 1997, respectively (Black et al. 2001). Although these levels are considered low and not expected to pose a hazard for consumers, the authors of the study commented that some raw material producers were not effectively controlling the levels of 1,4-dioxane in certain products. A more recent survey reported by the Campaign for Safe Cosmetics (2007) found that the levels of 1,4-dioxane in cosmetic products that were tested were slightly lower than in the survey conducted by the FDA in the 1990s. The levels of 1,4-dioxane in these products ranged from 1.5 to 12 ppm in baby and children's products and from 2 to 23 ppm in adult products. A second survey released in March of 2009 had similar results. Thirty-two out of 48 consumer products had detectable levels of 1,4-dioxane, with levels ranging from 0.27 to 35 ppm (Campaign for Safe Cosmetics 2009). Other studies reported that household laundry detergents, shampoos, soaps, and skin cleansers were found to contain 1,4-dioxane at levels ranging from 6 to 160 ppm (Gelman Sciences 1989a, 1989b). In Denmark, cosmetic products and dishwashing detergent, which used polyethoxylated surfactants, contained 1,4-dioxane at levels ranging from 0.3 to 96 ppm and from 1.8 to 65 ppm, respectively (Rastogi 1990). The FDA has indicated that the levels of 1,4-dioxane found in their monitoring of cosmetics do not present a hazard to consumers (FDA 2009).

1,4-Dioxane has been reported to be a contaminant in other consumer products. For example, 1,4-dioxane was found to be an impurity at concentrations of 0.5 and 1–3% in two household adhesive products from the United States (NIH 2004). 1,4-Dioxane was detected in 2 of 62 samples of household adhesives at concentrations of 1.0 w/w% for boot cement and 2.8 w/w% for universal cement (EPA 1992). 1,4-Dioxane was identified, but not quantified, in the headspace gas phase of a sample of dishwashing detergent, but was not identified in 58 other consumer products tested (Kwon et al. 2007).

1,4-Dioxane is formed from the breakdown of diethylene glycol. In 1988, consumer anti-freeze products contained 1,4-dioxane at concentrations ranging from 100 to 3,400 ppb (Gelman Sciences 1989c).

Radiator fluids have been found to contain slightly higher levels of 1,4-dioxane at concentrations ranging from 10 to 22,000 ppb (Gelman Sciences 1989c).

1,4-Dioxane was detected in 39 household aerosol products from Japan. In each of these samples, TCA was detected. The range of 1,4-dioxane concentration was 0.17–2.25% (Mori et al. 1992). A good correlation between the contents of 1,4-dioxane and TCA suggest that TCA containing 3% of 1,4-dioxane was used historically in the manufacture of aerosol products. However, because the use of TCA has been phased out in the United States since 1996, current levels of 1,4-dioxane in aerosol products should be limited.

1,4-Dioxane was identified but not quantified in human feces obtained from a healthy male individual from the former Soviet Union (Dmitriev et al. 1985). However, no information was provided in this study on the possible source of 1,4-dioxane in this feces sample or whether or not the individual was occupationally exposed to 1,4-dioxane.

Krotosznski et al. (1979) found 1,4-dioxane in the expired air of 24.8% of the samples taken from 54 healthy humans. The geometric mean concentration was 0.253 μg/m^3. This concentration is significantly higher than those reported in the ambient air studies in New Jersey (see Section 6.4.1). However, no attempt was made to correlate the concentrations in the expired air with those found in the ambient air, nor was there an attempt to correlate these concentrations with life style or occupational exposures. Conkle et al. (1975) also found 1,4-dioxane (0.41 μg/hour) in the expired air of one out of eight volunteers. These authors speculated that 1,4-dioxane was a normal metabolic product, although neither this study nor the former monitoring study cited above had undertaken rigorous steps to prevent contamination with 1,4-dioxane during the analysis.

In 1989, 1,4-dioxane was detected in highway rest-stop radiator boil-over pools at concentrations ranging from <10 to 2,300 ppb (Gelman Sciences 1989a, 1989b).

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The primary routes of human exposure to 1,4-dioxane for the general population are inhalation of 1,4-dioxane in air, ingestion of contaminated food and drinking water containing 1,4-dioxane, and dermal contact with consumer products. Because 1,4-dioxane may be found in tap water, human exposure to 1,4-dioxane may also occur during activities such showering, bathing, and laundering.

A study conducted by the Centers for Disease Control and Prevention (CDC) collected human blood specimens in 2007–2008 from a geographically-diverse population of U.S. residents ≥12 years old. No detectable concentrations of 1,4-dioxane were found in 2,053 human blood specimens analyzed (Wang et al. 2009). The authors concluded that despite the potential for human exposure to 1,4-dioxane from consumer products, the low dermal penetrability is likely to result in a negligible internal dose.

Recent levels of 1,4-dioxane in air are not available. In 1984, the concentration of 1,4-dioxane ranged from 0.1 to 0.4 μg/m^3 in ambient air sampled from the United States. Assuming that an adult breathes approximately 20 m^3 of air per day, the inhalation exposure would be 2–8 μg of 1,4-dioxane per day. Current exposure from air is likely to be less than this value. Exposure may be somewhat higher for persons living near sources of 1,4-dioxane emission. Individuals employed at industrial facilities that produce, process, and use 1,4-dioxane will also have higher exposures. Similarly, 1,4-dioxane is taken into the body by ingestion of drinking water. Current levels of 1,4-dioxane in drinking water are not available. In the 1970s, drinking waters in the United States were reported to contain 1 μg/L of 1,4-dioxane (Kraybill 1978). Using this concentration and the consumption rate as 2 L/day, the 1,4-dioxane intake from drinking water would be 2 μg/day. Current exposure from drinking water may likely be less than this value. Recently, a Total Diet Study in Japan determined the intake of 1,4-dioxane in food based on the average intake of food in the Kanto area of Japan (Nishimura et al. 2004). The 1,4-dioxane content of 12 food groups ranged between 2 and 15 μg/kg inclusive. From these results, the total daily intake of 1,4-dioxane was calculated to be 0.440 μg. This study indicates that the amount of 1,4-dioxane intake contributed from food is very low. FDA has estimated the exposure to 1,4-dioxane from the use of polyethylene glycol mono-isotridecyl ether sulfate sodium salt as a surfactant in adhesives intended for use in contact with food. Based on a daily diet of 3 kg, exposure to 1,4-dioxane has been estimated to be 0.2 ppb of daily diet or 0.6 μg/person/day (FDA 1998). Since most consumer products (e.g., detergents, shampoos, and cosmetic products) containing 1,4-dioxane may be diluted with water prior to application, dermal exposure is expected to be small in comparison to other exposures such as air, drinking water, and food.

The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) administered by the National Occupational Health and Safety Commission of Australia developed a ‘worst-case scenario’ for daily intake (inhalation and dermal exposure) for 1,4-dioxane from consumer products (not including pharmaceuticals or food products). The average daily intake from these exposures was calculated at around 7 μg/kg, based on an assumed level of 30 ppm 1,4-dioxane in end-use products (NICNAS 1998).

Occupational exposure of individuals involved in the production, processing, or use of 1,4-dioxane may result from inhalation or dermal exposure (De Rosa et al. 1996). The National Occupational Exposure Survey (1981–1983) indicated that 86,489 individuals, including 30,542 women, potentially were exposed to 1,4-dioxane (NIOSH 1977). This estimate was derived from observations of the actual use of the compound (25% of total observations) and the use of trade name products known to contain the compound (75%). The National Occupation Hazard Survey conducted by NIOSH from 1972 to 1974 estimated that 334,000 individuals were occupationally exposed to 1,4-dioxane, including 100,000 individuals occupationally exposed as a result of 1,4-dioxane used as a stabilizer in TCA (NIOSH 1976). In 1977, NIOSH estimated that 2,500 individuals were occupationally exposed to 1,4-dioxane, in addition to the 100,000 individuals occupationally exposed to both TCA and 1,4-dioxane (NIOSH 1977). OSHA reported that as many as 466,000 individuals may be occupationally exposed to 1,4-dioxane.

Individuals employed at chemical plants may be exposed to 1,4-dioxane as solvent vapors (Buffler et al. 1978). Between the period of 1994–1996, a survey in Hiroshima Prefecture, Japan was conducted to determine the levels of solvent vapors in 196 workplace areas. The survey was repeated every 6 months during this 3-year period. 1,4-Dioxane was reported in 6 of 1,176 cases at median and maximum concentrations of 0.5 and 0.8 ppm, respectively. 1,4-Dioxane was only detected in work areas where degreasing, cleaning, and wiping operations had occurred (Yasugi et al. 1998). During 1979, industrial hygiene monitoring was conducted at several plants which produced alcohol ethoxysulfate salts (Shell Oil Co. 1988). Time-weighted-average concentrations of 1,4-dioxane in air samples collected for five different jobs and locations within these plants were at or below the detection limit of <0.1 ppm. A maximum TWA concentration of 0.4 ppm reported in this monitoring study. In another study,1,4-Dioxane and HEAA were detected in the urine of individuals occupationally exposed to 1,4-dioxane. Individuals were exposed to a time-weighted average concentration of 1.6 ppm 1,4-dioxane for 7.5 hours. The mean concentration of 1,4-dioxane and HEAA in urine samples from exposed individuals at the end of each workday were 3.5 and 414 μmol/L (0.31 and 36.5 mg/L), respectively (Young et al. 1976).

Individuals involved in the manufacture of ethoxylated chemicals may be exposed to 1,4-dioxane from its occurrence as a by-product, and in particular during the stripping process, which is carried out to remove 1,4-dioxane from certain ethoxylated chemicals (mainly surfactants and emulsifiers) (EC 2002). Because of large quantities of TCA were previously used, past occupational exposure to 1,4-dioxane (used as a

stabilizer) may have been significant, particularly in metal degreasing operations. As the manufacture of TCA is currently restricted, only limited exposure from this exposure source is expected to occur.

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula, then to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not have the judgment of adults to avoid hazards (NRC 1993).

Specific information on the exposure of children to 1,4-dioxane does not exist. As for adults in the general population, small exposures occur from the normal ingestion of food and drinking water, inhaling air, and dermal contact with contaminated consumer products (e.g., containing ethoxylated surfactants). Home exposures may result from the unintentional consumption of consumer products (e.g., baby shampoo, household detergents) containing 1,4-dioxane. However, the extent of this possible exposure route in the general population is unknown.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Individuals who consume drinking water from contaminated wells may be exposed to higher levels of 1,4-dioxane. For example, groundwater has been reported to be contaminated with 1,4-dioxane in the following locations: Ann Arbor, Michigan; San Jose, California; and Menlo Park, California (DeRosa et al. 1996; Mohr 2004). The extent of 1,4-dioxane exposure for these populations is not known.

Individuals employed in occupations involved in the manufacture, processing and handling, and use of 1,4-dioxane will have potentially higher exposures to this chemicals. In addition, individuals involved in analytical science and research and development activities, which may utilize 1,4-dioxane as a solvent, may be exposed to higher levels of 1,4-dioxane.

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 1,4-dioxane is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 1,4-dioxane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. Sufficient information regarding the chemical and physical properties (i.e., log K_{ow}, log K_{oc}, Henry's law constant, vapor pressure, etc.) of 1,4-dioxane is available to evaluate its environmental fate (see Table 4-2). There are no data needs at this time.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2007, became available in February of 2009. This database is updated yearly and should provide a list of industrial production facilities and emissions.

1,4-Dioxane is currently produced in the United States, although current production volumes are not available. Information on current and future production and importation levels of 1,4-dioxane are needed to determine whether the risk for human exposure to 1,4-dioxane is significant. Although 1,4-dioxane is not widely used in the home, environment, or most workplaces, it can be present as a contaminant in materials that are found or used in these environments and, therefore, human exposures to 1,4-dioxane can occur. 1,4-Dioxane is used primarily as a solvent. 1,4-Dioxane has been found as an impurity in cosmetics, household and industrial detergents, and pharmaceuticals due to its occurrence as a by-product

in ethoxylated emulsifiers. 1,4-Dioxane may be present as a contaminant of food. However, no information is available that quantifies actual levels of 1,4-dioxane in food. Water is the most likely media to be contaminated with significant quantities of 1,4-dioxane (EC 2003; Hartung 1989).

Pure or nearly pure 1,4-dioxane is disposed of by incineration. It is expected that 1,4-dioxane is completely destroyed by this method. Aqueous solutions of 1,4-dioxane are disposed in waste water treatment facilities. Because 1,4-dioxane is resistant to biodegradation, complete mineralization of this chemical is not efficient. Thus, there may be need to develop effective methods of disposal for aqueous solutions of 1,4-dioxane. Additional information is needed on the amounts of 1,4-dioxane disposed of by each method.

Environmental Fate. There are no data needs regarding the environmental fate of 1,4-dioxane. 1,4-Dioxane is miscible in water and partitions primarily to the aqueous media in the environment. 1,4-Dioxane has high mobility in soil and has the potential to migrate into groundwater. In air, 1,4-dioxane will degrade by reaction with OH radicals with a half-life of <1 day (EPA 2000b). 1,4-Dioxane has been found to be resistant to biodegradation in the environment (Alexander 1973; Dow Chemical Co. 1989; Fincher and Payne 1962; Heukelekian and Rand 1955; Mills and Stack 1954). 1,4-Dioxane is expected to persist in both water and soil.

Bioavailability from Environmental Media. 1,4-Dioxane is absorbed following inhalation, oral, and dermal contact (see Chapter 3). However, 1,4-dioxane is not bioconcentrated. Based on dermal penetration studies, dermal absorption of 1,4-dioxane is limited. No data needs have been identified at this time.

Food Chain Bioaccumulation. Because 1,4-dioxane is miscible in water, it is not bioconcentrated in plants, aquatic organisms, or animals. 1,4-Dioxane is not biomagnified to any extent in prey organisms. No data needs have been identified at this time.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of 1,4-dioxane in contaminated media at hazardous waste sites are needed so that the information obtained on levels of 1,4-dioxane in the environment can be used in combination with the known body burden of 1,4-dioxane to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

1,4-Dioxane has been detected in air, water, and foodstuff. Although historical data are available (e.g., 1980s and earlier), recent information on the levels of 1,4-dioxane in these media are not available. Reliable monitoring data for the levels of 1,4-dioxane in contaminated media at hazardous waste sites are needed so that the information obtained on levels of 1,4-dioxane in the environment can be used in combination with the known body burden of 1,4-dioxane to access the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites. Estimates have been made for human intakes of 1,4-dioxane from air and drinking water. However, these estimates are based on historical monitoring data which may not be representative of current levels of 1,4-dioxane in environmental media. It is unclear how extensive the human exposure to 1,4-dioxane is indoors and from consumer products. Additional data that determines current levels of 1,4-dioxane in air, drinking water, food, and consumer products are necessary to assess human exposure to 1,4-dioxane.

Exposure Levels in Humans. 1,4-Dioxane has been detected in the urine of individuals who are occupationally exposed to 1,4-dioxane (Young et al. 1976). Two studies conducted by Conkle et al. (1975) and Krotosznski et al. (1979) involving 54 and 8 volunteers, respectively, detected 1,4-dioxane in a small number of expired air samples collected from these volunteers, but the source of the measured 1,4-dioxane could not be determined because the studies did not adequately document lifestyle or occupation. No other biological monitoring studies have been done in populations surrounding hazardous waste sites or in the general population. This information is necessary for assessing the need to conduct health studies on these populations. No estimates have been made for human intake of 1,4-dioxane from various environmental media. This information is necessary for determining the routes of exposure to 1,4-dioxane from these various media.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. Children are exposed to 1,4-dioxane in the same manner as adults. Exposure and body burden studies on children would be useful. Children who take frequent bubble baths may be exposed to higher levels of 1,4-dioxane than adults due to possible contamination of ethoxylated surfactants found in some of these commercial products. Additional studies are needed to determine whether this is a significant exposure route for children. It is not known whether children are different in their weight-adjusted intake of 1,4-dioxane. Additional studies would help to determine if children are more or less exposed to 1,4-dioxane compared to adults.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

Exposure Registries. No exposure registries for 1,4-dioxane were located. This substance is not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for sub-registries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

Historically, 1,4-dioxane has been used as a stabilizer in TCA at concentrations up to 4%. Often 1,4-dioxane is present at sites where TCA has been found as a contaminant. TCA is currently one of the compounds for which a Subregistry has been established in the Volatile Organic Compounds (VOCs) Registry. The VOCs Registry is part of the National Exposure Registry (NER), which was created and is being maintained by the Agency for Toxic Substances and Disease Registry.

6.8.2 Ongoing Studies

The FEDRIP (2009) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 6.8.1.

Researchers at North Carolina State University are investigating the intermediates and pathways of the microbial degradation of several important ether pollutants including 1,4-dioxane. In addition, the key enzymes responsible involved in the biodegradation of these important pollutants are to be characterized and subsequently identify and characterize the genes encoding these enzymes.

7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring 1,4-dioxane, its metabolites, and other biomarkers of exposure and effect to 1,4-dioxane. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations, such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision are included.

Levels of 1,4-dioxane in environmental and biological samples are determined analytically by gas chromatography-mass spectrometry (GC-MS) or gas chromatography-flame ionization detection (GC-FID). The determination of 1,4-dioxane at parts-per-billion (ppb or μg/L) concentrations in samples where water is present (e.g., water, soil, sediment, and tissues) is difficult. The miscibility of 1,4-dioxane in water causes poor purging efficiency, resulting in the relatively high reporting limits.

The purge-and-trap technique also suffers from interferences by some substances. 1,4-Dioxane gives poor response with headspace sample introduction due to its low volatility from water. The partition coefficients for 1,4-dioxane lead to low recoveries in single contact liquid-liquid extraction (LLE), and very large solvent-to-water ratios are needed to achieve acceptable recoveries (Draper et al. 2000). Because of these limitations, alternative techniques have been developed to improve the determination of 1,4-dioxane. Methods have been developed to extract 1,4-dioxane from the aqueous phase using solid phase extraction (SPE) followed by desorption with an organic solvent, heated purge-and-trap with salting out, azeotropic distillation, and continuous LLE. Isotopic dilution has also been used to correct for variability in MS instrument response.

7.1 BIOLOGICAL MATERIALS

Methods for the specific analysis of 1,4-dioxane and its metabolites in biological tissues and fluids are limited. Since the human body rapidly metabolizes 1,4-dioxane to 1,4-dioxane-2-one and HEAA, the metabolites of 1,4-dioxane may be used as biomarkers of exposure to 1,4-dioxane (Young et al. 1976, 1977).

Using heated headspace technique, 1,4-dioxane was determined in blood or urine (e.g., 4–5 mg) by heating a sample in a sealed tube to 120 °C. Volatiles from this sample were then analyzed by gas chromatography with the limit of detection being 0.01–0.02 μg (Royal Society of Chemistry 1988).

Groves et al. (1997) described the analysis of organic vapors in exhaled breath, which could provide information about occupational exposures to 1,4-dioxane. Analysis was conducted using an array of four polymer-coated surface-acoustic-wave (SAW) sensors and an adsorbent preconcentrator for rapid breath analysis. The adsorbent used in the preconcentrator was a porous styrene-divinylbenzene resin. Limits of detection range from 3.7 to 10.2 μg/L for 1,4-dioxane.

Biomarkers of exposure to 1,4-dioxane are the urinary metabolites 1,4-dioxane-2-one and HEAA (Royal Society of Chemistry 1988). Young et al. (1976) described a method for detection of 1,4-dioxane and HEAA in urine. Urine samples were treated with hydrochloric acid/methanol to convert HEAA to its methyl ester. Samples were then directly injected into a GC-MS for simultaneous analysis of 1,4-dioxane and HEAA. The detection limits were 0.07 and 0.1 μg/mL, respectively.

Analytical methods for the determination of 1,4-dioxane and its metabolites in biological samples are given in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

NIOSH 1602 is used to determine the concentration of 1,4-dioxane in a 10-L air sample by GC-FID. Samples are collected by drawing air through a solid sorbent tube containing coconut shell charcoal. The flow rate is between 0.01 and 0.2 L/minute for a total sample size of 0.5–15 L. 1,4-Dioxane is eluted from the solid sorbent with agitation using carbon disulfide. The carbon disulfide eluent sample is then injected directly into the GC-FID. The detection limit is 0.01 mg per sample (NIOSH 1994). This method is similar to a GC-FID method described by Bozzelli et al. (1980), in which the air samples were pumped through two sorbent filters and 1,4-dioxane was extracted at elevated temperatures with either a solvent or steam. The detection limit reported in this study was 0.01 ppb. A portable selected ion flow tube-mass spectrometer (SIFT-MS) described by Francis et al. (2009), can provide real-time environmental monitoring of several air pollutants, including 1,4-dioxane. The limits of detection and quantification were reported as 530 and 740 parts per trillion by volume, respectively.

Table 7-1. Analytical Methods for Determining 1,4-Dioxane in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood or urine	Heat sample in a sealed tube to 120 °C; inject headspace into GC	GC	0.01–0.02 µg	No data	Royal Society of Chemistry 1988
Blood or urine	Blood plasma or urine samples were analyzed by direct injection into the GC	GC/MS	0.1 µg/mL (blood) 0.2 µg/mL (urine)	No data	Young et al. (1977)
Exhaled breath	Pre-concentrate on breath sample on porous styrene-divinylbenzene resin	Four polymer-coated surface-acoustic-wave (SAW) sensors	3.7–10.2 µg/L	No data	Groves et al. 1997
Urine	Samples treated with HCl/methanol to convert HEAA to its methyl ester; samples then directly injected into GC-MS	GC-MS	0.07 µg/mL (1,4-dioxane); and 0.1 µg/mL (HEAA)	No data	Young et al. 1976

GC = gas chromatography; HCl = hydrochloric acid; HEAA = β-hydroxyethoxyacetic acid; MS = mass spectrometry

EPA Method 8015B is used to determine the concentration of 1,4-dioxane in environmental samples by GC. Samples may be introduced into the GC by direct injection (e g., aqueous samples) including the concentration of analytes by azeotropic distillation (EPA Method 5031). Purge-and-trap and solvent extraction are not appropriate for this method. Detection of the analyte is achieved by using a FID. Method detection limits for 1,4-dioxane in water, groundwater, and leachate are 12, 15, and 16 μg/L, respectively. Method detection limits for 1,4-dioxane in solids (e.g., incinerator ash and kaolin) are 0.31 and 0.16 mg/kg, respectively. Using azeotropic microdistillation, recoveries for 1,4-dioxane in groundwater, leachate, incinerator ash, and kaolin were 96–124, 102–103, 48–106, and 48–105%, respectively (EPA 1996a).

EPA Method 8260B is used to determine 1,4-dioxane in a variety of solid waste matrices by GC. This method is applicable to nearly all types of samples, regardless of water content, including various air sampling trapping media, groundwater and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments. Samples may be introduced to the capillary GC column by direct injection following dilution, sample concentration by azeotropic distillation (EPA Method 5031), and closed system vacuum distillation (EPA Method 5032) for aqueous, soil, oil, and tissue samples. Detection of the analyte eluted from the capillary column is achieved by using MS. The estimated quantitation limit for 1,4-dioxane is somewhat instrument dependent and also dependant on the choice of sample preparation/introduction method. No information on the recoveries for 1,4-dioxane were provided for this method (EPA 1996b).

EPA Method 1624 is used to determine 1,4-dioxane in water and in municipal and industrial discharges by isotopic dilution GC-MS. In this method, isotopically labeled 1,4-dioxane-d_8 is added to the sample as an isotope dilution standard. The samples are then introduced into the GC using a purge-and-trap methodology. 1,4-Dioxane is separated by GC and detected by MS. The labeled compounds serve to correct for the variability of the analytical technique. The detection limit for this method is 10 μg/L (EPA 2001).

Draper et al. (2000) described a sensitive method for detection of 1,4-dioxane in drinking water. This method was based on continuous LLE of 1,4-dioxane from aqueous samples by dichloromethane. Extraction of 1,4-dioxane in dichloromethane was followed by analysis using GC-MS. Detection limits as low as 0.2 μg/L were achieved in this method.

Epstein et al. (1987) described two methods for the determination of 1,4-dioxane in water and in solids and sediments. In the first method, 1,4-dioxane is extracted from water and soil samples using a heated purge-and-trap system following salting out with sodium sulfate at 1.6 M. GC-MS is then used as the method of analysis. The detection limit reported for this method was 2 ppb, with recoveries averaging 85%. In the second method, 1,4-dioxane is adsorbed on coconut shell charcoal followed by desorption with carbon disulfide/methanol. Analysis of the desorbate is conducted by GC with flame ionization detection. The limit of quantitation is around 2 ppb with recoveries ranging from 63 to 129%.

Kadokami et al. (1990) described a method for analysis of 1,4-dioxane in water by GC-MS. Preconcentration of 1,4-dioxane is achieved by passing the aqueous sample through an activated carbon column, followed by elution with acetone-dichloromethane. The organic extract is then concentrated with a Kuderna-Danish concentrator, followed by direct injection into the GC-MS with a selective ion monitor. The method detection limit was reported to be 0.024 μg/L. Recoveries of 1,4-dioxane from organic-free water, seawater, and river water were 98–101, 102, and 101%, respectively. Kawata et al. (2001) described a similar method of analysis for 1,4-dioxane in water. However, in this method, the solid-phase extraction media was activated carbon fiber felt with acetone as the elution media. Analytical determination of 1,4-dioxane was accomplished by GC-MS detection. The method detection limit was reported to be 0.03 μg/L. Recoveries of 1,4-dioxane in groundwater and river water were 97 and 92%, respectively.

Analytical methods for the determination of 1,4-dioxane in environmental samples are given in Table 7-2.

7.3 OTHER SAMPLES

Several methods that may be used to determine 1,4-dioxane in food, consumer cosmetic products, and surfactant raw materials are available.

The concentration of 1,4-dioxane in food additives may be determined using the 1,4-dioxane limit test (Committee on Food Chemicals Codex 1996). 1,4-Dioxane is extracted from a sample placed in a closed-system vacuum distillation apparatus. The distillate is then analyzed using GC-FID. The detection limit was not specified for this method. Daniels et al. (1981) utilized a similar methodology in the analysis of 1,4-dioxane in the food additive, polysorbate 65. The detection limit was not specified. Recoveries for 1,4-dioxane concentrations ranging from 0.5 to 600 ppm were from 85 to 101%, respectively.

Table 7-2. Analytical Methods for Determining 1,4-Dioxane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Samples were collected by drawing air through a solid sorbent tube containing coconut shell charcoal; 1,4-dioxane eluted with agitation using carbon disulfide.	GC-FID	0.01 mg per sample	No data	NIOSH 1994 (NIOSH Method 1602)
Air	Samples were collected by pumping air through two filters: Tenax, a porous polymer and Spherocarb, a carbon molecular sieve. Solvent or steam extraction at elevated temperature was used to remove 1,4-dioxane from the filters.	GC-FID	0.01 ppb	No data	Bozzelli et al. (1980)
Air	Ions from samples are created using an ion source external to the flow tube; ions are then extracted from the ion source using a quadrupole mass filter and injected into a flowing carrier gas and analyzed via an ion detector.	SIFT-MS	530 ppt	No data	Francis et al. 2009
Drinking water	Continuous liquid-liquid extraction using dichloromethane	GC-MS	0.2 µg/L	No data	Draper et al. 2000
Water, seawater, and river water	Pre-concentration by passing aqueous sample through an activated carbon column followed by elution with acetone-dichloromethane; organic extract concentrated with a Kuderna-Danish concentrator	GC-MS with a selective ion monitor	0.024 µg/L	98–102%	Kadokami et al. 1990
Groundwater and river water	Solid-phase extraction using activated carbon fiber felt with acetone as eluent	GC-MS	0.03 µg/L	97%, 92%	Kawata et al. 2001

Table 7-2. Analytical Methods for Determining 1,4-Dioxane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water, groundwater, leachate	Direct injection or azeotropic distillation (i.e., EPA Method 5031)	GC-FID	12–16 µg/L	96–124% (ground-water), 102–103% (leachate)	EPA 1996a (EPA Method 8015B)
Water, and municipal and industrial discharges	Isotopically labeled 1,4-dioxane-d_8 is added to the sample as an isotope dilution standard	Purge-and-trap GC-MS	10 µg/L	No data	EPA 2001 (EPA Method 1624)
Water, and solids and sediments	Adsorbed on coconut shell charcoal followed by desorption with carbon disulfide/methanol	GC-FID	2 ppb (µg/L or µg/kg)	63–129%	Epstein et al. 1987
Water, and solids and sediments	Extracted from water and soil samples using a heated purge-and-trap system following salting out with sodium sulfate at 1.6 M	GC-MS	2 ppb (µg/L or µg/kg)	85%	Epstein et al. 1987
Groundwater and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments	Azeotropic distillation (i.e., EPA Method 5031) or closed system vacuum distillation (i.e., EPA Method 5032)	GC-MS	No data	No data	EPA 1996b (EPA Method 8260B)

1,4-dioxane-d_8 = deuterium labeled 1,4-dioxane (or $C_4D_8O_2$); EPA = Environmental Protection Agency; FID = flame ionization detector; GC = gas chromatography; kg = 10^3 grams; mg = 10^{-3} grams; µg = 10^{-6} grams; MS = mass spectrometry; NIOSH = National Institute for Occupational Safety and Health; ppb = parts per billion; ppt = parts per trillion; SIFT = selected ion flow tube

Stafford et al. (1980) described the determination of 1,4-dioxane in ethoxylated surfactants using a direct injection GC method. 1,4-Dioxane is extracted from the ethoxylated surfactants using chlorobenzene, which is then diluted and injected directly into the GC-FID. The detection limit was reported to be 0.5 mg/kg, with a recovery of approximately 100%. Rastogi (1990) reported a method for the identification and quantification of 1,4-dioxane in polyethoxylated surfactants using headspace GC-MS. Dichloromethane and 1,4-dioxane-d_8 are added to the surfactant sample in a closed vial, which is then heated at 80 °C for 16–18 hours. The headspace gases are sampled with a gas-tight syringe and injected into the GC-MS for quantitative analysis. The detection limit for this method was approximately 0.3 ppm with recoveries of 92–94%.

1,4-Dioxane may be quantified in commercial cosmetic products by reversed-phase high-performance liquid chromatography (Scalia et al. 1990). Cosmetic samples are extracted using solid-phase extraction cartridges. Samples are then analyzed directly on a reverse-phase column with spectrophotometric detection at 200 nm and acetonitrile-water as eluent. The limit of detection was reported to be 6.5 µg/g. The recovery of 1,4-dioxane was between 81.5 and 90.1% in the 30–90 µg/g range. Ghassempour et al. (1998) described a modified GC-MS method for determination of 1,4-dioxane in cosmetic products (i.e., polyoxyethylene compound). Cosmetic product samples are prepared by dissolution of the material in dichloromethane. Samples are then analyzed directly by injection into a programmable temperature vaporizer attached to GC-MS. The minimum detection limit was reported to be 1 ng/L for this method. However, as this value is very low, the detection limit is likely much higher than reported by the authors.

Analytical methods for the determination of 1,4-dioxane in food and food additives, cosmetics, and ethoxylated surfactant samples are given in Table 7-3.

7.4 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 1,4-dioxane is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 1,4-dioxane.

Table 7-3. Analytical Methods for Determining 1,4-Dioxane in Food and Food Additives, Cosmetics, and Ethoxylated Surfactant Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food, food chemicals	Extraction followed by closed-system vacuum distillation	GC-FID	No data	No data	Committee on Food Chemicals CODEX 1996
Food additives (e.g., polysorbate 65)	Extraction followed by closed-system vacuum distillation	GC-FID	No data	85–101%	Daniels et al. 1981
Ethoxylated surfactants	Extraction using chlorobenzene	GC-FID	0.5 mg/kg	~100%	Stafford et al. 1980
Polyethoxylated surfactants	Dichloromethane and 1,4-dioxane-d_8 added to sample in a closed vial which is then heated at 80°C for 16–18 hours	Headspace GC-MS	0.3 ppm	92–94%	Rastogi 1990
Commercial cosmetic products	None	Reversed-phase high-performance liquid chromatography	6.5 µg/g	81.5–90.1%	Scalia et al. 1990
Cosmetic products (i.e., polyoxyethylene compound)	Dissolution in dichloromethane	Programmable temperature vaporizer-GC-MS	1 ng/L	No data	Ghassempour et al. 1998

1,4-dioxane-d_8 = deuterium labeled 1,4-dioxane (or $C_4D_8O_2$); CODEX = Codex Alimentarius Commission; FID = flame ionization detector; g = grams; GC = gas chromatography; kg = 10^3 grams; MS = mass spectrometry; ng = 10^{-9} grams; ppm = parts per million; µg = 10^{-6} grams

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.4.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Existing methods do not appear to be sensitive enough to measure background levels of 1,4-dioxane and its metabolite, HEAA, in the population (Young et al. 1976). Standard methods for the determination of 1,4-dioxane and its metabolite, HEAA, are needed to determine whether the general population is exposed to 1,4-dioxane.

Effect. Existing methods appear to be sensitive enough to measure levels of 1,4-dioxane and its metabolite, HEAA, at levels at which biological effects may occur in humans (Young et al. 1976).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The purpose of analytical methods is to identify contaminated areas and to determine if contaminant levels constitute a concern for human health. The media that are of most concern for human exposure to 1,4-dioxane are drinking water, food, and cosmetic products. In water, there are methods sensitive enough to measure background levels in the environment down to the sub-ppb level (<1 μg/L) (Draper et al. 2000; Kadokami et al. 1990; Kawata et al. 2001). Standard methods are also available for measurement of 1,4-dioxane in air and water samples (EPA 1996a, 1996b; NIOSH 1994). Methods have also been reported for the determination of 1,4-dioxane in food and food additives (Committee on Food Chemicals Codex 1996; Daniels et al. 1981), cosmetics (Ghassempour et al. 1998; Scalia et al. 1990), and ethoxylated surfactant materials (Rastogi 1990; Stafford et al. 1980).

7.4.2 Ongoing Studies

The Environmental Health Laboratory Science Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of 1,4-dioxane and other volatile organic compounds in blood. These methods use purge-and-trap methodology, high

resolution gas chromatography, and magnetic sector mass spectrometry, which give detection limits in the low parts per trillion (ppt) range.

No ongoing studies on 1,4-dioxane were found as a result of a search of the Federal Research in Progress database (FEDRIP 2009).

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8. REGULATIONS, ADVISORIES, AND GUIDELINES

MRLs are substance specific estimates, which are intended to serve as screening levels used by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites.

ATSDR has derived an acute-duration inhalation MRL of 2 ppm for 1,4-dioxane based on a NOAEL of 20 ppm for sensory irritation and pulmonary function in humans (Ernstgård et al. 2006). An uncertainty factor of 10 was used for human variability.

ATSDR has derived an intermediate-duration inhalation MRL of 0.2 ppm for 1,4-dioxane based on an increased incidence of nasal lesions in rats exposed to 1,4-dioxane 6 hours/day, 5 days/week for 13 weeks (Kasai et al. 2008). The MRL was derived using BMD modeling of incidence data for nasal lesions in rats. The predicted exposure concentration associated with a 10% extra risk for nasal lesions (BMC_{10}) for nasal lesions in female rats was 40.39 ppm; the lower 95% confidence limit on this concentration ($BMCL_{10}$) was 27.99 ppm. An uncertainty factor of 30 was used (3 for using dosimetric adjustments and 10 for human variability).

ATSDR has derived a chronic-duration inhalation MRL of 0.03 ppm for 1,4-dioxane based on a LOAEL of 50 ppm for increased incidence of nasal lesions in male rats exposed to 1,4-dioxane 6 hours/day, 5 days/week for 2 years (Kasai et al. 2009). An uncertainty factor of 300 was used (3 for using dosimetric adjustments, 10 for using a LOAEL, and 10 for human variability).

ATSDR has derived an acute-duration oral MRL of 5 mg/kg/day for 1,4-dioxane based on a NOAEL of 516 mg/kg/day for developmental and maternal effects in rats (Giavini et al. 1985). The LOAEL was 1,033 mg/kg/day. An uncertainty factor of 100 was used (10 for animal to human extrapolation and 10 for human variability).

ATSDR has derived an intermediate-duration oral MRL of 0.5 mg/kg/day for 1,4-dioxane based on a NOAEL of 52 mg 1,4-dioxane/kg/day for liver effects in male rats (Kano et al. 2008). The LOAEL was 126 mg/kg/day in males and 185 mg/kg/day in females. An uncertainty factor of 100 was used (10 for animal to human extrapolation and 10 for human variability).

ATSDR has derived a chronic-duration oral MRL of 0.1 mg/kg/day for 1,4-dioxane based on a NOAEL of 9.6 mg 1,4-dioxane/kg/day for liver effects in male rats (Kociba et al. 1974). The LOAEL was 94 mg/kg/day in males and 148 mg/kg/day in females. An uncertainty factor of 100 was used (10 for animal to human extrapolation and 10 for human variability).

The EPA (IRIS 2011) has derived a reference dose (RfD) of 0.03 mg/kg/day for 1,4-dioxane based on a NOAEL of 9.6 mg/kg/day for liver and kidney toxicity in male Sherman rats in a 2-year drinking water study (Kociba et al. 1974). The EPA (IRIS 2011) also derived an oral slope factor of $1x10^{-1}$ $(mg/kg/day)^{-1}$ based on increased incidence of hepatocellular adenoma and carcinoma in female BDF_1 mice in a 2-year drinking water study (Kano et al. 2009).

The National Academy of Sciences (NAS) established a maximum specification of 10 ppm for 1,4-dioxane in the ingredient polysorbate, a food additive (NAS 2003). It is also listed as an indirect food additive [21 CFR 175.105] (FDA 2003). FDA considered the same level, 10 ppm, to be an acceptable limit for 1,4-dioxane, during its consideration of a spermicide, N-9, in a contraceptive sponge product (prior to at least 1997) (FDA 1997). FDA also set a limit on 1,4-dioxane at 10 ppm in approving glycerides and polyglycerides for use as excipients in products such as dietary supplements (FDA 2006). (This regulation is located at 21 CFR 172.736.)

The international and national regulations, advisories, and guidelines regarding 1,4-dioxane in air, water, and other media are summarized in Table 8-1.

Table 8-1. Regulations, Advisories, and Guidelines Applicable to 1,4-Dioxane

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	Group 2B[a]	IARC 1999
WHO	Air quality guidelines	No data	WHO 2000
	Water quality guidelines	No data	WHO 2004
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (8-hour TWA)	20 ppm[b]	ACGIH 2011
NIOSH	REL (30-minute ceiling TWA)	1 ppm[c]	NIOSH 2004
	IDLH	500 ppm	
EPA	Hazardous air pollutant		EPA 2004d 42 USC 7412
OSHA	PEL (8-hour TWA) for general industry	100 ppm[b]	OSHA 2004c 29 CFR 1910.1000, Table Z-1
	PEL (8-hour TWA) for construction industry	100 ppm[b]	OSHA 2004b 29 CFR 1926.55, Appendix A
	PEL (8-hour TWA) for shipyard industry	100 ppm[b]	OSHA 2004a 29 CFR 1915.1000, Table Z
b. Water			
EPA	Drinking water standards and health advisories		EPA 2004b
	1-Day HA for a 10-kg child	4.0 mg/L	
	10-Day HA for a 10-kg child	0.4 mg/L	
	10^{-4} cancer risk	0.3 mg/L	
c. Food			
FDA	Indirect food additive for use only as a component of adhesives		FDA 2003 21 CFR 175.105
d. Other			
ACGIH	Carcinogenicity classification	Group A3[d]	ACGIH 2011
EPA	Carcinogenicity classification	Likely to be carcinogenic to humans	IRIS 2011
		No data	
	RfC	0.03 mg/kg/day	
	RfD		
	Oral slope factor	$1x10^{-1}$ $(mg/kg/day)^{-1}$	
	Drinking water unit risk	$2.9x10^{-6}$ $(\mu g/L)^{-1}$	

Table 8-1. Regulations, Advisories, and Guidelines Applicable to 1,4-Dioxane

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
	Community right-to-know; toxic chemical release reporting; effective date	01/01/1987	EPA 2004e 40 CFR 372.65
EPA	Superfund, Emergency Planning, and Community Right-To-Know Programs; designated as a hazardous substance pursuant to Section 112 of the Clean Air Act and Section 3001 of RCRA		EPA 2004a 40 CFR 302.4
	Reportable quantity	100 pounds	
	Hazardous waste identification	U108	EPA 2004c 40 CFR 261, Appendix VIII
NTP	Carcinogenicity classification	Reasonably anticipated to be a human carcinogen	NTP 2011
STATE			
a. Air			
No data			
b. Water			
California	Drinking water guidelines	3 µg/L	HSDB 2010
Florida	Drinking water guidelines	5 µg/L	
Maine	Drinking water guidelines	32 µg/L	
Massachusetts	Drinking water guidelines	50 µg/L	
c. Food			
No data			
d. Other			
No data			

[a]Group 2B: Possibly carcinogenic to humans.
[b]Skin designation: Potential significant contribution to the overall exposure by the cutaneous route, including mucous membranes and the eyes, either by contact with vapors, or of probable greater significance, by direct skin contact with the substance.
[c]Potential occupational carcinogen.
[d]Group A3: Confirmed animal carcinogen with unknown relevance to humans.

ACGIH = American Conference of Governmental Industrial Hygienists; CFR = Code of Federal Regulations; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HA = Health Advisory; HSDB = Hazardous Substances Data Bank; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; RfC = reference concentration; RfD = reference dose; TLV = threshold limit values; TWA = time-weighted average; USC = United States Codes; WHO = World Health Organization

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

Incidence—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Organophosphate or Organophosphorus Compound—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar

ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not necessarily mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that

are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Environmental Medicine, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology and Environmental Medicine, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-62, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 1,4-Dioxane
CAS Number: 123-91-1
Date: August 2011
Profile Status: Final
Route: [X] Inhalation [ ] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 8
Species: Human

Minimal Risk Level: 2 [ ] mg/kg/day [X] ppm

Reference: Ernstgård L, Iregren A, Sjögren B, et al. 2006. Acute effects of exposure to vapours of dioxane in humans. Human Exp Toxicol 25:723-729.

Experimental design: The acute-duration inhalation MRL is based on a NOAEL of 20 ppm for eye and respiratory effects in volunteers. In that study, six male and six female volunteers were exposed to 0 or 20 ppm 1,4-dioxane vapor for 2 hours under dynamic conditions. Each subject was exposed on two separate occasions to 0 or 20 ppm. End points monitored included self-rated symptoms on a visual analogue scale that measured discomfort of the eyes, nose and throat, breathing difficulty, solvent smell, headache, fatigue, nausea, dizziness and 'feeling of intoxication'. Rating was performed before, during (3, 60, and 118 minutes), and after exposure (20 and 180 minutes). Respiratory function was assessed by spirometry before exposure, immediately after, and 3 hours after exposure ceased. The specific parameters measured included vital capacity, forced vital capacity, forced expiratory volume in 1 second, peak expiratory flow, and forced expiratory flow at 25, 50, and 75% of the force vital capacity. Also assessed was nasal swelling before, immediately after, and 3 hours after exposure. Eye blinking was monitored throughout the exposure period by electromyography. Also, two inflammatory markers, high sensitivity C reactive protein and interleukin 6, were measured in blood before and 3 hours after exposure.

Effects noted in study and corresponding doses: Exposure to 1,4-dioxane under the conditions of the study did not significantly affect any of the end points monitored except the perception of smell of the chemical, which increased significantly after 3, 60, and 118 minutes if exposure. The NOAEL of 20 ppm was divided by an uncertainty factor of 10 (for human variability) to yield the MRL of 2 ppm. An adjustment to 24-hour exposure was not necessary because the first effects observed, as shown by Young et al. (1977), are local irritation effects that are not time-dependent.

Dose and end point used for MRL derivation: 20 ppm; NOAEL for eye and respiratory effects in humans.

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[ ] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: Not applicable.

Other additional studies or pertinent information which lend support to this MRL: Support for the acute-duration inhalation MRL of 2 ppm is provided by a study by Young et al. (1977) in which four healthy male volunteers were exposed to 50 ppm 1,4-dioxane for 6 hours under dynamic airflow conditions. Prior to the study, the subjects provided a complete history and underwent tests including chest x-ray, EKG, respiratory function tests, a conventional battery of 12 blood chemistry tests plus triglyceride and creatinine determinations, and complete hematological and urine analyses. Except for the chest x-ray, the tests were repeated 24 hours and 2 weeks after the exposure. The tests conducted 24 hours and 2 weeks after exposure did not reveal any exposure-related abnormalities, although no data were provided in the study. Eye irritation was a frequent and the only complaint throughout the exposure. Tolerance to the odor of 1,4-dioxane occurred during exposure. Two of the subjects could not perceive the odor after 4 and 5 hours in the chamber. The 50 ppm exposure level constitutes a minimal LOAEL for eye irritation, although there was no control experiment, and possible low humidity in the exposure chamber (not addressed in the report) might have contributed to the eye irritation.

Other studies with volunteers also support the findings of Ernstgård et al. (2006) and Young et al. (1977). For example, Silverman et al. (1946) exposed 12 subject to various concentrations of 1,4-dioxane for only 15 minutes and determined a NOAEL of 200 ppm for eye and nose irritation; the LOAEL was 300 ppm. Wirth and Klimmer (1936) reported that slight mucous membrane irritation started to take place in volunteers at exposure concentrations about 278 ppm for a few minutes (unspecified) and that at 1,390 ppm for several minutes, the subjects described prickling in the nose and scratchiness and dryness in the throat. Fairley et al. (1934) reported a NOAEL of 2,000 ppm (only level tested) for respiratory and ocular effects in six subjects exposed to 1,4-dioxane for only 3 minutes. Finally, Yant et al. (1930) described slight eye, nose, and throat irritation in a group of five subjects exposed to 1,600 ppm (only level tested) 1,4-dioxane for only 10 minutes. The available studies in animals used exposure concentrations that often caused death among the animals and were much higher than the concentrations tested by Young et al. (1977).

Agency Contact (Chemical Manager): Sharon Wilbur

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 1,4-Dioxane
CAS Number: 123-91-1
Date: August 2011
Profile Status: Final
Route: [X] Inhalation [ ] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 19
Species: Rat

Minimal Risk Level: 0.2 [ ] mg/kg/day [X] ppm

Reference: Kasai T, Saito M, Senoh H, et al. (2008). Thirteen-week inhalation toxicity of 1,4-dioxane in rats. Inhalation Toxicol 20: 961-971.

Experimental design: Groups of F344 DuCrj rats (10/sex/group) were exposed to target concentrations of 0 (clean air), 100, 200, 400, 800, 1,600, 3,200, or 6,400 ppm 1,4-dioxane vapors 6 hours/day, 5 days/week, for 13 weeks (Kasai et al. 2008). End points evaluated included mortality, clinical signs (daily), body weight and food consumption (once per week), hematology, clinical chemistry and urinalysis at termination, and gross and microscopic pathology of all major organs and tissues.

Effects noted in study and corresponding doses: All rats in the 6,400 ppm group died during the first week of the study. Examination of these rats showed that death was primarily caused by renal failure, as judged by marked necrosis observed in the renal tubules. Lung congestion was also observed in males and females from this exposure group. No abnormal clinical signs were observed during the study. Terminal body weight was reduced in all treated groups except the 100 ppm group, but not in a dose-related manner; the final weight reduced more than 10% relative to controls only in females exposed to 3,200 ppm. Data on food consumption were not provided. Changes in organ weight were limited to the liver, kidneys, and lungs and consisted in increases in relative organ weight generally in the high-dose groups of up to 15% relative to controls; data on absolute organ weights were not provided. Significant changes (although within normal values) in hematology and clinical chemistry parameters were limited to the 3,200 ppm groups and consisted of increases in mean corpuscular volume and serum ALT in males, decreases in glucose and triglycerides in males, and increases in red blood cell count, hemoglobin, hematocrit, and AST and ALT serum activities in females. Histologically, exposure to 1,4-dioxane affected principally the respiratory tract, in particular the nasal cavity of males and females. Significant nuclear enlargement of the respiratory epithelium was seen in all exposed groups. The incidences in males were 0/10 in the control group and 7/10, 9/10, 7/10, 10/10, 10/10, and 10/10 in exposed groups up to 3,200 ppm, respectively. The corresponding incidences in females were 0/10, 5/10, 9/10, 10/10, 10/10, 10/10, and 10/10. Severity of the lesion was dose-related. Significant nuclear enlargement of the olfactory epithelium started at 200 ppm (5/10 in males and 6/10 in females). Similar lesions in the trachea and bronchus appeared only in the high-exposure groups. The nuclear enlargement was characterized by the epithelial cells having a round to oval or elongated nucleus at least 4 times larger in diameter than normal. Significantly increased incidence of vacuolic change started in males at 400 ppm (0/10, 1/10, 3/10, 6/10, 10/10, 9/10) and in females at 800 ppm (0/10, 1/10, 2/10, 3/10, 7/10, 9/10, 10/10), while atrophy of the olfactory epithelium started in females at 800 ppm (0/10, 0/10, 2/10, 3/10, 5/10, 5/10, 4/10); incidence of atrophy of the olfactory epithelium in males was not presented. Significant single cell necrosis and centrilobular swelling occurred in the liver of males exposed to 3,200 ppm 1,4-dioxane; females in this exposure group showed only centrilobular swelling. Significant kidney changes were seen only in females from the 3,200 ppm exposure group and consisted of hydropic changes in the proximal tubule. No treatment-related lesions were reported in any other tissue or organ examined. Although

nuclear enlargement of the respiratory and olfactory epithelium occurred at lower exposure levels than other nasal lesions, it was not selected as the critical effect for MRL derivation on the grounds that the toxicological significance of the lesion is uncertain. There is some evidence suggesting that this alteration may represent a preneoplastic lesion. As discussed by Kasai et al. (2008), nuclear enlargement occurred as an early histopathological change in the respiratory tract of rats simultaneously exposed to sulfur dioxide and treated intraperitoneally with several N-nitrosamines known to induce nasal tumors in rats (Fowlie et al. 1990). In addition, studies have shown a good correlation between *in vivo* carcinogenicity and the extent of nuclear enlargement in HeLa cells *in vitro* (Grant and Grasso 1978). Since MRLs are not based on a consideration of cancer effects, nuclear enlargement is not considered a suitable basis of an MRL.

Dose and end point used for MRL derivation: $BMCL_{10}$ of 27.99 ppm for lesions in the olfactory epithelium of the nasal cavity in male rats.

[ ] NOAEL [ ] LOAEL [X] $BMCL_{10}$

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 3 for extrapolation from animals to humans with dosimetric adjustment
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: The intermediate-duration inhalation MRL was calculated using EPA's methodology (EPA 1994) for a category 3 gas, as explained in detail in Section 2.3 (derivation of the intermediate-duration inhalation MRL). A duration adjustment (6/24 hours x 5/7 days) seemed appropriate in the absence of information regarding whether Haber's Law is applicable under the experimental conditions of the study.

The MRL is derived as follows:

$$BMCL_{10[HEC]} = BMCL_{10[ADJ]} \times (H_{b/g}A / H_{b/g}H)$$

where:
$BMCL_{10[ADJ]}$ = 27.99 ppm x 6/24 hours x 5/7 days = 4.998 ppm and
$H_{b/g}A$ = animal blood:air partition coefficient = 1,861 (Sweeney et al. 2008)
$H_{b/g}H$ = human blood:air partition coefficient = 1,666 (Sweeney et al. 2008)

$$(H_{b/g}A / H_{b/g}H) = 1{,}861/1{,}666 = 1.117$$

Because the ratio of the partition coefficients is higher than 1, a default value of 1 is used in accordance with EPA's RfC methodology (EPA 1994).

$$BMCL_{[HEC]} = 4.998 \text{ ppm} \times 1 = 4.998 \text{ ppm}$$

Other additional studies or pertinent information which lend support to this MRL: Only one additional intermediate-duration inhalation study that exposed several animal species to high concentrations of 1,4-dioxane and monitored limited end points is available (Fairley et al. 1934). In that study, rats, mice,

guinea pigs, and rabbits were exposed to airborne 1,4-dioxane 3 hours/day, 5 days/week for periods of up to 12 weeks. At termination, examination of the animals revealed moderate to severe liver and kidney toxicity occurring at all exposure levels in all of the species tested. The lowest exposure level was 1,000 ppm. In a 2-year inhalation study, nasal alterations in both the respiratory and olfactory epithelium were reported in male rats (females were not tested) exposed to ≥50 ppm 1,4-dioxane vapors 6 hours/day, 5 days/week (Kasai et al. 2009). In the chronic-duration inhalation study in rats conducted by Torkelson et al. (1974), no interim histopathological evaluations were performed. In that study, rats were exposed 111 ppm 1,4-dioxane 7 hours/day (1 hour longer than Kasai et al. [2008]), 5 days/week for 2 years. Although Torkelson et al. (1974) reported that there were no treatment-related gross or microscopic lesions in the tissues examined and explicitly mention that there were no nasal tumors, the nasal cavity was not listed among the tissues and organs that were subjected to microscopic examination.

Agency Contact (Chemical Manager): Sharon Wilbur

BENCHMARK MODELING FOR CHANGES IN THE OLFACTORY EPITHELIUM IN RATS

Incidence data (Table A-1) for vacuolic change in the olfactory epithelium in male and female rats and of atrophy of the olfactory epithelium in female rats exposed to 1,4-dioxane vapors (Kasai et al. 2008) were analyzed using the BMD/BMC approach for MRL derivation. Models in the EPA Benchmark Dose Software (BMDS version 2.1.1) (Gamma, Logistic, Log-logistic, Multi-stage, Probit, Log-probit, Weibull models) were fit to the nasal lesions data to determine potential points of departure for the MRL. Adequate model fit is judged by three criteria: goodness-of-fit ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest benchmark concentration (BMCL, the lower limit of a one-sided 95% confidence interval on the BMC) is selected as the point of departure when differences between the BMCLs estimated from these models are more than 3-fold; otherwise, the BMCL from the model with the lowest Akaike's information criterion (AIC) is chosen. In accordance with EPA (2000a) guidance, BMCs and BMCLs associated with an extra risk of 10% are calculated for all models. Attempts to model the incidence of vacuolic change in the olfactory epithelium from male rats were unsuccessful in that none of the models could fit the data and/or had overall largest scaled residuals that exceeded the maximum value criteria of 2 (Table A-2). However, all models provided adequate fits after the highest dose was dropped. The best fit was provided by a Multistage (1-degree) model with a lowest predicted exposure concentrations associated with a 10% extra risk (BMC_{10}) of 40.39 ppm and a corresponding lower 95% confidence limit on this concentration ($BMCL_{10}$) of 27.99 ppm. Graphic representation of the fit is presented in Figure A-1. The best fit for the incidence data for vacuolic change in the olfactory epithelium from female rats was provided also by a Multistage (1-degree) model; the BMC_{10} and $BMCL_{10}$ values were 80.30 and 56.78 ppm, respectively (Table A-3); the dose-response curve is shown in Figure A-2. The best fit for the incidence data for atrophy of the olfactory epithelium in female rats was provided by a LogLogistic model; the BMC_{10} and $BMCL_{10}$ values were 172.57 and 103.81 ppm, respectively (Table A-4); the graphic representation of the fit is shown in Figure A-3. In order to be protective of human health, the lowest $BMCL_{10}$ of 27.99 ppm from the incidence of vacuolic change in the olfactory epithelium of male rats is selected as point of departure for MRL derivation. The $BMCL_{10}$ of 27.99 ppm was converted to a HEC ($BMCL_{10[HEC]}$) using the EPA cross-species dosimetric methodology (EPA 1994) for a category 3 gas, as explained in detail in Section 2.3.

Table A-1. Incidence Data for Vacuolic Change and Atrophy in the Nasal Cavity Olfactory Epithelium in F344 Rats Exposed to 1,4-Dioxane

Exposure concentration (ppm)						
0	100	200	400	800	1,600	3,200
Male rats (vacuolic change)						
0/10	1/10	3/10	6/10	10/10	10/10	9/10
Female rats (vacuolic change)						
0/10	1/10	2/10	3/10	7/10	9/10	10/10
Female rats (atrophy)						
0/10	0/10	2/10	3/10	5/10	5/10	4/10

Source: Kasai et al. 2008

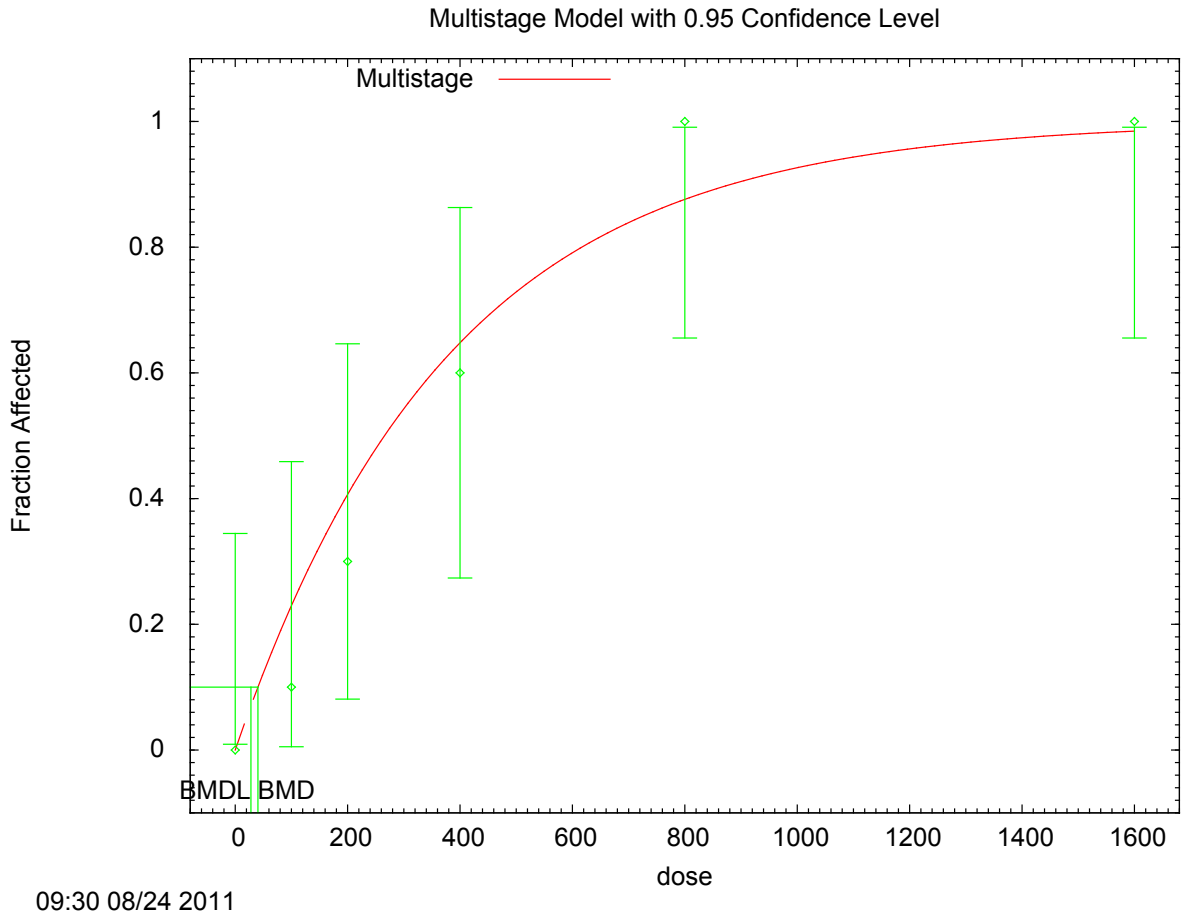
Table A-2. Model Predictions for the Incidence of Vacuolic Change in the Olfactory Epithelium In Male Rats Exposed to 1,4-Dioxane

Model	DF	χ^2	χ^2 Goodness of fit p-value[a]	Scaled residuals[b] Dose below BMC	Dose above BMC	Overall largest	AIC	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
All doses									
Gamma[c]	6	43.56	0	0.00	-0.60	-6.30	52.92	ND	ND
Logistic	5	316.7	0	-1.10	-0.07	-17.55	66.26	ND	ND
LogLogistic[d]	5	9.35	0.10	0.00	-0.15	-2.70	49.16	ND (LS)	ND (LS)
LogProbit[d]	6	11.88	0.06	0.00	-0.33	-3.04	48.51	ND	ND
Multistage (1-degree)[e]	6	43.56	0	0.00	-0.60	-6.30	52.92	ND	ND
Multistage (2-degree)[e]	6	43.56	0	0.00	-0.60	-6.30	52.92	ND	ND
Multistage (3-degree)[e]	6	43.56	0	0.00	-0.60	-6.30	52.92	ND	ND
Multistage (4-degree)[e]	6	43.56	0	0.00	-0.60	-6.30	52.92	ND	ND
Multistage (5-degree)[e]	6	43.56	0	0.00	-0.60	-6.30	52.92	ND	ND
Multistage (6-degree)[e]	6	43.56	0	0.00	-0.60	-6.30	52.92	ND	ND
Probit	5	56.22	0	-1.42	-0.30	-6.40	71.55	ND	ND
Weibull[c]	6	43.56	0	0.00	-0.60	-6.30	52.92	ND	ND
Highest dose dropped									
Gamma[c]	4	0.79	0.94	0.25	0.09	-0.61	37.29	112.53	47.46
Logistic	4	1.09	0.90	0.07	0.65	0.65	37.70	140.81	91.82
LogLogistic[d]	4	1.65	0.80	0.48	0.06	-0.82	38.47	121.09	64.79
LogProbit[d]	4	1.48	0.83	0.50	-0.09	-0.78	38.16	118.46	66.52
Multistage (1-degree)[e,f]	***5***	***3.09***	***0.69***	***0.00***	***-0.98***	***1.19***	***38.86***	***40.39***	***27.99***
Multistage (2-degree)[e]	4	0.42	0.98	0.05	0.20	-0.45	36.76	103.17	42.53
Multistage (3-degree)[e]	4	0.22	0.99	0.00	-0.16	0.30	36.45	86.98	39.84
Multistage (4-degree)[e]	3	0.16	0.98	0.00	-0.22	0.28	38.35	82.28	37.99
Multistage (5-degree)[e]	3	0.12	0.99	0.00	-0.19	0.25	38.30	84.93	37.02
Probit	4	0.79	0.94	0.09	0.57	0.57	37.26	131.72	86.59
Weibull[c]	4	0.52	0.97	0.19	0.23	-0.51	36.87	111.01	49.07

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
[f]Selected model.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; ND = not determined, goodness-of-fit criteria, p<0.10; ND (LS) = not determined; largest scaled residual >2

Figure A-1. Fit of Multistage 1 Degree Polynomial Model to Data on 1,4-Dioxane, Incidence of Vacuolic Change in the Olfactory Epithelium of Male Rats Exposed via Inhalation for 13 Weeks



Source: Kasai et al. 2008

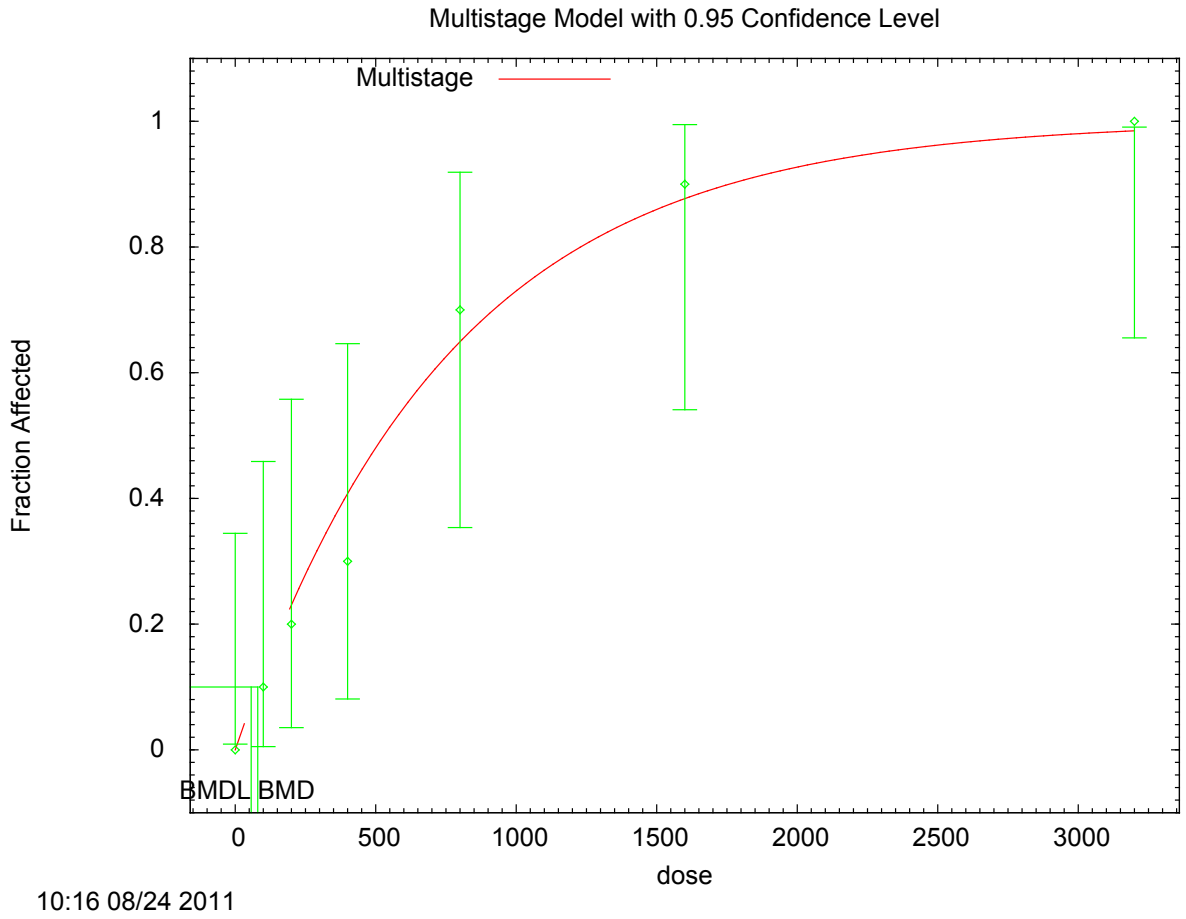
Table A-3. Model Predictions for the Incidence of Vacuolic Change in the Olfactory Epithelium in Female Rats Exposed by Inhalation to 1,4-Dioxane Vapor for 13 Weeks

Model	DF	χ^2	χ^2 Goodness of fit p-value[a]	Scaled residuals[b]: Dose below BMC	Scaled residuals[b]: Dose above BMC	Scaled residuals[b]: Overall largest	AIC	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
Gamma[c]	5	0.46	0.99	0.22	0.14	-0.51	51.97	119.11	58.89
Logistic	5	2.48	0.78	0.35	0.18	-0.97	54.53	238.59	165.80
LogLogistic[d]	5	1.30	0.93	0.59	0.25	-0.75	53.05	141.58	64.86
LogProbit[d]	5	1.35	0.93	0.64	0.12	-0.81	53.00	136.97	95.47
Multistage (1-degree)[e,f]	***6***	***0.90***	***0.99***	***0.00***	***-0.22***	***0.39***	***50.52***	***80.30***	***56.78***
Multistage (2-degree)[e]	5	0.39	1.00	0.04	0.09	-0.41	51.86	104.27	59.36
Multistage (3-degree)[e]	5	0.39	1.00	0.04	0.09	-0.41	51.86	104.27	59.36
Multistage (4-degree)[e]	4	0.40	0.98	0.04	0.09	0.42	53.86	103.74	59.36
Multistage (5-degree)[e]	4	0.39	0.98	0.03	0.08	0.43	53.85	103.04	59.32
Multistage (6-degree)[e]	4	0.39	0.98	0.03	0.08	0.43	53.85	102.80	59.27
Probit	5	2.59	0.76	0.33	0.19	-0.96	54.71	230.67	165.00
Weibull[c]	5	0.42	0.99	0.19	0.14	-0.47	51.90	116.72	59.17

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
[f]Selected model.

AIC = Akaike Information Criterion; BMC = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMCL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom

Figure A-2. Fit of Multistage 1 Degree Polynomial Model to Data on 1,4-Dioxane, Incidence of Vacuolic Change in the Olfactory Epithelium of Female Rats Exposed via Inhalation for 13 Weeks



Source: Kasai et al. 2008

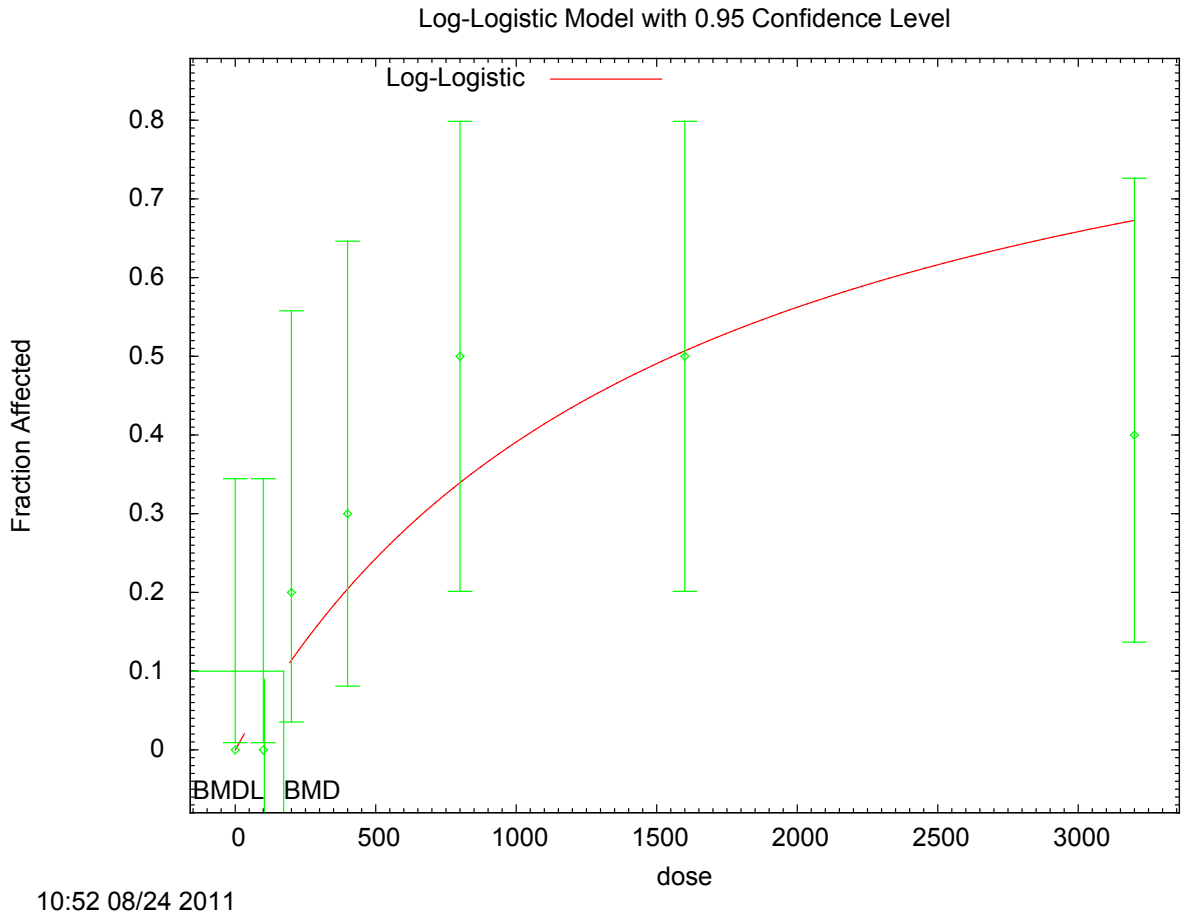
Table A-4. Model Predictions for the Incidence of Atrophy of the Olfactory Epithelium in Female Rats Exposed by Inhalation to 1,4-Dioxane Vapor for 13 Weeks

Model	DF	χ^2	χ^2 Goodness of fit p-value[a]	Scaled residuals[b] Dose below BMC	Scaled residuals[b] Dose above BMC	Scaled residuals[b] Overall largest	AIC	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
Gamma[c]	5	9.15	0.10	0.77	1.05	-1.71	77.39	336.54	193.55
Logistic	5	10.35	0.07	1.84	1.08	1.84	80.74	898.33	580.22
LogLogistic[d,e]	***6***	***6.47***	***0.37***	***-0.80***	***0.86***	***-1.84***	***71.99***	***172.57***	***103.81***
LogProbit[d]	5	11.48	0.04	1.17	1.78	-1.87	80.50	ND	ND
Multistage (1-degree)[f]	5	9.15	0.10	0.77	1.05	-1.71	77.39	336.54	193.55
Multistage (2-degree)[f]	5	9.15	0.10	0.77	1.05	-1.71	77.39	336.54	193.55
Multistage (3-degree)[f]	5	9.15	0.10	0.77	1.05	-1.71	77.39	336.54	193.55
Multistage (4-degree)[f]	5	9.15	0.10	0.77	1.05	-1.71	77.39	336.54	193.55
Multistage (5-degree)[f]	5	9.15	0.10	0.77	1.05	-1.71	77.39	336.54	193.55
Multistage (6-degree)[f]	5	9.15	0.10	0.77	1.05	-1.71	77.39	336.54	193.55
Probit	5	10.26	0.07	1.85	1.06	-1.48	80.52	846.81	557.27
Weibull[c]	5	9.15	0.10	0.77	1.05	-1.71	77.39	336.54	193.55

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residuals at doses immediately below and above the BMD; also the largest residual at any dose.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Selected model.
[f]Betas restricted to ≥0.

AIC = Akaike Information Criterion; BMC = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMC (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); DF = degrees of freedom; ND = not determined, goodness-of-fit criteria, p<0.10

Figure A-3. Fit of Log Logistic Model to Data on 1,4-Dioxane, Incidence of Atrophy of the Olfactory Epithelium in Female Rats Exposed via Inhalation for 13 Weeks



Source: Kasai et al. 2008

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 1,4-Dioxane
CAS Number: 123-91-1
Date: August 2011
Profile Status: Final
Route: [X] Inhalation [ ] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 27
Species: Rat

Minimal Risk Level: 0.03 [ ] mg/kg/day [X] ppm

Reference: Kasai T, Kano H, Umeda Y, et al. 2009. Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male. Inhal Toxicol 21:889-897.

Experimental design: Groups of male F344/DuCrj rats (50/group) were exposed whole-body to target concentrations of 0, 50, 250, or 1,250 ppm 1,4-dioxane vapors 6 hours/day, 5 days/week for 104 weeks; controls were exposed to clean air. End points evaluated included clinical signs and mortality (daily) and body weight and food consumption (once /week for the first 14 weeks, every 4 weeks thereafter). All rats were subjected to complete necropsy. Blood was collected at termination for clinical chemistry and hematology tests; urinary pH was measured in the last week of the study. All major organs were removed, weighed, and examined for macroscopic lesions. All major tissues and organs, including the entire respiratory tract, were examined microscopically.

Effects noted in study and corresponding doses: Survival rates in rats exposed to 250 ppm tended to decrease relative to controls, but the difference with controls was not statistically significant. Exposure to 1,250 ppm 1,4-dioxane significantly reduced ($p<0.05$) survival rate beginning on week 91. Terminal survival rate was 37/50, 37/50, 29/50, and 25/50 in the control, low-, mid-, and high-exposure groups, respectively. The decreased survival rates were attributed to increased number of deaths due primarily to peritoneal mesotheliomas, although nasal tumors contributed to the causes of death. Terminal body weight was reduced 6.3% in the high-exposure group. Food consumption was not affected by exposure to 1,4-dioxane. Significant increases in relative liver (27%) and lung (2%) weights were reported in the high-exposure group but there was no clear dose-response relationship. Significant changes in hematology and clinical chemistry tests included reduced hemoglobin (13%), MCV (6%), MCH (8%), increased serum AST (46%), ALT (95%), AP (15%), and γ-GTP (6–7-fold); urinary pH was reduced 7%. All of these changes were restricted to the high-exposure group. Treatment-related pre- and nonneoplastic lesions occurred in the nasal cavity, liver, and kidney. All exposed groups had significant increases in nuclear enlargement of the respiratory epithelium (0/50, 50/50, 48/50, 38/50), nuclear enlargement of the olfactory epithelium (0/50, 48/50, 48/50, 45/50), atrophy of olfactory epithelium (0/50, 40/50, 47/50, 48/50), and respiratory metaplasia of the olfactory epithelium (11/50, 34/50, 49/50, 48/50). Significant increases in liver lesions (centrilobular nuclear enlargement, acidophilic cell foci, basophilic cell foci, spongiosis hepatis, and centrilobular necrosis) occurred in the high-exposure group. Significant increases in nuclear enlargement of the proximal kidney tubule occurred in the mid- and high-exposure groups; significantly increased incidence of hydropic changes in the proximal tubule occurred in the high-exposure group. No significant changes occurred in other organs or tissues. The lowest exposure concentration tested, 50 ppm 1,4-dioxane, is a LOAEL for nasal lesions (atrophy of the olfactory epithelium), a NOAEL was not defined in this study.

The results of Kasai et al. (2009) clearly show that the nasal cavity was the most sensitive tissue following 2 years of exposure to 1,4-dioxane vapors. As discussed in the derivation of the intermediate-duration inhalation MRL for 1,4-dioxane, nuclear enlargement will not be considered a suitable basis for derivation of an MRL because it may represent a pre-neoplastic lesion. Incidences of atrophy (0/50, 40/50, 47/50, and 48/50) and respiratory metaplasia (11/50, 34/50, 49/50, and 48/50) of the olfactory epithelium were also significantly elevated at all exposure levels tested. Of these two lesions, the atrophy of the olfactory epithelium was selected as the critical effect for MRL derivation because it showed a higher incidence rate at the LOAEL than respiratory metaplasia. Because the incidence of this lesion at the lowest exposure level (50 ppm) was close to the maximal response level (80% of 50-ppm animals showed this lesion), BMD analysis of the data was not conducted. This decision is in accordance with guidelines stating that studies in which responses are at or near the maximal response level are not considered adequate for BMD analysis (EPA 2000a).

Dose and end point used for MRL derivation: 50 ppm; LOAEL for atrophy of the olfactory epithelium of the nasal cavity in male rats.

[ ] NOAEL [X] LOAEL

Uncertainty Factors used in MRL derivation:

[X] 10 for use of a LOAEL
[X] 3 for extrapolation from animals to humans with dosimetric adjustment
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? Not applicable.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: The chronic-duration inhalation MRL was calculated using EPA's methodology (EPA 1994) for a category 3 gas, as explained in detail in Section 2.3 (derivation of the intermediate-duration inhalation MRL). A duration adjustment (6/24 hours x 5/7 days) seemed appropriate in the absence of information regarding whether Haber's Law is applicable under the experimental conditions of the study.

The MRL is derived as follows:

$$LOAEL_{[HEC]} = LOAEL_{[ADJ]} \times (H_{b/g}A / H_{b/g}H)$$

where:
$LOAEL_{[ADJ]}$ = 50 ppm x 6/24 hours x 5/7 days = 8.9286 ppm and
$H_{b/g}A$ = animal blood:air partition coefficient = 1,861 (Sweeney et al. 2008)
$H_{b/g}H$ = human blood:air partition coefficient = 1,666 (Sweeney et al. 2008)

$$(H_{b/g}A / H_{b/g}H) = 1{,}861/1{,}666 = 1.117$$

Because the ratio of the partition coefficients is higher than 1, a default value of 1 is used in accordance with EPA's RfC methodology (EPA 1994).

$$LOAEL_{[HEC]} = 8.9286 \text{ ppm} \times 1 = 8.9286 \text{ ppm}$$

Other additional studies or pertinent information which lend support to this MRL: In a study conducted by Torkelson et al. (1974), groups of Wistar rats (288/sex) were exposed to 1,4-dioxane vapors at a

concentration of 0.4 mg/L (111 ppm) 7 hours/day, 5 days/week for 2 years. Controls were exposed to filtered room air. End points examined included clinical signs, eye and nasal irritation, skin condition, respiratory distress, and tumor formation. Hematological parameters (hemoglobin, red blood cell count, total and differential leukocyte counts, corpuscular volume) were determined after 16 and 23 months of exposure. Blood collected at termination was used also for determination of clinical chemistry parameters (serum ALT and alkaline phosphatase activity, BUN, total protein). Liver, kidneys, and spleen were weighed and the major tissues and organs were processed for microscopic examination. Exposure to 1,4-dioxane vapors had no significant effect on mortality or body weight gain and induced no signs of eye or nasal irritation or respiratory distress. Slight but statistically significant changes in hematological and clinical chemistry parameters were within the normal physiological limits and were considered of no toxicological importance. Organ weights were not significantly affected. Microscopic examination of organs and tissues did not reveal treatment-related effects. It should be noted, however, that the tissues from the nasal cavity were not listed among the tissues that were subjected to microscopic examination by Torkelson et al. (1974); therefore, the possibility exists that nasal lesions were present but were not detected. This possibility is strengthened by the results of Kasai et al. (2008) that reported a significant incidence of nasal lesions in rats following inhalation exposure to 100 ppm 1,4-dioxane for 13 weeks.

Agency Contact (Chemical Manager): Sharon Wilbur

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 1,4-Dioxane
CAS Number: 123-91-1
Date: August 2011
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 18
Species: Rat

Minimal Risk Level: 5 [X] mg/kg/day [ ] ppm

Reference: Giavini E, Vismara C, Broccia MA. 1985. Teratogenesis study of dioxane in rats. Toxicol Letters 26:85-88.

Experimental design: Groups of 17–20 pregnant Sprague-Dawley rats were treated with 0, 0.25, 0.5, or 1 mL 1,4-dioxane/kg/day (0, 258, 516, or 1,033 mg 1,4-dioxane/kg/day based on a specific gravity of 1.034) by gavage in water on Gds 6–15. Food consumption was determined daily and body weight was monitored every 3 days. Sacrifices were conducted on Gd 21 and the number of corpora lutea, implantations, resorptions, and liver fetuses was recorded. The fetuses were weighed and inspected for external malformations and half were examined for visceral abnormalities; the other half were examined for skeletal malformations.

Effects noted in study and corresponding doses: Rats treated with 1,033 mg 1,4-dioxane/kg/day gained 18% less weight than controls during treatment days, although the difference was not statistically significant. Food consumption was slightly (5%) but significantly ($p<0.05$) reduced in these rats during treatment. The average fetal weight in the high-dose group was slightly but significantly ($p<0.01$) lower than in controls. Also, a slight but significant ($p<0.05$) reduction in sternum ossification was seen in high-dose fetuses. There were no significant effects on the number of implantations and live fetuses, post-implantation loss, or incidence of malformations. Based on the reduced maternal and fetal body weight and reduced sternum ossification, a maternal and developmental LOAEL of 1,013 mg 1,4-dioxane/kg/day can be defined; the maternal and developmental NOAEL is 516 mg/kg/day. Attempts made to apply dose-response models to the data were unsuccessful, as no adequate fits of EPA BMDS models to the data were obtained; therefore, the NOAEL/LOAEL approach was used for MRL derivation.

Dose and end point used for MRL derivation: 516 mg/kg/day; NOAEL for developmental and maternal effects in rats.

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? A conversion was done from mL of 1,4-dioxane to mg of 1,4-dioxane using the specific gravity of 1,4-dioxane.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: Not applicable.

Other additional studies or pertinent information which lend support to this MRL: JRBC (1998) conducted a 2-week drinking water study in F344 rats and B6C3F$_1$ mice and reported that the most sensitive effect was an increased incidence of nuclear enlargement of the olfactory epithelium in male and female rats receiving doses of approximately 1,010 and 1,040 mg 1,4-dioxane/kg/day, respectively; the corresponding NOAELs were 370 and 400 mg/kg/day. The use of the nasal lesions as the point of departure for MRL derivation was precluded by recent data strongly suggesting that these lesions in rats are due to direct contact of the drinking water containing 1,4-dioxane with nasal epithelium while the rats drink the water(Sweeney et al. 2008). Increased incidence of hepatocyte swelling and vacuolation and hydropic changes in the renal proximal tubule were also reported in male and female rats dosed with 2,960 and 2,750 mg 1,4-dioxane/kg/day, respectively; the corresponding NOAELs were 1,010 and 1,040 mg/kg/day. Although the NOAELs for liver and kidney changes could have been considered as points of departure for MRL derivation, several study limitations, including the lack of statistical analysis of the results due to the fact that only 2 or 3 animals (out of 10/group) were examined, and the fact that end points such as hematology, clinical chemistry, clinical signs, and gross examinations were not conducted or reported, severely compromise the interpretation of the results.

Agency Contact (Chemical Manager): Sharon Wilbur

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 1,4-Dioxane
CAS Number: 123-91-1
Date: August 2011
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 22
Species: Rat

Minimal Risk Level: 0.5 [X] mg/kg/day [ ] ppm

Reference: Kano et al. 2008. Thirteen-week oral toxicity of 1,4-dioxane in rats and mice. J Toxicol Sci 33:141-153.

Experimental design: The intermediate-duration oral MRL is based on a NOAEL of 52 mg 1,4-dioxane/ kg/day for liver effects in rats. Groups of F344/DuCrj rats (10/sex/group) were administered 1,4-dioxane in the drinking water in concentrations of 0, 640, 1,600, 4,000, 10,000, or 25,000 ppm for 13 weeks (0, 52, 126, 274, 657, or 1,554 mg/kg/day in males; 0, 83, 185, 427, 756, or 1,614 mg/kg/day in females, estimated by the investigators). End points evaluated included clinical signs (daily), food (once a week) and water consumption (daily), body weight (once a week), complete hematology and clinical chemistry tests (at termination), urinalysis (at termination), organ weights, gross necropsy and histopathology.

Effects noted in study and corresponding doses: One female in the 1,614 mg/kg/day group died. Body weight gain was reduced at 756 mg/kg/day (12%) and 1,614 mg/kg/day (21%) in females and at 1,554 mg/kg/day (21%) in males. Food consumption was reduced 13% in females at 1,614 mg/kg/day. Water consumption was reduced in a dose-related manner in all male groups and in females at ≥126 mg/kg/day. Hematology tests showed significant increases in erythrocyte counts, hemoglobin, hematocrit, and neutrophils, and a decrease in lymphocytes in males at 1,554 mg/kg/day, and decreases in mean corpuscular volume and platelets in females at 1,614 mg/kg/day. Total protein and albumin were decreased in males at ≥274 mg/kg/day and in females at ≥427 mg/kg/day. Serum AST, ALT, AP, and LAP activities, and levels of cholesterol, triglycerides, sodium, and glucose were significantly elevated in high dose males and females. Urinary pH was decreased in males at ≥274 mg/kg/day and in females at ≥756 mg/kg/day. Absolute and relative kidney weights were increased in females at ≥231 mg/kg/day. Nuclear enlargement of the respiratory epithelium occurred in males at ≥126 mg/kg/day and in females at ≥185 mg/kg/day; nuclear enlargement of the olfactory and tracheal epithelium occurred in males at ≥274 mg/kg/day and in females at ≥427 mg/kg/day. Swelling of the central area of the liver was observed in males at ≥126 mg/kg/day and in females at ≥756 mg/kg/day, and vacuolar changes in the liver occurred in males at ≥657 mg/kg/day and in females at 1,614 mg/kg/day. The incidences of swelling of the central area of the liver in males were 0/10, 0/10, 9/10, 10/10, 10/10, and 10/10 in the control, 52, 126, 274, 657, and 1,554 mg/kg/day dose groups, respectively. Nuclear enlargement of the proximal tubule of the kidneys was seen in males at ≥657 mg/kg/day and in females at ≥756 mg/kg/day. Hydropic changes in the proximal tubule of the kidneys and vacuolar changes in the brain occurred in high-dose males and females (1,554 and 1,614 mg/kg/day, respectively). The study LOAEL was 126 mg/kg/day for liver effects in male rats. Limitations of the study include the lack of reporting on clinical signs and gross necropsy. To derive the MRL, the NOAEL of 52 mg/kg/day for liver effects in males was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability), yielding an intermediate-duration oral MRL of 0.5 mg/kg/day. The steepness of the dose-response relationship for liver lesions rendered the data set inadequate for BMD analysis.

Dose and end point used for MRL derivation: 52 mg/kg/day; NOAEL for liver effects in rats.

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? The conversion was done by the investigators, and the doses listed are means of ranges provided by the investigators.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: Not applicable.

Other additional studies or pertinent information which lend support to this MRL: A study by Lundberg et al. 1987) supports the liver findings of Kano et al. (2008). The study used male Sprague-Dawley rats (8–11/group) that were treated with 100 or 1,000 mg 1,4-dioxane/kg by gavage in saline 5 days/week for 7 weeks. One week after the last treatment, the rats were killed and the livers were processed for microscopic examination. The livers of high-dose rats showed enlarged foamy hepatocytes mainly in midzonal regions. The foamy appearance was due to vacuoles shown to contain fat. No treatment-related histopathological alterations were observed in the liver at the 100 mg/kg/day dose level. Also supporting the findings from Kano et al. (2008) is a report by Stott et al. (1981) who found that repeated dosing of rats with 1,000 mg 1,4-dioxane/kg/day for 7 or 11 weeks produced hepatocyte swelling and histopathology. Similar findings were reported in an earlier study in which rats were treated with doses of approximately 1,428 mg 1,4-dioxane/kg/day in the drinking water for 34 days (Fairley et al. 1934).

Agency Contact (Chemical Manager): Sharon Wilbur

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 1,4-Dioxane
CAS Number: 123-91-1
Date: August 2011
Profile Status: Final
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 39
Species: Rat

Minimal Risk Level: 0.1 [X] mg/kg/day [ ] ppm

Reference: Kociba RJ, McCollister SB, Park C, et al. 1974. 1,4-Dioxane. I. Results of a 2-year ingestion study in rats. Toxicol Appl Pharmacol 30:275-286.

Experimental design: Groups of Sherman rats (60/sex/dose level) were treated with 1,4-dioxane in the drinking water at levels of 0 (controls), 0.01, 0.1, or 1% for 716 days. Based on body weight and water consumption data, the investigators estimated that the water provided doses of 1,4-dioxane of 0, 9.6, 94, and 1,015 mg/kg/day for males and 0, 19, 148, and 1,599 mg/kg/day for females. Blood samples were collected from controls and high-dose rats during the 4th, 6th, 12th, and 18th months of the study and at termination. Additional end points evaluated included clinical signs, body weight, organ weights, and gross and microscopic examination of major tissues and organs.

Effects noted in study and corresponding doses: Treatment with 1,4-dioxane significantly increased mortality in high-dose males and females beginning at about 2–4 months of treatment. These rats showed degenerative changes in both the liver and kidneys. Body weight gain was significantly reduced in high-dose animals from the beginning of the study. Microscopic lesions were restricted to the liver and kidneys from the mid- and high-dose groups. The liver lesions consisted of various degrees of hepatocellular degeneration and necrosis and evidence of hepatic regeneration as indicated by hepatocellular hyperplastic nodule formation. The NOAEL for liver effects was 9.6 mg/kg/day in males and 19 mg/kg/day in females. The LOAELs were 94 mg/kg/day in males and 148 mg/kg/day in females. The kidneys showed tubular epithelial degeneration and necrosis, and there was evidence of renal tubular regeneration as indicated by increased tubular epithelial regenerative activity (≥94 mg/kg/day in males and ≥148 mg/kg/day in females). There were no compound-related alterations in hematological parameters at any time point. The MRL of 0.1 mg/kg/day was calculated by dividing the male rat NOAEL of 9.6 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability). The lack of quantitative information regarding incidences of non-neoplastic lesions precludes the use of BMD methodology for MRL derivation.

Dose and end point used for MRL derivation: 9.6 mg/kg/day; NOAEL for liver effects in rats.

[X] NOAEL [ ] LOAEL

Uncertainty Factors used in MRL derivation:

[ ] for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? A conversion was done by the investigators.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: Not applicable.

Other additional studies or pertinent information which lend support to this MRL: The NOAEL and LOAEL for liver effects from Kociba et al. (1974) are supported by the results of Kano et al. (2009). In that study, groups of F344/DuCrj rats (50/sex/dose level) received 1,4-dioxane in the drinking water for 104 weeks. 1,4-Dioxane was administered at levels of 0, 200, 1,000, and 5,000 ppm for 2 years (0, 11, 55, and 274 mg/kg/day for males; 0, 18, 83, and 429 mg/kg/day for females). End points evaluated included clinical signs, food and water consumption, body and organ weights, and gross and microscopic examination of major organs and tissues. Terminal body weight was reduced 9% in high-dose males (274 mg/kg/day) and 20% in high-dose females (429 mg/kg/day). In males, relative liver weight was significantly increased at 55 mg/kg/day (14%) and 274 mg/kg/day (72%). A significant increased incidence of mixed cell foci was observed in the liver from male rats dosed with ≥55 mg 1,4-dioxane/kg/day. Increased incidence of acidophilic and mixed cell foci were reported in the liver from high-dose females (429 mg/kg/day). In addition, both high-dose male (274 mg/kg/day) and female (429 mg/kg/day) rats had significantly increased incidence of nuclear enlargement and squamous cell metaplasia of the respiratory epithelium; females dosed with ≥83 mg 1,4-dioxane/kg/day also showed significantly increased incidence of nuclear enlargement of the nasal olfactory epithelium.

The NCI (1978) bioassay in Osborne-Mendel rats used somewhat higher dose levels than Kociba et al. (1974) and Kano et al. (2009), but did not observe liver lesions in male rats dosed with 240 mg 1,4-dioxane/kg/day, a dose level that caused liver hyperplasia in male F344 rats dosed with 81 mg/kg/day or that caused hepatocyte degeneration in Sherman rats dosed with 94 mg/kg/day. Since the dosing method was the same in the three studies, the drinking water, the different results may reflect differences in strain sensitivity.

Agency Contact (Chemical Manager): Sharon Wilbur

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APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system, which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

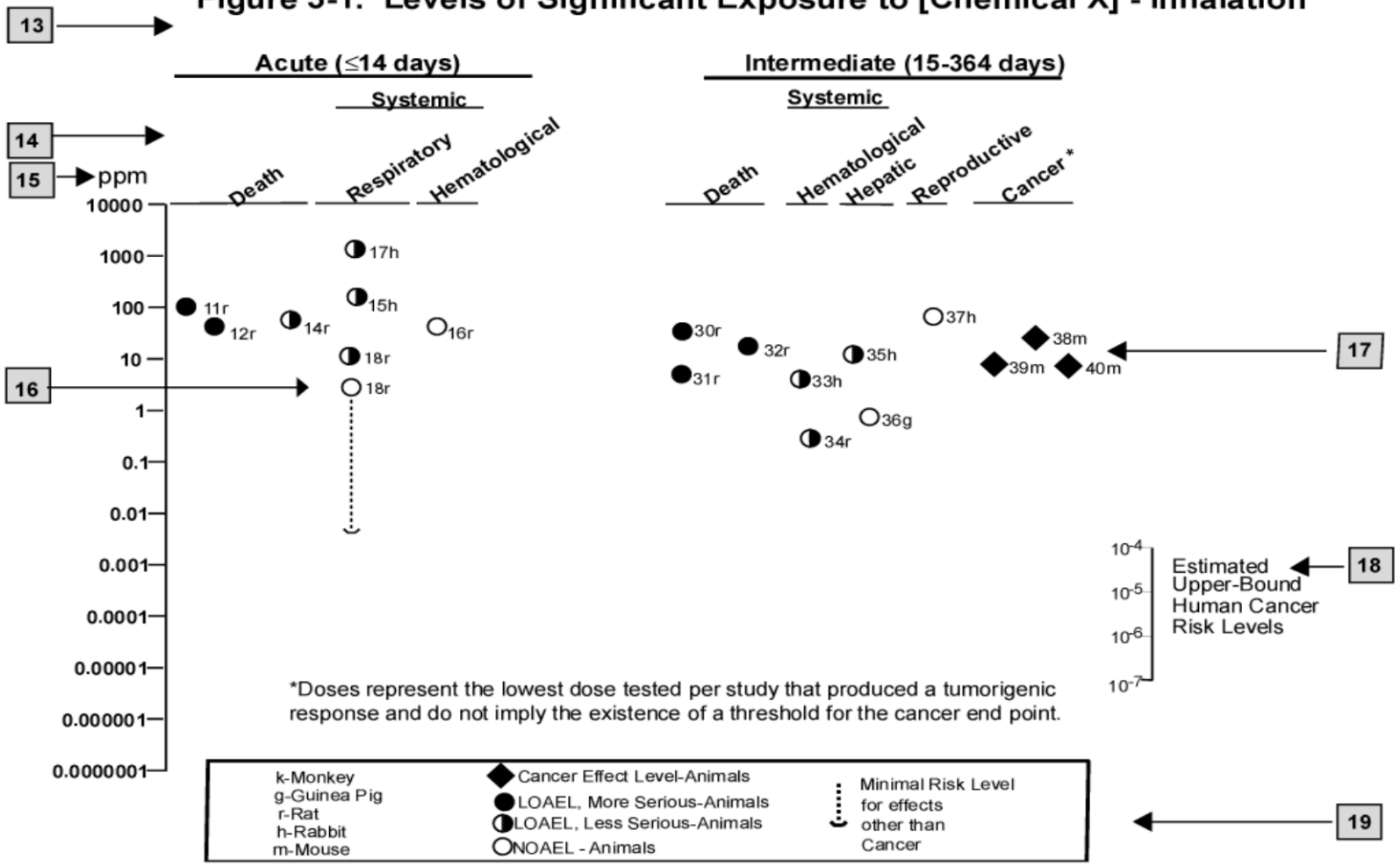
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	Serious (ppm)	Reference
2 → INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 → Systemic	↓	↓	↓	↓	↓		↓
18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
4 → CHRONIC EXPOSURE							
Cancer					11		
					↓		
38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of $5x10^{-3}$ ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



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APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD	benchmark dose
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation
DOT/UN/ NA/IMCO	Department of Transportation/United Nations/ North America/Intergovernmental Maritime Dangerous Goods Code

DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans,trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor

MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances
OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon

PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

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APPENDIX D. HEALTH ADVISORY

Health Advisory - An Overview for the Public

1,4-Dioxane

August 2007

Why is 1,4-dioxane currently a potential health concern?

Conflicting reports regarding 1,4-dioxane exposure from use of some bath and cosmetic products

Recent reports in the media about 1,4-dioxane contamination of children's bath products prompted ATSDR to reexamine its recommendations to families on reducing risks of exposure to 1,4-dioxane. **Note:** The acute effects described in this document are not likely to occur at concentrations of 1,4-dioxane that are normally found in the U.S. environment.

Why has the Agency for Toxic Substances and Disease Registry (ATSDR) provided this health advisory for 1,4-dioxane?

ATSDR provides trusted health information to the public

ATSDR's mission is to serve the public by using the best science, taking responsive public health actions, and providing trusted health information to prevent harmful exposures and disease related exposures to toxic substances.

What is 1,4-dioxane?

1,4-Dioxane is used in manufacturing and in household products

1,4-Dioxane (also called dioxane) is produced in large amounts (between 10 million and 18 million pounds in 1990) by three companies in the United States. Companies use dioxane:

- for a solvent for paper, cotton, and textile processing
- for chemical manufacturing, and
- in automotive coolant liquid.

APPENDIX D

How are people exposed to 1,4-dioxane?

Transmission through inhalation, ingestion, or skin contact

1,4-Dioxane enters the body when people breathe air or consume water or food contaminated with 1,4-dioxane. People can also be exposed following contact with cosmetics, shampoo, or bubble bath that contain certain ingredients in which 1,4-dioxane may be a contaminant. 1,4-Dioxane does not remain in the body because it breaks down into chemicals that are removed quickly.

Where is 1,4-dioxane found ?

Food	Traces of 1,4-dioxane can be ingested from: • some food supplements • food containing residues from packaging adhesives • food sprayed with pesticides containing 1,4-dioxane as a solvent or inert ingredient
Ground Water	A few communities' water supplies are contaminated with 1,4-dioxane. Information on the concentrations of 1,4-dioxane in groundwater, surface waters and drinking water are limited.
Household products	1,4-Dioxane may be present as a trace contaminant in household products such as: • shampoo • liquid dishwashing soap • baby lotion • hair lotions • bath foam • and other cosmetic products
Industrial solvents	1,4-Dioxane is primarily used as an industrial solvent in several manufacturing processes.
Spermicidal agents	1,4-Dioxane is found in some over-the-counter spermicidal sponges.

What are the health effects of 1,4-dioxane exposure?

Effects of 1,4-dioxane on human health and the environment depend on how much 1,4-dioxane is present and the length and frequency of exposures. **Note:** The acute effects described below are not likely to occur at concentrations of 1,4-dioxane that are normally found in the U.S. environment.

Short-term exposure to 1,4-dioxane

- Breathing: 1,4-Dioxane for short periods of time causes irritation of the eyes, nose and throat in humans. Exposure to large amounts of 1,4-dioxane can cause kidney and liver damage.
- Accidental worker exposure to large amounts of 1,4-dioxane has resulted in several deaths. Symptoms associated with these industrial deaths suggest 1,4-dioxane causes adverse nervous system effects.

Long-term exposure to 1,4-dioxane

- Animal studies: Laboratory studies show that repeated exposure to large amounts of 1,4-dioxane in drinking water, in air, or on the skin causes liver and kidney damage in animals Laboratory studies also show that oral exposure to 1,4-dioxane over a lifetime causes cancer in animals. Skin exposure of animals to 1,4-dioxane has shown that it can increase the cancer-causing properties of other chemicals.
- Human studies: There is little specific information regarding the non-cancer outcomes in workers following repeatedly breathing small amounts of 1,4-dioxane over long periods of time.
- Cancer classifications: (based on inadequate evidence in humans and sufficient evidence in animals):
    - Department of Health and Human Services (HHS) considers 1,4-dioxane as reasonably anticipated to be a human carcinogen.
    - Environmental Protection Agency (EPA) established that 1,4-dioxane is a probable human carcinogen.
    - International Agency for Research on Cancer (IARC) has determined that 1,4-dioxane is possibly carcinogenic to humans.

Reproductive health/infants and 1,4-dioxane

- Miscarriage and stillbirths: There are studies that show elevated rates of spontaneous abortion and stillbirths associated with occupational exposure to a combination of chemicals that included 1,4-dioxane, but the role of 1,4-dioxane, if any, is unknown.
- Breast milk transfer: A nursing mother exposed to a high amount of 1,4-dioxane might pass it to the infant through her breast milk. This concern is based on scientific models, not on actual data from the breast milk of women exposed to 1,4-dioxane.

Is there a medical test to show whether I've been exposed to 1,4-dioxane?

1,4-Dioxane and its breakdown products can be measured in your blood and urine

1,4-Dioxane and its breakdown products can be measured in your blood and urine, and positive results indicate you have been exposed to 1,4-dioxane. The tests are not routinely available at your doctor's office because they require special equipment, but the doctor can collect the samples and send them to a special laboratory. The tests need to be conducted within days after the exposure because 1,4-dioxane and its breakdown products leave the body fairly rapidly. These tests do not predict whether exposure to 1,4-dioxane will produce harmful health effects.

What levels of 1,4-dioxane are considered acceptable by regulatory agencies?

1,4-Dioxane levels in food set by the Food and Drug Administration (FDA)

- The National Academy of Sciences (NAS) specified a maximum limit of 10 ppm (parts per million) for 1,4-dioxane in the ingredient polysorbate, a food additive (NAS 2003).
- FDA also set a limit on 1,4-dioxane at 10 ppm in approving glycerides and polyglycerides in products such as dietary supplements. This regulation is located at 21 CFR 172.736. The FDA regulation for 1,4-dioxane as an indirect food additive is also 10 ppm and refers to its use as an adhesive component in packaging material.

1,4-Dioxane levels in cosmetics-voluntary cooperation

- FDA's regulatory legal authority over the cosmetics is different from other products regulated by the agency such as drugs, biologics, and medical devices. Consequently, FDA must rely, in part, on voluntary industry cooperation.
- Whereas the press has recently reported that FDA recommends 10 ppm for 1,4-dioxane in cosmetic products, the FDA does not have a recommendation for 1,4-dioxane in cosmetic products.

1,4-Dioxane levels in ground water

- The Environmental Protection Agency (EPA) recommends that the levels of 1,4-dioxane in drinking water that children drink for 1 day not exceed 4 milligrams per liter (mg/L) or 0.4 mg/L, if they drink water for 10 days. However, EPA has not established a federal drinking water standard (maximum contaminant level or MCL).

What do studies show about the levels of 1,4-dioxane in shampoos and bubble baths?

Note: Much of the information in this section is from: Black RE, Hurley FJ, Havery DC. 2001. Occurrence of 1,4-dioxane in cosmetic raw materials and finished cosmetic products. J AOAC Int 84(3):666-670.

1979: 1,4-Dioxane identified in raw materials used in the manufacture of cosmetic products

In 1979-1980, the FDA urged the cosmetic industry to monitor their raw materials for 1,4-dioxane.

1980s- Downward trend in levels of 1,4-dioxane.

The results of surveys suggested a downward trend in the levels of 1,4-dioxane in cosmetic finished products analyzed between 1981 and 1984. Changes in the manufacturing process may be responsible for the apparent trend. FDA surveys were then suspended in 1984 but were resumed in 1992.

1990s- Levels increase

Ninety-nine products were analyzed between 1992 and 1997. The products analyzed since 1994 focused on children's shampoos because the process used in their manufacturing was linked to 1,4-dioxane. The downward trend in the levels of 1,4-dioxane previously observed in products analyzed in the 1980s was no longer evident in the products analyzed in the 1990s. Of particular concern were levels of 1,4-dioxane observed in children's shampoos analyzed in 1994/95 manufactured by two companies. 1,4-Dioxane was frequently present at levels in excess of 85 ppm.

Can high levels of 1-4-dioxane be avoided in cosmetics, bath products and shampoos?

High levels can be avoided

The low levels of 1,4-dioxane observed in some raw materials and finished products demonstrate that with current technology, excessive levels of 1,4-dioxane are avoidable. Continued periodic monitoring of cosmetic ingredients and cosmetic finished products for the presence of 1,4-dioxane is necessary.

What can I do to ensure that my family is not exposed to 1,4-dioxane?

Check ingredients listed on product packaging

Given the expanding range of consumer products that may contain 1,4-dioxane as a contaminant, families should exercise caution in selecting products that do not clearly specify the ingredients that contain 1,4-dioxane.

The ingredients that may be listed on cosmetics, detergents, and shampoos include:

- polyethylene glycol (PEG),
- polyethylene,
- polyoxyethylene,
- or oxynol-

These ingredients are most likely to contain 1,4-dioxane.

Where can I find more information regarding 1,4-dioxane?

Document	Source
ATSDR ToxFAQs	http://www.atsdr.cdc.gov/toxfaqs/TF.asp?id=954&tid=199
EPA dioxane fact sheets	http://www.epa.gov/opptintr/chemfact/dioxa-sd.txt http://www.epa.gov/chemfact/dioxa-fs.pdf
FDA: Cosmetics	Cosmetic Handbook. 1992. U.S. Food and Drug Administration. Center for Food Safety and Applied Nutrition. FDA/IAS Booklet: 1992.
FDA: Food Additives	FDA's website at http://www.fda.gov/Food/FoodIngredientsPackaging/ucm115333.htm.
National Industrial Chemicals Notification and Assessments System	http://www.nicnas.gov.au/publications/car/pec/pec7/pec7_full_report_pdf.pdf This is a full public report on 1,4-dioxane from the National Industrial Chemicals Notification and Assessments Scheme.

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