# TOXICOLOGICAL PROFILE FOR HEXACHLOROBUTADIENE

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**
**Public Health Service**
**Agency for Toxic Substances and Disease Registry**

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The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

## UPDATE STATEMENT

Toxicological profiles are revised and republished as necessary, but no less than once every three years. For information regarding the update status of previously released profiles, contact ATSDR at:

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Division of Toxicology/Toxicology Information Branch
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# FOREWORD

The Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) extended and amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed the Agency for Toxic Substances and Disease Registry (ATSDR) to prepare 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 Environmental Protection Agency (EPA). The revised list of the 275 most hazardous substances was published in the Federal Register on October 28, 1992 (57 FR 48801). For prior versions of the list of substances, see Federal Register notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); and October 17, 1991 (56 FR 52166).

Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. Each profile must include the following:

(A) The examination, summary, and interpretation of available toxicological information and epidemiological evaluations on a hazardous substance in order to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects.

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects.

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

This toxicological profile is prepared in accordance with guidelines developed by ATSDR and 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 is intended to succinctly characterize the toxicological and adverse health effects information for the hazardous substance being described. Each profile identifies and reviews the key literature (that has been peer-reviewed) that describes a hazardous substance's toxicological properties. Other pertinent literature is also presented, but 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.

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. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protect public health will be identified by ATSDR and EPA. The focus of the profiles is on health and toxicological information; therefore, we have included this information in the beginning of the document.

*Foreword*

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

This profile reflects our assessment of all relevant toxicological testing and information that has been peer reviewed. It has been reviewed by scientists from ATSDR, the Centers for Disease Control and Prevention (CDC), and other federal agencies. It has also been reviewed by a panel of nongovernment peer reviewers 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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**THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:**

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

2. 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 endpoints.

3. 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.

4. Quality Assurance Review. The Quality Assurance Branch assures that consistency across profiles is maintained, identifies any significant problems in format or content, and establishes that Guidance has been followed.

# PEER REVIEW

A peer review panel was assembled for hexachlorobutadiene. The panel consisted of the following members:

1. Dr. Arthur Gregory, Private Consultant, Sterling Virginia.
2. Dr. Shane Que Hee, Associate Professor, Department of Environmental Health Sciences, UCLA School of Public Health, Los Angeles, California.
3. Dr. Renate Reimschuessel, Assistant Professor, University of Maryland School of Medicine, Baltimore, Maryland.

These experts collectively have knowledge of hexachlorobutadiene'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. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

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 Statement was prepared to give you information about hexachlorobutadiene and to emphasize the human health effects that may result from exposure to it. The Environmental Protection Agency (EPA) has identified 1,350 hazardous waste sites as the most serious in the nation. These sites comprise the "National Priorities List" (NPL): Those sites which are targeted for long-term federal cleanup activities. Hexachlorobutadiene has been found in at least 45 of the sites on the NPL. However, the number of NPL sites evaluated for hexachlorobutadiene is not known. As EPA evaluates more sites, the number of sites at which hexachlorobutadiene is found may increase. This information is important because exposure to hexachlorobutadiene may cause harmful health effects and because these sites are potential or actual sources of human exposure to hexachlorobutadiene.

When a substance is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. This 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 substances containing the substance or by skin contact with it.

If you are exposed to a substance such as hexachlorobutadiene, many factors will determine whether harmful health effects will occur and what the type and severity of those health effects will be. These factors include the dose (how much), the duration (how long), the route or pathway by which you are exposed (breathing, eating, drinking, or skin contact), the other chemicals to which you are exposed, and your individual characteristics such as age, gender, nutritional status, family traits, life-style, and state of health.

# 1. PUBLIC HEALTH STATEMENT

## 1.1 WHAT IS HEXACHLOROBUTADIENE?

Hexachlorobutadiene, also known as HCBD, perchlorobutadiene, or Dolen-Pur-, is a colorless liquid. It does not evaporate or burn easily. Hexachlorobutadiene has a turpentine-like odor. Most people will begin to smell a mild to pungent odor if the compound is present in air at 1 part hexachlorobutadiene per million parts of air (ppm). It is not known how it tastes or at what level people can taste it.

Hexachlorobutadiene does not occur naturally in the environment. It is formed during the processing of other chemicals such as tetrachloroethylene, trichloroethylene, and carbon tetrachloride. Hexachlorobutadiene is an intermediate in the manufacture of rubber compounds and lubricants. It is used as a fluid for gyroscopes, a heat transfer liquid, or a hydraulic fluid. Outside of the United States it is used to kill soil pests.

More information on the properties and uses of hexachlorobutadiene is found in Chapters 3 and 4.

## 1.2 WHAT HAPPENS TO HEXACHLOROBUTADIENE WHEN IT ENTERS THE ENVIRONMENT?

Hexachlorobutadiene is released to the environment in air, water, and soil, mainly as a result of its disposal following industrial use. Most of the hexachlorobutadiene wastes are destroyed by burning; some are released to the air in this process. It is not known what happens to hexachlorobutadiene after it enters the air. Based on the information we have on similar compounds, it may be broken down by sunlight and react with gases in the atmosphere. It is not known what chemicals are formed by these reactions or if the compounds formed are harmful. Based on the properties of similar compounds, one-half of the hexachlorobutadiene in the air is expected to be broken down to other chemicals within 60 days.

# 1. PUBLIC HEALTH STATEMENT

Hexachlorobutadiene may be released to water during disposal of factory waste. It is not known what happens to it in water or how long it remains there. Hexachlorobutadiene that is present in water may pass into the air or soil in small amounts. Small amounts of hexachlorobutadiene may be released to soil as a result of disposal of industrial wastes containing it. It is not known what happens to hexachlorobutadiene after it contacts soil. Because hexachlorobutadiene binds to most soils, it is expected to remain there for some time. The hexachlorobutadiene present in sandy soils may move through the soil to underground water, However, no information was found on how much reaches the underground water or how long it stays in the water. Hexachlorobutadiene can build up in fish and shellfish, where waters are contaminated. It is not known if hexachlorobutadiene builds up in plants.

More information on what happens to hexachlorobutadiene in the environment may be found in Chapters 4 and 5.

## 1.3 HOW MIGHT I BE EXPOSED TO HEXACHLOROBUTADIENE?

You may be exposed to hexachlorobutadiene by breathing contaminated air, eating contaminated food, drinking contaminated water, or by direct skin contact with this chemical. People working in the industrial facilities where hexachlorobutadiene is formed or used may be exposed. Concentrations found in outside air were 2-3 parts hexachlorobutadiene per trillion parts of air (ppt). Levels were much higher in or near industrial facilities where hexachlorobutadiene is formed or used. One survey detected air concentrations ranging from 22 to 43,000 ppt in a production facility. No information is available on how many workers are potentially exposed to hexachlorobutadiene.

Although hexachlorobutadiene is not very soluble in water, small amounts may be found in some public drinking water (less than 1 part hexachlorobutadiene per billion parts water [ppb]). It may also be found in underground water near hazardous waste sites. Hexachlorobutadiene has no agricultural or food chemical uses in the United States.

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Levels ranging from 0.1 to 4.7 milligrams per kilogram have been found in fish and shellfish because the compound is present in some surface water.

Exposure at waste sites is most likely to occur from the landfill disposal of waste by-products originating from chlorinated hydrocarbon manufacture.

More information on how you may be exposed to hexachlorobutadiene is found in Chapter 5.

## 1.4 HOW CAN HEXACHLOROBUTADIENE ENTER AND LEAVE MY BODY?

Hexachlorobutadiene may enter your body through the lungs when you breathe air contaminated with it. It also may enter your body if you drink water or eat food contaminated with hexachlorobutadiene. With the exception of fish and shellfish, however, hexachlorobutadiene has not been found in food. The amount of hexachlorobutadiene that enters your body by these routes depends on how much of the chemical you eat or drink.

What happens to hexachlorobutadiene when you breathe vapors of the compound is not known, but it most likely moves across your lungs into your bloodstream. In animal studies, most of the hexachlorobutadiene is changed by the body into more toxic compounds. It is not known how rapidly hexachlorobutadiene and its breakdown products are removed from your body through your urine and feces. Some is expected to remain in your body fat for long periods.

More information on how hexachlorobutadiene enters and leaves the body is given in Chapter 2.

## 1.5 HOW CAN HEXACHLOROBUTADIENE AFFECT MY HEALTH?

In one study of workers at a solvent production plant who breathed hexachlorobutadiene for long periods, the compound was shown to affect the function of the liver. Because the workers were also exposed to other solvents (carbon tetrachloride and perchloroethylene), it

is not certain if this effect was caused by hexachlorobutadiene alone. Studies in mice showed that brief exposure to high concentrations of hexachlorobutadiene irritate the nose. The effects of breathing low levels of hexachlorobutadiene are not known.

Ingestion of hexachlorobutadiene damaged the kidneys of rats and mice and, to a lesser extent, the liver of rats. These effects occurred after both short- and long-term exposures at very low dose levels. Young rats were affected more than adult rats. The kidneys of female rats appeared to be affected more than those of males. On the other hand, the liver of male rats was affected, but the liver of female rats was not. It is not clear if the differences between the sexes might be seen in humans. Kidney, brain, and liver damage were also seen in rabbits after contact of their skin with the compound for a short period.

Hexachlorobutadiene decreased fetal body weight in rats, but did not affect fetal development or impair their ability to produce offspring. The lungs, heart, brain, blood, muscles, and skeleton in rats or mice were not damaged after short- or long-term exposure.

Studies in rats indicate that hexachlorobutadiene may increase the risk of kidney cancer if exposures occur for long periods. The International Agency for Research on Cancer (IARC) has determined that hexachlorobutadiene is not classifiable as to its carcinogenicity in humans, but indicated that there was limited evidence that hexachlorobutadiene was carcinogenic in rats. EPA has determined that hexachlorobutadiene is a possible human carcinogen.

## 1.6 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO HEXACHLOROBUTADIENE?

Exposure to hexachlorobutadiene can be determined by measuring the chemical or its breakdown products in blood, urine, or fat. These tests are not usually performed in most doctors' offices because special equipment is needed. Samples can be collected and sent to special laboratories to determine if you were exposed to hexachlorobutadiene. These tests

cannot determine how much of the chemical you were exposed to or if adverse health effects will occur as a result of the exposure.

More information on how hexachlorobutadiene can be detected in your body is found in Chapters 2 and 6.

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

The federal government has develped guidelines and standards to protect the public from excess exposure to hexachlorobutadiene. EPA has recommended guidelines on how much hexachlorobutadiene can be present in drinking water for specific periods of time without causing adverse health effects in humans. EPA recommends that exposures in children should not exceed 0.3 milligrams per liter of water (mg/L) for 10-day periods, or 0.1 mg/L for more than 7 years. If adults are exposed for long periods (more than 7 years), EPA recommends that exposure levels should not exceed 0.4 mg/L.

Hexachlorobutadiene has been named a hazardous substance by EPA. If quantities equal to or greater than 1 pound are released to the environment, the National Response Center of the federal government must be notified immediately.

The Occupational Safety and Health Administration (OSHA) recommends that exposure to hexachlorobutadiene not exceed 0.02 ppm for an 8-hour workday over a 40-hour workweek. This limit is not enforced by the federal government, but it is the law in at least 25 states.

The National Institute for Occupational Safety and Health (NIOSH) classifies hexachlorobutadiene as a potential occupational carcinogen. Because there is potential for effects following contact of the chemical with the skin, measures should be taken to minimize skin exposure.

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More information on federal government guidelines and standards on hexachlorobutadiene is found in Chapter 7.

## 1.8 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:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE, E-29
Atlanta, Georgia 30333
(404) 639-6000

This agency can also provide you with information on the location of occupational and environmental health clinics. These clinics specialize in the recognition, evaluation, and treatment of illness resulting from exposure to hazardous substances.

# 2. HEALTH EFFECTS

## 2.1 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 of the toxicology of hexachlorobutadiene. 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.

## 2.2 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 (LOAEL) 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 hexachlorobutadiene are indicated in Table 2-2. Because cancer effects could occur at lower exposure levels, Figures 2-l and 2-2 also show ranges 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 (Minimal Risk Levels or MRLs) have been made for hexachlorobutadiene. 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 1990h), 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 A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

### 2.2.1 Inhalation Exposure

#### 2.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to hexachlorobutadiene.

In animals, all mice that were exposed to vapors of 50 ppm hexachlorobutadiene for 5 days died, but no deaths occurred at 10 ppm (NIOSH 1981).

#### 2.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, musculoskeletal , or dermal/ocular effects in humans or animals after inhalation exposure to hexachlorobutadiene. Limited data are available on hepatic effects in humans and on the respiratory and renal effects of hexachlorobutadiene in animals. These effects are discussed below. The highest NOAEL value and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

**Respiratory Effects.** Respiratory rates were decreased in mice exposed to vapors of hexachlorobutadiene at concentrations of 155 ppm or greater for 15 minutes. The authors characterized the responses as a reaction to nasal irritation (de Ceaurriz et al. 1988). Nasal irritation and respiratory difficulty was also reported in rats exposed to vapors at a concentration of 250 ppm for 2 days (4 hours/day) or 100 ppm for 12 days (6 hours/day) (Gage 1970). Breathing difficulty occurred even with exposure to 25 ppm for 15 days (6 hours/day).

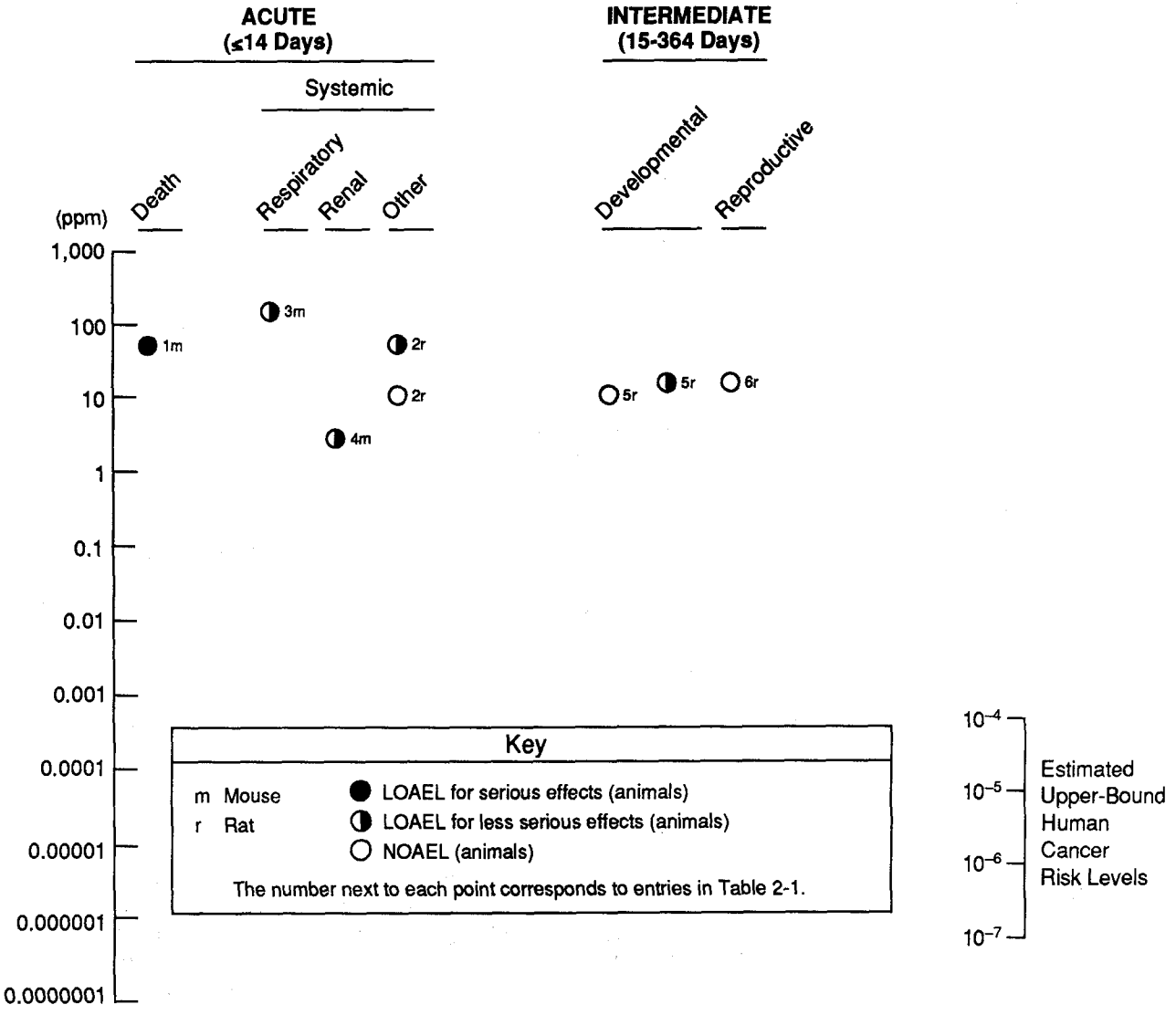
## TABLE 2-1. Levels of Significant Exposure to Hexachlorobutadiene - Inhalation

| Key to figure[a] | Species | Route | Exposure duration/ frequency | System | NOAEL (mg/kg/day) | LOAEL (effect) Less serious (mg/kg/day) | LOAEL (effect) Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|---|
| ACUTE EXPOSURE | | | | | | | | |
| Death | | | | | | | | |
| 1 | Mouse | | 5 d 7hr/d | | | | 50 (100% mortality) | NIOSH 1981 |
| Systemic | | | | | | | | |
| 2 | Rat | | 5 d 7hr/d | Other | 10 | 50 (body weight reduced 14%) | | NIOSH 1981 |
| 3 | Mouse | | 15 min | Resp | | 155 (decreased respiratory rate 36%) | | de Ceaurriz et al. 1988 |
| 4 | Mouse | | 4 hr | Renal | | 2.75 (damaged cortical proximal tubules) | | de Ceaurriz et al. 1988 |
| INTERMEDIATE EXPOSURE | | | | | | | | |
| Developmental | | | | | | | | |
| 5 | Rat | | Gd 6-20 6hr/d | | 10 | 15 (fetal body weight reduced 9.5% in males and 12.5% in females) | | Saillenfait et al. 1989 |
| Reproductive | | | | | | | | |
| 6 | Rat | | 15 d Gd 6-20 6hr/d | | 15 | | | Saillenfait et al. 1989 |

[a]The number corresponds to entries in Figure 2-1.

d = day(s); Gd = gestation day; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory

## FIGURE 2-1 Levels of Significant Exposure to Hexachlorobutadiene – Inhalation



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**Hepatic Effects.** Although the liver is not a major target of hexachlorobutadiene toxicity, there is some indication that it may be adversely affected following exposure in humans. Serum bile acids (deoxycholic acid, glycinedeoxycholic acid, taurine-chenodeoxycholic acid, and total deoxycholate) increased following chronic exposure in workers to estimated exposure levels of 0.005-0.02 ppm (Driscoll et al. 1992). It should be noted that the workers were also potentially exposed to other solvents (carbon tetrachloride and perchloroethylene). For this reason, and the fact that data are absent on morphological changes as well as other effects on liver function, the practical importance of this finding is reduced.

**Renal Effects.** Mice that were exposed to vapors of hexachlorobutadiene (2.75-25 ppm) for 4 hours showed an increase (4-91%) in the number of damaged cortical renal tubules (de Ceaurriz et al. 1988). Degeneration of the tubule midsection resulted from exposures to 250 ppm hexachlorobutadiene for 4 hours on each of 2 consecutive days (Gage 1970). Damage (not specified) to renal proximal tubules was also reported in rats exposed to vapors at a concentration of 25 ppm for 15 days (6 hours/day); the kidneys were pale and enlarged. At a higher concentration (100 ppm), degeneration of renal cortical tubules with epithelial regeneration occurred after 12 days of exposure (Gage 1970). Quantitative data on renal effects were limited.

**2.2.1.3 Immunological Effects**

No studies were located regarding immunological effects in humans or animals after inhalation exposure to hexachlorobutadiene.

**2.2.1.4 Neurological Effects**

No studies were located regarding neurological effects in humans or animals after inhalation exposure to hexachlorobutadiene.

**2.2.1.5 Reproductive Effects**

No studies were located regarding reproductive effects in humans after inhalation exposure to hexachlorobutadiene.

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In animals, the frequency of abnormal sperm morphology did not increase significantly over controls in mice exposed to concentrations of 10 ppm hexachlorobutadiene (NIOSH 1981). When mice were exposed to 50 ppm (the only other concentration tested), all animals died during the 5 week posttreatment period. Thus, a reliable NOAEL value for reproductive effects cannot be identified for this study. When rat dams were exposed to vapors of hexachlorobutadiene (up to 15 ppm) during gestation (gestation days 6-20), the mean number of implantation sites, total fetal loss, resorptions and number of live fetuses were comparable to unexposed controls (Saillenfait et al. 1989).

Studies evaluating the genotoxic potential of hexachlorobutadiene indicate that hexachlorobutadiene does not affect fertility in male rats. In dominant lethal tests in rats, fertility indices, number of corpora lutea or implantations, or the frequency of early death did not differ between animals that inhaled vapors of hexachlorobutadiene at concentrations up to 50 ppm and their unexposed controls (NIOSH 1981).

**2.2.1.6 Developmental Effects**

No studies were located regarding developmental effects in humans after inhalation exposure to hexachlorobutadiene .

In animals, data are limited to one intermediate-duration study in which rats were exposed to vapors of hexachlorobutadiene at concentrations up to 15 ppm during gestation days 6-20 (Saillenfait et al. 1989). The only effect observed was a reduction ($p < 0.01$) in fetal body weights at concentrations of 15 ppm (highest dose tested). No fetotoxic effects were observed at concentrations of 10 ppm or less. Embryotoxicity was not observed at any dose tested and there were no exposure-related external, visceral, or skeletal anomalies. It should be noted that reduced maternal body weight gain was observed at the 15 ppm vapor concentration.

The highest NOAEL value and a LOAEL value for developmental toxicity in rats are recorded in Table 2-l and plotted in Figure 2-l.

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**2.2.1.7 Genotoxic Effects**

No studies were located regarding genotoxic effects in humans after inhalation exposure to hexachlorobutadiene.

Hexachlorobutadiene did not cause dominant lethal mutations in rats after inhalation of vapors at concentrations of 10 or 50 ppm for up to 5 days (NIOSH 1981). Similarly, there were no increases in the frequency of chromosomal aberrations in bone marrow cells of rats exposed to 10 ppm for up to 5 days (NIOSH 1981).

Other genotoxicity studies are discussed in Section 2.4.

**2.2.1.8 Cancer**

No studies were located regarding cancer in humans or animals after inhalation exposure to hexachlorobutadiene. However, EPA has derived an inhalation unit risk of 0.022 $(mg/m^3)^{-1}$ (IRIS 1993), based on oral exposure data (see Section 2.2.2.8). Exposure levels corresponding to excess cancer risks of $10^{-4}$ to $10^{-7}$ are shown in Figure 2-l.

**2.2.2 Oral Exposure**

**2.2.2.1 Death**

No studies were located regarding death in humans after oral exposure to hexachlorobutadiene.

Acute oral exposures to hexachlorobutadiene were lethal in rats. Young rats were more sensitive to compound exposure than adult rats. $LD_{50}$ values for adult rats were 580 mg/kg (males) and 200-400 mg/kg (females). The $LD_{50}$ values for weanling male and female rats were 65 and 46 mg/kg, respectively (Kociba et al. 1977a). Important experimental details of this study were not available for review.

Mice exposed to 1,000 and 3,000 ppm hexachlorobutadiene in their diet (19-36 mg/kg/day) died after 3-5 days (NTP 1991; Yang et al. 1989). Animals exposed to 30-300 ppm (3-49 mg/kg/day)

survived the 15 day exposure period. Survival was not reduced in rats exposed to 100 mg/kg/day hexachlorobutadiene for 30 days or at dose levels of 15.6 mg/kg/day for 13 weeks (Harleman and Seinen 1979) and 100 mg/kg/day (Kociba et al. 1971). Mice survived dose levels of up to 19.2 mg/kg/day for 13 weeks (NTP 1991). In lifetime studies, survival was reduced significantly in male rats exposed to hexachlorobutadiene at a dose level of 20 mg/kg/day (Kociba et al. 1977a). Although the cause of death was not reported, renal damage, a major effect manifested by this compound, may have been a contributing factor.

A LOAEL value for lethality in rats after chronic exposure is recorded in Table 2-2 and plotted in Figure 2-2.

**2.2.2.2. Systemic Effects**

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or dermal/ocular effects in humans after oral exposure to hexachlorobutadiene for any duration category.

Studies have been conducted in animals to evaluate the respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, and renal effects. These effects are discussed below. No studies were located on dermal/ocular effects. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

**Respiratory Effects.** Intermediate-duration (30-148 days) exposure to 20 mg/kg/day (Schwetz et al. 1977) or 100 mg/kg/day (Kociba et al. 1971) and lifetime exposures to 20 mg/kg/day (Kociba et al. 1977a) did not cause treatment-related lesions of the lungs or changes in lung weight in rats exposed to hexachlorobutadiene at dose levels of 20 mg/kg/day.

**Cardiovascular Effects.** Hexachlorobutadiene did not alter heart weights or cause treatment-related lesions of the heart in rats or mice exposed for intermediate durations (90-148 days) at dose levels of 19.2-20 mg/kg/day (NTP 1991; Schwetz et al. 1977; Yang et al. 1989) and 100 mg/kg/day (Kociba et al. 1971) or after lifetime exposure at dose levels of 20 mg/kg/day (Kociba et al. 1977a).

## TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral

| Key to figure[a] | Species | Route | Exposure duration/ frequency | System | NOAEL (mg/kg/day) | LOAEL (effect) Less serious (mg/kg/day) | LOAEL (effect) Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|---|
| ACUTE EXPOSURE | | | | | | | | |
| Systemic | | | | | | | | |
| 1 | Rat | (F) | 14 d | Hepatic | 35 | | | Harleman and Seinen 1979 |
| | | | | Renal | | 4.6 (proximal convoluted tubule degeneration) | | |
| | | | | Other | | 4.4 (body weight reduced 9.5% in females) | | |
| INTERMEDIATE EXPOSURE | | | | | | | | |
| Systemic | | | | | | | | |
| 2 | Rat | (GO) | 13 wk | Hepatic | 2.5 | 6.3 (increased cytoplasmic basophilia) | | Harleman and Seinen 1979 |
| | | | | Hemato | 15.6 | | | |
| | | | | Renal | 1 | 2.5 (degeneration proximal tubules) | | |
| | | | | Other | 2.5 | | 6.3 (body weight decreased 29% in females and 13% in males) | |
| 3 | Rat | (F) | 30 d | Resp | 100 | | | Kociba et al. 1971 |
| | | | | Cardio | 100 | | | |
| | | | | Gastro | 100 | | | |
| | | | | Hemato | 3 | 10 (increased hemoglobin concentration) | | |
| | | | | Hepatic | 10 | 30 (centrilobular *hepatocellular* swelling) | | |
| | | | | Renal | 10 | 30 (tubular degeneration, necrosis) | | |
| | | | | Other | 100 | (thyroid, parathyroid, pituitary and adrenal glands) | | |

## TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral *(continued)*

| Key to figure[a] | Species | Route | Exposure duration/ frequency | System | NOAEL (mg/kg/day) | LOAEL (effect) Less serious (mg/kg/day) | LOAEL (effect) Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|---|
| 4 | Rat | (F) | 10-18 wk | Renal | | | 15 (tubular degeneration, necrosis) | Harleman and Seinen 1979 |
| | | | | Other | | 15 (maternal body weight decreased 15%) | | |
| 5 | Rat | (F) | 4 wk | Hepatic | 1.25 | 5 (absolute liver weight decreased 41%) | 20 (increased plasma aspartate amino-transferase and bilirubin) | Jonker et al. 1993b |
| | | | | Renal | 1.25 | 5 (tubular cytomegaly and decreased plasma creatinine) | | |
| | | | | Other | 1.25 | 5 (body weight decreased 10% in males and 15% in females, decreased adrenal weight, 17%) | | |
| 6 | Rat | (F) | 148 d | Resp | 20 | | | Schwetz et al. 1977 |
| | | | | Cardio | 20 | | | |
| | | | | Gastro | 20 | | | |
| | | | | Hemato | 20 | | | |
| | | | | Musc/skel | 20 | | | |
| | | | | Hepatic | 20 | | | |
| | | | | Renal | 0.2 | 2 (tubular degeneration) | | |
| | | | | Other | 2 | 20 (body weight gain reduced 8-9% in males and 5-17% in females) | | |

## TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral *(continued)*

| Key to figure[a] | Species | Route | Exposure duration/ frequency | System | NOAEL (mg/kg/day) | LOAEL (effect) Less serious (mg/kg/day) | LOAEL (effect) Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|---|
| 7 | Mouse | (F) | 13 wk | Resp | 19.2 | | | NTP 1991, Yang et al. 1989 |
| | | | | Cardio | 19.2 | | | |
| | | | | Gastro | 19.2 | | | |
| | | | | Musc/skel | 19.2 | | | |
| | | | | Hepatic | 19.2 | | | |
| | | | | Renal | | 0.2[b] (tubular degeneration) | | |
| | | | | Derm/oc | 19.2 | | | |
| | | | | Other | 1.5 | 4.9 (body weight gain reduced 49% in males) | | |
| Neurological | | | | | | | | |
| 8 | Rat | (GO) | 13 wk | | 15.6 | | | Harleman and Seinen 1979 |
| 9 | Rat | (F) | 148 d | | 20 | | | Schwetz et al. 1977 |
| 10 | Rat | (F) | 10-18 wk | | 15 | | 150 (ataxia, demyelination and degeneration of femoral nerve fiber) | Harleman and Seinen 1979 |
| 11 | Rat | (F) | 30 d | | 100 | | | Kociba et al. 1971 |
| 12 | Mouse | (F) | 13 wk | | 19.2 | | | NTP 1991, Yang et al. 1989 |
| Developmental | | | | | | | | |
| 13 | Rat | (F) | 43 d Gd1-22; Ld1-21 | | 2 | 20 (neonatal weight decreased 13%) | | Schwetz et al. 1977 |
| 14 | Rat | (F) | 6 wk | | | 15 (reduced pup weight, 16-19%) | | Harleman and Seinen 1979 |

## TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral *(continued)*

| Key to figure[a] | Species | Route | Exposure duration/ frequency | System | NOAEL (mg/kg/day) | LOAEL (effect) Less serious (mg/kg/day) | LOAEL (effect) Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|---|
| Reproductive | | | | | | | | |
| 15 | Rat | (F) | 10-18 wk | | 15 | | 150 (infertility) | Harleman and Seinen 1979 |
| 16 | Rat | (F) | 148 d | | 20 | | | Schwetz et al. 1977 |
| 17 | Mouse | (F) | 13 wk | | 19.2 | | | NTP 1991, Yang et al. 1989 |
| CHRONIC EXPOSURE | | | | | | | | |
| Death | | | | | | | | |
| 18 | Rat | (F) | 2 yr | | | | 20 (increased mortality) | Kociba et al. 1977a |
| Systemic | | | | | | | | |
| 19 | Rat | (F) | 2 yr | Resp | 20 | | | Kociba et al. 1977a |
| | | | | Cardio | 20 | | | |
| | | | | Gastro | 20 | | | |
| | | | | Hemato | 20 | | | |
| | | | | Musc/skel | 20 | | | |
| | | | | Hepatic | 20 | | | |
| | | | | Renal | 0.2 | 2 (tubular hyperplasia) | | |
| | | | | Other | 2 | 20 (reduced mean body weight in males [8-20%] and females [5-12%]) | | |
| Neurological | | | | | | | | |
| 20 | Rat | (F) | 2 yr | | 20 | | | Kociba et al. 1977a |

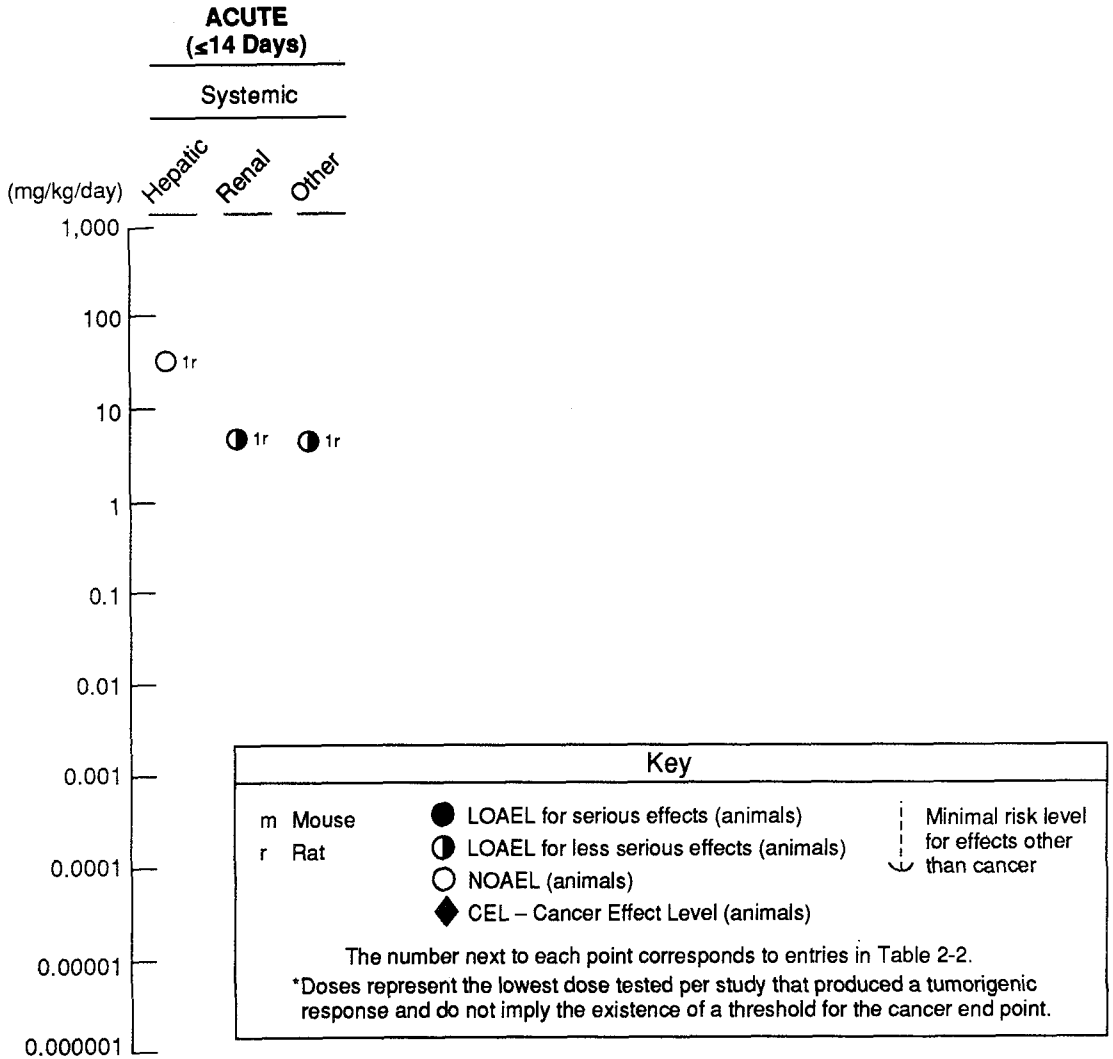
## TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral *(continued)*

| Key to figure[a] | Species | Route | Exposure duration/ frequency | System | NOAEL (mg/kg/day) | LOAEL (effect) Less serious (mg/kg/day) | LOAEL (effect) Serious (mg/kg/day) | Reference |
|---|---|---|---|---|---|---|---|---|
| Cancer | | | | | | | | |
| 21 | Rat | (F) | 2 yr | | | | 20 (CEL: kidney tumors) | Kociba et al. 1977a |

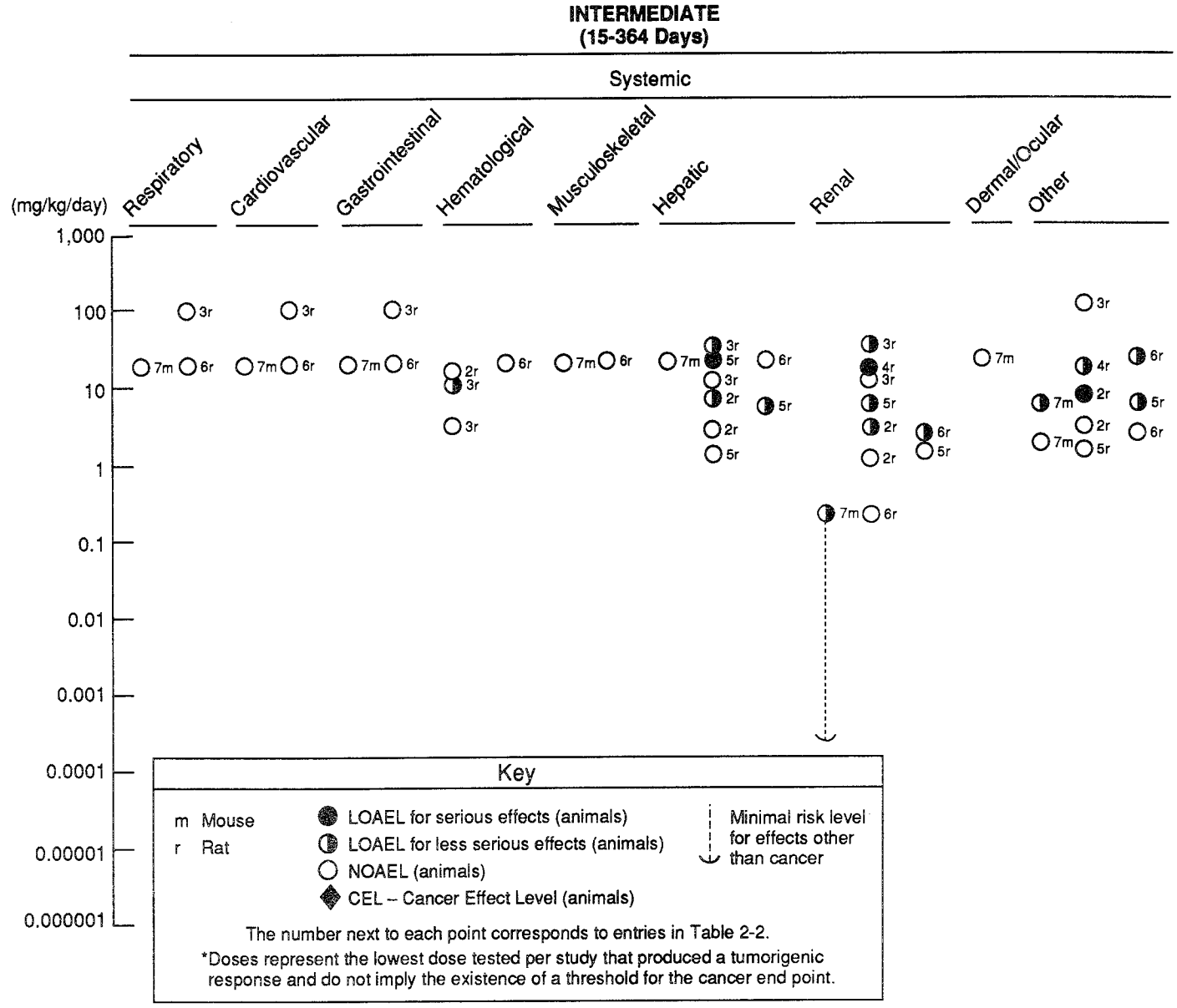
[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an intermediate oral minimal risk level (MRL) of 0.0002 mg/kg/day; dose divided by an uncertainty factor of 1,000 (10 for extrapolation from animals to humans, 10 for use of a LOAEL and 10 for human variability).

Cardio = cardiovascular; d = day(s); (F) = feed; Gastro = gastrointestinal; Gd = gestation day; (GO) = gavage - oil; Hemato = hematological; Ld = lactation day(s); LOAEL = lowest-observed-adverse-effect level; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); yr = year(s)

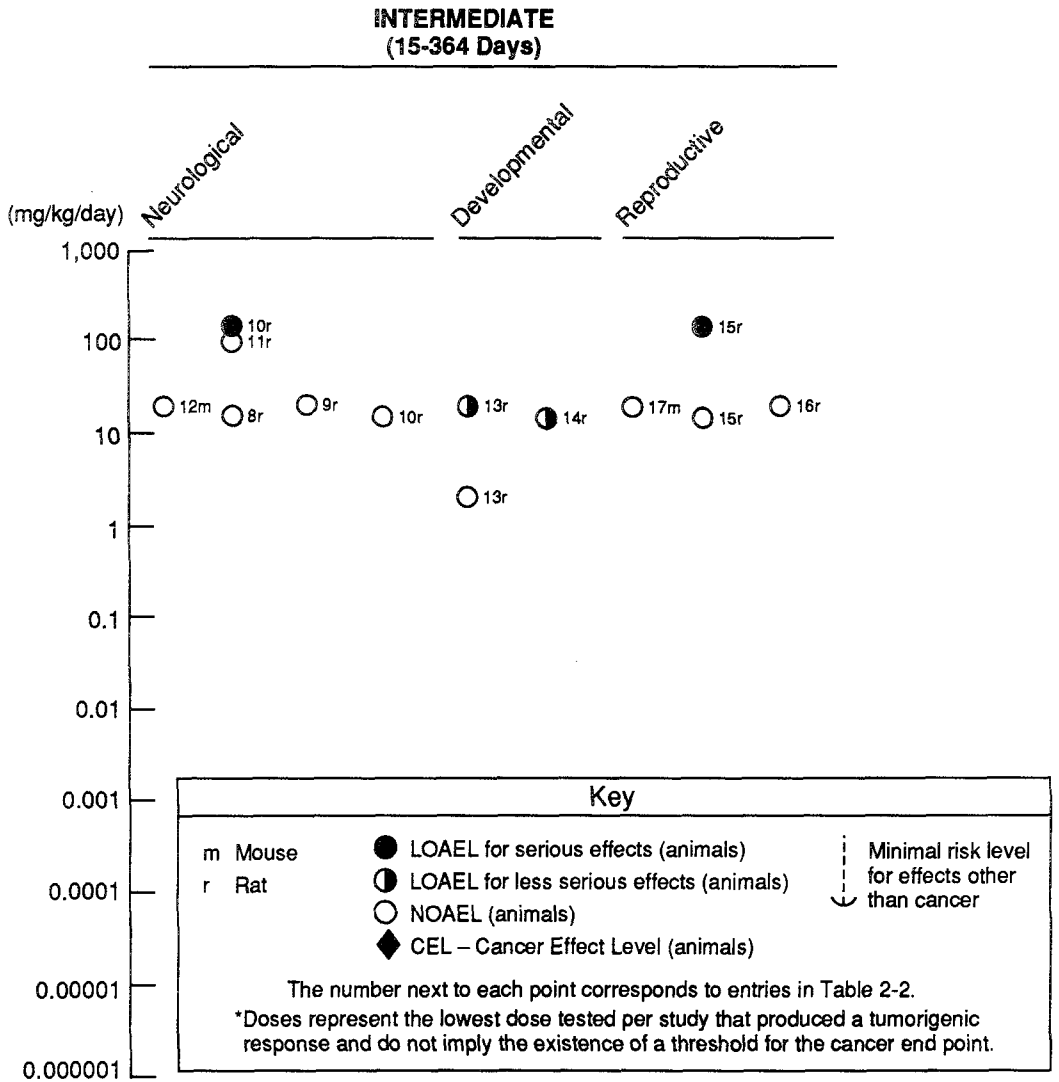
## FIGURE 2-2 Levels of Significant Exposure to Hexachlorobutadiene – Oral



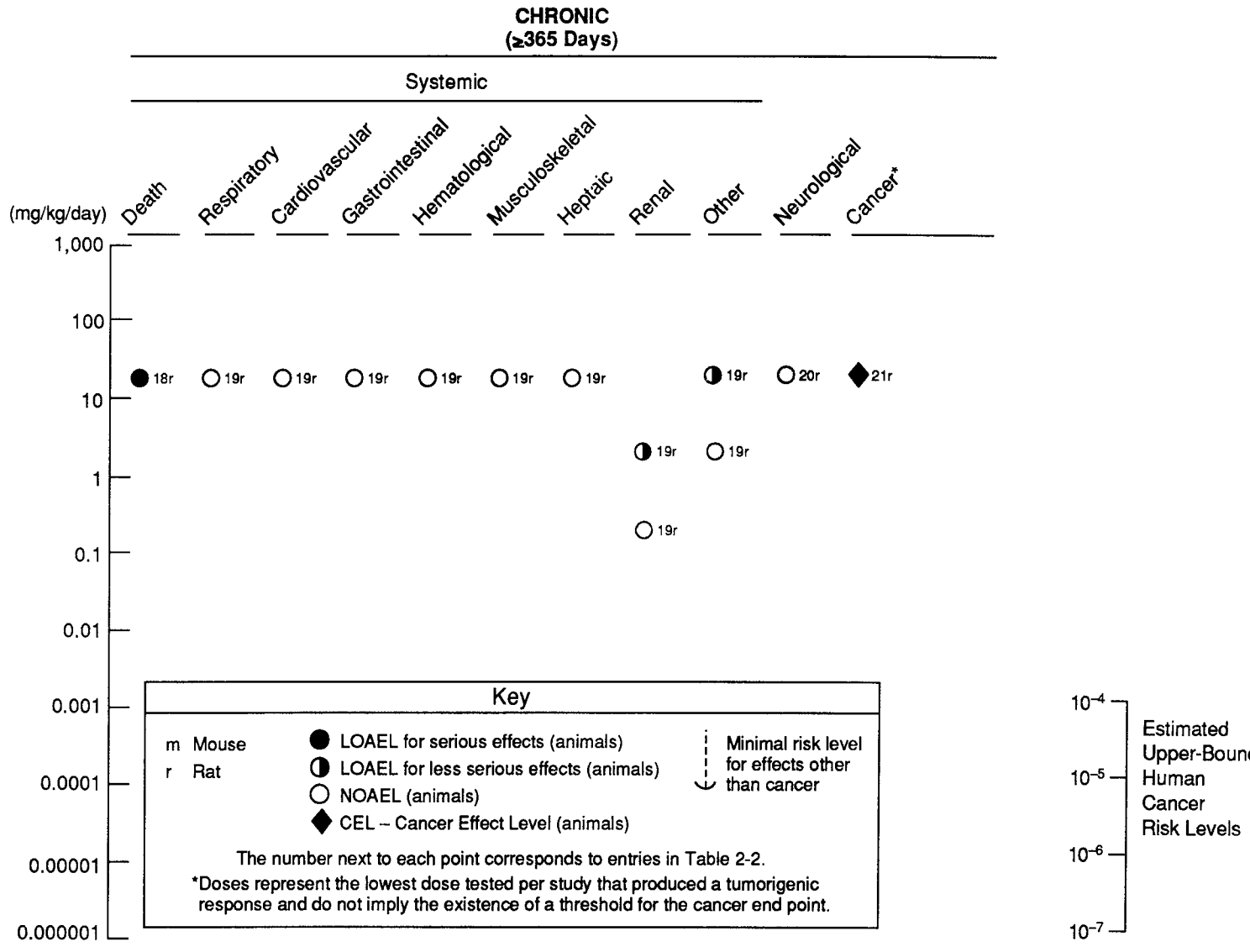
## FIGURE 2-2 Levels of Significant Exposure to Hexachlorobutadiene – Oral (Continued)



## FIGURE 2-2 Levels of Significant Exposure to Hexachlorobutadiene – Oral (Continued)



## FIGURE 2-2 Levels of Significant Exposure to Hexachlorobutadiene – Oral (Continued)



## 2. HEALTH EFFECTS

**Gastrointestinal Effects.** Intermediate-duration (30-148 days) exposure did not cause treatment-related histopathological lesions in the esophagus, stomach, small intestines, or large intestines in rats exposed to hexachlorobutadiene at dose levels up to 20 mg/kg/day (Schwetz et al. 1977) or levels up to 100 mg/kg/day (Kociba et al. 1971). Lifetime exposure at dose levels of 20 mg/kg/day (Kociba et al. 1977a) did not result in any effect on this system.

**Hematological Effects.** Evaluations of hematological parameters in rats revealed no treatment-related alterations in packed cell volume, red blood cell count, hemoglobin concentration, total white blood cell count, or differential white blood cell count in animals exposed to a dose level of 15.6 or 20 mg/kg/day after intermediate duration exposure (90-148 days) (Harleman and Seinen 1979; Schwetz et al. 1977). Similarly, one lifetime oral exposure (20 mg/kg/day) also did not cause hematological effects (Kociba et al. 1977a). However, in another study, hemoglobin concentration increased in rats at dose levels from 10-100 mg/kg/day, but not at 3 mg/kg/day. Other hematologic parameters, as mentioned above, were within normal values (Kociba et al. 1971).

**Musculoskeletal Effects.** No treatment-related lesions of the musculoskeletal system were observed in rats exposed to dose levels of 20 mg/kg/day hexachlorobutadiene for up to 148 days (Harleman and Seinen 1979; Schwetz et al. 1977) or 2 years (Kociba et al. 1977a).

**Hepatic Effects.** An animal study revealed that hexachlorobutadiene can affect the liver. However, the effects were less serious compared to effects in the kidney at the same dose. Liver weights were decreased in female rats fed 5 mg/kg/day or greater hexachlorobutadiene for 4 weeks (Jonker et al. 1993b). Histological examinations were not performed. However, evaluation of serum biochemical parameters in males revealed increased enzyme activity (aspartate aminotransferase, $p < 0.02$) and total bilirubin levels ($p < 0.02$) at doses of 20 mg/kg/day (highest dose tested). Cytoplasmic basophilia and liver weights were increased in male rats exposed to hexachlorobutadiene by gavage at dose levels of 6.3 mg/kg/day or greater for 13 weeks; treatment-related lesions were not observed in females (Harleman and Seinen 1979). In another study, hepatocellular swelling occurred at a dose level of 30 mg/kg/day and liver weights decreased at dose levels of 30-100 mg/kg/day or greater in female rats that were fed diets containing hexachlorobutadiene for 30 days (Kociba et al. 1971). Males were not evaluated concurrently. Although histological lesions were not observed in lifetime studies, urinary excretion of coproporphyrin increased at dose levels of 20 mg/kg/day, suggesting alterations in heme synthesis in the liver (Kociba et al. 1977a).

## 2. HEALTH EFFECTS

**Renal Effects.** The kidney appears to be the primary target organ following oral exposure to hexachlorobutadiene. Focal necrosis and increased urinary parameters (lactate dehydrogenase, N-acetyl-β-glucosaminidase) were reported in rats administered hexachlorobutadiene (100 mg/kg) for 24 hours (Jonker et al. 1993a). These effects were not seen at 10 mg/kg. In acute studies in which rats were fed hexachlorobutadiene (4.6 mg/kg/day) in the diet for 14 days, there was degeneration of tubular epithelial cells mainly confined to the straight limbs of the proximal tubules located in the outer zone of the medulla (Harleman and Seinen 1979). Similar effects were seen following intermediate-duration exposure in other species. Cell necrosis and regeneration were found as well as tubular epithelial cell degeneration in rats exposed to dose levels of 30-100 mg/kg/day for 30 days (Kociba et al. 1971). Diffuse tubular cytomegaly was observed in the cortex of the kidneys at dose levels of 5 mg/kg/day or greater in rats following oral exposure to hexachlorobutadiene for 4 weeks (Jonker et al. 1993b). Tubular degeneration also occurred in mice at dose levels from 0.2-19.2 mg/kg/day in females exposed to hexachlorobutadiene for 13 weeks (NTP 1991; Yang et al. 1989). Based on a value of 0.2 mg/kg/day, an intermediate oral MRL was calculated as described in the footnote in Table 2-2.

Kidneys were roughened and had a mottled cortex in males exposed to dose levels of 2 and 20 mg/kg/day for 148 days (Schwetz et al. 1977). Alterations in the kidney were also observed following longer exposures. For the most part, these effects were manifested as renal tubular hyperplasia which occurred at dose levels of 2 and 20 mg/kg/day, but not at a dose level of 0.2 mg/kg/day (Kociba et al. 1977a). Kidney weights were also affected. Intermediate- and chronic-duration oral exposures caused increased relative kidney weights or kidney/body weight ratios at dose levels from 2-20 mg/kg/day (Jonker et al. 1993b; Kociba et al. 1977a; NTP 1991; Schwetz et al. 1977; Yang et al. 1989).

Impaired kidney function accompanied morphological evidence of kidney damage. The ability to concentrate urine was significantly reduced in female rats at dose levels from 2.5-15.6 mg/kg/day for 13 weeks. The same effect was observed in males at 15 mg/kg/day (Harleman and Seinen 1979). On the other hand, alterations in various clinical chemistry indices (e.g., blood urea nitrogen, creatinine, y-glutarnyl transpeptidase, and alanine aminotransferase) were comparable to untreated controls in rats exposed to hexachlorobutadiene at dose levels up to 20 mg/kg/day up to 148 days (Harleman and Seinen 1979; Schwetz et al. 1977). Plasma urea levels decreased ($p < 0.05$) in female rats at dose

levels of 1.25 mg/kg/day or greater and in males at 20 mg/kg/day, while creatinine levels decreased ($p < 0.05$) in females at dose levels of 5 mg/kg/day or greater for 4 weeks (Jonker et al. 1993b).

#### 2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans after oral exposure to hexachlorobutadiene.

In animals, histological examination of lymphoid organs including the thymus and spleen did not reveal treatment-related lesions at dose levels up to 100 mg/kg/day rats (Harleman and Seinen 1979; Kociba et al. 1971, 1977a). Depletion and necrosis of lymphoid tissue in the lymph nodes, spleen, and thymus were noted in mice exposed to lethal doses of hexachlorobutadiene in the 2-week component of the NTP (1991) study. However no abnormalities in these tissues were seen after 13-week exposures to doses of up to 19.2 mg/kg/day (NTP 1991; Yang et al. 1989). Tests on effects of immune function have not been evaluated.

#### 2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to hexachlorobutadiene.

In animals, ataxia, and demyelination and degeneration of femoral nerve fibers were observed in rat dams exposed to dose levels of 150 mg/kg/day for up to 10 weeks (Harleman and Seinen 1979); however, no treatment-related brain lesions were seen following exposure to hexachlorobutadiene (Harleman and Seinen 1979; Kociba et al. 1971; NTP 1991; Schwetz et al. 1977; Yang et al. 1989). On the other hand, the mean brain/body weight ratio increased at dose levels of 10-100 mg/kg/day, but histopathological lesions were not seen at dose levels of 100 mg/kg/day or less (Kociba et al. 1971). Exposure to hexachlorobutadiene did not alter brain weights and there were no treatment-related histopathological lesions of the brain, spinal cord, and sciatic nerve in rats exposed to hexachlorobutadiene (20 mg/kg/day) for 2 years (Kociba et al. 1977a). Neurochemical and neurophysiological parameters have not been monitored.

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

**2.2.2.5 Reproductive Effects**

No studies were located regarding reproductive effects in humans after oral exposure to hexachlorobutadiene.

In animals, fertility was reduced 100% in Wistar-derived rat dams administered 150 mg/kg/day hexachlorobutadiene during a 10-week study. The mean litter size and the resorption rate did not differ significantly from controls in dams fed 15 mg/kg/day during an 18-week study (Harleman and Seinen 1979). The actual total exposure time for this study is not clear; the rats were exposed for at least 10 weeks at the high dose and 12 weeks (of an 18-week study) at the low dose. No determination of a reliable LOAEL or NOAEL value was possible for this study. In another study, fertility, gestation, viability, and lactation indices were comparable in treated and control groups of Sprague-Dawley rats at dose levels of 20 mg/kg/day for 148 days (Schwetz et al. 1977). No significant changes were seen in sperm count or incidence of abnormal sperm in mice exposed to hexachlorobutadiene (19 mg/kg/day) for 13 weeks (NTP 1991; Yang et al. 1989). Lifetime exposures up to 20 mg/kg/day did not reveal treatment-related lesions in the reproductive organs (Kociba et al. 1977a).

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

**2.2.2.6 Developmental Effects**

No studies were located regarding developmental effects in humans after oral exposure to hexachlorobutadiene.

In animal studies, rat dams were fed hexachlorobutadiene at dose levels of 15 mg/kg/day during gestation (as part of an 18-week study). Rat pup weights were reduced at birth and weaning. However, embryotoxicity and teratogenicity were not observed at this dose (Harleman and Seinen 1979). In another study, body weight was decreased ($p < 0.05$) on day 21 of lactation in rat pups

from dams exposed to hexachlorobutadiene at dose levels of 20 mg/kg/day throughout gestation and lactation; body weights were not reduced in pups from dams exposed to 2 mg/kg/day. No other signs of fetotoxicity were evident at doses up to 20 mg/kg/day. Teratogenic effects were not observed nor was hexachlorobutadiene embryotoxic at the doses tested (Schwetz et al. 1977).

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

**2.2.2.7 Genotoxic Effects**

No studies were located regarding genotoxic effects in humans after oral exposure to hexachlorobutadiene.

In animals, there is some evidence that hexachlorobutadiene interacts with genetic material. Male rats administered a single gavage dose of hexachlorobutadiene (20 mg/kg/day) showed a 40% increase in renal deoxyribonucleic acid (DNA) repair and 0.78 alkylations per million nucleotides (Stott et al. 1981). On the other hand, when hexachlorobutadiene was administered in the diet, it did not cause chromosomal aberrations in rat bone marrow cells (Schwetz et al. 1977).

Other genotoxicity studies are discussed in Section 2.4.

**2.2.2.8 Cancer**

No studies were located regarding carcinogenic effects in humans after oral exposure to hexachlorobutadiene.

Studies in rats reported renal tubular adenomas and adenocarcinomas in male and female animals at doses of 20 mg/kg/day (Kociba et al. 1977a). Metastasis to the lungs was observed. Combined incidences of renal tubular neoplasms in males (9/39, 23 %) and in females (6/40, 15 %) increased ($p < 0.05$) over controls (males-l/90, females-0/90, 0%). The tumor incidence was not increased in the 0.2 and 2 mg/kg/day dose groups but there were some indications of hyperplasia in animals exposed to 2 mg/kg/day. The EPA (1990f) evaluated these data and calculated a human potency factor of $7.8 \times 10^{-2}$ $(mg/kg/day)^{-1}$ ($q_1$*), representing a 95% upper confidence limit of extra lifetime

human risk. Based on this value, cancer risk levels of $10^{-4}$, $10^{-5}$, and $10^{-6}$ correspond to exposures of 0.001, 0.0001, 0.00001 mg/kg/day.

### 2.2.3 Dermal Exposure

#### 2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to hexachlorobutadiene.

During the 14 day observation period some rabbits (2-8) died after 8-hour exposure to doses of 775-1,550 mg/kg applied directly to shaved skin (3.2 $cm^2$), but no deaths occurred in the 388 mg/kg dose group. The author calculated an $LD_{50}$ of 1,116 mg/kg from these data (Duprat and Gradiski 1978). Central nervous system depression was evident, as manifested by stupor. Some animals were weak and anorexic, while others showed signs of dyspnea and cyanosis. The lungs, liver, and kidneys were congested in animals that died. Death was reportedly due to respiratory or cardiac failure.

A LOAEL value for lethality in rabbits after acute-duration exposure is recorded in Table 2-3.

#### 2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal/ocular, or other effects in humans after dermal exposure to hexachlorobutadiene. Liver, kidney, and dermal/ocular effects were reported in animals. These effects are discussed below. All LOAEL values for systemic effects in rabbits after acute-duration exposure are recorded in Table 2-3.

**Hepatic Effects.** Hydropic changes, fatty degeneration, and glycogen reduction were observed in rabbits after exposure of the skin to 388 mg/kg or greater hexachlorobutadiene for 8 hours (Duprat and Gradiski 1978). These effects were reversible within 3 weeks.

## TABLE 2-3. Levels of Significant Exposure to Hexachlorobutadiene - Dermal

| Species | Exposure duration/ frequency | System | NOAEL (mg/kg) | LOAEL (effect) Less serious (mg/kg) | LOAEL (effect) Serious (mg/kg) | Reference |
|---|---|---|---|---|---|---|
| ACUTE EXPOSURE | | | | | | |
| Death | | | | | | |
| Rabbit | Once 8 hr | | | | 116 (LD50) | Duprat and Gradiski 1978 |
| Systemic | | | | | | |
| Rabbit | Once 8 hr | Hepatic | | 388 (fatty degeneration, hydropic changes) | | Duprat and Gradiski 1978 |
| | | Renal | | 388 (tubular necrosis) | | |
| | | Derm/oc | | 388 (cutaneous necrosis) | | |

Derm/oc = dermal/ocular; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; mg/kg = milligrams per kilogram; NOAEL = no-observed-adverse-effect level

**Renal Effects.** Acute-duration dermal exposure in rabbits caused tubular necrosis 24 hours after exposure at dose levels 388 mg/kg or greater (Duprat and Gradiski 1978). The effects were partly reversible, as evident by epithelial regeneration 2 and 5 weeks after exposure.

**Dermal/Ocular Effects.** Skin necrosis was evident at the site of application in rabbits after exposure of the skin to dose levels of 388 mg/kg hexachlorobutadiene for 8 hours (Duprat and Gradiski 1978). However, most skin lesions had healed within 2 weeks.

**2.2.3.3 lmmunological Effects**

No studies were located regarding immunological effects in humans or animals after dermal exposure to hexachlorobutadiene.

**2.2.3.4 Neurological Effects**

No studies were located regarding neurological effects in humans after dermal exposure to hexachlorobutadiene.

Rabbits exposed to doses of 388-1550 mg/kg applied to shaved skin exhibited evidence of aneral nervous system depression (stupor) during exposure and in the l-2 hour period after exposure (Duprat and Gradiski 1978).

No studies were located regarding the following health effects in humans or animals after dermal exposure:

**2.2.3.5 Developmental Effects**

**2.2.3.6 Reproductive Effects**

**2.2.3.7 Genotoxic Effects**

Genotoxicity studies are discussed in Section 2.4.

2. HEALTH EFFECTS

#### 2.2.3.8 Cancer

No studies were located regarding carcinogenic effects in humans after dermal exposure to hexachlorobutadiene.

Hexachlorobutadiene did not produce skin papillomas, carcinomas, or tumors at distant sites in mice after application of dose levels of 2-6 mg/mouse for 440-594 days (Van Duuren et al. 1979). Data from this exploratory study are not sufficient to rule out carcinogenic effects via dermal exposure.

## 2 . 3 TOXICOKINETICS

In analogy with other unsaturated chlorinated compounds, hexachlorobutadiene absorption presumably occurs across the lipid portion of the intestinal matrix rather than by active or protein-facilitated transport. After absorption, most of the hexachlorobutadiene is carried to the liver where it is conjugated with glutathione and excreted in the bile. Mono- and bis-substituted glutathione conjugates are formed. The bile also contains the cysteinyl/glycinyl and cysteinyl derivatives of the glutathione conjugate. Biliary metabolites are resorbed from the intestines and undergo enterohepatic recirculation.

Hexachlorobutadiene and its metabolites preferentially distribute to the kidney, liver, adipose deposits, and possibly the brain. Some hexachlorobutadiene metabolites inhibit mitochondrial metabolism and react with DNA, resulting in cell death or tumorigenesis. Hexachlorobutadiene metabolites are excreted in the urine and feces. There is limited degradation to carbon dioxide which is exhaled from the lungs.

### 2.3.1 Absorption

#### 2.3.1.1 Inhalation Exposure

No studies were located regarding absorption in humans or animals after inhalation exposure to hexachlorobutadiene. The occurrence of effects after exposure (de Ceaurriz et al. 1988; Gage 1970) indicate that absorption does occur.

2. HEALTH EFFECTS

### 2.3.1.2 Oral Exposure

No studies were located regarding absorption in humans after oral exposure to hexachlorobutadiene. There have also been no direct studies of absorption in animals although data on excretion and distribution provide information which suggests that absorption does occur from the gastrointestinal tract (Reichert et al. 1985). In animals, absorption is rapid and virtually complete at low doses of hexachlorobutadiene (1 mg/kg). At a higher dose (50 mg/kg), unmetabolized hexachlorobutadiene is found in the fecal matter (Reichert et al. 1985).

When Alderley Park rats were given a single dose of 200 mg/kg of radiolabeled hexachlorobutadiene and sacrificed at 2, 4, 8, and 16 hours, an autoradiogram of longitudinal sections of whole animals sacrificed 4 hours after dosing demonstrated that the label was concentrated in the intestines. The intestinal label was determined to be 85% unmodified, unabsorbed hexachlorobutadiene. At 8 hours, the intestinal concentration of the label was no longer apparent as hexachlorobutadiene was absorbed and distributed to the tissues (Nash et al. 1984).

Most of the data pertaining to oral administration of hexachlorobutadiene utilized triglycerides (corn oil or tricaprylin) as a gavage dosing medium. Because of its high lipophilicity and low water solubility, it is likely that the absorption of hexachlorobutadiene from an aqueous solution would differ from that from a triglyceride media. When 1 mg/kg hexachlorobutadiene in tricaprylin was administered to female Wistar rats 30.61% was excreted in the urine over 72 hours (Reichert et al. 1985), while when the same dose in aqueous polyethylene glycol solution was given to male Sprague Dawley rats, only 18% was in the urine (Payan et al. 1991). These data suggest that absorption from the lipid solvent was greater than that with the aqueous solvent.

### 2.3.1.3 Dermal Exposure

No studies were located regarding absorption in humans after dermal exposure to hexachlorobutadiene. In animals, pure hexachlorobutadiene (388-1,550 mg/kg) applied to the skin of rabbits was completely absorbed in 8 hours (Duprat and Gradiski 1978).

2. HEALTH EFFECTS

### 2.3.2 Distribution

Hexachlorobutadiene has been identified in samples of human adipose tissue (Mes et al. 1985). The tissue samples were obtained from cadavers and, thus, no data were available pertaining to the route of exposure.

#### 2.3.2.1 Inhalation Exposure

No studies were located regarding distribution in humans or animals after inhalation exposure to hexachlorobutadiene.

#### 2.3.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to hexachlorobutadiene. In animals, 5-14 % of ($^{14}C$) radiolabeled hexachlorobutadiene was retained in the tissues and carcass 72 hours after compound administration (Dekant et al. 1988a; Reichert et al. 1985). The kidney (outer medulla), liver, and adipose tissue appeared to concentrate hexachlorobutadiene label when single doses of up to 200 mg/kg ($^{14}C$) hexachlorobutadiene in corn oil were administered by gavage (Dekant et al. 1988a; Nash et al. 1984; Reichert et al. 1985). In one report, the brain was also determined to contain a relatively high concentration of label 72 hours after exposure (Reichert et al. 1985). Label in the kidney 72 hours after exposure was more extensively covalently bound to proteins than that in the liver (Reichert et al. 1985).

Levels of label in the liver, kidney, and plasma were determined for the donor and recipient rats when secretions from bile duct cannulated donor rats, given a dose of 100 mg/kg hexachlorobutadiene were infused directly into the bile duct of nonexposed recipient rats, and thereby into their intestines (Payan et al. 1991). In the donor rats, after 30 hours, the kidney contained 0.26% of the dose, the liver 0.11%, and the plasma 0.013% from the intestinally absorbed material. In the recipient rats, the kidney contained 0.15% of the dose, the liver 0.97%, and the plasma 0.009% from the resorbed biliary metabolites. For each tissue the level of label from resorbed metabolites was about two-thirds of that from the original dose. The kidneys contained more of the label than the liver in both instances, clearly identifying the kidneys as a target organ.

**2.3.2.3 Dermal Exposure**

No studies were located regarding distribution in humans or animals after dermal exposure to hexachlorobutadiene.

**2.3.2.4 Other Exposure Routes**

In a study using doses of 0.1 and 300 mg/kg intraperitoneally-administered hexachlorobutadiene, the label was found in the liver, kidney, and adipose tissue. Very little of the label was found in the brain, lung, heart, and muscle tissue at 48 hours after dosing (Davis et al. 1980). The reported levels in the brain in this study differ from those reported at 72 hours following oral administration (Reichert et al. 1985). This may indicate that there is a gradual deposition of labeled hexachlorobutadiene and/or its metabolites in the brain lipids over time.

**2.3.3 Metabolism**

**2.3.3.1 Inhalation Exposure**

No studies were located regarding metabolism in humans or animals after inhalation exposure to hexachlorobutadiene.

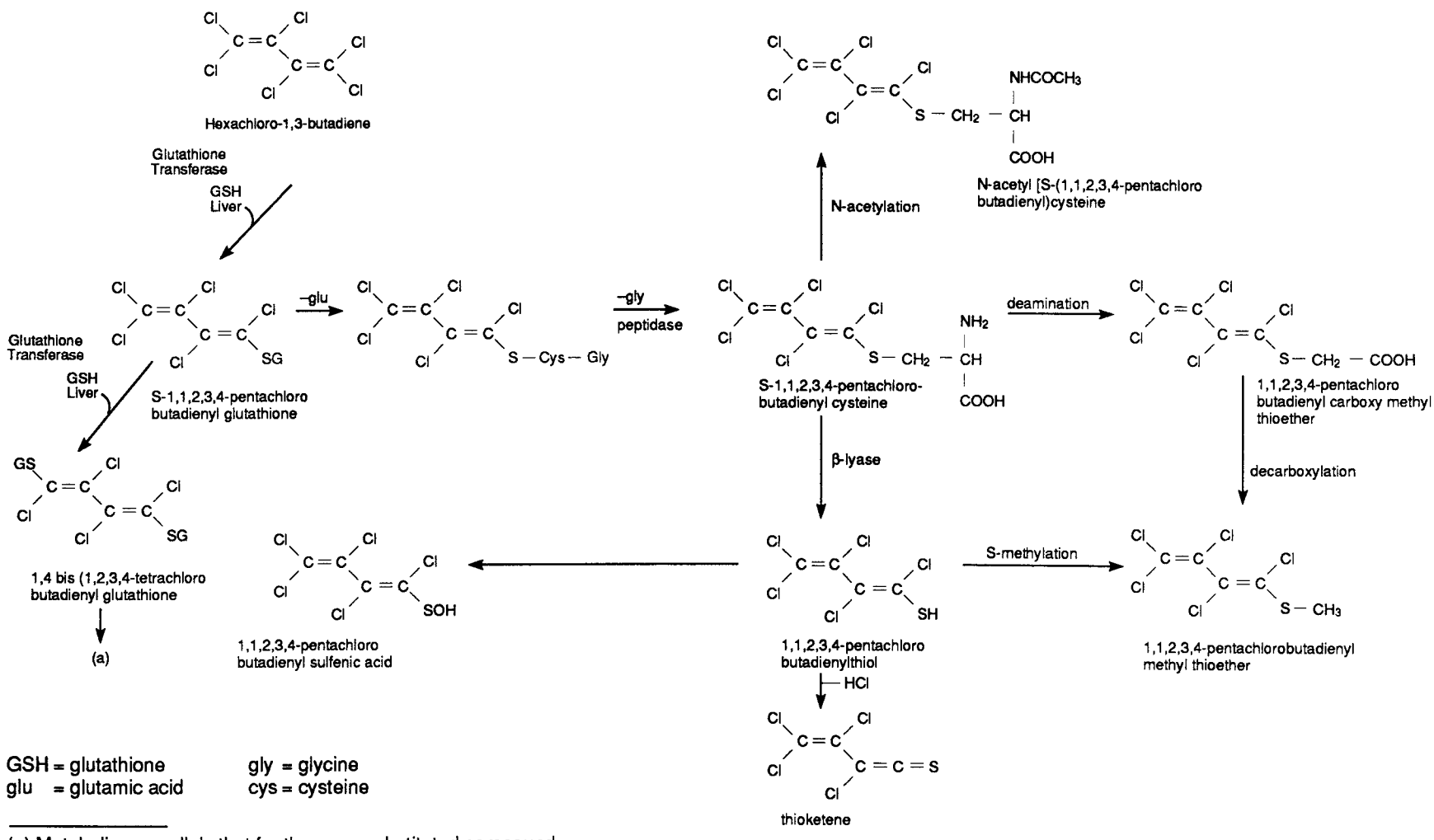
**2.3.3.2 Oral Exposure**

No studies were located regarding metabolism in humans after oral exposure to hexachlorobutadiene.

There is a considerable amount of information available concerning the metabolism of hexachlorobutadiene in animals. Figure 2-3 presents a proposed metabolic pathway for hexachlorobutadiene. This pathway is based on the metabolites identified in urine and bile using chromatographic techniques.

Most of the absorbed hexachlorobutadiene is transported via the portal circulation to the liver where it is conjugated with glutathione (Garle and Fry 1989). In rat livers both mono- and di-substituted conjugates have been identified (Jones et al. 1985), whereas mice appear to produce only the

# FIGURE 2-3 Proposed Pathways for Hexachlorobutadiene Metabolism*




GSH = glutathione gly = glycine
glu = glutamic acid cys = cysteine

(a) Metabolism parallels that for the monosubstituted compound

*Adapted from: Dekant et al. 1991; Jaffe et al. 1983; Nash et al. 1984; Wolf et al. 1984; Jones et al. 1985; Reichert et al. 1985; Reichert and Schutz 1986; Wild et al. 1986

monosubstituted conjugate (Dekant et al. 1988a). There was a dose-related decrease in hepatic levels of glutathione following exposure to hexachlorobutadiene, and pretreatment of experimental animals with agents that interfere with glutathione synthesis or conjugation reactions decreased the amount of glutathione conjugate that can be synthesized (Gietl and Anders 1991). There appears to be no oxidation of the hexachlorobutadiene by the mixed function oxidase system enzymes prior to conjugation (Garle and Fry 1989).

Glutathione conjugate is excreted with the bile into the intestinal tract. A portion of the material is hydrolyzed with the removal of glutamate or glutamate and glycine from the glutathione tripeptide to form the cysteine derivative or the cysteineiglycine derivative (Gietl et al. 1991; Gietl and Anders 1991; Nash et al. 1984). In one study, the glutathione conjugate accounted for 40 % of the label in the bile and the cysteine derivative for 15% of the label. Another 45% of the label was present as unidentified compounds (Nash et al. 1984).

The conversion of the glutathione conjugate to its cysteinyl derivative is mediated, at least in part, by enzymes in the intestinal epithelial cells. S-(Pentachlorobutadienyl)glutathione and S-(pentachlorobutadienyl)-L-cysteine are partially reabsorbed from the intestines and transported to the liver and subsequently to the body tissues (Gietl et al. 1991). Only a portion of the reabsorbed material is taken up by the liver for additional metabolism. When liver uptake of the glutathione conjugate was measured using perfused rat livers, the maximum uptake observed was 39% (Koob and Dekant 1992). A portion of this material was re-excreted in bile without any metabolic modification. The cysteine conjugate, acetylated cysteine conjugate, and six bis-substituted metabolites were synthesized from the glutathione conjugate and excreted in bile. Two of the bis-substituted metabolites were identified as the bis-1,4-glutathione conjugate and the bis-1,4-cysteine conjugate.

The cysteine conjugate was taken up by the liver to a greater extent than the glutathione conjugate (Koob and Dekant 1992). Up to 79% of the cysteine conjugate was absorbed, but this metabolite appeared to be toxic to the liver and caused decreased bile flow within 20 minutes. There were only small portions of the cysteine derivative and acetylated cysteine derivative in the bile. Bis-substituted derivatives, including the 1-cysteinyl-4-glutathionyl tetrachlorobutadiene, bis-1,4-cysteinyl tetrachlorobutadiene, and 1-cysteinyl-4-cysteinyl glycine tetrachlorobutadiene, were formed.

2. HEALTH EFFECTS

Additional processing of the hexachlorobutadiene metabolites produces the compounds identified in the urine (1,1,2,3-tetrachlorobutenoic acid, 1,1,2,3,4-pentachloro-1:3-butadienyl sulfenic acid, N-acetyl-S-l, 1,2,3,4-pentachlorobutadienyl-L-cysteine, S-l, 1,2,3,4-pentachlorobutadienylmercaptoacetic acid, 1,1,2,3,4-pentachlorobutadiene methylthioether, and 1,1,2,3,4-pentachlorobutadiene carboxymethylthioether) (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985).

A very small portion of the absorbed hexachlorobutadiene is oxidized to carbon dioxide. This pathway can be saturated since an increase in the hexachlorobutadiene dose does not cause a corresponding increase in excretion of labeled carbon dioxide (Dekant et al. 1988b; Payan et al. 1991; Reichert et al. 1985).

**2.3.3.3 Dermal Exposure**

No studies were located regarding metabolism in humans or animals after dermal exposure to hexachlorobutadiene.

**2.3.4 Excretion**

**2.3.4.1 Inhalation Exposure**

No studies were located regarding excretion in humans or animals after inhalation exposure to hexachlorobutadiene.

**2.3.4.2 Oral Exposure**

No studies were located regarding excretion in humans after oral exposure to hexachlorobutadiene.

In animals, hexachlorobutadiene and its metabolites are excreted in exhaled air, urine, and feces. In studies where radiolabeled ($^{14}C$) hexachlorobutadiene was administered at doses of 1, 30, 50, or 100 mg/kg, 4-8% of the dose was removed from the body in the exhaled air as unmetabolized hexachlorobutadiene and carbon dioxide within the 72 hours after compound administration (Dekant et al. 1988b; Payan et al. 1991; Reichert et al. 1985).

## 2. HEALTH EFFECTS

With single doses ranging from 1 to 200 mg/kg $^{14}C$ hexachlorobutadiene, the percent of the label in the urine ranged from 4.5 to 30.6% with the highest percentage associated with the lowest dose (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). At the higher doses urinary excretion values of 5 to 10% were common (Nash et al. 1984; Reichert and Schutz 1986). Some of the hexachlorobutadiene label excreted in the urine originates from the biliary metabolites reabsorbed from the intestinal tract and processed by the kidneys for excretion.

The contribution of reabsorbed biliary metabolites to urinary excretion is reflected in the differences in urinary excretion of label from bile duct cannulated rats and noncannulated rats. When a dose of 1 mg/kg hexachlorobutadiene in polyethylene glycol solution was given to bile duct cannulated male rats, the urine contained 11% of the label after 72 hours; in noncannulated rats given the same dose it contained 18 % of the label (Payan et al. 1991). When a dose of 100 mg/kg was given, the urine of the cannulated rats contained 7 % of the label and the urine of the noncannulated rats contained 9 % after 72 hours.

Metabolites identified in the urine include: S-( 1,1,2,3,4-pentachlorobutadienyl)glutathione; S-(1,1,2,3,4-pentachlorobutadienyl) cysteine; 1,1,2,3 ,-tetrachlorobutenoic acid; 1,1,2,3,4-pentachloro-1: 3-butadienyl sulfenic acid; N-acetyl-S- 1,1,2,3,4-pentachlorobutadienyl)-L-cysteine; S-pentachlorobutadienyl-mercaptoacetic acid; 1,1,2,3,4-pentachlorobutadiene methylthioether and 1,1,2,3,4-pentachlorobutadiene carboxymethylthioether (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985).

Fecal excretions contained unmetabolized, unabsorbed hexachlorobutadiene plus a portion of the hepatic metabolites excreted with the bile. At the lower doses almost all of the label in the feces originated with the biliary metabolites, whereas at the higher doses there was also some unabsorbed hexachlorobutadiene in the fecal matter (Dekant et al. 1988b). In rats given 200 mg/kg, feces collected during the 5-day period contained a total of 39 % of the dose. Only 5 % was excreted in the first 2 days after dosing. In another study, the feces and contents of the gastrointestinal tract contained 62 % of a 1 mg/kg dose and 72 % of a 100 mg/kg dose (Payan et al. 1991). The only metabolite that had been identified in the feces is S-( 1,1,2,3,4-pentachlorobutadienyl) glutathione (Dekant et al. 1988b), although unidentified metabolites were also present and most likely included the cysteine derivatives.

In one study where a single 200 mg/kg dose was given to rats by gavage, 35% of the label was found in the bile in the first 2 days after dosing. The biliary label was equally distributed over the 2 days of collection. In a different study, 66% of a 1 mg/kg dose was excreted in the bile of bile duct cannulated rats in 72 hours and 58 % of a 100 mg/kg dose (Payan et al. 1991).

Secretions from bile duct cannrulated rats given a dose of 100 mg/kg hexachlorobutadiene were infused directly into the bile duct of nonexposed rats (Payan et al. 1991). The levels of label in the urine, bile, and feces of both the donor and recipient rats were measured 30 hours after dosing. The label in the urine and bile of the recipient rats represented label that was reabsorbed from the gastrointestinal tract. It was determined that 80% of the biliary metabolites were reabsorbed and only 20% remained in the feces and gastrointestinal tract.

**2.3.4.3 Dermal Exposure**

No studies were located regarding excretion in humans or animals after dermal exposure to hexachlorobutadiene.

**2.3.4.4 Other Exposure Routes**

The distribution of radiolabel in excreta was measured in male rats for the 72 hour period after intravenous administration of doses of 1 or 100 mg/kg (Payan et al. 1991). At both doses about 8% of the radiolabel was exhaled. The amount of label in the urine was 21% of the low dose and 9% of the high dose; the amount in the feces was 59% of the low dose and 72% of the high dose. In a parallel study, the fecal, urinary, and biliary excretions were measured for rats with cannulated bile ducts. The urine contained 6-7 % of the dose and the feces less than 0.5 % for both doses. The bile contained 89% of the 1 mg/kg dose and 72% of the 100 mg/kg dose.

**2.3.5 Mechanism of Toxicity**

Much of the data related to the mechanism of hexachlorobutadiene toxicity indicate that the intermediates produced by modification of the S- 1,1,2,3,4-pentachlorodienyl cysteine derivative are responsible for the observed effects on the proximal tubules of the nephrons. The cysteine derivative is formed from the hexachlorobutadiene conjugate in the liver, intestines, and/or kidney through the

action of γ glutamyl transferase which removes the glutamate from the glutathione tripeptide followed by the action of a peptidase that removes the glycine from the carboxy terminus.

The cysteine derivative is further metabolized to simpler sulphur derivatives through the action of β-lyase. β-Lyase is present in the rodent liver, intestines, and kidneys (MacFarlane et al. 1989; Jones et al. 1988). In the kidney, the highest concentration of β-lyase is located in the pars recta of the proximal tubule, the same area that is damaged by hexachlorobutadiene. It should be noted that β-lyase has been detected in the entire proximal segment (Jones et al. 1988). It is present in both the cytosol and mitochondria and is pyridoxal phosphate dependent (MacFarlane et al. 1989). It degrades the cysteine conjugate to pyruvate, ammonia, and one or more reactive thiols (Dekant et al. 1990b; Schnellmann et al. 1987). A highly reactive thioketene (Figure 2-3) may form as an intermediate and cause local tissue damage (Dekant et al. 1991; Koob and Dekant 1992).

The effects of the cysteine conjugate on the activity of the cells of the proximal tubules was evaluated in cells from New Zealand white rabbits (Schnellmann et al. 1987). These studies indicate that the metabolites of the cysteine conjugate alter the action of the mitochondria in a two phase process. The first phase apparently causes an uncoupling of oxidative phosphorylation thereby preventing the generation of ATP. The deficiency of ATP in turn limits ATP dependent active transport in the tubules, inhibiting reabsorption processes. In the second phase, inhibition of cytochrome c-cytochrome oxidase activity and electron transport occur (Schnellmann et al. 1987). These changes result in cell damage as reflected in a decrease in the cellular retention of lactate dehydrogenase approximately 1 hour after exposure.

The carcinogenic properties of hexachlorobutadiene are proposed to result from binding of the sulfenic acid degradation product or a thioketene intermediate to cellular DNA (Dekant et al. 1990b; Henschler and Dekant 1990). Cell necrosis is thought to stimulate replication of cells with altered DNA, enhancing tumorigenesis.

## 2.4 RELEVANCE TO PUBLIC HEALTH

Data regarding the effects of exposure to hexachlorobutadiene on humans are sparse. Serum bile acids were increased in workers exposed to vapor concentrations of 0.005-0.02 ppm. These effects could not be attributed to hexachlorobutadiene alone because the workers were also potentially

exposed to other chemicals (carbon tetrachloride and perchloroethylene) and there were no background data on employee health, smoking habits, alcohol consumption, or other confounding variables. Animals have been studied more extensively. Although there are studies available on the systemic (respiratory) effects following inhalation, data are not sufficient to identify a reliable NOAEL value by this route. Much of the available data involve oral exposures in rats. The primary health effect associated with intermediate-duration and chronic-duration oral exposures to hexachlorobutadiene is kidney damage, which occurred in this organ at doses of 0.2 to 20 mg/kg/day. The liver was affected to a lesser extent and effects occurred at doses of 6.3 or 15.6 mg/kg/day. Acute dermal studies, although limited, confirm the toxic effects of hexachlorobutadiene on these organs.

Hexachlorobutadiene did not adversely affect reproduction in animals except at high doses (150 mg/kg/day for 10 weeks). Although there was some evidence of fetotoxicity in animals after inhalation (10 ppm) or oral (15 mg/kg/day) exposure, embryolethality and teratogenicity were not detected. Oral studies in animals indicate that hexachlorobutadiene may increase the risk of renal cancer at dose levels of 20 mg/kg/day. The effects of hexachlorobutadiene are most pronounced after repeated chronic exposure to low doses, suggesting that effects are cumulative. For this reason, there is greater concern for populations living near hazardous waste sites, where exposure to low levels may occur for long periods of time, than for acute exposure scenarios.

**Minimal Risk Levels for Hexachlorobutadiene**

**Inhalation MRLs**

Inhalation MRLs have not been derived for any duration category due to the lack of sufficient data to identify a target organ and reliable NOAEL values.

**Oral MRLs**

- An MRL of 0.0002 mg/kg/day has been derived for intermediate-duration oral exposure to hexachlorobutadiene.

This MRL was derived using a LOAEL value of 0.2 mg/kg/day, based on the presence of kidney damage in female mice (NTP 1991). Tubular cell degeneration and regeneration in the renal cortex were found in treated animals.

Other intermediate-duration oral studies confirm that the kidney is the primary target organ following oral exposure to hexachlorobutadiene. Renal damage, characterized as tubular hyperplasia, tubular epithelial degeneration, and tubular regeneration, was seen at dose levels of 2 mg/kg/day or greater (Harleman and Seinen 1979; Schwetz et al. 1977). A chronic study in rats reported renal tubular hyperplasia at dose levels of 2 mg/kg/day or greater (Kociba et al. 1977a).

No data were located on the effects of acute-duration oral exposure in humans. Two studies are available on the acute oral toxicity in animals. A LOAEL value of 4 mg/kg/day (based on kidney effects) was reported in one study in rats exposed to hexachlorobutadiene for 14 days (Harleman and Seinen 1979). On the other hand, a NOAEL value of 10 mg/kg was reported for kidney effects in a 24-hour rat study (Jonker et al. 1993a). Neither of these studies was considered suitable for the MRL determination because of the small numbers of animals evaluated.

No data were located on the effects of chronic-duration exposure in humans. A chronic-duration study in animals revealed tubular hyperplasia in rats at dose levels of 2 mg/kg/day or greater (Kociba et al. 1977a), but not at 0.2 mg/kg/day, the LOAEL for kidney effects from the intermediate-duration study in mice. Because the intermediate-duration MRL protects against chronic exposures, a chronic MRL has not been proposed.

**Death.** No studies were located regarding lethality in humans. Hexachlorobutadiene reduced survival in rats following acute- and chronic-duration exposures. Young rats may be more sensitive than adult rats. Acute oral doses of 580 mg/kg (male) and 200-400 mg/kg (female) were lethal to 50% of adult rats. Death occurred at lower dose levels in weanling females (46 mg/kg) and males (65 mg/kg) (Kociba et al. 1977a). However, in a rat reproduction study in which dams received intermediate-duration oral exposures to hexachlorobutadiene at doses of 20 mg/kg/day during gestation (days 1 to 22) and lactation (days l-21), pup survival was not affected even at doses that were maternally toxic (Schwetz et al. 1977). Acute-duration dermal exposures (775 mg/kg) can also reduce survival (50%) in animals (Duprat and Gradiski 1978). Based on these considerations,

lethality may be of concern in humans following exposure to hexachlorobutadiene. The basis for differential susceptibility between adult and young rats may be due to metabolic differences or differences in disposition of hexachlorobutadiene. As discussed in Section 2.3.2, hexachlorobutadiene distributes to body fat. The smaller fraction of fat in the newborn reduces the amount of sequestered hexachlorobutadiene; therefore, more of the compound may reach target organs (Hook et al. 1983).

**Systemic Effects**

***Respiratory Effects.*** No studies were located regarding respiratory effects in humans. The only data available indicating respiratory effects were reports of irritation of the nasal cavity in mice after acute (15 minutes) inhalation of vapors of hexachlorobutadiene at concentrations of 155 ppm or greater (de Ceaurriz et al. 1988). The importance of this finding to human health is uncertain.

***Cardiovascular Effects.*** No studies were located regarding cardiovascular effects in humans. In animals, intermediate-duration or chronic-duration oral exposure to hexachlorobutadiene at dose levels up to 100 mg/kg/day did not cause treatment-related lesions of the heart in rats or mice (Kociba et al. 1971, 1977a; NTP 1991; Schwetz et al. 1977; Yang et al. 1989). On the other hand, heart weights decreased significantly in mice at doses of 16.8 mg/kg/day (NTP 1991; Yang et al. 1989) or 65 mg/kg/day or greater in rats (Kociba et al. 1971). There were no histopathological lesions. Because treatment-related lesions were not observed even at doses higher than those causing other organ toxicity, cardiovascular toxicity may not be an area of concern in humans following exposure to hexachlorobutadiene.

***Gastrointestinal Effects.*** No studies were located regarding gastrointestinal effects in humans. Intermediate-duration (up to 100 mg/kg/day) or chronic-duration oral (20 mg/kg/day) exposure to hexachlorobutadiene did not cause treatment-related lesions of the gastrointestinal tract in rats (Kociba et al. 1971, 1977a; Schwetz et al. 1977). Because histological lesions were not observed even at doses higher than those causing other organ toxicity, gastrointestinal toxicity may not be an area of concern in humans following exposure to hexachlorobutadiene.

***Hematological Effects.*** No studies were located regarding hematological effects in humans. Animal studies evaluating the hematological effects of hexachlorobutadiene involved mainly intermediate-duration and chronic-duration oral exposures up to 20 mg/kg/day in rats (Harleman and Seinen 1979;

Kociba et al. 1977a; Schwetz et al. 1977). There was increased hemoglobin concentration in rats after intermediate-duration oral exposure (10 mg/kg/day or greater) (Kociba et al. 1971). However, hematocrit, red blood cell, and differential leucocyte counts were comparable to untreated controls. For this reason, hematological effects may not be an area of major concern in humans following exposure to hexachlorobutadiene.

***Musculoskeletal Effects.*** No studies were located regarding musculoskeletal effects in humans. Intermediate-duration or chronic-duration oral exposure to hexachlorobutadiene at dose levels up to 20 mg/kg/day did not cause treatment-related lesions of the musculoskeletal system in rats (Harleman and Seinen 1979; Kociba et al. 1977a; Schwetz et al. 1977). Because histological lesions were not observed even at doses higher than those causing other organ toxicity, musculoskeletal effects may not be an area of concern in humans following exposure to hexachlorobutadiene.

***Hepatic Effects.*** Data in humans are limited to one study which reported significant dose-related increases in the concentration of serum bile acids in workers after inhalation exposure to hexachlorobutadiene (0.005-0.02 ppm) (Driscoll et al. 1992). The practical importance of this finding is reduced because workers were also potentially exposed to other solvents (carbon tetrachloride and perchloroethylene) and background information on other confounding variables was minimal. No studies were located regarding other hepatic effects in humans.

In animals, liver damage may occur after oral exposure to hexachlorobutadiene; however, the effects are less severe than those associated with renal damage. Intermediate-duration oral exposures caused liver damage in male rats at dose levels of 6.3 mg/kg/day or greater (Harleman and Seinen 1979; Kociba et al. 1971). Histological lesions were not found in female rats. Relative liver weights were increased in female rats, but occurred at higher dose levels (15 mg/kg/day) than in male rats (6.3 mg/kg/day). In another study, liver weights were decreased in female rats at dose levels of 5 mg/kg/day or greater following exposure to hexachlorobutadiene for 4 weeks (Jonker et al. 1993b). In the same study, serum biochemical parameters (aspartate aminotransferase activity and total bilirubin) were increased at doses of 20 mg/kg/day. Urinary excretion of coproporphyrin increased at dose levels of 20 mglkglday in lifetime studies; however, histopathological lesions were not found (Kociba et al. 1977a).

2. HEALTH EFFECTS

Dermal studies in which rabbits received applications of hexachlorobutadiene (775 mg/kg or greater) directly to the skin identified hepatocyte damage and fatty degeneration of the centrilobular area as effects of exposure. Effects were reversible within 3 weeks (Duprat and Gradiski 1978). Studies using intraperitoneal injection support findings of morphological alterations in the liver. A single intraperitoneal dose of hexachlorobutadiene (100 mg/kg or greater) caused disruption of mitochondria in periportal hepatocytes which resulted in an influx of water and ions into the cell without effects on the sodium pump (Lock et al. 1982, 1985). Alterations in serum biochemical parameters have been reported following intraperitoneal injection. Alkaline phosphatase activity increased ($p < 0.05$) at doses of 52 mg/kg/day or greater (Bai et al. 1992). Aspartate aminotransferase activity and total bilirubin levels decreased ($p < 0.05$) at a dose of 104 mg/kg/day (highest dose tested).

***Renal Effects.*** No studies were located regarding renal effects in humans. Acute-, intermediate- and chronic-duration oral studies in rats and mice revealed that the kidney is the primary target organ for hexachlorobutadiene toxicity. Acute exposure (24 hours) to hexachlorobutadiene (100 mg/kg or greater) caused focal necrosis and an increase in plasma creatinine levels (Jonker et al. 1993a). When rats were exposed to hexachlorobutadiene (5 mg/kg/day) for 4 weeks, tubular cytomegaly was reported (Jonker et al. 1993b). Tubular cell degeneration and regeneration in the renal cortex occurred in female mice at dose levels of 0.2 mg/kg/day for 13 weeks (NTP 1991; Yang et al. 1989). Tubular degeneration and cell necrosis occurred in rats after short duration exposures (30-148 days) at dose levels of 20 or 30 mg/kg/day (Harleman and Seinen 1979; Kociba et al. 1971; Schwetz et al. 1977).

Lifetime exposures at dose levels from 2.5 mg/kg/day revealed renal hyperplasia in rats (Kociba et al. 1977a). For the most part, kidney lesions were more pronounced in females and occurred at lower dose levels. Morphological changes were found in female rats in the 2.5 mg/kg/day dose groups, whereas comparable alterations were first seen in male rats at dose levels of 6.3 mg/kg/day. Kidney weights or kidney/body weight ratios were increased at dose levels causing morphological damage (Harleman and Seinen 1979; Kociba et al. 1977a; NTP 1991; Schwetz et al. 1977; Yang et al. 1989). Results of evaluations of impairment in kidney functions were consistent with morphological alterations. The capacity to concentrate urine was reduced in female rats at dose levels of 2.5 mg/kg/day and in males at 15 mg/kg/day (Harleman and Seinen 1979). Although histological lesions were not evident at the low dose in this study, kidney damage has been reported at comparable dose levels following chronic oral exposures (Kociba et al. 1977a).

## 2. HEALTH EFFECTS

Biochemical indices (blood urea nitrogen, creatinine) were comparable to controls at dose levels up to 20 mg/kg/day in some studies (Harleman and Seinen 1979; Kociba et al. 1977a; Schwetz et al. 1977). However, blood urea levels increased significantly at dose levels of 200 mg/kg (highest dose tested) and creatinine levels increased at 100 mg/kg in an acute study (24 hours) (Jonker et al. 1993a). Similarly, urinary lactate dehydrogenase and N-acetyl-β−glucosaminidase increased significantly in the 24 hours after exposure to a single dose of 100 mg/kg or greater. In a different study, blood creatinine levels decreased in females at dose levels of 5 mg/kg/day or greater, while blood urea levels decreased at doses of 1.25 mg/kg/day or greater hexachlorobutadiene for 4 weeks (Jonker et al. 1993b). Although statistically significant differences in biochemical parameters were found, the importance of these effects is reduced because the effects were not completely dose-related. Accordingly, these parameters may not be reliable indicators of renal damage following hexachlorobutadiene exposure.

Kidney damage was also seen following an 8 hour dermal exposure in rabbits that received applications of hexachlorobutadiene (775 mg/kg or greater). The effects were reversible within 3-5 weeks (Duprat and Gradiski 1978). Intraperitoneal studies revealed patterns of damage similar to those for other routes and duration categories. Species differences were reported. Renal tubular necrosis was evident in rats and was confined mainly to the straight limb of the proximal tubules involving the medulla. Effects were evident within 24 hours at dose levels of 100 mg/kg (Lock and Ishmael 1979). At higher (200 mg/kg) doses, necrosis was evident by 8 hours (Ishmael et al. 1982). Studies in mice also revealed that hexachlorobutadiene causes damage to the proximal tubules of the kidney; effects were observed at lower dose levels (50 mg/kg) than in rats and were observed in both the cortex and the medulla *(*Ishmael et al. 1984). It was also noted that active regeneration of the tubular epithelium was evident by 5 days after treatment, and by 14 days, tubular morphology had returned to normal (Ishmael et al. 1984).

Metabolites of hexachlorobutadiene (glutathione conjugate, cysteine conjugate, and its N-acetyl cysteine conjugate) produced effects at lower doses than the parent compound after intraperitoneal injection and there was differential susceptibility between sexes (Ishmael and Lock 1986). A single intraperitoneal dose of 25 mg/kg of the conjugates caused minimal to moderate necrosis in males and severe necrosis in females. On the other hand, a comparable dose caused no effect in males and females after exposure to the parent compound (Ishmael and Lock 1986).

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One or two hexachlorobutadiene metabolites appear to cause some of the compound-induced renal damage (see Section 2.3.5) and are more toxic than the parent compound, causing comparable lesions in the kidneys at lower doses. These active thioacylating metabolites are capable of modifying DNA, as indicated by the isolation of sulfur-containing nucleides from hydrolyzed DNA from renal cells exposed to the hexachlorobutadiene cysteine derivative (Vamvakas et al. 1988b).

Overall, the kidney is highly susceptible to the toxicity of hexachlorobutadiene, in contrast to other organs, due to the activity of β-lyase and other mercapturic acid processing enzymes (Vamvakas et al. 1988b). The greater sensitivity of females may be due to differences in renal enzymes responsible for the tissue levels of the active metabolites (Hook et al. 1983). Based on data in animals, renal toxicity is a major concern in humans who may be chronically exposed to this material from hazardous waste sites or other sources.

*Dermal/Ocular Effects.* No studies were located regarding dermal/ocular effects in humans. Acute-duration dermal exposure caused skin necrosis in rabbits; however, effects were reversible within 2 weeks (Duprat and Gradiski 1978). Nasal irritation resulted from 15 minute exposure to vapor concentrations of 155 ppm (de Ceaurriz et al. 1988). No dermal/ocular effects were seen following intermediate- or chronic-duration dermal exposure in rabbits. Based on acute effects in rabbits, hexachlorobutadiene may pose some risk to humans following skin contact with the chemical depending on the area exposed. Inhalation of vapors may cause irritation of the nasal mucosa.

Immunological Effects. No studies were located regarding immunological effects in humans. Oral intermediate or chronic exposures to hexachlorobutadiene did not produce treatment-related histopathological lesions in lymphoid tissue (spleen or thymus) in mice (NTP 1991; Yang et al. 1989) or rats (Harleman and Seinen 1979; Kociba et al. 1971, 1977a) after 13 weeks of exposure. Necrosis of lymphoid tissue did occur in the spleen, lymphoids, and thymus of mice exposed to lethal doses (NTP 1991; Yang et al. 1989). No data are available on immunological effects following acute-duration oral exposure. In the absence of tests that evaluate impairment of immune functions, firm conclusions cannot be made about the potential for hexachlorobutadiene to affect immune processes in humans.

Neurological Effects. No studies were located regarding neurological effects in humans. Intermediate oral exposure to hexachlorobutadiene caused damage to the nervous system in rats.

## 2. HEALTH EFFECTS

Ataxia associated with demyelination and fragmentation of femoral nerve fibers was reported in adult female rats that received 150 mg/kg hexachlorobutadiene. Effects were not seen at lower dose levels (15 mg/kg/day) (Harleman and Seinen 1979). No neurological effects were reported following chronic oral exposures (Kociba et al. 1977a). Dermal application of 388-1550 mg/kg induced stupor in rabbits during the 8-hour exposure period and for the 2-hour period immediately after exposure (Duprat and Gradiski 1978). Although neurological symptoms were not present in all studies, these data, and the fact that hexachlorobutadiene has been found in brain tissue, suggest neurological effects may occur in humans following hexachlorobutadiene exposure.

**Reproductive Effects.** No studies were located regarding reproductive effects in humans. Acute-duration inhalation exposure to hexachlorobutadiene (10 ppm) did not adversely affect sperm morphology in mice (NIOSH 1981). In a developmental toxicity study, the mean number of implantation sites, total fetal loss, and live fetuses per litter in rat dams exposed to 15 ppm hexachlorobutadiene during gestation were comparable to unexposed controls (Saillenfait et al. 1989). No data were found on intermediate- or chronic-duration inhalation exposure in mice. Intermediate-duration oral exposure did not adversely affect fertility, gestation, viability, and lactation indices in rats at dose levels of 20 mg/kg/day (Schwetz et al. 1977). Similarly, hexachlorobutadiene did not adversely affect mean litter size and resorption rate in rats fed 15 mg/kg/day hexachlorobutadiene (Harleman and Seinen 1979). There were no histological lesions in the gonads or accessory sex organs after intermediate- or chronic-duration exposures (Kociba et al. 1977a; Schwetz et al. 1977). Based on these data and the fact that the compound has not been detected in reproductive tissue, hexachlorobutadiene does not appear to pose a significant risk to human reproduction.

**Developmental Effects.** No studies were located regarding developmental effects in humans. In intermediate-duration inhalation animal studies, fetal body weight was reduced in rat pups following exposure of dams to hexachlorobutadiene vapors at concentrations of 15 ppm for 15 days (Saillenfait et al. 1989). After intermediate oral exposure in rat dams administered hexachlorobutadiene (during gestation and lactation) at dose levels of 20 mg/kg/day, body weights decreased on lactation day 21 (Schwetz et al. 1977) and pup weights were reduced at dose levels of 15 mg/kg/day for 6 weeks (during gestation and lactation) during an 18 week study (Harleman and Seinen 1979). In both cases, no other fetotoxic effects were reported. Similar results were found in rat pups of dams administered a single dose of hexachlorobutadiene by intraperitoneal injection at dose levels of 10 mg/kg (Hardin et al. 1981). Because the fetotoxic effects occurred at concentrations that were also maternally toxic

and the fact that embryolethality and major malformations were not observed, it is not likely that low levels of hexachlorobutadiene will pose any significant risk to renal development and function in humans.

**Genotoxic Effects.** No studies were located regarding genotoxic effects in humans. For the most part, acute inhalation studies evaluating chromosomal damage in rats and gene mutation in Drosophila revealed that hexachlorobutadiene is not mutagenic (NIOSH 1981). On the other hand, results of oral studies in rats do not agree suggesting differences in activation and detoxification. Hexachlorobutadiene can affect genetic material as evident by the induction of DNA repair and alkylation (Stott et al. 1981) (Table 2-4). The compound did not cause chromosomal aberrations in rat bone marrow cells (Schwetz et al. 1977).

Several *in vitro* assays have been evaluated; however, results were mixed, suggesting differences in activation and detoxification mechanisms (Table 2-5). In bacterial assay systems employing *Salmonella typhimurium,* hexachlorobutadiene was not mutagenic either in the presence or absence of metabolic activation (DeMeester et al. 1980; Haworth et al. 1983; Reichert et al. 1983; Stott et al. 1981; Vamvakas et al. 1988a) or in the presence of activation (Roldan-Arjona et al. 1991). On the other hand, results were positive in other bacterial assays employing S. typhimurium (Reichert et al. 1984; Roldan-Arjona et al. 1991; Vamvakas et al. 1988a). Certain metabolites of hexachlorobutadiene have also been evaluated. Monooxidation products of hexachlorobutadiene were mutagenic in *Salmonella* with and without metabolic activation (Reichert et al. 1984). Similarly, monooxidation products induced unscheduled DNA synthesis as well as morphological transformations in cultured Syrian hamster embryo fibroblasts (Schiffmann et al. 1984). However, results did not agree for hexachlorobutadiene in an *in vitro* unscheduled DNA synthesis assay employing rat hepatocytes (Stott et al. 1981). Studies of cysteine conjugates of hexachlorobutadiene reported that N-acetyl-S-pentachlorobutadienyl-L-cysteine (mercapturic acid) and D,L-homocysteinate derivatives were mutagenic in *S. typhimurium,* while mercaptoacetic acid and methylthioether derivatives were inactive (Wild et al. 1986). In other tests employing *S. typhimurium,* one cysteine conjugate was mutagenic both with and without activation (Dekant et al. 1986). Overall, results suggest that genotoxicity may not be a major factor in the toxicity of hexachlorobutadiene in humans. On the other hand, some influence of genetic mechanisms cannot be ruled out since there was limited evidence of renal DNA repair and alkylation (Stott et al. 1981).

## TABLE 2-4. Genotoxicity of Hexachlorobutadiene *In Vitro*

| Species (test system) | End point | Results | | Reference |
|---|---|---|---|---|
| | | With activation | Without activation | |
| Prokaryotic organisms: | | | | |
| *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) | Gene mutation | - | - | Haworth et al. 1983 |
| *S. typhimurium* (TA98, TA100) | Gene mutation | - | - | Reichert et al. 1983 |
| *S. typhimurium* (TA98, TA100, TA2638) | Gene mutation | + | - | Vamvakas et al. 1988a |
| *S. typhimurium* (TA98, TA100, TA2638) | Gene mutation | +[a] | +[a] | Vamvakas et al. 1988a |
| *S. typhimurium* (TA98, TA100, TA2638) | Gene mutation | -[b] | -[b] | Vamvakas et al. 1988a |
| *S. typhimurium* (TA100) | Gene mutation | + | - | Reichert et al. 1984 |
| *S. typhimurium* (TA100) | Gene mutation | +[c] | +[c] | Reichert *et al.* 1984 |
| *S. typhimurium* (TA100) | Gene mutation | +[d] | +[d] | Wild et al. 1986 |
| *S. typhimurium* (TA100) | Gene mutation | -[e] | -[e] | Wild et al. 1986 |
| *S. typhimurium* (TA98, TA100, TA1530, TA1535, TA1538) | Gene mutation | - | - | DeMeester et al. 1980 |
| *S. typhimurium* (TA98, TA100, TA2638) | Gene mutation | +[f] | Not tested | Dekant et al. 1986 |
| *S. typhimurium* | - | - | - | Stott et al. 1981 |
| *S. typhimurium* | Gene mutation | - | + | Roldan-Arjona et al. 1991 |
| Mammalian cells: | | | | |
| Syrian hamster embryo fibroblast | Unscheduled DNA synthesis | + | + | Schiffmann et al. 1984 |

## TABLE 2-4. Genotoxicity of Hexachlorobutadiene *In Vitro (continued)*

| Species (test system) | End point | Results | | Reference |
|---|---|---|---|---|
| | | With activation | Without activation | |
| Syrian hamster embryo fibroblast | Morphological transformation | + | + | Schiffmann et al. 1984 |

- = negative result; + = positive result; DNA = deoxyribonucleic acid

[a] Conjugates of hexachlorobutadiene - 1-(glutathion-S-yL)-1,2,3,4,4-pentachloro-1,3-butadiene
[b] Conjugates of hexachlorobutadiene - 1,4-(bis-glutathion-S-yL)-1,2,3,4-tetrachloro-1,3-butadiene and 1,4-(bis-cystein-S-yL) -1,2,3,4-tetrachloro-1,3-butadiene
[c] Monooxidation product - perchloro-3-butenoic acid and perchloro-3-butenoic acid chloride
[d] Conjugates of hexachlorobutadiene - mercapturic acid and methyl-N-acetyl-S-pentachlorobutadienyl-D-L-homocysteinate
[e] Conjugates of hexachlorobutadiene - S-pentachlorobutadienyl-mercapto acetic acid and pentachlorobutadienyl-methylthioether
[f] Conjugate of hexachlorobutadiene - S-1,2,3,4,4-pentachlorobuta-1,3-dienylcysteine

## TABLE 2-5. Genotoxicity of Hexachlorobutadiene *In Vivo*

| Species (test system) | End point | Results | Reference |
|---|---|---|---|
| Mammalian cells: | | | |
| Rat | Dominant lethality | - | NIOSH 1981 |
| Rat (bone marrow cells) | Chromosomal aberration | - | NIOSH 1981 |
| Rat (kidney cells) | DNA alkylation | + | Stott et al. 1981 |
| | DNA repair | + | Stott et al. 1981 |
| Rat (bone marrow cells) | Chromosomal aberration | - | Schwetz et al. 1977 |
| Eukaryotic organism: | | | |
| Insect: | | | |
| Drosophila | Gene mutation (sex-linked recessive lethal) | - | NIOSH 1981 |

- = negative result; + = positive result; DNA = deoxyribonucleic acid

**Cancer.** No studies were located regarding the carcinogenic potential of hexachlorobutadiene in humans. It is carcinogenic in rats after lifetime oral exposures. The incidence of adenomas and adenocarcinomas in the kidney increased over control levels at doses of 20 mg/kg/day. Two neoplasms metastasized to the lungs (Kociba et al. 1977a). The IARC (1979) evaluated the carcinogenic potential of hexachlorobutadiene and concluded there was limited evidence that hexachlorobutadiene is carcinogenic in rats. EPA considers hexachlorobutadiene to be a possible human carcinogen (Group C) (IRIS 1993).

Several studies have assessed the mechanism of hexachlorobutadiene-induced renal tumorigenesis (see Section 2.3.5). The carcinogenic properties of hexachlorobutadiene may result from binding of intermediary metabolites to cellular DNA (Dekant et al. 1990b; Henschler and Dekant 1990). In addition, the occurrence of renal tubular regeneration suggests that cell repair processes which induce the replication of cells with altered DNA may be a factor in the tumorigenesis process. Based on carcinogenic effects in rats, exposure to hexachlorobutadiene may pose some risk for development of kidney tumors in humans.

## 2.5 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 hexachlorobutadiene are discussed in Section 2.5.1.

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 not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by hexachlorobutadiene are discussed in Section 2.5.2.

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 2.7, Populations That Are Unusually Susceptible.

### 2.5.1 Biomarkers Used to Identify or Quantify Exposure to Hexachlorobutadiene

Human exposure to hexachlorobutadiene can be determined by measuring the parent compound in blood and adipose tissue (Bristol et al. 1982; Mes et al. 1985). Data in animals are limited, but do suggest that hexachlorobutadiene can be detected in urine and exhaled air. Approximately 4-31% of the administered radioactivity was detected in the urine of mice or rats within 72 hours following the administration of single oral doses of $^{14}$C-hexachlorobutadiene (l-200 mg/kg) (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). No information was located on how long before it can no longer be detected. Unmetabolized hexachlorobutadiene was detected in exhaled air after animals were given doses of l-100 mg/kg (Dekant et al. 1988b; Payan et al. 1991; Reichert et al. 1985).

Cysteine conjugates of hexachlorobutadiene are converted to thio derivatives (e.g., 1,1,2,3,4-Pentachlorobutadiene methylthioether and 1,1,2,3,4-pentachlorobutadiene carboxy methylthioether) which have been detected in urine (Reichert et al. 1985). Accordingly, tests to determine concentrations of

these sulfur derivatives in urine may be useful in determining if exposure to hexachlorobutadiene has occurred.

### 2.5.2 Biomarkers Used to Characterize Effects Caused by Hexachlorobutadiene

Data are sparse regarding biomarkers of the effects of hexachlorobutadiene in humans. Workers chronically exposed to the compound (along with carbon tetrachloride and perchloroethylene) had increased serum bile acids (Driscoll et al. 1992). Because the workers were also exposed to other chemicals, effects reported cannot be attributed to hexachlorobutadiene alone.

As discussed in Section 2.2, renal damage is the primary toxic effect associated with exposure to hexachlorobutadiene in animals (Harleman and Seinen 1979; Kociba et al. 1971, 1977a; NTP 1991; Schwetz et al. 1977). Because hexachlorobutadiene-induced renal damage is mostly to the proximal convoluted tubules, tests to determine increases in urine glucose creatinine and alkaline phosphatase activity, as well as molecular weight pattern of proteins excreted in the urine, may be useful indicators of kidney damage. Urine volume and specific gravity may be evaluated as part of the overall assessment of kidney damage after exposure to hexachlorobutadiene. Excretion of urinary coproporphyrin was increased in animals at dose levels that did not induce renal tumors. This parameter may be useful in the overall assessment of potential exposure to hexachlorobutadiene. The characteristics renal damage associated with hexachlorobutadiene exposure may also occur with other compounds (e.g., S-C 1,2-dichlorovinyl cysteine and mercuric chloride). Therefore, these parameters are not specific for hexachlorobutadiene exposure. Additional information concerning biomarkers for effects on the immune, renal, and hepatic systems can be found in the CDC/ATSDR Subcommittee Report on Biological Indicators of Organ Damage (CDC/ATSDR 1990), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990).

## 2.6 INTERACTIONS WITH OTHER CHEMICALS

Several studies have been conducted to assess factors which influence the toxicity of hexachlorobutadiene. Most of these studies have involved effects of mixed function oxidase activity (MFO) on renal toxicity. The administration of MFO inhibitors including SKF-525A (Lock and Ishmael 1981) and piperonyl butoxide (Davis 1984; Hook et al. 1982) did not alter

hexachlorobutadiene-induced renal damage. Similar results were reported in tests evaluating MFO inducers such as phenobarbital (Lock and Ishmael 1981), β-naphthoflavone, isosafrole, and Aroclor 1254 (Hook et al. 1982). Renal toxicity was not exacerbated by prior exposure to ketonic solvents (Hewitt and Brown 1984).

There are reports of interactions of hexachlorobutadiene with other chemicals. Combined administration of minimally toxic doses of hexachlorobutadiene with mercuric chloride and potassium dichromate for 24 hours caused synergistic effects as evident by marked increases in urinary (6-24 hour) alkaline phosphatase, lactate dehydrogenase and N-acetyl-β-glucosaminidase activities, as well as more severe tubular necrosis than caused by treatment with hexachlorobutadiene alone (Jonker et al. 1993a). Antagonistic effects were evident as characterized by smaller increases in urinary γ-glutamyl transferase activity compared to treatment with hexachlorobutadiene alone. Combined administration of the same chemicals did not cause additive interactions regarding biochemical parameters or histopathological changes in the kidney (Jonker et al. 1993a). An additional study revealed that when animals are treated for 4 weeks with minimally toxic doses of hexachlorobutadiene in combination with other chemicals (mercuric chloride, δ-limonene, and lysinoalanine), there is an increase in growth retardation and renal toxicity (renal weight, urine concentrating ability, and renal structure) in male rats but not in females (Jonker et al. 1993b).

## 2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to hexachlorobutadiene than will most persons exposed to the same level of hexachlorobutadiene in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting endproduct metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

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Studies in animals revealed that hexachlorobutadiene causes damage to the proximal tubules of the kidney and, to a lesser extent, to the liver. Accordingly, people with preexisting kidney and liver damage may have compromised organ functions and are expected to be more vulnerable to chemical insult than people with normal kidney and liver functions. Infants are more likely to be affected following exposure to hexachlorobutadiene than adults. Studies in animals showed that young rats were more sensitive to the acute lethal effects of hexachlorobutadiene than adults. This greater susceptibility in newborns can be attributed to immature organ systems.

## 2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to hexachlorobutadiene. 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 hexachlorobutadiene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

### 2.8.1 Reducing Peak Absorption Following Exposure

Exposure to hexachlorobutadiene can occur by inhalation of vapors, ingestion, and dermal contact. The compound can cause kidney damage and, to a lesser extent, liver damage after ingestion or if it comes in contact with skin.

Information regarding methods for reducing absorption following exposure to hexachlorobutadiene was obtained primarily from the HSDB. No other sources were available. If inhalation of hexachlorobutadiene has occurred, movement of the patient to fresh air is recommended. No specific treatment is available; however, patients are usually monitored for respiratory distress, respiratory tract irritation, bronchitis and pneumonia. If there has been substantial ingestion of the compound, syrup of ipecac is administered within 30 minutes of ingestion to induce vomiting. Syrup of ipecac is usually not given in cases of severe central nervous system depression or coma because there is risk of pulmonary aspiration. The absorption of hexachlorobutadiene may be reduced following oral exposure by binding the compound in the gastrointestinal tract. Activated charcoal in the form of aqueous suspension or sorbitol slurry may be administered for this purpose. However, if syrup of ipecac is given within 1 hour of ingestion of activated charcoal, it is not effective. Another suggested

treatment following oral ingestion of hexachlorobutadiene may be a cathartic, such as magnesium or sodium sulfate to speed fecal excretion. Lipids are not usually given by mouth because this may increase absorption. In cases where hexachlorobutadiene has been splashed into the eyes, irrigation with copious amounts of water for 15 minutes has been recommended. In order to minimize absorption through the skin, areas of skin that have come in contact with the compound should be washed with soap and water.

### 2.8.2 Reducing Body Burden

No information was located on the retention of hexachlorobutadiene or its metabolites in humans. In animals, the compound and its metabolites were detected in urine and areas extent in breath within 3 days after exposure. Adverse effects were seen within 24 hours, suggesting that the compound and its metabolites are toxic while retained in the body.

There are no specific treatments for reducing the body burden following absorption of hexachlorobutadiene. As discussed in Section 2.3, there is extensive reabsorption and enterohepatic recirculation of biliary metabolites, which are thought to play a major role in the nephrotoxicity of the compound. One approach to reducing body burden may involve the administration of compounds that would decrease reabsorption of biliary metabolites. Activated charcoal may be used for this purpose.

### 2.8.3 Interfering with the Mechanism of Action for Toxic Effects

No information is available on treatment methods that employ substances that interfere with the mechanism of toxicity of hexachlorobutadiene. Studies in animals indicate that hexachlorobutadiene exerts its effects on the proximal tubules of the kidney. The major portion of the toxicity of hexachlorobutadiene results from initial formation of glutathione conjugates of the compound and the subsequent uptake of the glutathione-derived conjugates by renal tissues through an organic transport mechanism. Thus, prevention of transport of conjugate anions may reduce the toxicity associated with exposure to hexachlorobutadiene. Use of a uricosuric agent such as probenecid may be an effective treatment. In animals, probenecid blocked the accumulation of a mercapturic acid derivative of hexachlorobutadiene, in renal tissue, the extent of covalent binding of radioactivity to renal protein, and the nephrotoxicity (Lock and Ishmael 1985).

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## 2.9 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 hexachlorobutadiene 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 hexachlorobutadiene.

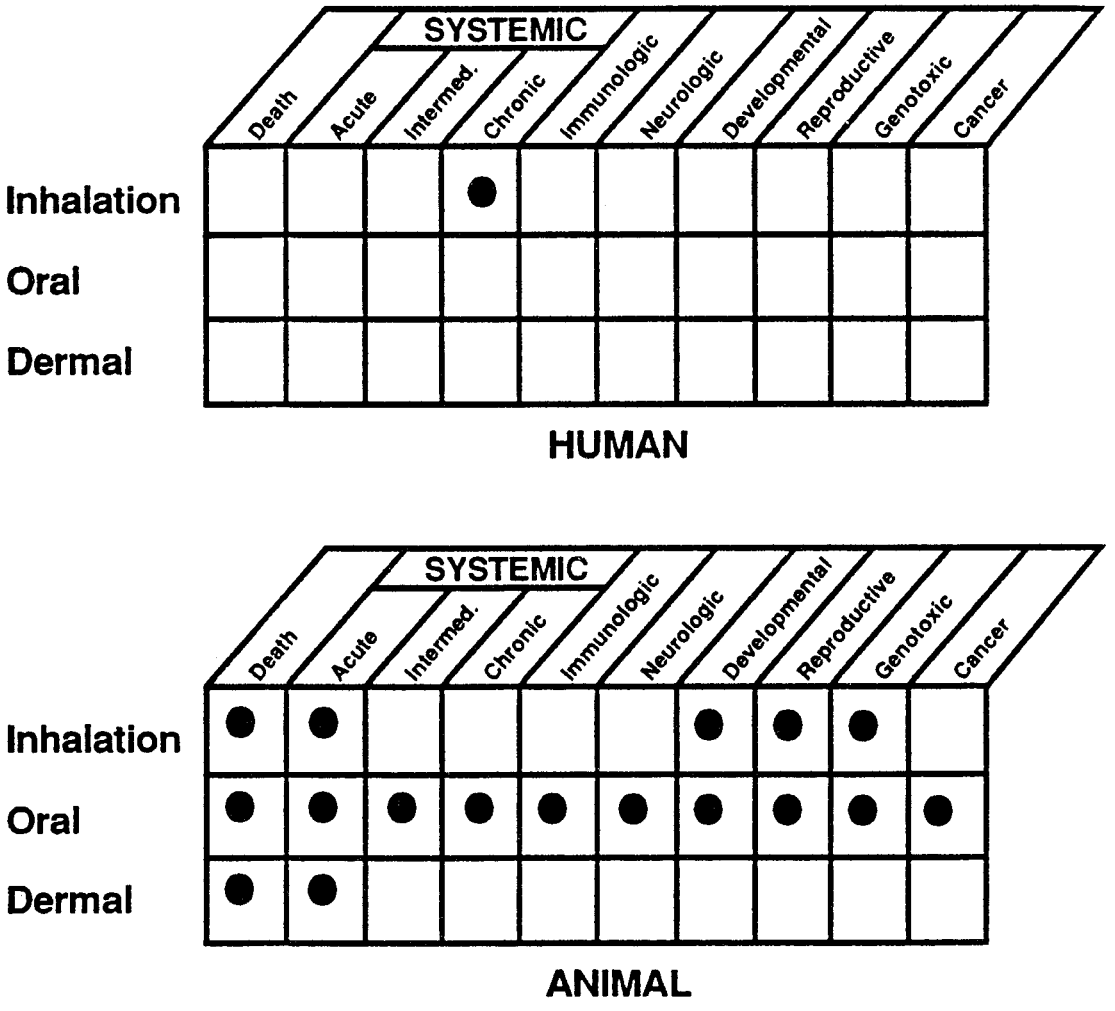
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean 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.

### 2.9.1 Existing Information on Health Effects of Hexachlorobutadiene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to hexachlorobutadiene are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of hexachlorobutadiene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as “data needs. ” A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 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 2-4, information was located regarding health effects of hexachlorobutadiene in humans after inhalation exposure but not after oral or dermal exposure.

**FIGURE 2-4. Existing Information on Health Effects of Hexachlorobutadiene**




● Existing Studies

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In animals, information exists on lethality, acute systemic effects (respiratory), reproductive and developmental effects, and cancer following inhalation exposure, but none was found on other systemic effects after acute, intermediate, or chronic exposure. Much of the information in animals focused on oral exposure. Lethality, systemic effects after acute, intermediate and chronic exposures, immunotoxicity, developmental effects, reproductive effects, genotoxicity, and cancer have been evaluated. Reports on dermal exposure after direct application to the skin involved lethality, acute systemic effects (kidney, liver, dermal/ocular), and cancer.

### 2.9.2 Identification of Data Needs

**Acute-Duration Exposure.** No data are available on the effects of hexachlorobutadiene in humans after acute exposure by inhalation, oral, and dermal routes. Hexachlorobutadiene (50 ppm) was lethal in mice after acute (5 days) inhalation exposure and caused irritation of the nasal cavities following 15 minute exposures to concentrations of 15 ppm or greater (de Ceaurriz et al. 1988; NIOSH 1981). Because sufficient data are not available to determine target organs or determine critical effect levels, an acute inhalation MRL cannot be determined.

In one acute-duration (24-hour) oral study in rats, hexachlorobutadiene caused focal necrosis of the kidneys and increased urinary biochemical parameters at doses of 100 mg/kg (Jonker et al. 1993a). Another acute-duration (14 days) oral exposure study revealed that hexachlorobutadiene caused renal tubular epithelial degeneration in rats at dose levels of 4.6 mg/kg/day or greater but no effects were seen in the liver up to doses of 35 mg/kg/day (Harleman and Seinen 1979). The number of animals in both of these studies were small and, thus, the data were not suitable for derivation of an MRL.

Acute-duration dermal exposure to hexachlorobutadiene (388 mg/kg) caused liver and kidney damage in rabbits. For the most part, these effects were reversible within 2-5 weeks (Duprat and Gradiski 1978). In the same dermal study, some rabbits died within 24 hours after exposure to 775 mg/kg hexachlorobutadiene applied directly to the skin for 8 hours, but no deaths occurred at dose levels of 388 mL/kg (Duprat and Gradiski 1978). However, due to the lack of an appropriate methodology for the development of dermal MRLs, no dermal MRLs were derived. Although the vapor pressure of hexachlorobutadiene limits vapor concentration in the air, short-term inhalation exposures are possible and worthy of investigation. There is potential for oral exposures in populations living near

hazardous waste sites; therefore, additional short-term animal studies by oral routes may be useful to more thoroughly assess the potential human health risk.

**Intermediate-Duration Exposure.** No data are available on the effects of hexachlorobutadiene in humans after intermediate-duration inhalation, oral, or dermal exposures. In animals, data on inhalation exposure are limited to one developmental toxicity study in rats in which maternal body weights were reduced at a concentration (15 ppm) that was also fetotoxic (Saillenfait et al. 1989). Oral studies revealed kidney damage in female mice at dose levels of 0.2 mg/kg/day (NTP 1991; Yang et al. 1989). This LOAEL was used to derive an intermediate-duration oral MRL of 0.0002 mg/kg/day .

Liver damage was evident in male rats at dose levels of 6.3 mg/kg/day but not at dose levels of 2.5 mg/kg/day for 13 weeks (Harleman and Seinen 1979). Treatment-related histopathological hepatic lesions were not seen in females. Some serum biochemical parameters (aspartate aminotransferase and total bilirubin), were increased at doses of 20 mg/kg/day for 4 weeks (Jonker et al. 1993b). No data are available on the effects of hexachlorobutadiene in animals after intermediate-duration dermal exposure. Inhalation exposure to vaporous hexachlorobutadiene can occur when this material is exposed to the environment. Studies of toxicity from material absorbed through the lungs are justified.

**Chronic-Duration Exposure and Cancer.** Data in humans are limited to one study that reported increases in serum bile acids in workers chronically exposed to vapors of hexachlorobutadiene (0.005-0.02 ppm). Because workers were also potentially exposed to other chemicals (carbon tetrachloride and perchloroethylene), these effects cannot be attributed to hexachlorobutadiene exposure alone. No studies are available on the effects of hexachlorobutadiene in humans after oral or dermal exposure.

In animals, a chronic-duration oral rat study showed that the kidney was the target organ following chronic exposure to hexachlorobutadiene. Kidney damage as well as evidence of impaired kidney function were evident in female rats at dose levels of 2 mg/kg/day, but not at 0.2 mg/kg/day (Kociba et al. 1977a). Since the intermediate-duration oral MRL is protective against affects on the kidney following lifetime exposure, a chronic MRL was not derived. Data are not available to derive a

chronic inhalation MRL. This data need can be evaluated after the results of the suggested acute- and intermediate-duration research becomes available.

No epidemiological studies of hexachlorobutadiene are available. The occurrence of renal tumors after chronic oral exposure in rats suggests carcinogenicity may be an area of concern following occupational exposure to hexachlorobutadiene and long-term exposures from waste sites.

**Genotoxicity.** No information is available on the genotoxic effects of hexachlorobutadiene in humans. Following exposure to hexachlorobutadiene, results were negative in *in vivo* rat and *Drosophila* tests that evaluated gene mutation and chromosomal damage (NIOSH 1981). The results were negative in *in vitro* tests evaluating gene mutation (De Meester et al. 1980; Haworth et al. 1983; Reichert et al. 1983; Vamvakas et al. 1988a) and DNA repair in *Salmonella* tests (with and without metabolic activation), and positive in mammalian assay systems using Syrian hamster cells (Schiffmann et al. 1984); the overall results were not consistent. Studies of hexachlorobutadiene metabolites have indicated that some of the cysteine derived metabolites are mutagenic while others are not (Wild et al. 1986). Additional studies of the genotoxicity of intermediary metabolites are needed.

**Reproductive Toxicity.** No data are available on the reproductive toxicity of hexachlorobutadiene in humans. Hexachlorobutadiene did not cause adverse reproductive effects in mice or rats after inhalation or oral exposures, even at dose levels causing kidney and liver damage (Harleman and Seinen 1979; Kociba et al. 1977a; NIOSH 1981; NTP 1991; Saillenfait et al. 1989; Schwetz et al. 1977). No data are available on the reproductive toxicity of hexachlorobutadiene after dermal exposure. Based on existing data in animals, it does not appear that exposure to the compound would pose any significant risk to human reproduction.

**Developmental Toxicity.** No studies were located regarding developmental effects in humans. Inhalation and oral studies in rat pups revealed that hexachlorobutadiene is fetotoxic, but not embryotoxic or teratogenic, at dose levels that are also maternally toxic (Harleman and Seinen 1979; Saillenfait et al. 1989; Schwetz et al. 1977). Additional oral studies in another species would be useful in clarifying the apparent lack of significant effects of hexachlorobutadiene on development. Systemic toxicity studies in rabbits suggest there is potential for dermal absorption to

hexachlorobutadiene (Duprat and Gradiski 1978). However, the toxicokinetics of this compound by this route has not been evaluated.

**Immunotoxicity.** No data are available on the immunotoxicity of hexachlorobutadiene in humans following inhalation, oral, or dermal exposure. Data in animals are limited to intermediate and chronic oral studies which examined histological lesions of lymphoid tissue (spleen or thymus) in mice and rats. These studies did not reveal treatment-related lesions except at lethal doses (Harleman and Seinen 1979; Kociba et al. 1971, 1977a; NTP 1991; Schwetz et al. 1977; Yang et al. 1989). Additional studies to evaluate immune function via the oral route would be useful to determine whether this system is susceptible to hexachlorobutadiene toxicity.

**Neurotoxicity.** No data are available on the neurotoxicity of hexachlorobutadiene in humans after inhalation, oral, or dermal exposure. Histological lesions were not found in the brain in rats (Harleman and Seinen 1979; Kociba et al. 1971; Schwetz et al. 1977) or mice (NTP 1991; Yang et al. 1989). Brain weights were increased after intermediate-duration oral exposure (Kociba et al. 1971). However, such increases were attributed to decreases in body weight gain. Neurological effects were not seen after chronic-duration oral exposure in rats (Kociba et al. 1977a). A comprehensive battery of neurophysiological and neurochemical tests has not been performed and is needed to provide a more thorough assessment of the potential for hexachlorobutadiene to affect the nervous system in humans.

**Epidemiological and Human Dosimetry Studies.** Information is not available on the adverse health effects of hexachlorobutadiene in humans. Data on rats and mice identified the kidney as the target organ following oral exposure (Harleman and Seinen 1979; Kociba et al. 1971, 1977a; NTP 1991; Yang et al. 1989). Other studies involving inhalation or dermal exposures confirm this finding (de Ceaurriz et al. 1988; Duprat and Gradiski 1978). Well-conducted epidemiological studies are needed to determine if similar patterns of damage occur in humans. More importantly, evidence of cancer in animals is sufficient to cause concern for populations which may be exposed to low levels of hexachlorobutadiene for long periods of time.

**Biomarkers of Exposure and Effect.** There is no single biological indicator of exposure to hexachlorobutadiene. Various tests of renal function and biochemical changes associated with renal damage may be measured to detect effects resulting from short-term, intermediate, and long-term

exposure. Because similar effects can also occur following exposure to other substances, these tests are not specific for hexachlorobutadiene exposure. Although hexachlorobutadiene and its metabolites are excreted in urine, the metabolism of the compound has not been characterized in humans. Additional tests addressing the dose-response relationship between hexachlorobutadiene excretion in breath and the excretion of sulfur-containing metabolites in urine would prove valuable.

**Absorption, Distribution, Metabolism, and Excretion.** Data are available on the pharmacokinetics of hexachlorobutadiene in animals by the oral route, but not in humans. There are no data in humans or animals on exposures to hexachlorobutadiene by the inhalation or dermal routes. Because of the key role of the liver in producing the metabolites which are responsible for the nephrotoxicity of this compound, knowledge of the pharmacokinetics of inhalation and dermal exposures would be valuable. Oral studies reported the presence of the enzymes responsible for the glutathione conjugation reaction and the subsequent formation of derivatives in the liver, intestines, and kidney. It is not known at this time how hexachlorobutadiene is distributed and metabolized by inhalation and dermal routes. It is postulated that distribution and metabolism by these routes would be similar to that for the oral route.

**Comparative Toxicokinetics.** There are no data on metabolism of hexachlorobutadiene in humans. On the other hand, toxic metabolites and proposed mechanism of renal toxicity have been evaluated in animals employing both *in vivo* and *in vitro* test systems (Dekant et al. 1990b; Schneumann et al. 1987). It is not known if similar metabolic pathways and metabolites occur in humans.

**Methods for Reducing Toxic Effects.** Sufficient methods and treatments are available for reducing peak absorption of hexachlorobutadiene following oral exposure and for limiting the concentrations in the body tissues if absorption has occurred (HSDB 1993). However, antidotal methods have not been established that would be effective in treating overdoses of hexachlorobutadiene, based on interference with the mechanism of action of the compound. A key factor in the overall toxicity of hexachlorobutadiene is the accumulation of glutathione derived conjugates in renal tissue due to hexachlorobutadiene-induced impairment of organic ion transport and secretion. Further studies to identify ways to prevent or reduce accumulation in the target tissue are warranted.

### 2.9.3 On-going Studies

A study is being conducted by R.G. Schnellmann (University of Georgia) for the National Institute of Environmental Health Sciences to evaluate the mechanism of nephrotoxicity of halocarbons, including hexachlorobutadiene. The mechanism of how metabolites alter proximal tubular cellular physiology to produce toxicity is being investigated, with particular emphasis on the effects of metabolites on mitochondria (CRISP 1993).

# 3. CHEMICAL AND PHYSICAL INFORMATION

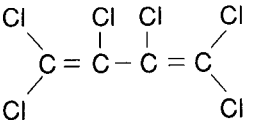
## 3.1 CHEMICAL IDENTITY

Information regarding the chemical identity of hexachlorobutadiene is located in Table 3-l.

## 3.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of hexachlorobutadiene is located in Table 3-2.

## TABLE 3-1. Chemical Identity of Hexachlorobutadiene

| Characteristic | Information | Reference |
|---|---|---|
| Chemical name | Hexachlorobutadiene | Montgomery and Welkom 1990 |
| Synonym(s) | Perchlorobutadiene; HCBD; 1,1,2,3,4,4-Hexachloro-1,3-butadiene; 1,3-Hexachlorobutadiene; Dolen-Pur; GP-40-66:120 | Montgomery and Welkom 1990<br>HSDB 1993 |
| Registered trade name(s) | No data | |
| Chemical formula | $C_4Cl_6$ | Montgomery and Welkom 1990 |
| Chemical structure |  | Montgomery and Welkom 1990 |
| Identification numbers: | | |
| CAS registry | 87-68-3 | Montgomery and Welkom 1990 |
| NIOSH RTECS | EJ0700000 | HSDB 1993 |
| EPA hazardous waste | U128 | HSDB 1993 |
| OHM/TADS | OHM 8100011 | HSDB 1993 |
| DOT/UN/NA/IMCO shipping | UN 2279 | HSDB 1993 |
| | IMCO 6.1 | HSDB 1993 |
| HSDB | 2870 | HSDB 1993 |
| NCI | No data | |

CAS = Chemical Abstracts Services; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances 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 3-2. Physical and Chemical Properties of Hexachlorobutadiene

| Property | Information | Reference |
|---|---|---|
| Molecular weight | 260.76 | Montgomery and Welkom 1990 |
| Color | Colorless | HSDB 1993 |
| Physical state | Liquid | Montgomery and Welkom 1990 |
| Melting point | -21°C | Montgomery and Welkom 1990 |
| Boiling point | 215°C | Montgomery and Welkom 1990 |
| Density: | | |
| at 20°C | 1.55 g/cm$^3$ | HSDB 1993 |
| Odor | Mild to pungent | Montgomery and Welkom 1990 |
| Odor threshold: | | |
| Water | No data | |
| Air | 12.0 mg/m$^3$ | Ruth 1986 |
| Solubility: | | |
| Water at 20°C | 2–2.55 mg/L | Montgomery and Welkom 1990 |
| Organic solvent(s) | Soluble in ethanol and ether | Montgomery and Welkom 1990 |
| Partition coefficients: | | |
| Log $K_{ow}$ | 4.78 | Montgomery and Welkom 1990 |
| Log $K_{oc}$ | 3.67 | Montgomery and Welkom 1990 |
| Vapor pressure at 25°C | 0.15 mmHg | Montgomery and Welkom 1990 |
| Henry's law constant: | 0.001–0.026 atm-m$^3$/mol | Montgomery and Welkom 1990 |
| Autoignition temperature | 610°C | Sax and Lewis 1989 |
| Flashpoint | Noncombustible | Montgomery and Welkom 1990 |
| Flammability limits | Noncombustible | Montgomery and Welkom 1990 |
| Conversion factors | 1 ppm = 10.5 mg/m$^3$<br>1 mg/m$^3$ = 0.095 ppm | ACGIH 1991 |
| Explosive limits | No data | |

# 4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

## 4.1 PRODUCTION

Hexachlorobutadiene was first prepared in 1877 by the chlorination of hexyl oxide (IARC 1979). Commercial quantities of hexachlorobutadiene have never been produced in the United States. The primary source of hexachlorobutadiene found in the United States is inadvertent production as a waste by-product of the manufacture of certain chlorinated hydrocarbons, such as tetrachloroethylene, trichloroethylene, and carbon tetrachloride (EPA 1980; Yang 1988). In 1982, EPA reported an annual volume of about 28 million pounds of hexachlorobutadiene inadvertently produced as a waste by-product from this source (EPA 1982b; HSDB 1993). Table 4-l summarizes information on U.S. companies that reported the production, import, or use of hexachlorobutadiene in 1990 based on the Toxics Release Inventory TRI90 (1992). The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive List.

## 4.2 IMPORT/EXPORT

Since 1974, most hexachlorobutadiene used commercially in the United States has been imported from Germany. Imported quantities remained fairly constant in the late 1970s, averaging about 500,000 pounds annually, but dropped to 145,000 pounds in 1981 (EPA 1980; 1982d). More recent information on the volume of imported hexachlorobutadiene is not available (NTP 1991).

## 4.3 USE

Hexachlorobutadiene is used as a chemical intermediate in the manufacture of rubber compounds (EPA 1982d). Lesser quantities of hexachlorobutadiene are used as a solvent, a fluid for gyroscopes, a heat transfer liquid, hydraulic fluid, and as a chemical intermediate in the production of chlorofluorocarbons and lubricants (EPA 1980; IARC 1979; Verschueren 1983). Small quantities are also used as a laboratory reagent (EPA 1982d). In the international market, Russia is reported to be one of the major users of hexachlorobutadiene, where it is used as a fumigant on grape crops.

**TABLE 4-1. Facilities that Manufacture or Process Hexachlorobutadiene[a]**

| Facility | Location[b] | Range of maximum amounts on site in pounds | Activities and uses |
|---|---|---|---|
| DOW CHEMICAL CO. | PITTSBURG, CA | 1,000-9,999 | As a byproduct |
| VULCAN CHEMICALS | WICHITA, KS | 1,000-9,999 | Produce; as a byproduct |
| VULCAN MATERIALS CO. CHEMICALS DIV. GEISMAR FACILITY | GEISMAR, LA | 10,000-99,999 | Produce; for on-site use/processing as a byproduct; as a reactant |
| DOW CHEMICAL CO. LOUISIANA DIV. | PLAQUEMINE, LA | 1,000-9,999 | Produce; as an impurity |
| PPG INDUSTRIES INC. | WESTLAKE, LA | 1,000,000-9,999,999 | Produce; as a byproduct |
| MALLINCKRODT SPECIALTY CHEMICALS CO. | SAINT LOUIS, MO | 100,000-999,999 | As a reactant |
| OCCIDENTAL CHEMICAL CORP. NIAGARA PLANT | NIAGARA FALLS, NY | 1,000-9,999 | As an impurity |
| DOW CHEMICAL CO. TEXAS OPERATIONS | FREEPORT, TX | 0-99 | As a byproduct; as an impurity; in ancillary or other uses |

[a]Derived from TRI90 (1992)
[b]Post Office state abbreviations

Hexachlorobutadiene is also used as a fumigant in France, Italy, Greece, Spain and Argentina (IARC 1979; NTP 1991). Prior to 1975, the largest domestic use of hexachlorobutadiene was for the recovery of "snift" (chlorine-containing) gas in chlorine plants (HSDB 1993). More recent information from U.S. chlorine producers indicates that hexachlorobutadiene is no longer used for this process (EPA 1982d; IARC 1979).

**4.4 DISPOSAL**

Waste streams resulting from the inadvertent production of hexachlorobutadiene as a byproduct of certain chlorinated hydrocarbons typically contain 33-80% hexachlorobutadiene. These wastes are disposed of by various methods. Over the last decade, disposal practices have shifted from landfilling to incineration. Incineration, which is considered the preferred method of disposal, reportedly achieves greater than 99.9% destruction efficiency (EPA 1982d). In 1982, approximately 68% of an estimated 27 million pounds of hexachlorobutadiene wastes were disposed of by incineration, 32% by deep well injection, and less than 0.2% by hazardous waste landfill operations (EPA 1982d).

The generation, treatment, storage and disposal of hexachlorobutadiene-containing wastes are subject to regulation under RCRA (see Chapter 7). Underground injection of hexachlorobutadiene is subject to permits issued under an Underground Injection Control program promulgated under the Safe Drinking Water Act (EPA 1982d).

According to TRI90 (1992), 84,345 pounds of hexachlorobutadiene were transferred to landfills and/or other treatment/disposal facilities and 958 pounds were sent to publicly-owned treatment works in 1990.

# 5. POTENTIAL FOR HUMAN EXPOSURE

## 5.1 OVERVIEW

There are no known natural sources of hexachlorobutadiene which contribute to environmental levels. The main source of hexachlorobutadiene in the United States is its production as a by-product of chlorinated hydrocarbon synthesis. An estimated 100,000 pounds of this by-product are released to the environment each year. The majority of hexachlorobutadiene-containing waste is disposed of by incineration, with lesser amounts disposed by deep well injection and landfill.

Literature data regarding the fate and transport of hexachlorobutadiene are limited. Much of the available information consists of modeling based on the physical and chemical properties of hexachlorobutadiene, and the monitoring data. These data indicate that hexachlorobutadiene will bind to soil particles and sediments, and is found in air and water bound to particulates. Some volatilization of hexachlorobutadiene from surface waters and soils may also occur. The bioconcentration of hexachlorobutadiene has been reported in fish and shellfish with considerable variability between species (EPA 1976; Oliver and Niimi 1983; Pearson and McConnell 1975).

Data regarding the transformation and degradation of hexachlorobutadiene are limited. Much of the available information consists of modeling based on the monitoring data and by analogy to structurally similar compounds. Hexachlorobutadiene may react with reactive oxygen species in air for which the half-life has been estimated to range from months to years. Under aerobic conditions, but not anaerobic conditions, hexachlorobutadiene undergoes complete biodegradation in water. The observations in water are believed to hold true for soils as well.

Low levels of hexachlorobutadiene can be detected in air, water, and sediment. Atmospheric levels of hexachlorobutadiene in rural and urban air samples typically range from 2 to 11 ppt, with a mean value of 2-3 ppt. Higher levels can be detected at areas near industrial and chemical waste disposal sites and production sites. Hexachlorobutadiene is infrequently detected in ambient waters, but has been detected in drinking water at levels of 2-3 ppt. Sediments contain higher levels of hexachlorobutadiene than the waters from which they were obtained. Foodstuffs generally do not

contain detectable levels of hexachlorobutadiene, with the exception of fish, in which concentrations of 0.1-4.7 mg/kg have been reported.

Hexachlorobutadiene has been detected in human adipose tissue and blood samples. These data indicate that exposure to hexachlorobutadiene does occur in humans, however route-specific estimates of hexachlorobutadiene exposure were not located. Based on monitoring data, individuals who work in hexachlorobutadiene-producing facilities, live at or near hazardous waste facilities, or consume large amounts of hexachlorobutadiene-contaminated fish may have above-average exposures to hexachlorobutadiene.

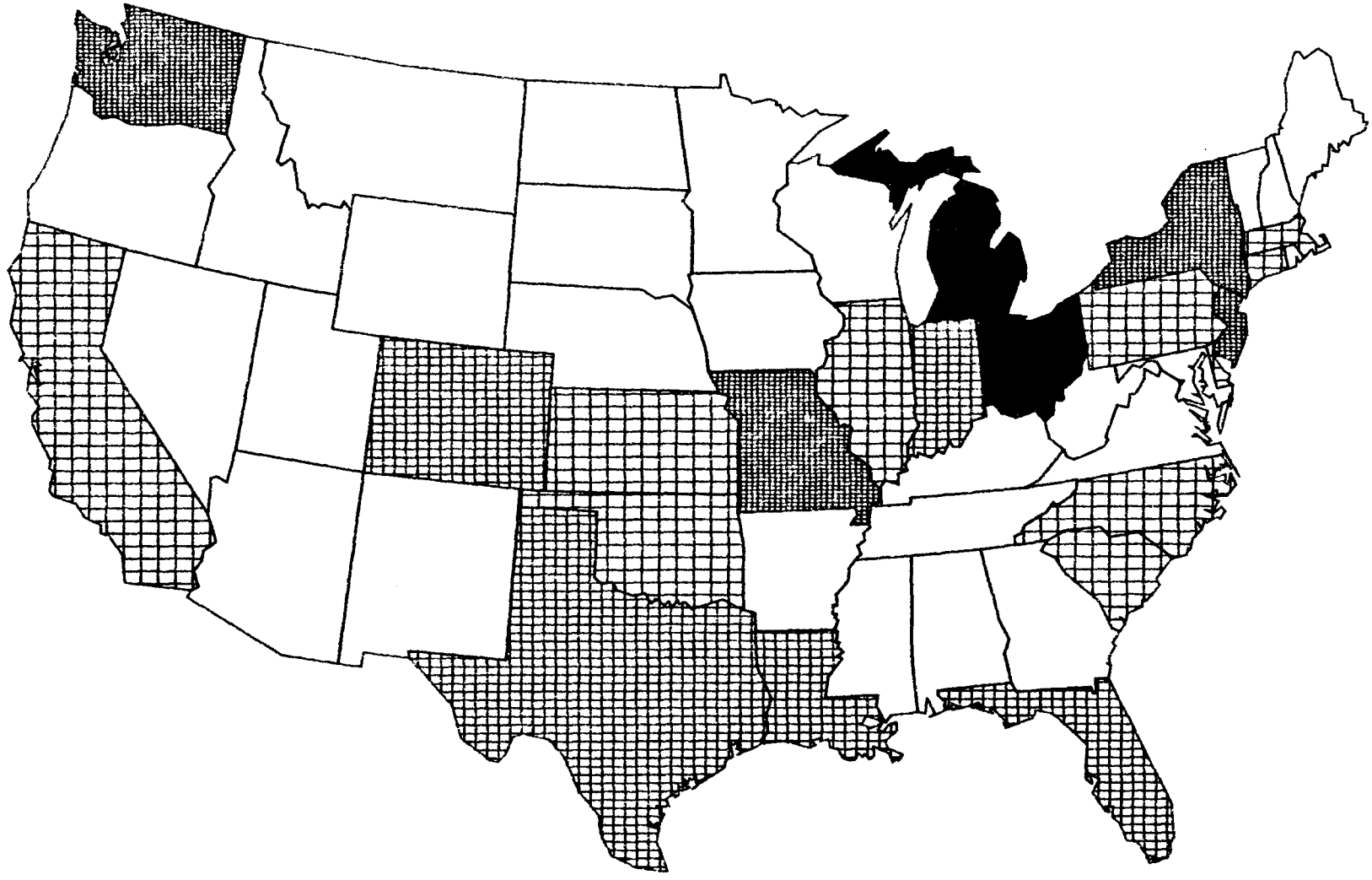
Hexachlorobutadiene has been identified in at least 45 of the 1,350 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 1993). However, the number of sites evaluated for hexachlorobutadiene is not known. The frequency of these sites within the United States can be seen in Figure 5-l.

## 5.2 RELEASES TO THE ENVIRONMENT

### 5.2.1 Air

There are no known natural sources of hexachlorobutadiene which contribute to environmental levels. The predominant source of hexachlorobutadiene is inadvertent production from the synthesis of certain chlorinated hydrocarbons (EPA 1982b). In 1975, the production of hexachlorobutadiene in the United States was estimated to be 8 million pounds, with 0.1 million pounds released to the environment (NSF 1975). Sixty-eight percent of the 27 million pounds of hexachlorobutadiene waste generated in the United States in 1982 was disposed of by incineration. This process typically obtains a 99.99% destruction efficiency, indicating that approximately 1,900 pounds were released to the atmosphere. According to TRI90 (1992), an estimated total of 4,906 pounds (2.2 metric tons) of hexachlorobutadiene, amounting to 82% of the total environmental release, was discharged to the air from manufacturing and processing facilities in the United States in 1990 (see Table 5-l). The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

FIGURE 5-1. FREQUENCY OF NPL SITES WITH HEXACHLOROBUTADIENE CONTAMINATION *




*Derived from HazDat 1993

**TABLE 5-1. Releases to the Environment from Facilities that Manufacture or Process Hexachlorobutadiene[a]**

| Facility | Location[b] | Reported amounts released in pounds | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Air | Underground injection | Water | Land | Total environment[c] | POTW[d] transfer | Off-site waste transfer |
| DOW CHEMICAL CO. | PITTSBURG, CA | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| VULCAN CHEMICALS | WICHITA, KS | 49 | 330 | 0 | 0 | 379 | 0 | 8 |
| VULCAN MATERIALS CO. CHEMICALSDIV. GEISMAR FACILITY | GEISMAR, LA | 842 | 0 | 10 | 0 | 852 | 0 | 295 |
| DOW CHEMICAL CO. LOUISIANA DIV. | PLAQUEMINE, LA | 51 | 0 | 0 | 0 | 51 | 0 | 0 |
| PPG INDUSTRIES INC. | WESTLAKE, LA | 3,345 | 0 | 705 | 0 | 4,050 | 0 | 73,022 |
| MALLINCKRODT SPECIALTY CHEMICALS CO. | SAINT LOUIS, MO | 550 | 0 | 0 | 0 | 550 | 940 | 7,000 |
| OCCIDENTAL CHEMICAL CORP. NIAGARA PLANT | NIAGARA FALLS, NY | 25 | 0 | 0 | 0 | 25 | 18 | 20 |
| DOW CHEMICAL CO. TEXAS OPERATIONS | FREEPORT, TX | 44 | 0 | 0 | 0 | 44 | 0 | 4,000 |

[a]*Derived from TRI90 (1992)*
[b]Post Office state abbreviations
[c]The sum of all releases of the chemical to air, land, water, and underground injection wells by a given facility.
[d]POTW = publicly owned treatment works

5. POTENTIAL FOR HUMAN EXPOSURE

### 5.2.2 Water

Hexachlorobutadiene may be released to underground and surface waters through discharge from industrial facilities, by leaching from industrial discharges, by leaching from landfills or soils, or by urban runoff. Hexachlorobutadiene was detectable in 1.6% of 1,190 industrial effluent samples reported in the EPA Storage and Retrieval (STORET) database (Staples et al. 1985). The median concentration for all samples, including nondetects was < 6 ppb. This chemical was also detected in leachate from an industrial landfill at a concentration of 0.109 ppm (Brown and Donnelly 1988) and from a hazardous waste site (Hauser and Bromberg 1982). In 1982, of the 27 million pounds of hexachlorobutadiene waste produced in the United States as a by-product of chlorinated hydrocarbon production, 9 million pounds was disposed of by deep well injection (EPA 1982b). According to TR190 (1992), an estimated total of 715 pounds (0.3 metric tons) of hexachlorobutadiene, amounting to 12% of the total environmental release, was discharged to the water from manufacturing and processing facilities in the United States in 1990 and 330 pounds (about 6%) was disposed of by underground injection (see Table 5-l).

### 5.2.3 Soil

Hexachlorobutadiene may be released to soil by disposal of wastes in landfill operations. In 1982, only 0.2% of the 27 million pounds of hexachlorobutadiene waste produced as a by-product of chlorinated hydrocarbon-synthesis was disposed of in landfill operations (EPA 1982b). These data indicate that the release to soil was approximately 54,000 pounds. According to TR190 (1992), no hexachlorobutadiene was discharged to the soil from manufacturing and processing facilities in the United States in 1990 (see Table 5-l). The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

## 5.3 ENVIRONMENTAL FATE

### 5.3.1 Transport and Partitioning

Hexachlorobutadiene can exist in the atmosphere as a vapor or adsorbed to airborne particulate matter. The atmospheric burden of hexachlorobutadiene has been estimated to be 3.2 and 1.3 million kg/year for the northern and southern hemispheres, respectively (Class and Ballschmiter 1987).

# 5. POTENTIAL FOR HUMAN EXPOSURE

Significant dispersion of hexachlorobutadiene has been confirmed by the detection of hexachlorobutadiene at areas which are far removed from release sources (Class and Ballschmiter 1987). A high partition coefficient (log $K_{oc}$) value of 3.67 (Montgomery and Welkom 1990) for hexachlorobutadiene indicates that adsorption to soils with high organic carbon content can occur. Wind erosion of contaminated surface soils can then lead to airborne hexachlorobutadiene-containing particulate matter. Levels of hexachlorobutadiene have been detected in fly ash from the incineration of hexachlorobutadiene-containing hazardous waste (Junk and Ford 1980). The transport of particulate matter is a function of particle size and wind speed, however no data were located regarding the transport of hexachlorobutadiene-containing particles in air.

Transport and partitioning of hexachlorobutadiene in water involves volatilization to the atmosphere and sorption to soil and sediments particulates. The high partition coefficient (log $K_{ow}$) of 4.78 (Montgomery and Welkom 1990) for hexachlorobutadiene leads to preferential partitioning to sediments and biota over water. Environmental surveys generally report higher levels of hexachlorobutadiene in sediments than in the waters that contain them (Elder et al. 1981; EPA 1976; Oliver and Charlton 1984). Hexachlorobutadiene has a vapor pressure of 0.15 mmHg (25°C) (Montgomery and Welkom 1990), indicating that volatilization from water occurs. Volatilization is reduced by adsorption to organic material in the water.

The transport and partitioning of hexachlorobutadiene in soils involve volatilization and adsorption. An estimated high partition coefficient (log $K_{oc}$) of 3.67 (Montgomery and Welkom 1990) for hexachlorobutadiene in soil indicates that soil adsorption can occur, particularly in soils with a high organic carbon content. Sorption was the predominant fate process for hexachlorobutadiene during anaerobic digestion of sludges (Govind et al. 1991). Data indicate that hexachlorobutadiene is mobile in sandy soils which have relatively low organic-carbon contents (Piet and Zoeteman 1980). Volatilization from surface soils is relatively low; binding to the organic carbon content of the soil further reduces hexachlorobutadiene release.

In rainbow trout the bioconcentration factor (BCF) was dependent on water concentration (Oliver and Niimi 1983). At low concentrations of 0.10 ng/L a BCF of 5,800 was obtained, compared to a value of 17,000 obtained with higher water concentrations of 3.4 ng/L. Hexachlorobutadiene preferentially accumulates in the liver of fish (Pearson and McConnell 1975). In mussels, the BCF was determined to be between 900 and 2,000 (Pearson and McConnell 1975). However, lower values were obtained

for algae, crayfish, and bass (160, 60, and 29, respectively) (EPA 1976). The EPA is reviewing new BCF data and has recommended a value of 392 (EPA 1989a).

### 5.3.2 Transformation and Degradation

#### 5.3.2.1 Air

No data were located regarding the transformation and degradation of hexachlorobutadiene in air. Based on the monitoring data, the tropospheric half-life of hexachlorobutadiene was estimated by one author to be 1.6 years in the northern hemisphere (Class and Ballschmiter 1987). However, analogy to structurally similar compounds such as tetrachloroethylene indicates that the half-life of hexachlorobutadiene may be as short as 60 days, predominantly due to reactions with photochemically produced hydroxyl radicals and ozone (Atkinson 1987; Atkinson and Carter 1984). Oxidation constants of $< 10^3$ and $6(m^{\bullet} hr)^{-1}$ were estimated for reactions with singlet oxygen and peroxy radicals, respectively (Mabey et al. 1982).

#### 5.3.2.2 Water

Data concerning the transformation and degradation of hexachlorobutadiene in waters are limited. Under aerobic conditions, hexachlorobutadiene underwent complete biodegradation after 7 days in water inoculated with domestic sewage (Tabak et al. 1981). Biodegradation of hexachlorobutadiene also occurred during anaerobic digestion of wastewater sludges, although sorption was the predominant fate process (Govind et al. 1991). However, biodegradation did not occur in anaerobic waters (Johnson and Young 1983). Based on monitoring data, the half-life of hexachlorobutadiene in rivers and lakes was estimated to be 3-30 days and 30-300 days, respectively (Zoeteman et at. 1980). Data regarding the hydrolysis or photolysis of hexachlorobutadiene in water were not located.

#### 5.3.2.3 Sediment and Soil

Data regarding the transformation and degradation of hexachlorobutadiene in soil were not located. However, based on the observation that hexachlorobutadiene was completely biodegraded in water under aerobic conditions (Tabak et al. 1981), biodegradation probably occurs in nonarid soils as well.

5. POTENTIAL FOR HUMAN EXPOSURE

## 5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

### 5.4.1 Air

In the United States, the reported average concentration of hexachlorobutadiene, based on 72 samples from urban and source dominated areas, was 36 ppt (0.38 μg/$m^3$) (Shah and Heyerdahl 1988; Shah and Singh 1988). Hexachlorobutadiene levels ranging from 2 to 11 ppt were reported in a number of cities (Pellizzari 1978; Singh et al. 1980; Singh et al. 1982). Higher levels of hexachlorobutadiene were reported in Niagara Falls, with concentrations of up to 37 ppt detected in ambient air levels and up to 38 ppt detected in the basement air of homes near industrial and chemical waste disposal sites (Pellizzari 1982).

Occupational exposures can be significantly higher for individuals who work at plants that produce chlorinated hydrocarbons. Maximum air levels off plant property, at a plant boundary, and within a plant were reported to be 22 ppt, 938 ppt, and 43,000 ppt, respectively (Li et al. 1976).

### 5.4.2 Water

Hexachlorobutadiene has been detected in some surface waters but the incidence of detection is low. It was detected in 0.2% of 593 ambient water samples in the STORET database with a median level for all samples of less than 10 ppb (Staples et al. 1985). Hexachlorobutadiene was detected in 1 of 204 surface water sites sampled across the United States with a concentration of 22 ppb (Ewing et al. 1977). Low levels of hexachlorobutadiene were detected in the Niagara River at 0.82 ppt (Oliver and Charlton 1984). Hexachlorobutadiene was not detected in rainwater (Pankow et al. 1984) or urban storm water runoff (Cole et al. 1984) in a number of U.S. cities. It has not been detected in open ocean waters; however, the coastal waters of the Gulf of Mexico were reported to contain 3-15 ppt (Sauer 1981).

Low levels of hexachlorobutadiene (less than 1 ppb) may be found in drinking water (EPA 1989a). Finished drinking water samples from two U.S. cities were found to contain 1.6 ppt and 2.7 ppt, respectively (Lucas 1984). Hexachlorobutadiene was also detected in groundwater at 6 of 479 waste disposal sites in the United States (Plumb 1991).

### 5.4.3 Sediment and Soil

Hexachlorobutadiene adsorbs to sediments in contaminated water. Sediments from the Niagara River were found to contain 2.9-l 1 µg/kg (Oliver and Charlton 1984). Sediments from the Great Lakes were reported to contains levels of hexachlorobutadiene typically ranging from 0.08 to 120 µg/kg (Fox et al. 1983; Oliver and Bourbonniere 1985; Oliver and Charlton 1984). Data regarding the levels of hexachlorobutadiene in soils were not located. Hexachlorobutadiene was not detectable in any of 196 sediment samples reported on the STORET database (Staples et al. 1985). The median detection limit was < 500 ppb.

### 5.4.4 Other Environmental Media

Hexachlorobutadiene was detected in several foodstuffs in the United Kingdom (McConnell et al. 1975) and Germany (Kotzias et al. 1975), but it was not detected in the United States in milk, eggs, or vegetables even when the samples were obtained from within a 25-mile radius of facilities producing chlorinated hydrocarbons (Yip 1976; Yurawecz et al. 1976). Fish samples from the Mississippi River were found to contain hexachlorobutadiene levels ranging from 0.1 to 4.7 mg/kg (Laska et al. 1976; Yip 1976; Yurawecz et al. 1976). Fish from the Great Lakes generally did not contain detectable levels of hexachlorobutadiene (Camanzo et al. 1987; DeVault 1985) with the exception of trouts from Lake Ontario, which were reported to contain 0.06-0.3 mg/kg (Oliver and Niimi 1983). Hexachlorobutadiene was not detectable in any of 51 biota samples reported on the STORET database (Staples et al. 1985).

Hexachlorobutadiene was not detected in sewage influents (Levins et al. 1979) or in sewage samples (EPA 1990g).

## 5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The general population can be exposed to low levels of hexachlorobutadiene in air, food, and water. Estimates of source or route-specific exposures to humans were not located. Hexachlorobutadiene has been detected in human adipose tissue with a concentration ranging from 0.8 to 8 µg/kg wet weight (McConnell et al. 1975; Mes et al. 1982). Higher concentrations were reported in human liver samples with values ranging from 5.7 to 13.7 µg/kg wet weight (McConnell et al. 1975). These data

indicate that exposure to hexachlorobutadiene occurs in humans, but do not identify sources or routes of exposure. Although exposure from foods is probably a minor route of exposure, people who consume large amounts of fish obtained from contaminated waters may be exposed to significant quantities of hexachlorobutadiene. Similarly, persons who live in source-dominated areas or work in plants that produce chlorinated hydrocarbons may be exposed to significant levels of hexachlorobutadiene in the air. No information was found on the number of workers potentially exposed to hexachlorobutadiene.

## 5.6 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

People who live in source-dominated areas (at or near hazardous waste sites or chlorinated hydrocarbon production plants) and workers in these areas are potentially exposed to high levels of hexachlorobutadiene. Individuals who consume large amounts of fish from contaminated waters may also be exposed to above-average levels of hexachlorobutadiene.

## 5.7 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 hexachlorobutadiene is available. Where adequate information is not available, ATSDR, in conjunction with the 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 hexachlorobutadiene.

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.

5. POTENTIAL FOR HUMAN EXPOSURE

### 5.7.1 Identification of Data Needs

**Physical and Chemical Properties.** The physical and chemical properties of hexachlorobutadiene are sufficient to make estimations on its fate in the environment. No data regarding the odor threshold of hexachlorobutadiene in water were located.

**Production, Import/Export, Use, Release, and Disposal.** Hexachlorobutadiene is not produced for commercial purposes in the United States, however small amounts are imported from Germany. Hexachlorobutadiene is mainly produced as a by-product of chlorinated hydrocarbon synthesis and is a primary component of "hex-wastes" (EPA 1982b). Its uses as a pesticide and fumigant have been discontinued. Hexachlorobutadiene is disposed chiefly by incineration, and to a lesser extent by deep well injection and landfill operations (EPA 1982b). More recent production and release data would be helpful in estimating human exposure to hexachlorobutadiene.

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to the EPA. The Toxics Release Inventory (TRI), which contains this information for 1990, became available in May of 1992. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

**Environmental Fate.** Much of the environmental fate information on hexachlorobutadiene consists of modeling based on its physical and chemical properties and its similarity to related compounds. Further studies which determine the extent to which hexachlorobutadiene volatilizes from surface waters and soils, and the effects of organic-carbon content on this process would be helpful. Studies which experimentally determine the specific reactions and rates which drive the degradation of hexachlorobutadiene in air, water, and soil would be valuable. Data are lacking on hexachlorobutadiene adsorption to soil or its biodegradation in this medium. More information on the fate of the compound in soil would be useful since this medium may be a pathway of exposure for populations living near emission sources.

**Bioavailability from Environmental Media.** Toxicity studies in animals indicate that absorption of hexachlorobutadiene through the gastrointestinal tract, respiratory tract, and skin can occur. Studies

which identify the relationship between absorption and the matrix of soils, sediments, and foods would be useful in establishing whether or not absorption is significantly affected by such factors.

**Food Chain Bioaccumulation.** Bioconcentration factors have been determined for algae, shellfish, and fish and exhibit a wide range (29-17,000) (EPA 1976; Oliver and Niimi 1983; Pearson and McConnell 1975). This wide range may be explained in part by species differences in metabolism or differences in concentrations tested. Studies also indicate that hexachlorobutadiene preferentially accumulates in the livers of fish. Further studies which might explain the wide range of BCF values would be helpful. No information was located regarding the bioaccumulation of hexachlorobutadiene in plants or aquatic organisms. More information is needed to determine the importance of terrestrial/aquatic food chain bioaccumulation as a potential human exposure pathway.

**Exposure Levels in Environmental Media.** Data are available on the occurrence of hexachlorobutadiene in air, water, and foodstuff. The majority of the monitoring data on hexachlorobutadiene are outdated and therefore more recent information on the levels typically found in the environment would allow for more accurate estimation of human exposures, and could also serve to indicate time-dependent trends when compared with older data. No data were located regarding the occurrence of hexachlorobutadiene in groundwater or soil.

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

**Exposure Levels in Humans.** Hexachlorobutadiene has been detected in human adipose tissues and blood (Bristol et al. 1982; Mes et al. 1985). Studies which establish a correlation between exposure levels in environmental media and the resulting levels in human tissues and excreta would be valuable in predicting exposures and corresponding health risks in humans who live at or near hazardous waste sites and who are likely to be exposed to hexachlorobutadiene.

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

**Exposure Registries.** No exposure registries for hexachlorobutadiene were located. This substance is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for subregistries 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.

### 5.7.2 On-going Studies

No on-going studies were located regarding the environmental fate or potential for human exposure to hexachlorobutadiene.

# 6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring hexachlorobutadiene, its metabolites, and other biomarkers of exposure and effect to hexachlorobutadiene. 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 are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

## 6.1 BIOLOGICAL MATERIALS

Gas chromatography (GC) with an electron-capture detector (ECD) and/or GC with detection by mass spectrometry (MS) have been used to measure hexachlorobutadiene concentrations in human blood and adipose tissue (Bristol et al. 1982; LeBel and Williams 1986; Mes et al. 1985) and in rat liver tissue (Wang et al. 1991). In gas chromatography, samples dissolved in a volatile solvent are injected into a heated column with a stationary phase consisting of silica coated with a liquid phase. An inert gas carries the sample through the column, and the partitioning of hexachlorobutadiene between the mobile and stationary phases gives it a characteristic retention time which is used to identify it. Electron-capture detectors use a radioactive source such as $^{63}$Ni to generate electrons that are captured by the chlorine atoms in hexachlorobutadiene. Reduction in electron flow by this capture produces a characteristic signal for hexachlorobutadiene. Identity of hexachlorobutadiene is confirmed by detection by mass spectroscopy, which provides specific identification by a characteristic ion fragmentation pattern.

Biological samples are prepared for analysis by extraction with organic solvents. This extract from blood may be used directly (Bristol et al. 1982; Kastl and Hermann 1983), but extracts from adipose or liver tissue are cleaned up by gel permeation chromatography (GPC), which separates

hexachlorobutadiene from higher molecular weight lipids, and/or by passage through a Florisil column which retains lipids and other contaminants (LeBel and Williams 1986; Mes et al. 1985). These methods provide 42- 122 % recovery and can detect < 1 μg/L hexachlorobutadiene in blood and 1 μg/kg hexachlorobutadiene in fat (Bristol et al. 1982; LeBel and Williams 1986; Mes et al. 1985; Wang et al. 1991). No information was located on methods for detection of hexachlorobutadiene metabolites or other biomarkers of hexachlorobutadiene exposure or effect.

Table 6-l summarizes the methods used for sample preparation and analysis of hexachlorobutadiene in biological samples.

## 6.2 ENVIRONMENTAL SAMPLES

Hexachlorobutadiene in environmental samples is also measured using GC coupled with ECD, MS, a halogen electrolytic conductivity detector (HECD), or a photoionization detector (PID) (APHA 1992a, 1992b; EPA 1982a, 1982c, 1986, 1989c, 1989d, 1990b, 1990d, 1990e). Several methods have been used for extraction of hexachlorobutadiene from environmental samples. Standard methods for analysis of air involve pumping the air through a material that will adsorb hexachlorobutadiene or through a cold trap to condense the hexachlorobutadiene (EPA 1990b; NIOSH 1990). Purge-and-trap methods are used to extract hexachlorobutadiene from water, soil, or solid waste (APHA 1992b; EPA 1989c, 1989d, 1989e, 1990e). Purge-and-trap methods involve bubbling an inert gas through the sample, trapping the hexachlorobutadiene in a tube containing a sorbent material, and then heating the sorbent tube and flushing the hexachlorobutadiene into a GC. Soil, sediment, and waste samples are mixed with water prior to purging (EPA 1990e). An alternative way to prepare water, soil, or solid waste samples for GC analysis is to extract with methylene chloride or some other organic solvent; for waste water, soil, and solid waste samples, the organic extracts are cleaned up by gel permeation chromatography (GPC) or Florisil adsorption chromatography (FAC) (APHA 1992a; EPA 1982a, 1982c, 1986). Purge-and-trap methods generally provide > 90% recovery, while organic extraction may have lower and more variable recovery rates (APHA 1992a, 1992b; EPA 1982a, 1982c, 1989c, 1990e).

Gas chromatographic methods with ECD and other detectors have a detection limit for hexachlorobutadiene of 0.02-0.05 μg/L in water (EPA 1982a, 1989c, 1989d, 1989e). Detection limits for soil and solid waste are usually higher, depending on matrix interferences, extraction, and

## TABLE 6-1. Analytical Methods for Determining Hexachlorobutadiene in Biological Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Blood | Extract with hexane | GC/ECD and GC/MS | <1 μg/L | 60–83 | Bristol et al. 1982 |
| Blood | Extract with hexane | GC/ECD | 18 ng/L | 85–122 | Kastl and Hermann 1983 |
| Adipose tissue | Extract with acetone/hexane, clean up with GPC and FAC | GC/ECD and GC/MS | No data | 42–67 | LeBel and Williams 1986 |
| Adipose tissue | Extract with benzene/acetone, precipitate fat, clean up with FAC | GC/ECD and GC/MS | 1 μg/kg [a] wet weight | No data | Mes et al. 1985 |
| Rat liver tissue | Homogenize with sodium sulfate, digest with perchloric acid/acetic acid, extract with hexane, clean up with concentrated sulfuric acid and Florisil®, elute with hexane | Capillary column | 0.0009 ppb | 87 | Wang et al. 1991 |

[a] Lowest concentration detected

ECD = electron capture detector; FAC = Florisil adsorption chromatography; GC = gas chromatography; GPC = gel permeation chromatography; MS = mass spectrometry

clean up procedures (EPA 1986, 1990e). Detection by MS is most specific because identification is based on the characteristic mass ion as well as the retention time. Newer MS methods can achieve detection limits of 0.04-0.11 μg/L in water, comparable to ECD (EPA 1982a, 1989e).

Table 6-2 summarizes some of the methods used for sample preparation and analysis of hexachlorobutadiene in environmental samples.

## 6. 3 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 hexachlorobutadiene is available. Where adequate information is not available, ATSDR, in conjunction with the 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 hexachlorobutadiene.

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.

### 6.3.1 Identification of Data Needs

**Methods for Determining Biomarkers of Exposure and Effect.** Hexachlorobutadiene can be measured in human blood and adipose tissue, with detection limits < 1 μg/L in blood and 1 μg/kg wet weight of adipose tissue (Bristol et al. 1982; LeBel and Williams 1986; Mes et al. 1985). No hexachlorobutadiene was detected in blood from controls or residents near a hazardous waste site (Bristol et al. 1982), indicating that the method was not sensitive enough to measure background levels of hexachlorobutadiene in the general population. It is likely that this method would be sensitive enough to measure levels at which biological effects occur. Hexachlorobutadiene was detected in adipose tissue of victims of accidental and nonaccidental deaths, with about twice as much

## TABLE 6-2. Analytical Methods for Determining Hexachlorobutadiene in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Air | Sorb on Amberlite XAD-2®, desorb with hexane | GC/ECD | 0.02 µg/sample | 85–100 | NIOSH 1990 |
| Indoor air | Collect in stainless steel canister, concentrate sample in cryogenic trap | GC/ECD or GC/MS | 0.2 ppbv | 90–110 | EPA 1990b |
| Water | Adjust to pH > 11, extract with methylene chloride, dry, concentrate | GC/MS | 0.9 µg/L | 24–116 | APHA 1992a |
| Water | Purge and trap | GC/PID and GC/HECD | No data | 98–99 | APHA 1992b |
| Water | Purge and trap | GC/MS | 0.04–0.11 µg/L | 88–91 | Eichelberger et al. 1990; EPA 1989e |
| Water | Purge and trap | GC/PID | 0.02 µg/L | No data | EPA 1989d |
| Water | Purge and trap | GC/PID and GC/HECD | 0.05–0.09 µg/L | 92–99 | EPA 1989c |
| Waste water | Extract with methylene chloride, dry, concentrate into hexane, clean up with FAC | GC/ECD | 0.05 µg/L | 86–106 | EPA 1982a |

## TABLE 6-2. Analytical Methods for Determining Hexachlorobutadiene in Environmental Samples *(continued)*

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Waste water | Extract with methylene chloride, dry, concentrate | GC/MS | 0.9 µg/L | 20–76 | EPA 1982c |
| Soil/solid waste | Extract with organic solvent, clean up with GPC | GC/MS | 0.66–50 mg/kg wet weight | No data | EPA 1986 |
| Soil/solid waste | Purge and trap | GC/MS | 0.05–2.5 mg/kg wet weight | 93–107 | EPA 1990e |

ECD = electron capture detection; FAC = florisil adsorption chromatography; GC = gas chromatography; GPC = gel permeation chromatography; HECD = halogen electrolytic conductivity detector; MS = mass spectroscopy; PID = photoionization detector; ppbv = parts per billion by volume

in accident than nonaccident victims (Mes et al. 1985). This indicates that the GC/ECD and GC/MS method is sensitive enough to measure background levels of hexachlorobutadiene in the general population as well as levels at which biological effects occur. No data were located concerning methods to measure hexachlorobutadiene metabolites in biological samples; such methods would be useful if it were established that hexachlorobutadiene metabolite levels were reliable markers of exposure to hexachlorobutadiene.

No data were located concerning methods to measure biological markers of hexachlorobutadiene effects. Research into biomarkers of effect would be most useful if performed in conjunction with development of sensitive, specific, and reliable methods for measuring the biomarker(s) of effect.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media.** Methods for detection of hexachlorobutadiene in air, water, soil, solid waste, and food are all based on gas chromatography (APHA 1992a, 1992b; EPA 1982a, 1982c, 1986, 1989c, 1989d, 1989e, 1990b, 1990e). Existing methods for analysis of air and water appear to be sufficiently sensitive, specific, and reliable to measure background levels in the environment. Matrix interference and contamination by co-eluting chemicals may limit the sensitivity and specificity of methods for analysis of hexachlorobutadiene in soil and solid waste (EPA 1986, 1990e). Supercritical fluid extraction, which uses carbon dioxide liquified above 31°C at high pressure, might provide efficient extraction of hexachlorobutadiene from large samples (Walters 1990). Supercritical fluid chromatography may provide an alternate approach to GC for analysis of hexachlorobutadiene and other compounds from complex environmental samples (Pospisil et al. 1991). An immunoassay for heptachlor has been developed which shows 1.6 % cross-reactivity with hexachlorobutadiene (Stanker et al. 1990). Development of an immunoassay specific for hexachlorobutadiene could provide a rapid, inexpensive, and sensitive method for detecting hexachlorobutadiene in environmental samples. No data were located concerning methods to measure hexachlorobutadiene degradation products in the environment. Degradation products are likely to be compounds that could be separated either by GC or by high performance liquid chromatography (HPLC) (for oxidized, polar degradation products). Mass spectrometry would be likely to be the most specific method to identify such products. Development of methods to measure hexachlorobutadiene degradation products would be useful for assessing the fate of hexachlorobutadiene in the environment.

### 6.3.2 On-going Studies

No information was located concerning on-going studies for improving methods of analysis of hexachlorobutadiene, its metabolites, or other biomarkers of exposure and effect to hexachlorobutadiene in biological materials or environmental samples.

## 7. REGULATIONS AND ADVISORIES

Because of its potential to cause adverse health effects in exposed people, a number of regulations and guidelines have been established for hexachlorobutadiene by various international, federal, and state agencies. These values are summarized in Table 7-l.

The ATSDR has calculated an intermediate-duration exposure oral MRL of 0.0002 mg/kg/day, based on a LOAEL of 0.2 mg/kg/day for the presence of kidney damage in female mice (NTP 1991).

The EPA has derived a chronic oral RfD of $2x10^{-4}$ mg/kg/day for hexachlorobutadiene (EPA 1993). This RfD is based on a LOAEL of 0.5 mg/kg/day for renal effects in mice exposed via the diet for 13 weeks (Yang et al. 1989). Since this RfD is currently under review by EPA and has been withdrawn from EPA's Integrated'Risk Information System (IRIS 1993), it is subject to change.

## TABLE 7-1. Regulations and Guidelines Applicable to Hexachlorobutadiene

| Agency | Description | Information | References |
|---|---|---|---|
| INTERNATIONAL | | | |
| IARC | Carcinogenic classification | Group 3[a] | IARC 1987 |
| NATIONAL | | | |
| Regulations: | | | |
| a. Air: | | | |
| OSHA | PEL TWA | 0.02 ppm (0.24 mg/m$^3$)[b] | OSHA 1989 (29 CFR 1910.1000) |
| EPA OAQPS | Hazardous Air Pollutant | Yes | Public Law 101-549, Section 112 |
| b. Water: | | | |
| EPA ODW | Monitoring requirements for unregulated contaminants | Yes | 40 CFR 141 |
| EPA OWRS | General permits under NPDES | Yes | 40 CFR 122 |
| | General Pretreatment Regulations for Existing and New Sources of Pollution | Yes | 40 CFR 403 |
| c. Other: | | | |
| EPA OERR | Reportable quantity | 1 lb. | EPA 1989b (40 CFR 302.4) |
| EPA OSW | Hazardous Waste Constituent (Appendix VIII) | Yes | EPA 1980 (40 CFR 261) |
| | Residue Concentration Limit from Burning Hazardous Waste in Boilers and Industrial Furnaces | $5x10^{-3}$ mg/kg | EPA 1991 (40 CFR 266.122) |
| | Groundwater Monitoring List (Appendix IX) | Yes | EPA 1987b (40 CFR 264) |
| | Land Disposal Restrictions | Yes | EPA 1990d (40 CFR 268) |
| EPA OTS | Toxic Chemical Release Reporting Rule | Yes | EPA 1988a (40 CFR 372) |
| | Health and Safety Data Reporting Rule | Yes | EPA 1988b (40 CFR 716.120) |
| | Preliminary Assessment Information Reporting Rule | Yes | EPA 1982b (40 CFR 712.30) |

**TABLE 7-1. Regulations and Guidelines Applicable to Hexachlorobutadiene *(continued)***

| Agency | Description | Information | References |
|---|---|---|---|
| Guidelines: | | | |
| a. Air: | | | |
| ACGIH | TLV TWA | 0.02 ppm (0.21 mg/m$^3$) Suspected occupational Carcinogen (skin) | ACGIH 1991 |
| NIOSH | REL, TWA | 0.02 ppm (0.24 mg/m$^3$) (skin) | NIOSH 1992 |
| b. Water: | | | |
| EPA ODW | Health Advisories | | EPA 1989b |
| | One-day (child) | 0.3 mg/L | |
| | Ten-day (child) | 0.3 mg/L | |
| | Longer-term (child) | 0.1 mg/L | |
| | Longer-term (adult) | 0.4 mg/L | |
| | Lifetime (adult) | 0.001 mg/L | |
| EPA OWRS | Ambient Water Quality Criteria Ingesting water and organisms: | 0.45 µg/L | EPA 1980 |
| | Ingesting organisms only: | 50 µg/L | |
| c. Other: | | | |
| EPA | RfD | | EPA 1993 |
| | (oral) | 2x10$^{-4}$ mg/kg/day[c] | |
| | Carcinogenic Classification | Group C[d] | IRIS 1993 |
| | Cancer slope factor ($q_1$*) | | |
| | $q_1$* (oral) | 7.8x10$^{-2}$ (mg/kg/day)$^{-1}$ | |
| | $q_1$* (inhalation) | 7.8x10$^{-2}$ (mg/kg/day)$^{-1}$ | |
| STATE | | | |
| Regulations and Guidelines: | | | |
| a. Air: | Acceptable ambient air concentrations | | NATICH 1991 |
| Connecticut | | 2.4 µg/m$^3$ (8 hour) | |
| Kansas | | 0.0455 µg/m$^3$ (1 year) | |
| Nevada | | 6 µg/m$^3$ (8 hour) | |
| New York | | 0.8 µg/m$^3$ (1 year) | |
| Oklahoma | | 0.00 | |
| South Carolina | | 1.20 µg/m$^3$ (24 hour) | |
| Texas | | 2.10 µg/m$^3$ (30 minutes) 0.210 µg/m$^3$ (annual) | |
| Vermont | | 0.045 µg/m$^3$ (annual) | |
| Virginia | | 2.10 µg/m$^3$ (24 hour) | |

**TABLE 7-1. Regulations and Guidelines Applicable to Hexachlorobutadiene** *(continued)*

| Agency | Description | Information | References |
|---|---|---|---|
| b. Water: | Drinking water quality standards | | FSTRAC 1990 |
| Kansas | | 4.5 µg/L | |
| Minnesota | | 1.4 µg/L | |

[a] Group 3: Not classifiable as to its carcinogenicity in humans.
[b] Due to a federal court decision, not enforceable as of 03/22/93 (Hanson 1993). There is no currently federally enforceable PEL for hexachlorobutadiene (EPA 1993 35338).
[c] Oral RfD withdrawn from IRIS on 05/01/93 as a result of further review. A new RfD summary is in preparation.
[d] Group C: possible human carcinogen.

ACGIH = American Conference of Governmental Industrial Hygienists; EPA = Environmental Protection Agency; IARC = International Agency for Research on Cancer; NIOSH = National Institute for Occupational Saftey and Health; NPDES = National Pollutant Discharge Elimination System; OAQPS = Office of Air Quality Planning and Standards; ODW = Office of Drinking Water; OERR = Office of Emergency and Remedial Response; OSHA = Occupational Safety and Health Administration; OSW = Office of Solid Waste; OTS = Office of Toxic Substances; OWRS = Office of Water Regulations and Standards; PEL = Permissible Exposure Limit; REL = Recommended Exposure Limit; RfD = Reference Dose; TLV = Threshold Limit Value; TWA = Time-Weighted Average

## 8. REFERENCES

ACGIH. 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices. 5th ed. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

*ACGIH. 1991. Threshold limit values for chemical substances and physical agents and biological exposure indices for 1991 to 1992. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

ACS. 1990. Chemcyclopedia 90. J.H. Kuney, ed. The American Chemical Society, Washington, DC:94.

Anders MW, Jakobson I. 1985. Biotransformation of halogenated solvents. Stand J Work Environ Health 11:23-32.

*APHA. 1992a. M-6410, extractable base/neutrals and acids. In: Greenburg AE, Clesceri LS, Eaton AD, eds. Standard methods for the examination of water and wastewater. 18th ed. Washington, DC: American Public Health Association, 6-77-6-89.

*APHA. 1992b. M-6230, volatile halocarbons. In: Greenburg AE, Clesceri LS, Eaton AD, eds. Standard methods for the examination of water and wastewater. 18th ed. Washington, DC: American Public Health Association, 6-55-6-57.

APHA. 1992c. M-6210, volatile organics. In: Greenburg AE, Clesceri LS, Eaton AD, eds. Standard methods for the examination of water and wastewater. 18th ed. Washington, DC: American Public Health Association, 6-26-6-46.

*Atkinson R. 1987. A structure-activity relationship for the estimation of rate constants for the gasphase reactions of OH radicals with organic compounds. Int J Chem Kinet 19:799-828.

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*Cited in text

## 8. REFERENCES

*Atkinson R, Carter WPL. 1984. Kinetics and mechanisms of the gas-phase reaction of ozone with organic compounds under atmospheric conditions. Chem Rev 84:437-70.

*ATSDR. 1989. Decision guide for identifying substance-specific data needs related to toxicological profiles. Agency for Toxic Substances and Disease Registry. Atlanta, GA.

Badaeva LN, Belousva AA, Ba:ida NA, et al. 1990. [Characteristics of the neuro- and immunotoxic effects of transplacental hexachlorobutadiene]. Gig Sanit Sept. 90:75-78. (Russian).

Badaeva LN, Ovsiannikova LM, Kiseleva NI, et al. 1985. [manifestation of neurotoxic effect of chlororganic pesticide hexachlorobutadiene postnatal period of ontogenesis in rats.] Arkh Anat Gist01 Em Briol Aug. 89:44-49. (Russian).

Baggett JM, Berndt WO. 1984. Renal and hepatic glutathione concentrations in rats after treatment with hexachloro-1,3-butadiene and citrinin. Arch Toxicol 56:46-49.

*Bai CL, Canfield PJ, Stacey NH. 1992. Effects of hexachloro-1,3-butadiene and 1,1,2,2-tetrachloroethylene on individual serum bile acids. Toxicology and Industrial Health 8: 19 l-203.

Ban M, de Ceaurriz J. 1988. Probenecid-induced protection against acute hexachloro-1,3-butadiene and methyl mercury toxicity to the mouse kidney. Toxicol Lett 40:71-76.

Barkley J, Bunch J, Bursey JT, et al. 1980. Gas chromatography mass spectrometry computer analysis of volatile halogenated hydrocarbons in man and his environment - A multimedia environmental study. Biomed Mass Spectrometry 7: 139-147.

*Barnes DG, Dourson M. 1988. Reference dose (RfD): Description and use in health risk assessments. Regul Toxicol Pharmacol 8:471-486.

Bauer S, Wolff I, Werner N, et al. 1992. Toxicological investigations in the semiconductor industry: I. Studies on the acute oral toxicity of a complex mixture of waste products from the aluminium plasma etching process. Toxicology and Industrial Health 8: 141-156.

## 8. REFERENCES

Bernard A, Lauwerys RR. 1991. Proteinuria: changes and mechanisms in toxic nephropathies. Toxiocology 21: 373-405.

Berndt WO, Mehendale HM. 1978. Effect of hexachlorobutadiene on renal function in the rat. Toxicol Appl Pharmacol 45 :296-297.

Berndt WO, Mehendale HM. 1979. Effects of hexachlorobutadiene (HCBD) on renal function and renal organic ion transport in the rat. Toxicology 14:55-65.

*Bristol DW, Crist HL, Lewis RG, et al. 1982. Chemical analysis of human blood for assessment of environmental exposure to semivolatile organochlorine chemical contaminants. J Anal Toxicol 6:269-275.

*Brown KW, Donnelly KC. 1988. An estimation of the risk associated with the organic constituents of hazardous and municipal waste landfill leachates. Hazardous Waste and Hazardous Materials 5: l-30.

*Camanzo J, Rice CP, Jude DJ. 1987. Organic priority pollutants in nearshore fish from 14 Lake Michigan tributaries and embayments, 1983. J Great Lakes Res 13 :296-309.

*CDC/ATSDR. 1990. Biomarkers of organ damage or dysfunction for the renal, hepatobiliary and immune systems. Atlanta, GA: CDC/ATSDR Subcommittee on Biomarkers of Organ Damage and Dysfunction, Centers for Disease Control, Agency for Toxic Substances and Disease Registery. Summary report, August 27, 1990.

*Class T, Ballschmiter K. 1987. Global baseline pollution studies. Freznius Z. Anal Chem 327: 198-204.

Class T, Ballschmiter K. 1986. Chemistry of organic traces in air VI: Distribution of chlorinated Cl - C4 hydrocarbons in air over the northern and southern Atlantic ocean. Chemosphere 15:413-427.

## 8. REFERENCES

*Cole RH, Frederick RE, Healy RE, et al. 1984. Preliminary findings of the priority pollutants monitoring project of the nationwide urban runoff program. J WPCF 56:898-908.

*CRISP. 1993. Computer Retrieval of Information on Scientific Projects. National Institutes of Health, Division of Research Grants, Bethesda, MD. May 13, 1993.

*Davis ME. 1984. Changes of hexachlorobutadiene nephrotoxicity after piperonyl butoxide treatment. Toxicology 30:217-225.

Davis ME. 1988. Effects of AT-125 on the nephrotoxicity of hexachloro-1,3-butadiene in rats. Toxicol Appl Pharmacol 95 :44-52.

*Davis ME, Berndt WO, Mehendale HM. 1980. Disposition and nephrotoxicity of hexachloro-1,3-butadiene. Toxicology 16: 179-191.

de Ceaurriz J, Ban M. 1990. Role of γ-glutamyltranspeptidase and β-lyase in the neptrhotoxicity of hexachloro-1,3-butadiene and methyl mercury in mice. Toxicol Lett 50:249-256.

*de Ceaurriz J, Gagnaire F, Ban M, et al. 1988. Assessment of the relative hazard involved with airborne irritants with additional hepatotoxic or nephrotoxic properties in mice. J Appl Toxicol 8:417-422.

*De Meester C, Mercier M, Poncelet F. 1980. Mutagenic activity of butadiene, hexachlorobutadiene, and isoprene. Ind Environ Xenobio Pot Int Conf 195-203.

*Dekant W, Schrenk D, Vamvakas S, et al. 1988a. Metabolism of hexachloro-1,3-butadiene in mice: *in vivo* and *in vitro* evidence for activation by glutathione conjugation. Xenobiotica 18:803-816.

*Dekant W, Urban G, Gorsman C, et al. 1991. Thioketene formation from γ-haloalkenyl 2-nitrophenyl disulfides: models for biological reactive intermediates of cytotoxic S-conjugates. J Am Chem Soc 113:5120-5122.

## 8. REFERENCES

*Dekant W, Vamvakas S, Anders MW. 1990a. Review section: Bioactivation of hexachlorobutadiene by glutathione conjugation. Food Chem Toxicol 28:285-293.

*Dekant W, Vamvakas S, Berthold K, et al. 1986. Bacterial β-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene and hexachlorobutadiene. Chem Biol Interactions 60:31-45.

*Dekant W, Vamvakas S, Henschler D, et al. 1988b. Enzymatic conjugation of hexachloro-1,3-butadiene with gultathione. Formation of l-(Glutathion-S-yl)-1,2,3,4,4-pentachlorobuta-l,3-diene and 1,4-bis(glutathion-S-yl)-l,2,3,4-tetrachlorobuta-l,3-diene. Drug Metabolism and Disposition 16:701-706.

*Dekant W, Vamvakas S, Koob M, et al. 1990b. A mechanism of haloalkene-induced renal carcinogenesis. Environ Health Perspect 88: 107-l 10.

*DeVault DS. 1985. Contaminants in fish from Great Lakes harbors and tributary mouths. Arch Environ Contam Toxicol 14:587-594.

*Driscoll TR, Hamdan HH, Wang G, et al. 1992. Concentrations of individual serum or plasma bile acids in workers exposed to chlorinated aliphatic hydrocarbons. Br J Ind Med 49:700-705.

*Duprat P, Gradiski D. 1978. Percutaneous toxicity of hexachlorobutadiene. Acta Pharmacol Toxicol 43:346-353.

*Eichelberger JW, Bellar TA, Donnelly JP, et al. 1990. Determination of volatile organics in drinking water with USEPA method 524.2 and the ion trap detector. J Chromatogr Sci 28:460-467.

*Elder V, Proctor B, Hites R. 1981. Organic compounds found near dump sites in Niagara Falls, New York. Environ Sci Technol 15: 1237-1243.

*EPA. 1976. An ecological study of hexachlorobutadiene (HCBD). Washington, DC: U.S. Environmental Protection Agency, Office of Toxic Substances. EPA/560/6-76-010.

## 8. REFERENCES

*EPA. 1980. Ambient water quality criteria document for hexachlorobutadiene. Washington, DC: U.S. Environmental Protection Agency, Office of Water Regulations and Standards. EPA 440/5-80-053.

*EPA. 1982a. Chlorinated hydrocarbons-method 612. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory.

*EPA. 1982b. U.S. Environmental Protection Agency. Federal Register 47:26992-27008.

*EPA. 1982c. Test method. Base/neutrals and acids methods 625. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory.

*EPA. 1982d. U.S. Environmental Protection Agency. Federal Register 47:58029-58030.

*EPA. 1984. Health effects assessment for hexachlorobutadiene. Washington, DC: U.S. Environmental Protection Agency, Office of Emergency and Remedial Response, Office of Solid Waste and Emergency Response. EPA/540/1-86-053.

*EPA. 1986. Gas chromatography/mass spectrometry for semivolatile organics: capillary column technique-method 8270. In: Test methods for evaluating solid waste. SW-846. Washington, DC: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response.

*EPA. 1987a. U.S. Environmental Protection Agency. Part II. Federal Register. 52:25690-25717.

*EPA, 1987b. U.S. Environmental Protection Agency. Part II. Federal Register. 52:25942-25953.

EPA. 1987c. Measurement of hydrolysis rate constants for evaluation of hazardous waste land disposal: Vol. 1. Data on 32 chemicals. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/S3-86/043.

## 8. REFERENCES

EPA. 1987d. Measurement of hydrolysis rate constants for evaluation of hazardous waste land disposal: Vol. 2. Data on 54 chemicals. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/S3-87/019.

*EPA. 1988a. U.S. Environmental Protection Agency. Part II. Federal Register. 53:4500-4539.

*EPA. 1988b. U.S. Environmental Protection Agency. Part V. Federal Register 53:38642-38654.

*EPA. 1988c. U.S. Environmental Protection Agency. Part II. Federal Register 53:4500-4532.

EPA. 1988d. Measurement of hydrolysis rate constants for evaluation of hazardous waste land disposal: Vol. 3. Data on 70 chemicals. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/S3-88/028.

EPA. 1988e. Interim protocol for measuring hydrolysis rate constants in aqueous solutions. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/S3-88/014.

*EPA. 1989a. Ambient water quality criteria document addendum for hexachlorobutadiene. Cincinnati, OH: U.S. Environmental Protection Agency, Office of Water Regulations and Standards. Final draft ECAO-CIN-652.

*EPA. 1989b. Health advisory for hexachlorobutadiene. Washington, DC: U.S. Environmental Protection Agency, Office of Water. PB91-160663.

*EPA. 1989c. Measurement of volatile organic compounds in water by purge and trap capillary column gas chromatography with photoionization and electrolytic conductivity detectors in series - method 502.2. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory.

*EPA. 1989d. Measurement of volatile aromatic and unsaturated organic compounds in water by purge and trap gas chromatography - method 503.1. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory.

## 8. REFERENCES

*EPA. 1989e. Measurement of purgeable organic compounds in water by capillary column gas chromatography/mass spectrometry - method 524.2. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory.

*EPA. 1990a. Application of open-tubular columns to SW-846 GC methods. In: Test methods for evaluating solid waste. SW-846. Washington, DC: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response. EPA/600/S4-90/021.

*EPA. 1990b. Method IP-1A (In: Compendium of methods for the determination of air pollutants in indoor air. Washington, DC: U.S. Environmental Protection Agency, Atmospheric Research and Exposure Assessment Laboratory, EPA/600/S4-90/1010.

*EPA. 199Oc. Method 8120A. Chlorinated hydrocarbons by gas chromatography. Cincinnati, OH: U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory.

*EPA. 1990d. U.S. Environmental Protection Agency. Part II. Federal Register 55:22520-22720.

*EPA. 1990e. Volatile organic compounds by gas chromatography/mass spectrometry (GC/MS): capillary column technique-method 8260. In: Test methods for evaluating solid waste. SW-846. Washington, DC: U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response.

*EPA. 1990f. Pathway analysis of chemical hydrolysis for 14 RCRA chemicals. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/M-89/009.

*EPA. 199Og. U.S. Environmental Protection Agency. Part III. Federal Register 503:47229.

*EPA. 1990h. Interim methods for the development of inhalation reference doses. Washington, DC: U.S. Environmental Protection Agency, Office of Heath and Environmental Assessment. EPA/600/8-90/066A.

*EPA. 1991. U.S. Environmental Protection Agency. Part III. Federal Register 56:7134-7240.

## 8. REFERENCES

*EPA. 1993. U.S. Environmental Protection Agency. Health effects summary tables. Supplement No. 1 to the March 1993 annual update. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. EPA 540-R-93-058.

*Ewing BB, Chian ESK. 1977. Monitoring to detect previously unrecognized pollutants in surface waters. Appendix: organic analysis data. National Technical Information Service. EPA-560/6-77-015A.

Flynn LR, Bass SW, Meierer RE. 1991. Headspace screening/capillary column GC/MS analysis for volatile organics: validation studies and applications. Waste Testing and Quality Assurance 106-l 14.

*FSTRAC. 1990. Summary of state and federal drinking water standards and guidelines. Washington, DC: Federal-State Toxicology and Regulatory Alliance Committee, Chemical Communication Subcommittee. 7: 17.

Ford JH, Evans J. 1985. Distribution of 5'-nucleotidase in the tissues of sheep and the effect of kidney and liver lesions on the activity of enzyme in plasma and urine. Res Vet Sci 39: 103-109.

*Fox ME, Carey JH, Oliver BG. 1983. Compartmental distribution of organochlorine contaminants in the Niagara River and the western basin of Lake Ontario. J Great Lakes Res 9:287-294.

*Gage JC. 1970. The subacute inhalation toxicity of 109 individual chemicals. Brit J Industr Med 27: l-8.

*Garle MJ, Fry J. 1989. Detection of reactive metabolites *in vitro.* Toxicology 54:101-110.

Gartland KP, Beddell CR, Lindon JC, et al. 1991. Computer patter recognition analysis of PMR spectroscopic data from urine obtained from rats in various nephrotoxicity states. Proceedings, Fourth International Symposium on Nephrotoxicity, Guilford, England, UK 1989. Marcel Dekker, Inc., New York, NY 519-524.

Gartland KP, Bonner FW, Nicholson JK. 1989. Investigations into the biochemical effects of region-specific nephrotoxins. Mol Pharmacol 35:242-250.

## 8. REFERENCES

Gartland KP, Sweatman BC, Beddell CR, et al. 1991. Proton nuclear magnetic resonance urinalysis studies on the biochemical effects of a nephrotoxic dose of uranyl nitrate in the Fischer 344 rat. Proceedings, Fourth International Symposium on Nephrotoxicity, Guilford, England, UK 1989. Marcel Dekker, Inc., New York, NY 525530.

*Gietl YS, Anders MW. 1991. Biosynthesis and biliary excretion of S-conjugates of hexachlorobuta-1,3-diene in the perfused rat liver. Drug Metabolism and Disposition 19:274-277.

*Gietl Y, Vamvakas S, Anders MW. 1991. Intestinal absorption of S-(pentachlorobutadienyl)glutathione and S-(pentachlorobutadienyl)-L-cysteine, the glutathione and cysteine S-conjugates of hexachlorobuta-1,3-diene. Drug Metabolism and Disposition 19:703-707.

*Govind R, Flaherty PA, Dobbs RA. 1991. Fate and effects of semivolatile organic pollutants during anaerobic digestion of sludge. Water Res 25:547-556.

Gradiski D, Duprat P, Magadur JL, et al. 1975. [Etude toxicologique experimentale de l'hexachlorobutadiene]. Eur J Toxicol 8: 180-187. (French).

Green T, Odum J. 1985. Structure/activity studies of the nephrotoxic and mutagenic action of cysteine conjugates of chloro- and fluoroalkenes. Chem Biol Interactions 54: 15-3 1.

*Hardin BD, Bond GP, Sikov MR, et al. 1981. Testing of selected workplace chemicals for teratogenic potential. Stand J Work Environ Health 7:66-75.

*Harleman JH, Seinen W. 1979. Short-term toxicity and reproduction studies in rats with hexachloro-( 1,3)-butadiene . Toxicol Appl Pharmacol 47: 1-14.

*Hauser TR, Bromberg SM. 1982. EPA's monitoring program at Love Canal 1980. Environmental Monitoring and Assessment 2:249-271.

*Haworth S, Lawlor T, Mortelmans K, et al. 1983. Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen Suppl 1: 3-142.

## 8. REFERENCES

*HazDat. 1993. Agency for Toxic Substances and Disease Registery (ATSDR), Atlanta, GA. October, 1993.

*Henschler D, Dekant W. 1990. Nephrocarcinogenic xenobiotics. Toxicol Lett 53 : 105-110.

*Hewitt WR, Brown EM. 1984. Nephrotoxic interactions between ketonic solvents and halogenated aliphatic chemicals. Fund Appl Toxicol 4:902-908.

*Hook JB, Ishmael J, Lock EA. 1983. Nephrotoxicity of Hexachloro-1:3-butadiene in the rat: The effect of age, sex, and strain. Toxicol Appl Pharmacol 67:122-131.

*Hook JB, Rose MS, Lock EA. 1982. The nephrotoxicity of hexachloro-1:3-butadiene in the rat: Studies of organic anion and cation transport in renal slices and the effect of monooxygenase inducers. Toxicol Appl Pharmacol 65: 373-382.

*HSDB. 1992. Hazardous Substances Data Bank. National Library of Medicine, National Toxicology Information Program, Bethesda, MD. January 20, 1992.

*HSDB. 1993. Hazardous Substances Data Bank. National Library of Medicine, National Toxicology Information Program, Bethesda, MD. May 13, 1993.

*IARC. 1979. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 20: Hexachlorobutadiene. World Health Organization, Lyon, France.

*IARC. 1987. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. Suppl 7. Overall evaluations of carcinogenicity: Updating of IARC monographs volumes l-42. World Health Organization, International Agency for Research on Cancer, Lyon, France, 64.

*IRIS. 1992. Integrated Risk Information System. U.S. Environmental Protection Agency, Washington, DC. March 26, 1992.

*IRIS. 1993. Integrated Risk Information System. U.S. Environmental Protection Agency, Washington, DC. May 14, 1993.

## 8. REFERENCES

*Ishmael J, Lock EA. 1986. Nephrotoxicity of hexachlorobutadiene and its glutathione-derived conjugates. In: Presented at the Fourth International Symposium of the Society of Toxicologic Pathologists, June 5-7, 1985. Washington, DC. Toxicol Patho1 14:258-262.

*Ishmael J, Pratt I, Lock EA. 1982. Necrosis of the pars recta ($S_3$ segment) of the rat kidney produced by hexachloro-1,3-butadiene. J Pathol 138:99-l 13.

*Ishmael J, Pratt I, Lock EA. 1984. Hexachloro-1,3-butadiene-induced renal tubular necrosis in the mouse. J Pathol 142:195-203.

*Jaffe DR, Hassall CD, Brendel K. 1983. *In vivo* and *in vitro* nephrotoxicity of the cysteine conjugate of hexachlorobutadiene. J Toxicol Environ Health 11:857-867.

*Johnson LD, Young JC. 1983. Inhibition of anaerobic digestion by organic priority pollutants. J Water Pollut Control Fed 55: 1141-l 149.

*Jones TW, Gerdes RG, Ormstad K, et al. 1985. The formation of both a mono- and bis-substituted glutathione conjugate of hexachlorobutadiene by isolated hepatocytes and following *in vivo* administration to the rat. Chem Biol Interactions 56:251-267.

*Jones TW, Chen Q, Schaeffer V, et al. 1988. Immunohistochemical localization of glutamine transaminase K, a rat kidney cysteine conjugate β-lyase, and the relationship to the segment specificity of cysteine conjugate nephrotoxicity. Ma1 Pharm 341621-627.

*Jonker D, Jones MA, van Bladeren PJ, et al. 1993a. Acute (24 hr) toxicity of a combination of four nephrotoxicants in rats compared with the toxicity of the individual compounds. Food Chem Toxicol 3 1:45-52.

*Jonker D, Woutersen RA, van Bladeren PJ, et al. 1993b. Subacute (4-wk) oral toxicity of a combination of four nephrotoxins in rats: comparison with the toxicity of the individual compounds. Food Chem Toxicol 3 1: 125-136.

## 8. REFERENCES

*Junk GA, Ford CS. 1980. A review of organic emissions from selected combustion processes. Chemosphere 9: 187-230.

Kaminksy R, Kaiser KL, Hites RA. 1983. Fates of organic compounds from Niagara Falls dumpsites in Lake Ontario. J Great Lakes Res 9:183-189.

*Kastl PE, Hermann EA. 1983. Quantitative gas chromatographic determination of hexachloro-1,3-butadiene in whole rat blood at part per trillion levels. Journal of Chromatography 280:390-393.

Keith LH, Garrison AW, Allen FR, et al. 1976. Identification of organic compounds in drinking water from thirteen U.S. cities. In: Identification & analysis of organic pollutants in water. Ann Arbor, MI: Ann Arbor Science Publishers, Inc., 329-373.

Kennedy GL, Graepel GJ. 1991. Acute toxicity in the rat following either oral or inhalation exposure. Toxicol Lett 56:317-326.

Khalturin GU, Chudin VA, Andriushkeeva NI. 1984. [Hexachlorobutadiene distribution in rats during chronic intragastric adminstration.] Gig Sanit Oct. 10:91. (Russian).

King JW. 1989. Fundamentals and applications of supercritical fluid extraction in chromatographic science. J Chromatog Sci 27:355-364.

Kluwe WM, McNish R, Smithson K, et al. 1981. Depletion by l,2-dibromoethane, 1,2-dibromo-3-chloropropane, tris(2,3-dibromopropyl)phosphate, and hexachloro-1,3-butadiene of reduced nonprotein sulfhydryl groups in target and non-target organs. Biochem Pharmacol 30:2265-2271.

Knocke WR, Conley L, van Benschoten JE. 1992. Impact of dissolved organic carbon on the removal of iron during water treatment. Water Res 26:1515-1522.

*Kociba RJ Gehring PJ, Humiston CG, et al. 1971. Toxicologic study of female rats administered hexachlorobutadiene or hexachlorobenzene for thirty days. The Dow Chemical Company, Midland, Michigan.

## 8. REFERENCES

*Kociba RJ, Keyes DG, Jersey GC. 1977a. Results of a two year chronic toxicity study with hexachlorobutadiene in rats. Am Ind Hyg Assoc J 38589-602.

Kociba RJ, Schwetz BA, Keyes DG. 1977b. Chronic toxicity and reproduction studies of hexachlorobutadiene in rats. Environ Health Perspect 21:49-53,

*Koob M, Dekant W. 1992. Biotransformation of the hexachlorobutadiene metabolites 1-(glutathion-S-yl)-pentachlorobutadiene and l-(cystein-*S*-yl)-pentachlorobutadiene in the isolated perfused rat liver. Xenobiotica 22:125-138.

*Kotzias D, Klein W, Korte F. 1975. [Beitrage Zur Gkologischen Chemie CVI Vorkommen Von Xenobiotika Im Sickerwasser Von Mtilldeponien.] Chemosphere 5:301-306. (German)

Kuehl DW, Leonard EN, Welch KJ, et al. 1980. Identification of hazardous organic chemicals in fish from the Ashtabula River, Ohio, and Wabash River, Indiana. J Assoc Off Anal Chem 63: 1238-1244.

Kuo CH, Hook JB. 1983. Effects of age and sex on hexachloro-1,3-butadiene toxicity in the Fischer 344 rat. Life Sci 33:517-523.

*Laska AL, Bartell CK, Laseter JL. 1976. Distribution of hexachlorobenzene and hexachlorobutadiene in water, soil, and selected aquatic organisms along the lower Mississippi River, Louisiana. Bull Environ Contam Toxicol 15:535-542.

Lau YL, Oliver BG, Krishnappan BG. 1989. Transport of some chlorinated contaminants by the water, suspended sediments, and bed sediments in the St. Clair and Detroit Rivers. Environ Toxicol Chem 8:293-301.

*LeBel GK, Williams DT. 1986. Pesticide and industrial chemical residues: Determination of helogenated contaminants in human adipose tissue. J Assoc Off Anal Chem 69:451-458.

*Levins P, Adams J, Brenner P, et al. 1979. Sources of toxic pollutants found in influents to sewage treatment plants VI. Integrated interpresentation. NTIS PB81-219685.

## 8. REFERENCES

Ligocki M, Levenberger C, Pankow J. 1985. Trace organic compounds in rain-II. Gas scavenging of neutral organic compounds. Atmos Environ 19: 1609-1617.

*Li RT, Going JE, Spigarelli JL. 1976. Sampling and analysis of selected toxic substances. Task 1B. Hexachlorobutadiene. NTIS PB-253.941.

Lock EA. 1989. Mechanism of nephrotoxic action due to organohalogenated compounds. Toxicol Lett 46:93-106.

*Lock EA, Ishmael J. 1979. Original investigations: The acute toxic effects of hexachloro-1:3-butadiene on the rat kidney. Arch Toxicol 43:47-57.

*Lock EA, Ishmael J. 1981. Hepatic and renal nonprotein sulfhydryl concentration following toxic doses of hexachloro-1,3-butadiene in the rat: The effect of Aroclor 1254, Phenobarbitone, or SKF 525A treatment. Toxicol Appl Pharmacol 57:79-87.

*Lock EA, Ishmael J. 1985. Effect of the organic acid transport inhibitor probenecid on renal cortical uptake and proximal tubular toxicity of hexachloro-1,3-butadiene and its conjugates. Toxicol Appl Pharmacol 81: 32-42.

Lock EA, Ishmael J, Hook JB. 1984. Nephrotoxicity of hexachloro-1,3-butadiene in the mouse: The effect of age, sex, strain, monooxygenase modifiers, and the role of glutathione. Toxicol Appl Pharmacol 72:484-494.

*Lock EA, Ishmael J, Pratt I. 1982. Hydropic change in rat liver induced by hexachloro-1 : 3-butadiene. J Appl Toxicol 6:315-320.

*Lock EA, Pratt IS, Ishmael J. 1985. Hexachloro-1,3-butadiene-induced hydropic change in mouse liver. J Appl Toxicol 5:74-79.

Lopez-Avila V, Beckert WF, Billets S. 1991. Supercritical fluid extraction and its application to environmental analysis. Waste Testing and Quality Assurance 141-153.

## 8. REFERENCES

*Lucas SV. 1984. GCYMS (gas chromatography-mass spectrometry) analysis of organics in drinking water concentrates and advanced waste treatment concentrates, Volume I. NTIS PB85-128221.

*Mabey WR, Smith JH, Pod011 RT, et al. 1982. Aquatic fate process data for organic priority pollutants. Report to U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC, by SRI International, Menlo Park, CA. EPA 440/4-81-014.

*MacFarlane M, Foster JR, Gibson GG, et al. 1989. Cysteine conjugate β-lyase of rat kidney cytosol: Characterization, immunocytochemical localization, and correlation with hexachlorobutadiene nephrotoxicity. Toxicol Appl Pharmacol 98: 185-197.

Malins DC, McCain BB, Brown DW, et al. 1984. Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. Environ Sci Technol 18:705-713.

Markwell RD, Comrel DW, Gabric AJ. 1989. Bioaccumulation of lipophilic compounds from sediments by oligochaetes. Wat Res 22: 1443-1450.

*McConnell G, Ferguson DM, Pearson CR. 1975. Chlorinated hydrocarbons and the environment. Endeavor 34: 13-18.

Mes J. - 1981. Experiences in human milk analysis for halogenated hydrocarbon residues. Inter J Environ Anal Chem 9:283-299.

*Mes J, Davies DJ, Turton D. 1982. Polychlorinated biphenyl and other chlorinated hydrocarbon residues in adipose tissue of Canadians. Bull Environ Contam Toxicol 28:97- 104.

*Mes J, Davies DJ, Turton D. 1985. Environmental contaminants in human fat: A comparison between accidental and nonaccidental causes of death. Ecotoxicol Environ Safety 10:70-74.

*Montgomery JH, Welkom LM. 1990. Groundwater chemicals desk reference. Chelsea, MI: Lewis Publications, Inc. 334-336.

## 8. REFERENCES

*NAS/NRC. 1989. Bilogic markers in reproductive toxicology. National Academy of Sciencs of National Research Council. Washington, DC: National Academy Press, 15-35.

*Nash JA, King LJ, Lock EA, et al. 1984. The metabolism and disposition of hexachloro-1:3-butadiene in the rat and its relevance to nephrotoxicity. Toxicol Appl Pharmacol 73: 124-137.

*NATICH. 1991. National Air Toxics Information Clearing House: NATICH database report on state, local and EPA air toxics activities. Report to U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards. Research Triangle Park, NC, by Radian Corporation, Austin, TX. EPA-450/3-91-018.

*NIOSH. 1981. Tier II mutagenic screening of 13 NIOSH priority compounds: Individual compound report hexachloro- 1,3-butadiene . Cincinnati, OH: National Institute for Occupational Safety and Health, PB83-152397.

*NIOSH. 1990. Hexachlorobutadiene - method P&CAM 307-update. In: NIOSH Manual of Analytical Methods. 3rd Ed. 4th Supplement. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Services, Centers for Disease Control, National Institute for Occupational Safety and Health.

*NIOSH. 1992. Recommendations for occupational safety health, compendium of policy documents and statements. U.S. Department of Health and Human Services, Centers for Disease Control, NIOSH, Cincinnati, OH.

*NSF. 1975. Research program on hazard priority ranking of manufactured chemicals (Chemicals l-20) 9-A-l. NTIS PB-263161.

*NTP. 1991. National Toxicology Program. Toxicity studies of hexachloro-1,3-butadiene in $B6C3F_1$ mice (feed studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH publication no. 91-3120.

Oliver, BG. 1985. Desorption of chlorinated hydrocarbons from spiked and anthropogenically contaminated sediments. Chemosphere 14: 1087-l 106.

## 8. REFERENCES

*Oliver BG, Bourbonniere RA. 1985. Chlorinated contaminants in surficial sediments of Lakes Huron, St. Clair and Erie: implications regarding sources along the St. Clair and Detroit rivers. J Great Lakes Res 11:366-372.

*Oliver BG, Charlton MN. 1984. Chlorinated organic contaminants on settling particulates in the Niagara River vicinity of Lake Ontario. Environ Sci Technol 18:903-908.

*Oliver BG, Niimi AJ. 1983. Bioconcentrations of chlorobenzenes from water by rainbow trout: correlations with partition coefficients and environmental residues. Environ Sci Technol 10: 148-152.

Oliver BG, Nicol KD. 1985. Field testing of a large volume liquid-liquid extraction device for halogenated organics in natural waters. Intern J Environ Anal Chem 25:275-285.

Oliver BG, Niimi AJ. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario Ecosystem. Environ Sci Technol 22:388-397.

Oliver BG, Charlton MN, Durham RW. 1989. Distribution, redistribution, and geochronology of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in Lake Ontario Sediments. Environ Sci Technol 23 :200-208.

*OSHA. 1989. Occupational Safety and Health Administration: Part III. Federal Register 54:2332-2959. January 19.

*OTA. 1990. Neurotoxicity: Identifying and controlling poisons of the nervous system. Washington, DC: Office of Technology Assessment, U.S. Congress. OTA-BA-436.

Pankow JF, Rosen ME. 1988. Determination of volatile compounds in water by purging directly to a capillary column with whole column cryotrapping. Environ Sci Technol 22:398-405.

*Pankow JF, Isabelle LM, Asher WE. 1984. Trace organic compounds in rain. 1. Sampler design and analysis by adsorption/thermal desorption (ATD). Environ Sci Technol 18:3 10-3 18.

## 8. REFERENCES

Patterson JW, Kodukala PS. 1981. Emission and effluent control: biodegradation. Chem Eng Prog 77:48-55.

Payan JP, Beydon D, Fabry JP, et al. 1993. Partial contribution of biliary metabolites to nephrotoxicity, renal content and excretion of [$^{14}$C]hexachloro-l,3-butadiene in rats. J Appl Toxicol 13:19-24.

*Payan JP, Fabry JP, Beydon D, et al. 1991. Biliary excretion of hexachloro-1,3-butadiene and its relevance to tissue uptake and renal excretion in male rats. J Appl Toxicol 11:437-442.

*Pearson CR, McConnell G. 1975. Chlorinated $C_1$ and $C_3$ hydrocarbons in the marine environment. Proc R Sot Lond Biol 189:305-332.

*Pellizzari ED. 1978. Quantification of chlorinated hydrocarbons in previously collected air samples. EPA-450/3-78-l 12.

*Pellizzari ED. 1982. Analysis for organic vapor emissions near industrial and chemical waste disposal sites. Environ Sci Technol 16:781-785.

Pereira WE, Rostad CE, Chlou CT, et al. 1988. Contamination of estuarine water, biota, and sediment by halogenated organic compounds: A field study. Environ Sci Technol 22:772-778.

*Piet GJ, Zoeteman BC. 1980. Organic water quality changes during sand bank and dune filtration of surface waters in the Netherlands. J Amer Water Works Assoc 72:400-404.

*Plumb RH. 1991. The occurrence of appendix IX organic constituents in disposal site ground water. Ground Water Monitoring Review 157-164.

Poncelet F, Duverger-van Bogaert M, Lambotte-Vandepaer M, et al. 1984. Mutagenicity, carcinogenicity, and teratogenicity of industrially important monomers. Kirch-Volders M, ed. New York, Plenum Press, p. 205-279.

## 8. REFERENCES

*Pospisil PA, Marcus MF, Kobus MA. 1991. The application of supercritical fluid capillary chromatography to the analysis of appendix-VIII and IX compounds. Waste Testing and Quality Assurance 154-169.

Poteryaeva G. 1966. [The effect of hexachlorobutadiene on off-springs of albino rats.] Gig Sanit 31:33. (Russian)

Pratt IS, Lock EA. 1988. Deacetylation and further metabolism of the mercapturic acid of hexachloro-1,3-butadiene by rat kidney cytosol *in vitro.* Arch Toxicol 62:341-345.

Public Law 101-541. 1990. Clean Air Act Amendments of 1990. Environmental Reporter 352-353.

Reichert D. 1983. Metabolism and disposition of hexachloro(l,3)butadiene in rats. In: Hayes AW, Schnell RC, Miya TS, eds. Developments in the Science and Practice of Toxicology, Elsevier Science Publishers, 411-414.

*Reichert D, Schutz S. 1986. Mercapturic acid formation is an activation and intermediary step in the metabolism of hexachlorobutadiene. Biochem Pharmacol 35 : 1271-1275.

*Reichert D, Neudecker T, Schutz S. 1984. Mutageneicity of hexachlorobutadiene, perchlorobutenoic acid and perchlorobutenoic acid chloride. Mutat Res 137: 89-93.

*Reichert D, Neudecker T, Spengler U. 1983. Mutagenicity of dichloroacetylene and its degradation products trichloroacetyl chloride, trichloroacryloyl chloride and hexachlorobutadiene. Mutat Res 117:21-29.

*Reichert D, Schutz S, Metzler M. 1985. Excretion pattern and metabolism of hexachlorobutadiene in the rats: Evidence for metabolic activation by conjugation reactions. Biochem Pharmacol 34:499-505.

Rodriguez OM, Desideri PG, Lepri L, et al. 1991. Simultaneous separation and determination of hydrocarbons and organochlorine compounds by using a two-step microcolumn. J Chromatogr 555:221-228.

## 8. REFERENCES

*Roldan-Arjona T, Garcia-Pedrajas MD, Luque-Romero FL, et al. 1991. An association between mutagenicity of the Ara test of *salmonella typhimurium* and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons. Mutagenesis 6: 199-205.

Rosenfeld JK, Plumb RH. 1991. Ground water contamination at wood treatment facilities. Ground Water Monitoring Review XI: 133-140.

*Ruth JH. 1986. Odor thresholds and irritation levels of several chemical substances: A review. J Am Ind Hyg Assoc 47:A-142-A151.

*Saillenfait AM, Bonnet P, Guenier JP, et al. 1989. Inhalation teratology study on hexachloro-1,3-butadiene in rats. Toxicol Lett 47:235-240.

*Sauer TC. 1981. Volatile organic compounds in open ocean and coastal surface waters. Organic Geochemistry 3:91-101.

*Sax NI, Lewis RJ. 1989. Dangerous properties of industrial materials. 7th ed. New York, NY: Van Nostrand Reinhold, p. 2687.

“Schiffmann D, Reichert D, Henschler D. 1984. Induction of morphological transformation and unscheduled DNA synthesis in Syrian hamster embryo fibroblasts by hexachlorobutadiene and its putative metabolite pentachlorobutenoic acid. Cancer Lett 23 :297-305.

*Schnellmann RG, Lock EA, Mandel LJ. 1987. A mechanism of S-(1,2,3,4,4-pentachloro-1,3-butadienyl)-L-cysteine toxicity to rabbit renal proximal tubules. Toxicol Appl Pharmacol 90:513-521.

Schrenk D, Dekant W. 1989. Short communication. Covalent binding of hexachlorobutadiene metabolites to renal and hepatic mitochondrial DNA. Carcinogenesis 10: 1139-l 141.

Schwartz BS, Ford DP, Bolla KI, et al. 1990. Solvent-associated decrements in olfactory function in paint manufacturing workers. Am J Ind Med 18:697-706.

## 8. REFERENCES

Schwetz BA, Norris JM, Kociba RJ. 1974. Reproduction study in Japanese quail fed hexachlorobutadiene for 90 days. Toxicol Appl Pharmacol 30:255-265.

*Schwetz BA, Smith FA, Humiston CG. 1977. Results of a reproduction study in rats fed diets containing hexachlorobutadiene. Toxicol Appl Pharmacol 42: 387-398.

*Shah JJ, Heyerdahl EK. 1988. National ambient volatile organic compounds (VOCs) data base update. EPA/600/3-38/010a.

*Shah JJ, Singh HB. 1988. Distribution of volatile organic chemicals in outdoor and indoor air. Environ Sci Technol 22: 1381-1388.

Shen TT. 1982. Air quality assessment for land disposal of industrial wastes. Environ Management 6:297-305.

*Singh HB, Sales LJ, Smith A, et al. 1980. Atmospheric measurements of selected hazardous organic chemicals. Menlo Park, CA: SRI International. Project No. 7774:6.

*Singh HB, Sales LJ, Stiles RE. 1982. Distribution of selected gaseous organic mutagens and suspect carcinogens in ambient air. Environ Sci Technol 16:872-880.

*Stanker LH, Watkins B, Vanderlaan M, et al. 1990. Analysis of heptachlor and related cyclodiene insecticides in food products. In: ACS Symposium Series 451. Immunoassays for trace chemical analysis. Washington, DC; American Chemical Society, 108-123.

*Staples CA, Werner AF, Hoogheem TJ. 1985. Assessment of priority pollutant concentrations in the United States using Storet database. Environ Toxicol Chem 4: 131-142.

Stein VB, Narang RS. 1984. Chlorinated compounds and phenols in tissue, fat, and blood from rats fed industrial waste site soil extract: methods and analysis. J Assoc Off Anal Chem 67:111-116.

Stonard MD, Gore CW, Oliver JA, et al. 1987. Urinary enzymes and protein patterns as indicators of injury to different regions of the kidney. Fund Appl Toxicol 9:339-351.

## 8. REFERENCES

*Stott WT, Quast JF, Watanabe PG. 1981. Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. Toxicol Appl Pharmacol 60:287-300.

*Tabak HH, Quave SA, Mashni CI, et al. 1981. Biodegradability studies with organic priority pollutant compounds. J Water Pollut Control Fed 53: 1503-1518.

Theiss JC, Stoner GD, Shimkin MB, et al. 1977. Test for carcinogenicity of United States drinking waters by pulmonary tumor response in strain A mice. Cancer Res 37:2717-2720.

Thomann RV. 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ Sci Technol23:699-707.

*TRI90. 1992. Toxic Chemical Release Inventory. National Library of Medicine, National Toxicology Information Program, Bethesda, MD.

U.S. Air Force. 1988. Interactions amond drinking and ground water contaminants on renal and hepatic function. Washington, DC. AFOSR-TR-88-0717.

*Vamvakas S, Kordowich FJ, Dekant W, et al. 1988a. Mutagenicity of hexachloro-1,3-butadiene and its S-conjugates in the Ames test-role of activation by the mercapturic acid pathway in its nephrocarcinogenicity. Carcinogenesis 9:907-910.

*Vamvakas S, Muller DA, Dekant W, et al. 1988b. DNA-binding of sulfur-containing metabolites from $^{35}$S-(pentachlorobutadienyl)-L-cysteine in bacteria and isolated renal tubular cells. Drug Metabolic Interact 6: 349-358.

Van de Meent D, den Hollander HA, Pool WG, et al. 1986. Organic micropollutants in Dutch coastal waters. Wat Sci Tech 18:73-81.

*Van Duuren BL, Goldschmidt BM, Loewengart G, et al. 1979. Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. JNCI 63: 1433-1439.

## 8. REFERENCES

*Verschueren K. 1983. Handbook of environmental data on organic chemicals. Van Nostrand Reinhold Company, New York, NY: pp. 717-718.

*Walters SM. 1990. Clean-up techniques for pesticides in fatty foods. Anal Chim Acta 236:77-82.

*Wang F, Ji Z, Wang D. 1991. A rapid pretreatment procedure for determining trace organochlorine compounds in biological samples by capillary gas chromatography. Microchemical Journal 44 : 67-7 1.

*Wild D, Schutz S, Reichert D. 1986. Mutagenicity of the mercapturic acid and other S-containing derivatives of hexachloro-1,3-butadiene. Carcinogenesis 7:431-434.

*Wolf CR, Berry PN, Nash JA, et al. 1984. Role of microsomal and cytosolic glutathione Stransferases in the conjugation of hexachloro-1:3-butadiene and its possible relevance to toxicity. J Pharmacol Exp Ther 228 : 202-208.

*Yang RS. 1988. Hexachloro-1,3-butadiene: toxicology, metabolism, and mechanisms of toxicity. Rev Environ Contam Toxicol 101: 121-137.

*Yang RS, Abdo KM, Elwell MR. 1989. Subchronic toxicology studies of hexachloro-1,3-butadiene (HCBD) in B6C3$F_1$ mice by dietary incorporation. JEPTO 9:323-332.

*Yip G. 1976. Survey of hexachloro-1,3-butadiene in fish, eggs, milk, and vegetables. J Assoc Off Anal Chem 59:559-561.

*Yurawecz MP, Dreifuss PA, Kamps LR. 1976. Determination of hexachloro-1,3-butadiene in spinach, eggs, fish, and milk by electron capture gas-liquid chromatography. J Assoc Off Anal Chem 59:552-558.

*Zoeteman BC, Harmsen K, Linders JB, et al. 1980. Persistent organic pollutants in river water and groundwater in the Netherlands. Chemosphere 9:231-249.

## 9. GLOSSARY

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

**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 a 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.

**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.

**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.

**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.

**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.

# 9. GLOSSARY

**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 occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

**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.

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

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

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

***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 which 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 is expected to have caused death in humans or animals.

# 9. GLOSSARY

**Lethal Dose$_{(50)}$ (LD$_{50}$) -** The dose of a chemical which 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 dose 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.

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

**Minimal Risk Level -** An estimate of daily human exposure to a dose of a chemical that is likely to be without an appreciable risk of adverse noncancerous effects over a specified duration of exposure.

**Mutagen -** A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

**Neurotoxicity -** The occurrence of adverse effects on the nervous system following exposure to chemical.

**No-Observed-Adverse-Effect Level (NOAEL) -** The dose of 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.

**Permissible Exposure Limit (PEL) -** An allowable exposure level in workplace air averaged over an 8-hour shift.

# 9. GLOSSARY

**$q_1$* -** 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 $\mu g/m^3$ for air).

**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 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 CERCLA. Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 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.

**Short-Term Exposure Limit (STEL) -** The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

**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.

# 9. GLOSSARY

**Threshold Limit Value (TLV) -** A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

**Time-Weighted Average (TWA) -** An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

**Toxic Dose ($TD_{50}$) -** 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.

**Uncertainty Factor (UF) -** A factor used in operationally deriving the RfD 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 LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

# APPENDIX A

# USER'S GUIDE

## Chapter 1

### Public Health Statement

This chapter of the profile is a health effects summary written in nontechnical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or substance 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 substance.

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

### Tables and Figures for Levels of Significant Exposure (LSE)

Tables (2-1, 2-2, and 2-3) and figures (2-l and 2-2) are used to summarize health effects by duration of exposure and end point and to illustrate graphically levels of exposure associated with those effects. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs) for Less Serious and Serious health effects, or Cancer Effect Levels (CELs). In addition, these tables and figures illustrate differences in response by species, Minima1 Risk Levels (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. The LSE tables and figures can be used 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.

The legends presented below demonstrate the application of these tables and figures. A representative example of LSE Table 2-l and Figure 2-l are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

### LEGEND

**See LSE Table 2-1**

(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. 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 Table 2-1, 2-2, and 2-3, respectively). LSE figures are limited to the inhalation (LSE Figure 2-1) and oral (LSE Figure 2-2) routes.

(2). Exposure Duration Three exposure periods: acute (14 days or less); intermediate (15 to 364 days); and chronic (365 days or more) are presented within each route of exposure. In this example, an inhalation study of intermediate duration exposure is reported.

APPENDIX A

(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.

(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 define a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in Figure 2-l).

(5) Species The test species, whether animal or human, are identified in this column. Species

(6) Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen 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 [substance x] via inhalation for 13 weeks, 5 days per week, for 6 hours per day.

(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 in this study.

(8) NOAEL A No-Observed-Adverse-Effect Level (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 Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest exposure level used in the study that caused a hannful 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 "Less Serious" respiratory effect reported in key number 18 (hyperplasia) occurred at a LOAEL of 10 ppm.

(10) Reference The complete reference citation is given in Chapter 8 of the profile.

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiological studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses which did not cause a measurable increase in cancer.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

**LEGEND**

**See LSE Figure 2-1**

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure levels for particular exposure duration.

**TABLE 2-1. Levels of Significant Exposure to [Chemical x] - Inhalation**

| Key to figure[a] | Species | Exposure frequency/ duration | System | NOAEL (ppm) | LOAEL (effect) Less serious (ppm) | LOAEL (effect) Serious (ppm) | Reference |
|---|---|---|---|---|---|---|---|
| INTERMEDIATE EXPOSURE | | | | | | | |
| Systemic | | | | | | | |
| 18 | Rat | 13 wk 5d/wk 6hr/d | Resp | 3[b] | 10 (hyperplasia) | | Nitschke et al. 1981 |
| CHRONIC EXPOSURE | | | | | | | |
| Cancer | | | | | | | |
| 38 | Rat | 18 mo 5d/wk 7hr/d | | | | 20 (CEL, multiple organs) | Wong et al. 1982 |
| 39 | Rat | 89-104 wk 5d/wk 6hr/d | | | | 10 (CEL, lung tumors, nasal tumors) | NTP 1982 |
| 40 | Mouse | 79-103 wk 5d/wk 6hr/d | | | | 10 (CEL, lung tumors, hemangiosarcomas) | NTP 1982 |

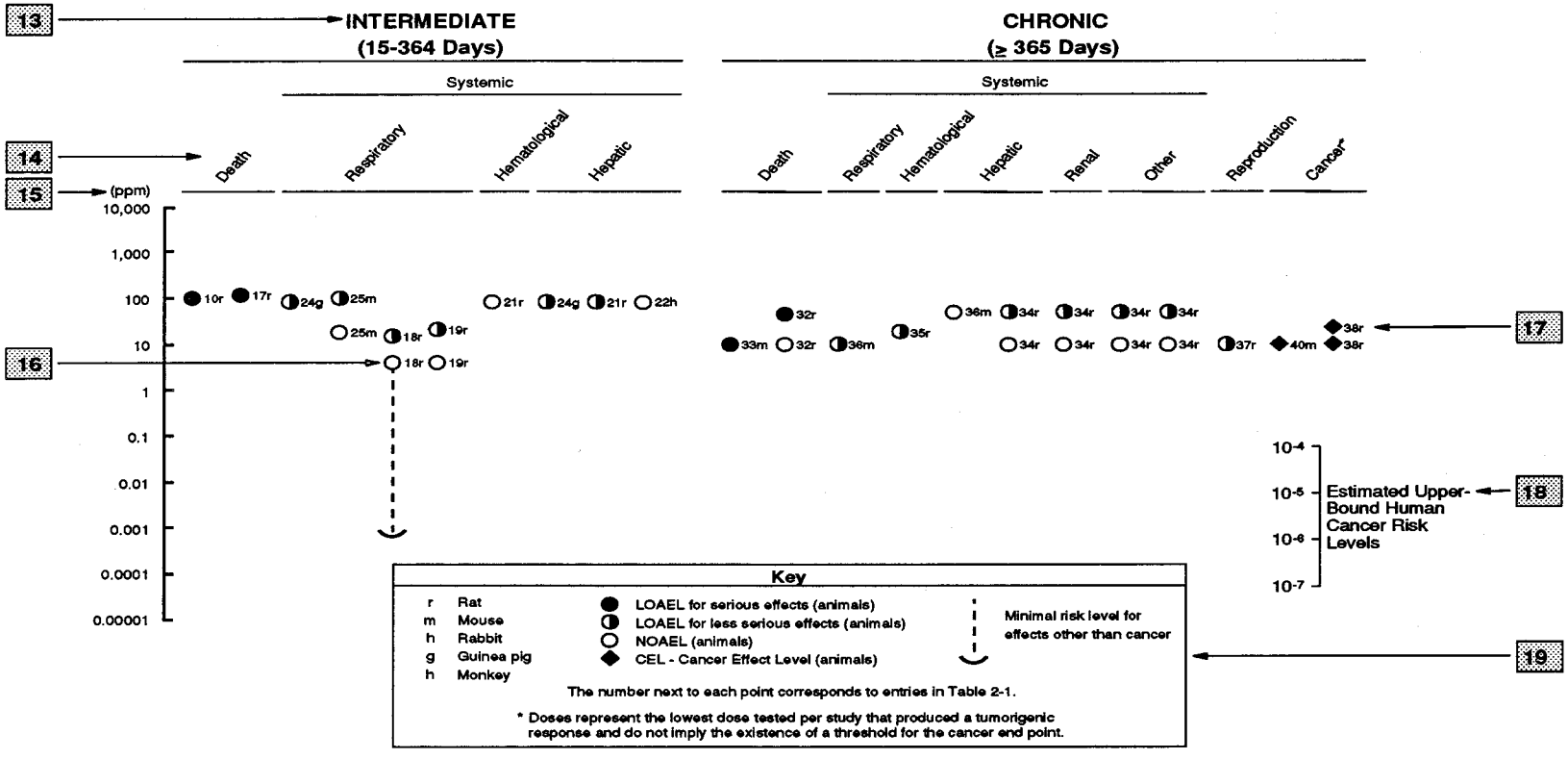
[a] The number corresponds to entries in Figure 2-1.

[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x $10^{-3}$ 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).

CEL = cancer effect level; d = day(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

SAMPLE

## FIGURE 2-1. Levels of Significant Exposure to [Chemical X] - Inhalation



(13) Exposure Duration The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14) Health Effect These are the categories of health effects for which reliable quantitative data exist. The same health effects appear in the LSE table.

(15) Levels of Exposure Exposure levels for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure levels are reported on the log scale "y" axis. Inhalation exposure is reported in mg/m3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, 18r NOAEL is the critical end point for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates a NOAEL for the test species (rat). 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 38r is one of three studies for which Cancer Effect Levels (CELs) were derived. The diamond symbol refers to a CEL for the test species (rat). 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 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.

**Chapter 2 (Section 2.4)**

**Relevance to Public Health**

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicological, epidemiological, 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 section discusses health effects by end point. Human data are presented first, then animal data. Both are organized by route of exposure (inhalation, oral, and dermal) and by duration (acute, intermediate, and chronic*)*. *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

APPENDIX A

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. MRLs for noncancer end points if derived, and the end points from which they were derived are indicated and discussed in the appropriate section(s).

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Identification of Data Needs section.

**Interpretation of Minimal Risk Levels**

Where sufficient toxicologic information was available, MRLs were derived. MRLs are specific for route (inhalation or oral) and duration (acute, intermediate, or chronic) of exposure. Ideally, MRLs can be derived from all six exposure scenarios (e.g., Inhalation - acute, -intermediate, -chronic; Oral - acute, -intermediate, - 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. They should help physicians and public health officials determine the safety of a community living near a substance emission, given the concentration of a contaminant in air or the estimated daily dose received via food or water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicological information on which the number is based. Section 2.4, “Relevance to Public Health,” contains basic information known about the substance. Other sections such as 2.6, “Interactions with Other Chemicals” and 2.7, “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 used by the Environmental Protection Agency (EPA) (Barnes and Dourson 1988; EPA 1989a) to derive reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the 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 effects (e.g., systemic, neurological, and developmental). In order to compare NOAELs and LOAELs for specific end points, all inhalation exposure levels are adjusted for 24hr exposures and all intermittent exposures for inhalation and oral routes of intermediate and chronic duration are adjusted for continuous exposure (i.e., 7 days/week). If the 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 NOAEL that does not exceed any adverse effect levels. The NOAEL is the most suitable end point for deriving an MRL. When a NOAEL is not available, a Less Serious LOAEL can be used to derive an MRL, and an uncertainty factor of (1, 3, or 10) is employed. MRLs are not derived from Serious LOAELs. Additional uncertainty factors of (1, 3, or 10 ) are used for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and (1, 3, or 10) are used for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. Generally an uncertainty factor of 10 is used; however, the MRL Workgroup reserves the right to use uncertainty factors of (1, 3, or 10) based on scientific judgement. The product is then divided into the adjusted 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 LSE Tables.

# APPENDIX B

# ACRONYMS, ABBREVIATIONS, AND SYMBOLS

| | |
|---|---|
| ACGIH | American Conference of Governmental Industrial Hygienists |
| ADME | Absorption, Distribution, Metabolism, and Excretion |
| atm | atmosphere |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| BCF | bioconcentration factor |
| BSC | Board of Scientific Counselors |
| C | Centigrade |
| CDC | Centers for Disease Control |
| CEL | Cancer Effect Level |
| CERCLA | Comprehensive Environmental Response, Compensation, and Liability Act |
| CFR | Code of Federal Regulations |
| CLP | Contract Laboratory Program |
| cm | centimeter |
| CNS | central nervous system |
| d | day |
| DHEW | Department of Health, Education, and Welfare |
| DHHS | Department of Health and Human Services |
| DOL | Department of Labor |
| ECG | electrocardiogram |
| EEG | electroencephalogram |
| EPA | Environmental Protection Agency |
| EKG | see ECG |
| F | Fahrenheit |
| $F_1$ | first filial generation |
| FAO | Food and Agricultural Organization of the United Nations |
| FEMA | Federal Emergency Management Agency |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| fpm | feet per minute |
| ft | foot |
| FR | Federal Register |
| g | gram |
| GC | gas chromatography |
| gen | generation |
| HPLC | high-performance liquid chromatography |
| hr | hour |
| IDLH | Immediately Dangerous to Life and Health |
| IARC | International Agency for Research on Cancer |
| ILO | International Labor Organization |
| in | inch |
| 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 |
| $LC_{Lo}$ | lethal concentration, low |
| $LC_{50}$ | lethal concentration, 50% kill |
| $LD_{Lo}$ | lethal dose, low |
| $LD_{50}$ | lethal dose, 50% kill |
| LOAEL | lowest-observed-adverse-effect level |
| LSE | Levels of Significant Exposure |
| m | meter |
| mg | milligram |
| min | minute |
| mL | milliliter |
| mm | millimeter |
| mmHg | millimeters of mercury |
| mmol | millimole |
| mo | month |
| mppcf | millions of particles per cubic foot |
| MRL | Minimal Risk Level |
| MS | mass spectrometry |
| NIEHS | National Institute of Environmental Health Sciences |
| NIOSH | National Institute for Occupational Safety and Health |
| NIOSHTIC | NIOSH's Computerized Information Retrieval System |
| ng | nanogram |
| nm | nanometer |
| NHANES | National Health and Nutrition Examination Survey |
| nmol | nanomole |
| NOAEL | no-observed-adverse-effect level |
| NOES | National Occupational Exposure Survey |
| NOHS | National Occupational Hazard Survey |
| NPL | National Priorities List |
| NRC | National Research Council |
| NTIS | National Technical Information Service |
| NTP | National Toxicology Program |
| OSHA | Occupational Safety and Health Administration |
| PEL | permissible exposure limit |
| pg | picogram |
| pmol | picomole |
| PHS | Public Health Service |
| PMR | proportionate mortality ratio |
| ppb | parts per billion |
| ppm | parts per million |
| ppt | parts per trillion |
| REL | recommended exposure limit |
| RfD | Reference Dose |
| RTECS | Registry of Toxic Effects of Chemical Substances |
| sec | second |
| SCE | sister chromatid exchange |
| SIC | Standard Industrial Classification |
| SMR | standard mortality ratio |

APPENDIX B

| | |
|---|---|
| STEL | short term exposure limit |
| STORET | STORAGE and RETRIEVAL |
| TLV | threshold limit value |
| TSCA | Toxic Substances Control Act |
| TRI | Toxics Release Inventory |
| TWA | time-weighted average |
| U.S. | United States |
| UF | uncertainty factor |
| yr | year |
| WHO | World Health Organization |
| wk | week |

| | |
|---|---|
| $>$ | greater than |
| $\geq$ | greater than or equal to |
| $=$ | equal to |
| $<$ | less than |
| $\leq$ | less than or equal to |
| % | percent |
| $\alpha$ | alpha |
| $\beta$ | beta |
| $\delta$ | delta |
| $\gamma$ | gamma |
| $\mu$m | micron |
| $\mu$g | microgram |

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