TOXICOLOGICAL PROFILE FOR
BENZENE

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

September 1997

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UPDATE STATEMENT

A Toxicological Profile for benzene was released in April 1993. This edition supersedes any previously released draft or final profile.

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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FOREWORD

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

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

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

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance 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; and

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

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

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

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*Legislative Background

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed 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 EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on April 29, 1996 (61 FR 18744). 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); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); and February 28, 1994 (59 FR 9486). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Green Border Review. Green Border review assures 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 end points.

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.

PEER REVIEW

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

1. Dr. Rogene Henderson, Biochemist, Lovelace Inhalation Toxicology Research Institute, Kirtland AFB, Albuquerque, New Mexico;

2. Dr. Carroll Snyder, Director, Laboratory of Inhalation Carcinogenesis and Toxicity, A.J. Lanza Laboratories, New York University, Tuxedo, New York; and

3. Dr. Gisela Witz, Professor, University of Medicine & Dentistry New Jersey (UMDNJ), Robert Wood Johnson Medical School, Department of Environmental and Community Medicine, Piscataway, New Jersey.

These experts collectively have knowledge of benzene's physical and chemical properties, toxico-kinetics, 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 public health statement tells you about benzene and the effects of exposure.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are the sites targeted for long-term federal cleanup. Benzene has been found in at least 816 of the 1,428 current or former NPL sites. However, it's unknown how many NPL sites have been evaluated for this substance. As more sites are evaluated, the sites with benzene may increase. This information is important because exposure to this substance may harm you and because these sites may be sources of exposure.

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 are exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance or by skin contact.

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

1.1   WHAT IS BENZENE?

Benzene, also known as benzol, is a colorless liquid with a sweet odor. Benzene evaporates into air very quickly and dissolves slightly in water. Benzene is highly flammable. Most people can begin to smell benzene in air at 1.5-4.7 parts of benzene per million parts of air (ppm) and smell benzene in water at 2 ppm. Most people can begin to taste benzene in water at 0.5-4.5 ppm. Benzene is found in air, water, and soil.

Benzene found in the environment is from both human activities and natural processes. Benzene was first discovered and isolated from coal tar in the 1800s. Today, benzene is made mostly from petroleum sources. Because of its wide use, benzene ranks in the top 20 in production volume for chemicals produced in the United States. Various industries use benzene to make other chemicals, such as styrene (for Styrofoam ® and other plastics), cumene (for various resins), and cyclohexane (for nylon and synthetic fibers). Benzene is also used for the manufacturing of some types of rubbers, lubricants, dyes, detergents, drugs, and pesticides. Natural sources of benzene, which include volcanoes and forest fires, also contribute to the presence of benzene in the environment. Benzene is also a part of crude oil and gasoline and cigarette smoke. For more information on the nature and uses of benzene, see Chapters 3 and 4.

1.2   WHAT HAPPENS TO BENZENE WHEN IT ENTERS THE ENVIRONMENT?

Benzene is commonly found in the environment. Industrial processes are the main sources of benzene in the environment. Benzene levels in the air can increase from emissions from burning coal and oil, benzene waste and storage operations, motor vehicle exhaust, and evaporation from gasoline service stations. Since tobacco smoke contains high levels of benzene, tobacco smoke is another source of benzene in air. Industrial discharge, disposal of products containing benzene, and gasoline leaks from underground storage tanks can release benzene into water and soil.

Benzene can pass into air from water and soil surfaces. Once in the air, benzene reacts with other chemicals and breaks down within a few days. Benzene in the air can attach to rain or snow and be carried back down to the ground.

Benzene in water and soil breaks down more slowly. Benzene is slightly soluble in water and can pass through the soil into underground water. Benzene in the environment does not build up in plants or animals. For more information on what happens to benzene after it gets into the environment, see Chapters 4 and 5.

1.3   HOW MIGHT I BE EXPOSED TO BENZENE?

Most people are exposed to a small amount of benzene on a daily basis. You can be exposed to benzene in the outdoor environment, in the workplace, and in the home. Exposure of the general population to benzene is mainly through breathing air that contains benzene. The major sources of benzene exposure are tobacco smoke, automobile service stations, exhaust from motor vehicles, and industrial emissions. Vapors (or gases) from products that contain benzene, such as glues, paints, furniture wax, and detergents can also be a source of exposure. Auto exhaust and industrial emissions account for about 20% of the total nationwide exposure to benzene. About 50% of the entire nationwide exposure to benzene results from smoking tobacco or from exposure to tobacco smoke. The average smoker (32 cigarettes per day) takes in about 1.8 milligrams (mg) of benzene per day. This is about 10 times the average daily intake of nonsmokers.

Background levels of benzene in air range from 2.8 to 20 parts of benzene per billion parts of air (ppb) (1 ppb is 1,000 times less than 1 ppm and equals about 3 micrograms of benzene in a cubic meter of air [$\mu g/m^3$]). People living in cities or industrial areas are generally exposed to higher levels of benzene in air than those living in rural areas. Benzene levels in the home are usually higher than outdoor levels. People living around hazardous waste sites, petroleum refining operations, petrochemical manufacturing sites, or gas stations may be exposed to higher levels of benzene in air.

For most people, the level of exposure to benzene through food, beverages, or drinking water is not as high as through air. Typical drinking water contains less than 0.1 ppb benzene. Benzene has been detected in some bottled water, liquor, and food. Leakage from underground gasoline storage tanks or from landfills and hazardous waste sites containing benzene can result in benzene contamination of well water. People with benzene-contaminated tap water can be exposed from drinking the water or eating foods prepared with the water. In addition, exposure can result from breathing in benzene while showering, bathing, or cooking with contaminated water.

Individuals employed in industries that make or use benzene may be exposed to the highest levels of benzene. As many as 238,000 people may be occupationally exposed to benzene in the United States. These industries include benzene production (petrochemicals, petroleum refining, and coke and coal chemical manufacturing), rubber tire manufacturing, and storage or transport of benzene and petroleum products containing benzene. Other workers who may be exposed to benzene because of their occupations include steel workers, printers, rubber workers, shoe makers, laboratory technicians, firefighters, and gas station employees. For more information on how you might be exposed to benzene, see Chapter 5.

1.4 HOW CAN BENZENE ENTER AND LEAVE MY BODY?

Benzene can enter your body through your lungs when you breathe contaminated air. It can also enter through your stomach and intestines when your eat food or drink water that contains benzene. Benzene can enter your body through skin contact with benzene-containing products such as gasoline.

When you are exposed to high levels of benzene in air, about half of the benzene you breathe in leaves your body when you breathe out. The other half passes through the lining of your lungs and enters your bloodstream. Animal studies show that benzene taken in by eating or drinking contaminated foods behaves similarly in the body to benzene that enters through the lungs. A small amount will enter your body by passing through your skin and into your bloodstream during skin contact with benzene or benzene-containing products. Once in the bloodstream, benzene travels throughout your body and can be temporarily stored in the bone marrow and fat. Benzene is converted to products, called metabolites, in the liver and bone marrow. Some of the harmful effects of benzene exposure are believed to be caused by these metabolites. Most of the metabolites of benzene leave the body in the urine within 48 hours after exposure. For more information on how benzene can enter and leave your body, see Chapter 2.

1.5   HOW CAN BENZENE AFFECT MY HEALTH?

To protect the public from the harmful effects of toxic chemicals and to find ways to treat people who have been harmed, scientists use many tests.

One way to see if a chemical will hurt people is to learn how the chemical is absorbed, used, and released by the body; for some chemicals, animal testing may be necessary. Animal testing may also be used to identify health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method to get information needed to make wise decisions to protect public health. Scientists have the responsibility to treat research animals with care and compassion. Laws today protect the welfare of research animals, and scientists must comply with strict animal care guidelines.

After exposure to benzene, several factors determine whether harmful health effects will occur and if they do, what the type and severity of these health effects might be. These factors include the amount of benzene to which you are exposed and the length of time of the exposure. Most data involving effects of long-term exposure to benzene are from studies of workers employed in industries that make or use benzene. These workers were exposed to levels of benzene in air far greater than the levels normally encountered by the general population. Current levels of benzene in workplace air are much lower than in the past. Because of this reduction, and the availability of protective equipment such as respirators, fewer workers have symptoms of benzene poisoning.

Brief exposure (5-10 minutes) to very high levels of benzene in air (l0,000-20,000 ppm) can result in death. Lower levels (700-3,000 ppm) can cause drowsiness, dizziness, rapid heart rate, headaches, tremors, confusion, and unconsciousness. In most cases, people-will stop feeling these effects when they stop being exposed and begin to breathe fresh air.

Eating foods or drinking liquids containing high levels of benzene can cause vomiting, irritation of the stomach, dizziness, sleepiness, convulsions, rapid heart rate, coma, and death. The health effects that may result from eating foods or drinking liquids containing lower

levels of benzene are not known. If you spill benzene on your skin, it may cause redness and sores. Benzene in your eyes may cause general irritation and damage to your cornea.

Benzene causes problems in the blood. People who breathe benzene for long periods may experience harmful effects in the tissues that form blood cells, especially the bone marrow. These effects can disrupt normal blood production and cause a decrease in important blood components. A decrease in red blood cells can lead to anemia. Reduction in other components in the blood can cause excessive bleeding. Blood production may return to normal after exposure to benzene stops. Excessive exposure to benzene can be harmful to the immune system, increasing the chance for infection and perhaps lowering the body's defense against cancer.

Benzene can cause cancer of the blood-forming organs. The Department of Health and Human Services (DHHS) has determined that benzene is a known carcinogen. The International Agency for Cancer Research (IARC) has determined that benzene is carcinogenic to humans, and the EPA has determined that benzene is a human carcinogen. Long-term exposure to relatively high levels of benzene in the air can cause cancer of the blood-forming organs. This condition is called leukemia. Exposure to benzene has been associated with development of a particular type of leukemia called acute myeloid leukemia (AML).

Exposure to benzene may be harmful to the reproductive organs. Some women workers who breathed high levels of benzene for many months had irregular menstrual periods. When examined, these women showed a decrease in the size of their ovaries. However, exact exposure levels were unknown, and the studies of these women did not prove that benzene caused these effects. It is not known what effects exposure to benzene might have on the developing fetus in pregnant women or on fertility in men. Studies with pregnant animals show that breathing benzene has harmful effects on the developing fetus. These effects include low birth weight, delayed bone formation, and bone marrow damage.

The health effects that might occur in humans following long-term exposure to food and water contaminated with benzene are not known. In animals, exposure to food or water contaminated with benzene can damage the blood and the immune system and can even cause cancer. See Chapter 2 for more information on the health effects resulting from benzene exposure.

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

Several tests can show if you have been exposed to benzene. Some of these tests may be available at your doctor's office. All of these tests are limited in what they can tell you. The test for measuring benzene in your breath must be done shortly after exposure. This test is not very helpful for detecting very low levels of benzene in your body. Benzene can be measured in your blood. However, since benzene disappears rapidly from the blood, measurements may be accurate only for recent exposures. In the body, benzene is converted to products called metabolites. Certain metabolites of benzene, such as phenol, muconic acid, and S-phenyl-N-acetyl cysteine (PhAC) can be measured in the urine. The amount of phenol in urine has been used to check for benzene exposure in workers. The test is useful only when you are exposed to benzene in air at levels of 10 ppm or greater. However, this test must also be done shortly after exposure, and it is not a reliable indicator of how much benzene you have been exposed to, since phenol is present in the urine from other sources (diet, environment). Measurement of muconic acid or PhAC in the urine is a more sensitive and reliable indicator of benzene exposure. The measurement of benzene in blood or of metabolites in urine cannot be used for making predictions about whether you will experience any harmful health effects. Measurement of all parts of the blood and measurement of bone marrow are used to find benzene exposure and its health effects.

For people exposed to relatively high levels of benzene, complete blood analyses can be used to monitor possible changes related to exposure. However, blood analyses are not useful when exposure levels are low. For more information on tests for benzene exposure, see Chapters 2 and 6.

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

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

Regulations and recommendations can be expressed in not-to-exceed levels in air, water, soil,
or food that are usually based on levels that affect animals, then they are adjusted to help
protect people. Sometimes these not-to-exceed levels differ among federal organizations
because of different exposure times (an 8-hour workday or a 24-hour day), the use of
different animal studies, or other factors.

Recommendations and regulations are also periodically updated as more information becomes
available. For the most current information, check with the federal agency or organization
that provides it. Some regulations and recommendations for benzene include the following:

EPA has set the maximum permissible level of benzene in drinking water at 5 parts per
billion (ppb). Because benzene can cause leukemia, EPA has set a goal of 0 ppb for benzene
in drinking water and in water such as rivers and lakes. EPA estimates that 10 ppb benzene
in drinking water that is consumed regularly or exposure to 0.4 ppb benzene in air over a
lifetime could cause a risk of one additional cancer case for every 100,000 exposed persons.
EPA recommends a maximum permissible level of benzene in water of 200 ppb for shortterm
exposures (10 days) for children.

EPA requires that the National Response Center be notified following a discharge or spill into the environment of 10 pounds or more of benzene.

The Occupational Safety and Health Administration (OSHA) regulates levels of benzene in the workplace. The maximum allowable amount of benzene in workroom air during an 8-hour workday, 40-hour workweek is 1 part per million (ppm). Since benzene can cause cancer, the National Institute for Occupational Safety and Health (NIOSH) recommends that all workers likely to be exposed to benzene wear special breathing equipment (NIOSH 1974). For more information on federal regulations, see 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, Mails top E-29
> Atlanta, GA 30333

> * Information line and technical assistance

> Phone: (404) 639-6000
> Fax: (404) 639-6315 or 6324

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

* <u>To order toxicological profiles, contact:</u>

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Phone: (800) 553-6847 or (703) 487-4650

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

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for benzene. 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 derrnal 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 B). 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

Occupational or environmental exposure to benzene or benzene-containing materials usually occurs through the inhalation or dermal route. The main route of exposure is considered to be inhalation. Studies regarding human occupational or environmental inhalation exposure, in addition to experimental animal studies, are discussed in this section (Section 2.2.1).

2.2.1.l   Death

Case reports of fatalities due to acute benzene exposures have appeared in the literature since the early 1900s (Cronin 1924; Greenburg 1926; Hamilton 1922). Deaths occurred suddenly or within several hours after exposure (Avis and Hutton 1993; Cronin 1924; Greenburg 1926; Hamilton 1922; Winek et al. 1967). The benzene concentrations encountered by the victims were not often known. However, it has been estimated that 5-10 minutes of exposure to 20,000 ppm benzene in air is usually fatal (Flury 1928). Lethality in humans has been attributed to asphyxiation, respiratory arrest, central nervous system depression, or suspected cardiac collapse (Avis and Hutton 1993; Hamilton 1922; Winek and Collom 1971; Winek et al. 1967). Cyanosis, hemolysis, and congestion or hemorrhage of organs were reported in the cases for which there were autopsy reports (Avis and Hutton 1993; Greenburg 1926; Hamilton 1922; Winek et al. 1967). Death from various causes, including cancer, has been associated with chronic inhalation exposure to benzene (Li et al. 1994; Paxton et al. 1994a). In the study by Li et al. (1994), which was conducted during 1972-87 and was comprised of 74,828 benzene-exposed workers employed in 672 factories and 35,805 unexposed workers from 109 factories located in 12 cities in China, there were no significant differences found in the relative risks for total mortality and cancer mortality between male and female workers exposed to benzene. Relative risk estimates for total mortality were slightly higher for men than for women in the specific occupational categories but were not found to be significant in total mortality observed.

In animals, acute inhalation exposure to high concentrations of benzene has caused death. An inhalation LC_{50} value for rats was calculated as 13,700 ppm for a 4-hour exposure (Drew and Fouts 1974). Additionally, 4 of 6 rats died following a 4-hour exposure to 16,000 ppm benzene (Smyth et al. 1962). However, in a study by Green et al. (1981b), male CD-l mice exposed by inhalation to doses of benzene up to 4,862 ppm, 6 hours per day for 5 days showed no lethality. Lower doses (up to 400 ppm) for longer periods of time (2 weeks) did not cause death in mice (Cronkite et al. 1985). Lethality in monkeys and cats exposed to unspecified concentrations has been ascribed to ventricular fibrillation due to increased release of adrenaline (Nahum and Hoff 1934). Exposure of rabbits to 45,000 ppm of benzene for approximately 30 minutes caused narcosis that was followed by the death of all exposed animals (Carpenter et al. 1944). Furthermore, early deaths of rats and mice have occurred from intermediate and chronic exposure to air concentrations of 200 or 300 ppm of benzene in cancer studies (Cronkite et al. 1989; Farris et al. 1993; Maltoni et al. 1982a, 1983). Intermediate exposures (6 hours per day, 5 days per week for 50 days) of male CD-l mice to benzene at doses of 9.6 ppm caused no increase in mortality, although mice exposed to 302 ppm benzene under the same regimen for a total of 26 weeks showed mortality approaching 50% (Green et al. 1981b). Mortality was observed in 97% of the CBA/Ca mice exposed to 300 ppm benzene for 16 weeks, as compared to 20% mortality in sham-exposed mice (Cronkite 1986). In Sprague-Dawley rats that received 300 ppm benzene vapor for 6 hours per day, 5 days a week for 691 days, the calculated median survival time was shown to be 51 weeks as compared to 65 weeks for controls (Snyder et al. 1978a). However, Snyder et al. (1984) reported a median survival time of 546 days for male Sprague-Dawley rats exposed to 100 ppm benzene for 5 days per week, 6 hours per day for life, compared to 560 days for air-exposed controls. It is not clear whether the difference in survival was significant or due to benzene exposure since both controls and exposed rats experienced early mortality from respiratory infections. Companion studies were also conducted with AKR and C57BL mice exposed to 300 ppm benzene (Snyder et al. 1978a, 1980). The calculated median survival time for AKR and C57BL mice exposed to 300 ppm benzene was shown to be 11 and 41 weeks compared to 39 and 75 weeks, respectively, for controls. For AKR mice exposed to 100 ppm, the calculated median survival time was 39 weeks (number of deaths not shown) as compared to 41 weeks for controls (Snyder et al. 1980).

The LC_{50} value and all reliable LOAEL values for each species and duration category are recorded in Table 2- 1 and plotted in Figure 2- 1.

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation

Key to figure [a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
ACUTE EXPOSURE							
Death							
1	Human	1 d 5-10 min				20000 (death)	Flury 1928
2	Rat (Sprague-Dawley)	4 hr				13700 (LC₅₀)	Drew and Fouts 1974
3	Rat (NS)	4 hr				16000 (4/6 died)	Smyth et al. 1962
4	Rabbit (NS)	3.7-36.2 min				45000 (death in 36.2 min)	Carpenter et al. 1944
Systemic							
5	Human	1-21 d 2.5 - 8 hr/d	Resp		60 M (mucous membrane irritation, dyspnea)		Midzenski et al. 1992
			Hemato			60 M (leukopenia, anemia, thrombocytopenia, MCV elevation)	
			Dermal		60 M (skin irritation)		
6	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d	Bd Wt	300 F	2200 F (decreased maternal body weight)		Green et al. 1978
7	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d	Bd Wt	10 F	50 F (decreased maternal body weight and weight gain)		Kuna and Kapp 1981
8	Rat (Wistar)	7 d 8 hr/d	Hemato	50 F	100 F (leukopenia)		Li et al. 1986
9	Rat (Wistar)	15 min	Cardio			3526 M (ventricular arrhythmia)	Magos et al. 1990

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
10	Rat (CFY)	Gd 7-14 24 hr/d	Hepatic	125 F			Tatrai et al. 1980a
			Bd Wt			125 F (decreased maternal weight gain of <22.08% of controls)	
11	Rat (CFY)	Gd 7-14 24 hr/d	Hepatic	47	141 F (increased relative liver weight)		Tatrai et al. 1980b
			Bd Wt		47 F (decreased maternal weight gain)		
12	Rat (Sprague- Dawley)	2 wk 5 d/wk 6 hr/d	Hemato	30	300 (decrease in leukocytes, males; decrease in lymphocytes)		Ward et al. 1985
13	Mouse (BALB/c)	7 d 6 hr/d	Hemato	47 M	211 M (depressed WBC count)		Aoyama 1986
			Bd Wt	47 M	211 M (16% decrease in body weight)		
14	Mouse (BALB/c)	14 d 6 hr/d	Hemato		48 M (depressed WBC count)		Aoyama 1986
			Bd Wt	48 M	208 M (18% decrease in body weight)		
15	Mouse (DBA/2)	2 wks 6 hr/d 5 d/wk	Hemato			300 M (hematocrit decreased by 26%, leukocytes decreased by 80%, bone marrow cellularity decreased by 93%)	Chertkov et al. 1992
			Bd Wt		300 M (15% decrease)		
16	Mouse (Hale- Stoner)	11 d 5 d/wk 6 hr/d	Hemato			400 M (decreased erythrocytes & leukocytes)	Cronkite et al. 1982

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
17	Mouse (C57B1/6 BNL)	2 wk 5 d/wk 6 hr/d	Hemato	25		100 (decreased hematocrit, hemolytic anemia)	Cronkite et al. 1985
18	Mouse (Hale-Stoner)	2 d 5 d/wk 6 hr/d	Hemato		400 M (decreased CFU-E cells)		Cronkite et al. 1989
19	Mouse (CBA/Ca BNL)	2 d 5 d/wk 6 hr/d	Hemato		3000 M (neutropenia; reduced cellularity)		Cronkite et al. 1989
20	Mouse (C57BL/6BNL)	8 d 6 hr/d	Hemato		3000 F (decreased marrow cellularity)		Cronkite et al. 1989
21	Mouse (DBA/2J)	5 d 6 h/d	Hemato		10 M (50% decrease in CFU-E numbers)		Dempster and Snyder 1991
22	Mouse (C57B1/6)	2-8 d 24 hr/d	Hemato			100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980
23	Mouse (CD-1)	5 d 6 hr/d	Hemato	9.9 M		103 M (decreased marrow cellularity; granulocytopenia, lymphocytopenia; decreased polymorphonucleucytes)	Green et al. 1981
			Bd Wt	4862 M			
24	Mouse (Swiss Webster, C57B1/6J)	2 wk 4 d/wk 6 h/d	Hemato			300 M (reduced bone marrow cellularity and CFU-E development)	Neun et al. 1992
25	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d	Hemato	300 F	900 F (CFU-E depression)		Plappert et al. 1994a

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
26	Mouse (C57Bl/6J)	6 d 6 hr/d	Hemato		10 M (depressed lymphocyte counts; elevated RBC's)		Rozen et al. 1984
27	Mouse (NMRI)	2 wk 5 d/wk 8 hr/d	Hemato	10.5 M	21 M (increased micronucleated polychromatic erythrocytes; decreased granulopoietic stem cells)		Toft et al. 1982
28	Mouse (NMRI)	1-10 d 24 hr/d	Hemato			21 M (reduced bone marrow cellularity; increased polychromatic erythrocytes; decreased granulopoietic stem cells)	Toft et al. 1982
29	Mouse (NMRI)	1 wk	Hemato	14 M			Toft et al. 1982
30	Mouse (CD-1)	2 wk 5 d/wk 6 hr/d	Hemato	30		300 (anemia, decreased hemoglobin,erythrocytes and hematocrit; hypoplasia of bone marrow; leukopenia)	Ward et al. 1985
			Bd Wt	300			
31	Mouse (Swiss-Webster)	5 d 6 hr/d	Hemato	3 M	25 M (decrease in WBC count)		Wells and Nerland 1991
32	Rabbit	Gd 7-20 24 hr/d	Bd Wt	156.5 F	313 F (reduced maternal weight gain)		Ungvary and Tatrai 1985

Immunological/Lymphoreticular

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	Less serious (ppm)	Serious (ppm)	Reference
33	Rat (Wistar)	7 d 8 hr/d		50 F	100 F (leukopenia; increased leukocyte alkaline phosphatase)		Li et al. 1986

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
34	Mouse (BALB/c)	7 d 6 hr/d			47 M (depressed T- and B-lymphocytes; decreased spleen weight and WBC count)		Aoyama 1986
35	Mouse (BALB/c)	14 d 6 hr/d			48 M (depressed T- and B-lymphocytes; decreased spleen & thymus weights and WBC count		Aoyama 1986
36	Mouse (DBA/2)	2 wks 6 hr/d 5 d/wk				300 M (leukocytes decreased by 80%, bone marrow cellularity decreased by 93%)	Chertkov et al. 1992
37	Mouse (CBA/Ca)	2 wk 5 d/wk 6 hr/d		10	25 (lymphopenia)		Cronkite 1986
38	Mouse (Hale- Stoner)	11 d 5 d/wk 6 hr/d				400 M (decreased bone marrow cellularity)	Cronkite et al. 1982
39	Mouse (C57B1/6 BNL)	2 wk 5 d/wk 6 hr/d		10	25 (lymphopenia)		Cronkite et al. 1985
40	Mouse (CBA/Ca BNL)	2 d 5 d/wk 6 hr/d			3000 M (decreased lymphocytes, CFU-S content in marrow)		Cronkite et al. 1989
41	Mouse (C57B1/6)	2-8 d 24 hr/d				100 M (leukopenia; decrease in marrow cellularity)	Gill et al. 1980
42	Mouse (CD-1)	5 d 6 hr/d		9.9 M		103 M (decreased femoral marrow and splenic cellularities; reduced splenic granulocytes)	Green et al. 1981

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
43	Mouse (Swiss Webster, C57B1/6J)	2 wk 4 d/wk 6 h/d				300 M (reduced bone marrow cellularity)	Neun et al. 1992
44	Mouse (Hybrid)	5 d 5 d/wk 6 hr/d		100 F	300 F (increased helper lymphocytes)		Plappert et al. 1994a
45	Mouse (C57BL/6)	1-12 d 6 hr/d		10 M	30 M (Listeria infection, T and B lymphocyte depression)		Rosenthal and Snyder 1985
46	Mouse (C57Bl/6J)	6 d 6 hr/d			10 [b] M (decreased circulating lymphocytes and mitogen-induced blastogenesis of femoral T and B-lymphocytes)		Rozen et al. 1984
47	Mouse (NMRI)	2 wk 5 d/wk 8 hr/d		10.5 M	21 M (decreased granulopoietic stem cells)		Toft et al. 1982
48	Mouse (NMRI)	1-10 d 24 hr/d			21 M (decreased granulopoietic stem cells)		Toft et al. 1982
49	Mouse (CD-1)	2 wk 5 d/wk 6 hr/d		30		300 (leukopenia; lymphopenia; splenic periateriolar lymphoid sheath depletion, lymphoid depletion of mesenteric lymph nodes; thymic atrophy; myeloid hypoplasia of femoral marrow)	Ward et al. 1985
50	Mouse (Swiss-Webster)	5 d 6 hr/d		3 M	25 M (decrease in spleen weight and WBC count)		Wells and Nerland 1991

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
Neurological							
51	Human	30 min			300 (drowsiness, dizziness, headaches)		Flury 1928
52	Human	1-21 d 2.5 - 8 hr/d			60 M (dizzyness, nausea, headache, peculiar or strong odor, chemical taste, fatigue)		Midzenski et al. 1992
53	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		300 F	2200 F (lethargy)		Green et al. 1978
54	Mouse (C57BL)	1-14 d 5 d/wk 6 hr/d			100 M (increased milk licking: behavioral index)	3000 M (tremors; decreased grip strength)	Dempster et al. 1984
55	Mouse (CD1, C57BL/6J)	5 d 6 hr/d			300 (hyperactivity)	900 (narcosis)	Evans et al. 1981
56	Rabbit (NS)	3.7-36.2 min				45000 (narcosis, tremors, excitement, chewing, loss of pupillary & blink reflex; pupillary contraction & involuntary blinking)	Carpenter et al. 1944
Reproductive							
57	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		100 F			Coate et al. 1984
58	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		2200 F			Green et al. 1978
59	Rat (CFY)	Gd 7-14 24 hr/d		125 F			Tatrai et al. 1980a

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL				Reference
					Less serious (ppm)		Serious (ppm)		
60	Mouse (CF-1)	Gd 6-15 7 hr/d		500 F					Murray et al. 1979
61	Rabbit (New Zealand)	Gd 6-18 7 hr/d		500 F					Murray et al. 1979
62	Rabbit	Gd 7-20 24 hr/d		156.5 F			313 F	(increased abortions and resorptions)	Ungvary and Tatrai 1985
Developmental									
63	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d		40	100	(decreased fetal weight)			Coate et al. 1984
64	Rat (Sprague-Dawley)	Gd 6-15 6 hr/d			100	(increased incidence of missing sternebrae)			Green et al. 1978
65	Rat (Sprague-Dawley)	Gd 6-15 7 hr/d		10	50	(decreased fetal weight)			Kuna and Kapp 1981
66	Rat (CFY)	Gd 7-14 24 hr/d			125	(decreased mean fetal weight; increased fetal weight retardation; skeletal retardation; 17% decrease in mean placental weight)			Tatrai et al. 1980a
67	Rat (CFY)	Gd 7-14 24 hr/d			47	(decreased fetal weight; signs of skeletal retardation)	141	(significant increase in fetal mortality)	Tatrai et al. 1980b
68	Mouse (Swiss-Webster)	Gd 6-15 6 hr/d		10	20	(decreased circulating erythroid precursors, elevation of granulocytic precourser cells in neonates and 6-wk old offspring)			Keller and Snyder 1988

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL				Reference
					Less serious (ppm)		Serious (ppm)		
	Systemic								
78	Human	4 mo -1 yr	Hemato				150	(pancytopenia)	Aksoy and Erdem 1978
79	Human	4 mo -1 yr	Hemato				210	(pancytopenia, hypocellular to hypercellular bone marrow)	Aksoy et al. 1972
80	Human	1 yr	Hemato		40	(decrease in WBC counts in first 4 months)			Cody et al. 1993
81	Human	3.5 mo -19 y	Hemato				29	(aplastic anemia)	Yin et al. 1987c
82	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d	Hemato				500	(decreased WBC & lymphocytes; increased RBC & hemoglobin)	Dow 1992
83	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d	Bd Wt	300 F					Kuna et al. 1992
84	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d	Hemato	30			300	(decrease in leukocytes, slight decrease in marrow cellularity)	Ward et al. 1985
			Bd Wt	300					
85	Rat (Wistar)	204 d 5 d/wk 7 hr/d	Hemato		88 M (leukopenia)				Wolf et al. 1956
86	Mouse (C57BL)	24 wk 5 d/wk 6 hr/d	Hemato		10 M	(depressed splenic red cells)			Baarson et al. 1984
87	Mouse (C57BL)	9 wk 5 d/wk 6 hr/d	Hemato		10 M	(depressed peripheral red blood cells, CFU-E)			Baarson et al. 1984

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
88	Mouse (Hale-.Stoner)	9.5 wk 5 d/wk 6 hr/d	Hemato			400 M (decreased erythrocytes and leukocytes, decreased bone marrow cellularity)	Cronkite et al. 1982
89	Mouse (C57B1/ 6BNL)	4-16 wk 5 d/wk 6 hr/d	Hemato		300 (stem cell depression in bone marrow, reversible after 2-4 weeks)		Cronkite et al. 1985
90	Mouse (Hale-Stoner)	9.5 wk 5 d/wk 6 hr/d	Hemato		400 M (decreased CFU-E, BFU-E & CFU-GM cells)		Cronkite et al. 1989
91	Mouse (CBA/Ca BNL)	20 d 5 d/wk 6 hr/d	Hemato			316 M (neutropenia; reduced cellularity)	Cronkite et al. 1989
92	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato	25 M	316 M (decreased stem cells in bone marrow)		Cronkite et al. 1989
93	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d	Hemato		300 M (granulocytic hyperplasia in bone marrow)•		Farris et al. 1993
94	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d	Hemato Bd Wt	302 M		302 M (decreased WBC, RBC; altered RBC morphology)	Green et al. 1981
95	Mouse (CD-1)	50 d 5 d/wk 6 hr/d	Hemato Bd Wt	9.6 M 9.6 M			Green et al. 1981
96	Mouse (Kunming)	30 d 6 d/wk 2 hr/d	Hepatic Renal Bd Wt	12.52 M 12.52 M 12.52 M			Li et al. 1992

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)		LOAEL Serious (ppm)		Reference
97	Mouse (DBA/2, B6C3F1, C57B1/6)	13 wk 3 or 5 d/wk 6 hr/d	Hemato				300 M	(depressed rate of erythropoiesis, increased frequency of MN-PCE and MN-NCE)	Luke et al. 1988b
98	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d	Hemato	100 F	300 F	(slight anemia; BFU-E & CFU-E depression in bone marrow)			Plappert et al. 1994a
99	Mouse (Hybrid)	8 wk 5 d/wk 6 hr/d	Hemato				300 F	(decreased Hgb, Hct, erythrocyte counts)	Plappert et al. 1994b
100	Mouse (BDF1)	8 wk 5 d/wk 6 hr/d	Hemato		100 F	(BFU-E and CFU-E depression)			Seidel et al. 1989b
101	Mouse (NMRI)	8 wk	Hemato	14 M					Toft et al. 1982
102	Mouse (Hybrid)	6 or 7 wk 5 d/wk 6 h/d	Hemato				300 F	(decreased CFU-C, BFU-E & CFU-E)	Vacha et al. 1990
103	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d	Hemato	30			300	(pancytopenia, bone marrow hypoplasia)	Ward et al. 1985
			Bd Wt	300					
104	Rabbit (NS)	243 d 5 d/wk 7 hr/d	Hemato		80	(leukopenia)			Wolf et al. 1956
105	Pig (Duroc-Jersey)	3 wk 5 d/wk 6 hr/d	Hemato	20	100	(decreased peripheral WBC, & increased erythroid cells)			Dow 1992
106	Gn pig (NS)	32 or 269 d 5 d/wk 7 hr/d	Hemato		88	(leukopenia)			Wolf et al. 1956

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL				Reference
					Less serious (ppm)		Serious (ppm)		

Immunological/Lymphoreticular

107	Human	4 mo -1 yr					210	(pancytopenia, hypoplastic to hyperplastic bone marrow. enlarged spleen)	Aksoy et al. 1972
108	Human	1 yr			40	(decreased lymphocytes)			Cody et al. 1993
109	Rat (Sprague-Dawley)	3 wk 5 d/wk 6 hr/d					500	(decreased myeloid & lymphoid cells)	Dow 1992
110	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		30	300	(leukopenia and lymphopenia)			Ward et al. 1985
111	Rat (Wistar)	204 d 5 d/wk 7 hr/d			88	(leukopenia, increased spleen weight)			Wolf et al. 1956
112	Rat (NS)	20 wk 6 d/wk 4 hr/d			4570	(increased leukocyte alkaline phosphatase, decreased white blood cell count)			Yin et al. 1982
113	Mouse (C57B1)	24 wk 5 d/wk 6 hr/d			10 M	(decreased number of splenic lymphocytes)			Baarson et al. 1984
114	Mouse (C57B1/ 6BNL)	4-16 wk 5 d/wk 6 hr/d					300	(reduced bone marrow cellularity; stem cell depression, reversible after 2-4 weeks)	Cronkite et al. 1985
115	Mouse (CBA/Ca BNL)	20 d 5 d/wk 6 hr/d					316 M	(decreased lymphocytes, CFU-S content in marrow)	Cronkite et al. 1989

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
116	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d			300 M (granulocytic hyperplasia)		Farris et al. 1993
117	Mouse (C57B1/6)	6 wk 5 d/wk 6 hr/d				1000 (leukopenia, granulocytopenia, lymphocytopenia)	Gill et al. 1980
118	Mouse (CD-1)	50 d 6 hr/d 5 d/wk			9.6 M (increased splenic CFU-S)		Green et al. 1981
119	Mouse (CD-1)	26 wk 6 hr/d 5 d/wk				302 M (reduced marrow and spleen cellularity; decreased spleen weight)	Green et al. 1981
120	Mouse (CD-1)	50 d 5 d/wk 6 hr/d			9.6 M (increased spleen weight, total splenic nucleated cellularity & NRBC)		Green et al. 1981
121	Mouse (CD-1)	26 wk 5 d/wk 6 hr/d				302 M (lymphocytopenia, anemia, decreased spleen weight, decreased spleen & marrow cellularities)	Green et al. 1981
122	Mouse (Kunming)	30 d 6 d/wk 2 hr/d		3.13 M	12.52 M (26% decrease in relative spleen weight; decrease in myelocytes, premyelocytes, myeloblasts, and metamyelocytes in the bone marrow)		Li et al. 1992
123	Mouse (Hybrid)	8 wks 5 d/wk 6 hr/d		100 F	300 F (increased T4/T8 ratio)		Plappert et al. 1994a

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
124	Mouse (C57B1/6)	100 d 5 d/wk 6 hr/d				100 M (death in 9/10 mice due to depressed cell-mediated immunity)	Rosenthal and Snyder 1987
125	Mouse (C57B1/6)	20 d 5 d/wk 6 hr/d			10 M (delayed splenic lymphocyte reaction to foreign antigens evaluated in in vitro mixed lymphocyte reaction)		Rosenthal and Snyder 1987
126	Mouse (Hale- Stoner)	4-5 wk 5 d/wk 6 hr/d		50 F	200 F (suppressed antibody response to fluid tetanus toxoid)		Stoner et al. 1981
127	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (leukocyte & lymphocyte depression; bone marrow hypoplasia; lymphoid depletion in mesenteric lymph node, plasma cell infiltration of mandibular lymph node, splenic periarteriolar lymphoid sheath depletion)	Ward et al. 1985
128	Gn Pig (NS)	32 or 269 d 5 d/wk 7 hr/d			88 (leukopenia, increased spleen weight)		Wolf et al. 1956
129	Rabbit (NS)	243 d 5 d/wk 7 hr/d			80 (leukopenia)		Wolf et al. 1956
130	Pig (Duroc- Jersey)	3 wk 5 d/wk 6 hr/d		20 F	100 F (T-cell depression; decreased peripheral WBC; decreased total lymphocytes)		Dow 1992

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
Neurological							
131	Rat (Wistar)	3 wk 3-4 x 4 hr				929 M (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994
132	Mouse (H)	3 wk 3-4 x 2 hr				856 F (calculated 30% depression of evoked electrical activity)	Frantik et al. 1994
133	Mouse (Kunming)	30 d 6 d/wk 2 hr/d			0.78[c] M (increased rapid response)		Li et al. 1992
Reproductive							
134	Rat (Sprague-Dawley)	10 wk Gd 0-20 Ld 5-20 5 d/wk 6 hr/d		300 F			Kuna et al. 1992
135	Rat (Wistar)	93 d 5 d/wk 7-8 hr/d			6600 M (testicular weight increase)		Wolf et al. 1956
136	Mouse (CD-1)	13 wk 5 d/wk 6 hr/d		30		300 (bilateral cyst in ovaries; atrophy/degeneration of testes; decrease in spermatozoa; increase in abnormal sperm)	Ward et al. 1985
137	Rabbit (NS)	243 d 5 d/wk 7-8 hr/d			80 M (degeneration of germinal epithelium in testes)		Wolf et al. 1956
138	Gn pig (NS)	32 or 269 d 5 d/wk 7-8 hr/d			88 M (testicular weight increase)		Wolf et al. 1956

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
Cancer							
139	Human	3.5 mo -19 y				29 (CEL: humanlymphocytic leukemia)	Yin et al. 1987c
140	Rat (Sprague-Dawley)	15 wk 4-5 d/wk 4-7 hr/d				200 (CEL: hepatomas)	Maltoni et al. 1982a, 1983, 1985
141	Rat (Sprague-Dawley)	15 wk 5 d/wk 4-7 hr/d				200 (CEL: hepatomas; onset of Zymbal gland carcinoma)	Maltoni et al. 1982b, 1983, 1985
142	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				100 M (CEL: leukemia)	Cronkite 1986
143	Mouse (C57BL/ 6BNL)	4-16 wk 5 d/wk 6 hr/d				300 (CEL: thymic and non-thymic lymphoma)	Cronkite et al. 1984, 1985
144	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				300 (CEL: hepatomata, lymphomatous & myelogenous neoplasms; Harderian & Zymbal gland, squamous cell & mammary carcinoma, papillary adenocarcinoma of the lung)	Cronkite et al. 1989
145	Mouse (CBA/Ca BNL)	16 wk 5 d/wk 6 hr/d				100 M (CEL: hepatomata, lymphomatous & myelogenous neoplasms)	Cronkite et al. 1989
146	Mouse (CBA/Ca)	16 wk 5 d/wk 6 hr/d				300 M (CEL: lymphoma in 12%)	Farris et al. 1993

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
147	Mouse (C57BL, CD-1)	10 wk 5 d/wk 6 hr/d				1200 M (CEL: 46% lung adenoma on CD-1 mice)	Snyder et al. 1988

CHRONIC EXPOSURE

Death

148	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200 (61% died, versus 46% in controls)	Maltoni et al. 1982a
149	Rat (Sprague-Dawley)	691 d 5 d/wk 6 hr/d				300 M (median lifespan 51 wk vs 65 wk in controls)	Snyder et al. 1978a
150	Mouse (AKR, C57Bl)	life 5 d/wk 6 hr/d				300 M (median lifespan 11-41 wk vs 39-75 wk in controls)	Snyder et al. 1978a, Snyder et al. 1980

Systemic

151	Human	4 mo- 15 yr	Hemato			150 (pancytopenia)	Aksoy and Erdem 1978
152	Human	1-3 yr	Hemato			3 (anemia, lymphocytosis, thrombocytopenia, leukopenia, leukocytosis)	Doskin 1971
153	Human	NS	Hemato			24 (pancytopenia, hypoplastic bone marrow)	Erf and Rhoads 1939
154	Human	3-29 yr	Hemato		25 M (increased mean corpuscular volume)		Fishbeck et al. 1978
155	Human	0.5-5 yr	Hemato			11 (anemia, macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939
156	Human	1-25 yr	Hemato	20		75 (anemia and leukopenia)	Kipen et al. 1989

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL		Reference
					Less serious (ppm)	Serious (ppm)	
157	Human	1-21 yr	Hemato	0.53 M			Tsai et al. 1983
158	Human	>1 yr	Hemato		0.69 (leukopenia)		Xia et al. 1995
159	Human	>1 yr	Resp		33 M (sore throat; nasal irritation) 59 F (sore throat; nasal irritation)		Yin et al. 1987b
			Hemato	33 M 59 F			
			Renal	33 M 59 F			
			Ocular		33 M (eye irritation) 59 F (eye irritation)		
160	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d	Resp	300 M			Snyder et al. 1978a, 1984
			Hemato			100 M (anemia, leukopenia)	
			Hepatic	300 M			
			Renal	300 M			
			Bd Wt	100 M	300 M (unspecified decreased body weight gain relative to controls)		
161	Mouse (AKR/J, C57BL/6J)	lifetime 5 d/wk 6 hr/d	Resp	300 M			Snyder et al. 1978a, 1980
			Hemato			100 M (anemia, increased neutrophil levels; pancytopenia; bone marrow hypoplasia)	
			Hepatic	300 M			
			Renal	300 M			
			Bd Wt			300 M (59% decrease in weight gain)	

33

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL				Reference
					Less serious (ppm)		Serious (ppm)		
Immunological/Lymphoreticular									
162	Human	3-5 yr					11	(macrocytosis, thrombocytopenia)	Goldwater 1941, Greenburg et al. 1939
163	Human	1-25 yr		20	75	(leukopenia)			Kipen et al. 1989
164	Human	>1 yr			0.69	(leukopenia)			Xia et al. 1995
165	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d			100 M	(decreased lymphocyte counts, splenic hyperplasia)			Snyder et al. 1978a, 1984
166	Mouse (AKR, C57Bl)	lifetime 5 d/wk 6 hr/d			100 M	(lymphocytopenia, bone marrow hypoplasia)			Snyder et al. 1978a, 1980
167	Mouse (C57BL, CD-1)	lifetime 6 hr/d 5 d/wk			300 M	(lymphopenia)			Snyder et al. 1988
Cancer									
168	Human	4-15 yr					150	(CEL: leukemia)	Aksoy and Erdem 1978
169	Human	28 mo- 40 yr					1	(CEL: leukemia, lymphoma)	Aksoy et al. 1987
170	Human	1-10 yr					10 M	(CEL: leukemia)	Infante 1978 (Follow-up to Infante 1977)
171	Human	18 mo					0.3 M	(CEL: leukemia)	Ott et al. 1978
172	Human	1-14 yr					16 M	(CEL: leukemia)	Rinsky et al. 1981 (Follow-up to Infante et al. 1977a)

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

Key to[a] figure	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL			Reference
					Less serious (ppm)	Serious (ppm)		
173	Human	1-30 yr				200	(CEL: leukemia)	Vigliani and Forni 1976
174	Human	>1 yr				2	(CEL: chronic erythroid leukemia)	Yin et al. 1989 (Follow-up to Yin et al. 1987a)
175	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200	(CEL: hepatomas)	Maltoni et al. 1982a
176	Rat (Sprague-Dawley)	86 wk 5 d/wk 4-7 hr/d				200	(CEL: Zymbal gland carcinoma)	Maltoni et al 1982b
177	Rat (Sprague-Dawley)	104 wk 5 d/wk 4-7 hr/d				200	(CEL: hepatomas)	Maltoni et al. 1983, 1985
178	Rat (Sprague-Dawley)	lifetime 5 d/wk 6 hr/d				100 M	(CEL: Zymbal gland carcinoma, myelogenous leukemia, liver tumors)	Snyder et al. 1984
179	Mouse (AKR/J, C57BL6J)	lifetime 5 d/wk 6 hr/d				300 M	(CEL: hematopoietic neoplasms [8/40], including 6 thymic lymphomas)	Snyder et al. 1978a, 1980

Table 2-1. Levels of Significant Exposure to Benzene - Inhalation (continued)

| Key to[a] figure | Species/ (strain) | Exposure/ duration/ frequency | System | NOAEL (ppm) | LOAEL | | Reference |
					Less serious (ppm)	Serious (ppm)	
180	Mouse (C57BL, CD-1)	lifetime every 3rd wk 7 d/wk				300 M (CEL: 35% increase of Zymbal gland carcinomas in C57BL mice)	Snyder et al. 1988

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

[b]Used to derive an acute inhalation minimal risk level (MRL) of 0.05 ppm. Concentration was converted to a human equivalent concentration and divided by an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) and adjusted for intermittent exposure by multiplying by 6/24 (see Appendix A).

[c]Used to derive an intermediate inhalation MRL of 0.004 ppm. Concentration was converted to a human equivalent concentration and divided by an uncertainty factor of 90 (3 for use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) and adjusted for intermittent exposure by multiplying by 2/24 (see Appendix A).

AChE = acetylcholinesterase; Bd Wt = body weight; BFU-E = burst-forming colonies, erythroids; Cardio = cardiovascular; CEL = cancer effect level; CFU-E = colony-forming units-erythroid progenitor cells; CFU-G = colony-forming units - granulopoietic stem cells; CFU-GM - colony forming units - macrophages; CNS = central nervous system; d = day(s); F = female; Gd = gestational day; Hct = hematocrit; Hemato = hematological; Hgb = hemoglobin; hr = hour(s); LC_{50} = lethal concentration, 50% kill; Ld = lactational day(s); LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); MN-NCE = micronucleated normochromatic erythrocytes; MN-PCE = micronucleated polychromatic erythrocytes; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational exposure; RBC = red blood cell; WBC = white blood cell; wk = week(s); yr = year(s)

Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation

Acute (≤14 days)

Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation (cont.)

Acute (≤14 days)

(ppm)

Immunological/Lymphoreticular

Neurological

Reproductive

Developmental

100000

10000

1000

100

10

1

0.1

0.01

0.001

0.0001

0.00001

0.000001

56h

40m

53r 54m 58r
55m

38m 43m 44m 49m 51 53r 60m 62h 69m 71h 72h
36m 41m 42m 54m 55m 57r 59r 61h 66r 67r 70m
33r 34m 37m 39m 44m 45m 47m 49m 50m 52 55m 62h 63r 65r 68m 72h
33r 35m 42m 46m 48m 50m 64r 67r
37m 39m 45m 47m 50m 63r 65r 68m

Key

r	rat	■ LC$_{50}$ (animals)
m	mouse	● LOAEL for serious effects (animals)
g	guinea pig	◑ LOAEL for less serious effects (animals)
h	rabbit	○ NOAEL (animals)
p	pig	◆ CEL: cancer effect level (animals)

Minimal risk level for effects other than cancer

▲ LOAEL for serious effects (humans)

▲ LOAEL for less serious effects (humans)

△ NOAEL (humans)

▼ CEL: cancer effect level (humans)

The number next to each point corresponds to entries in Table 2-1.

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation (cont.)

Intermediate (15-364 days)

Systemic

(ppm)

Death *Hematological* *Hepatic* *Renal* *Body Weight*

1000 —

73r   74m   76m
75m   77m
78   79
82r   84r
88m   89m   90m   91m   93m   97m   99m   102m
92m   94m   98m   103m
103m

100 —
85m
80   84r
98m   100m   105p
104h   105p   106g

10 —
86m   87m   92m   95m   101m   103m
96m   96m
95m

1 —

0.1 —

0.01 —

0.001 —

0.0001 —

0.00001 —

0.000001 —

Key

r	rat
m	mouse
g	guinea pig
h	rabbit
p	pig

■ LC_{50} (animals)

● LOAEL for serious effects (animals)

◐ LOAEL for less serious effects (animals)

○ NOAEL (animals)

◆ CEL: cancer effect level (animals)

▲ LOAEL for serious effects (humans)

▲ LOAEL for less serious effects (humans)

△ NOAEL (humans)

▼ CEL: cancer effect level (humans)

⊔ Minimal risk level for effects other than cancer

The number next to each point corresponds to entries in Table 2-1.

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.
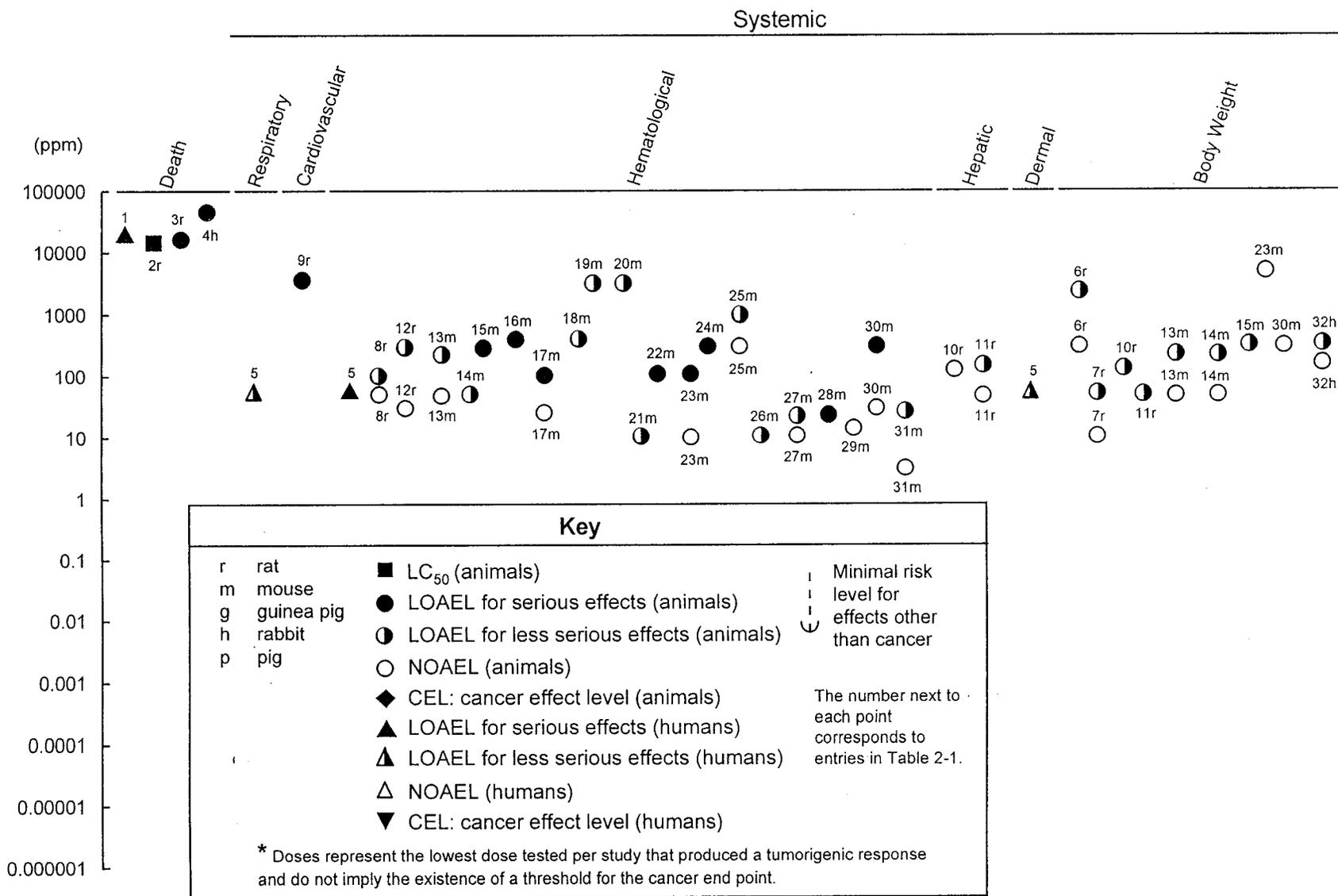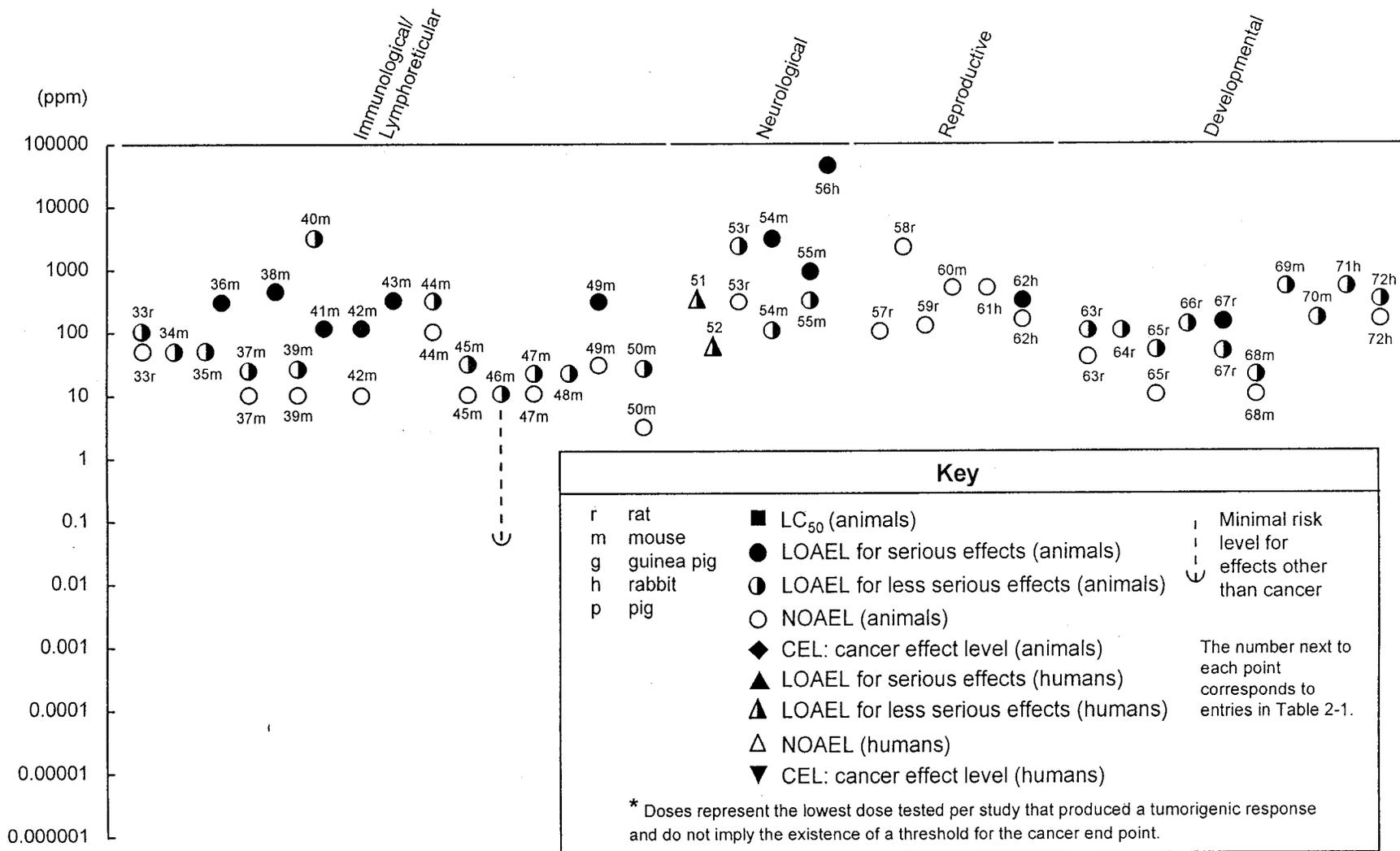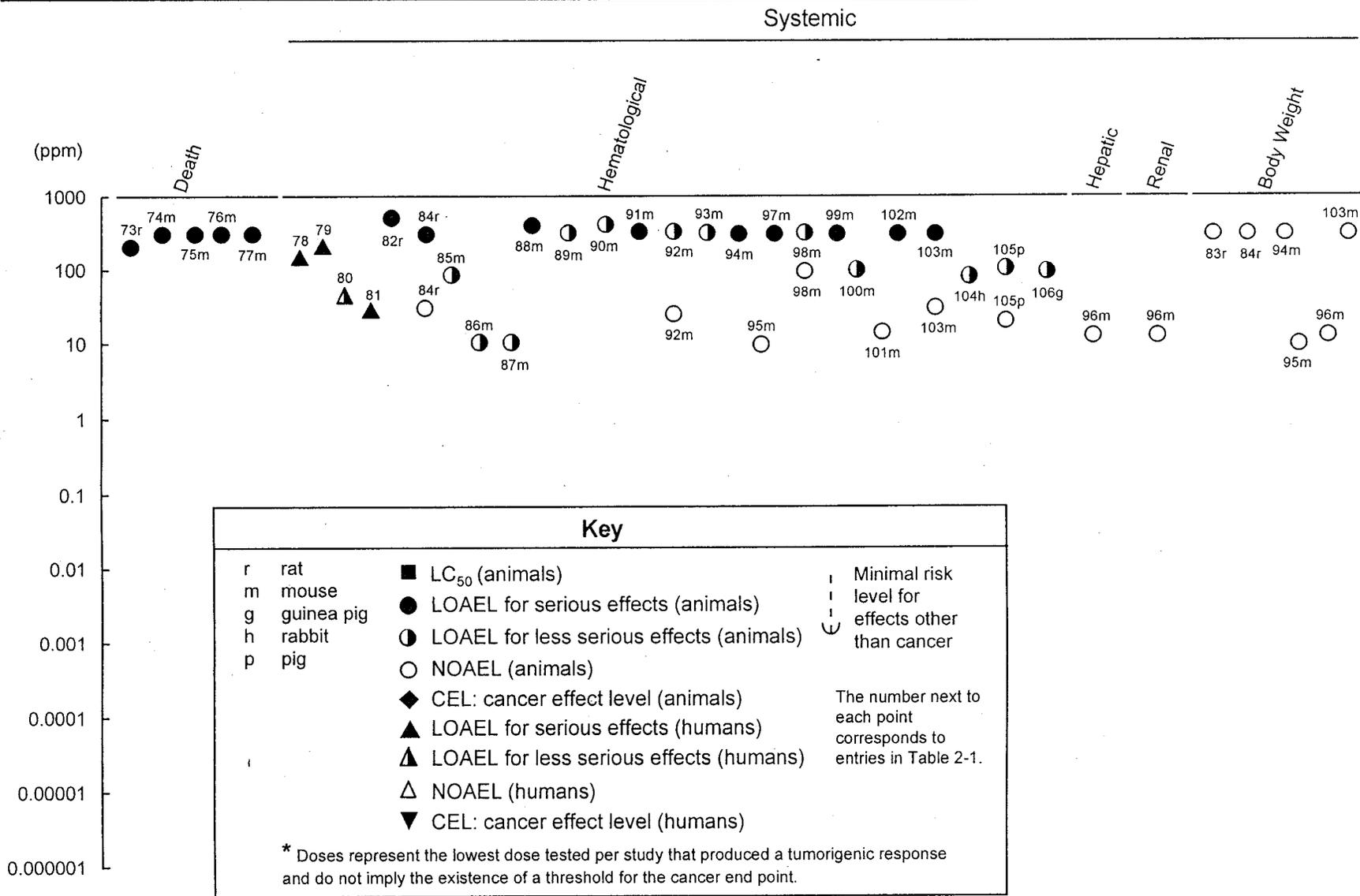
Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation (cont.)

Intermediate (15-364 days)

Figure 2-1. Levels of Significant Exposure to Benzene - Inhalation (cont.)

Chronic (≥365 days)

Systemic

(ppm)

Death Respiratory Hematological Hepatic Renal Ocular Body Weight Immunological/Lymphoreticular Cancer*

1000 —
149r 160r 160r 160r 161m 167m 173 175r 177r 179m
148r 150m 161m 151 156 161m 161m 160r 163 165r 168 176r 180m
159 153 156 159 160r 159 160r 159 166m 178r
152 154 155 162 163 170 172
157 158 164 169 174
171

100 —
10 —
1 —
0.1 —
0.01 —
0.001 —
0.0001 —
0.00001 —
0.000001 —

Key

r	rat
m	mouse
g	guinea pig
h	rabbit
p	pig

■ LC_{50} (animals)

● LOAEL for serious effects (animals)

◑ LOAEL for less serious effects (animals)

○ NOAEL (animals)

◆ CEL: cancer effect level (animals)

▲ LOAEL for serious effects (humans)

▲ LOAEL for less serious effects (humans)

△ NOAEL (humans)

▼ CEL: cancer effect level (humans)

Minimal risk level for effects other than cancer

The number next to each point corresponds to entries in Table 2-1.

10^{-4}
10^{-5}
10^{-6}
10^{-7}

Estimated Upper-Bound Human Cancer Risk Levels

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

2.2.1.2   Systemic Effects

No studies were located regarding endocrine, metabolic, or body weight effects in humans or gastrointestinal, musculoskeletal, endocrine, metabolic, or dermal effects in animals following inhalation exposure to benzene. Available data pertaining to systemic effects are presented below.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2- 1.

Respiratory Effects. Respiratory effects have been reported in humans after acute exposure to benzene vapors (Avis and Hutton 1993; Midzenski et al. 1992; Winek and Collum 1971; Winek et al. 1967; Yin et al. 1987b). Fifteen male workers employed in removing residual fuel from shipyard tanks were evaluated for benzene exposure (Midzenski et al. 1992). Mucous membrane irritation was noted in 80% and dyspnea was noted in 67% of the workers at occupational exposures of greater than 60 ppm for up to 3 weeks. Nasal irritation and sore throat were reported by male and female workers exposed to 33 and 59 ppm benzene, respectively, for more than 1 year (Yin et al. 1987b). After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic, edematous lungs (Avis and Hutton 1993). Acute granular tracheitis, laryngitis, bronchitis, and massive hemorrhages of the lungs were observed at autopsy of an 18-year-old male who died of benzene poisoning after intentional inhalation of benzene (Winek and Collum 1971). Similarly, acute pulmonary edema was found during the autopsy of a 16-year-old who died after sniffing glue containing benzene (Winek et al. 1967).

Snyder et al. (1978a, 1984) reported no treatment-related effects on lung tissue in male Sprague-Dawley rats exposed to 0, 100, or 300 ppm benzene 5 days per week, 6 hours per day for life. In addition, no adverse histopathological effects on lung tissue were observed in AKR/J or C57BL/65 mice exposed to 300 ppm benzene for life (Snyder et al. 1978a, 1980).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to benzene, although ventricular fibrillation has been proposed as the cause of death in some human poisonings (Avis and Hutton 1993; Winek and Collom 1971).

One animal study was found that investigated the effects of acute inhalation exposure to high concen-
trations of benzene vapor on the heart muscle of cats and monkeys (Nahum and Hoff 1934).
Information from the electrocardiograms indicated that exposure to benzene vapor caused extra
systoles and ventricular tachycardia of the prefibrillation type. Animals that had their adrenals and
stellate ganglias removed did not exhibit extra systoles or ventricular tachycardia. This study is
limited in that exact levels of exposure are not available. An additional study investigated the
influence of benzene inhalation on ventricular arrhythmia in the rat (Magos et al. 1990). Rats exposed
to 3,526-8,224 ppm of benzene in a closed chamber for 15 minutes exhibited an increased number of
ectopic ventricular beats.

Gastrointestinal Effects. Very few data are available describing gastrointestinal effects in
humans after inhalation exposure to benzene. In a case study involving the death of an l8-year-old
boy who intentionally inhaled benzene, the autopsy revealed congestive gastritis (Winek and Collom
1971). No other details or data were given.

Hematological Effects. Data regarding effects on the human hematological system following
acute inhalation exposure to benzene are scant, but indicate leukopenia, anemia, and thrombocytopenia
after more than 2 days occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992).
Epidemiological studies on persons exposed to various levels of benzene in the workplace for
intermediate and chronic periods of time also indicate hematological effects. These studies, described
in detail below, generally have limitations, such as concomitant exposure to other chemicals and lack
of appropriate control groups. In addition, a lack of adequate exposure data precludes a qualitative
determination of the relationship between severity of effects and exposure levels. However, sufficient
data are available to show that the hematopoietic system is a major target for benzene toxicity.
Studies that were conducted well and that show effects linked to specific exposure levels are presented
in Table 2-l and Figure 2-l. Effects on leukocytes, lymphocytes, and bone marrow are also discussed
in Section 2.2.1.3.

The human studies described below show that inhalation exposure to benzene for several months to
several years results in pancytopenia or other deficits in the relative numbers of circulating blood cells.
Continued exposure to benzene can also result in aplastic anemia or leukemia.

Pancytopenia is the reduction in the number of all three major types of blood cells: erythrocytes (red blood cells), thrombocytes (platelets), and leukocytes (white blood cells). In adults, all three major types of blood cells are produced in the red bone marrow of the vertebrae, sternum, ribs, and pelvis. The red bone marrow contains immature cells, known as multipotent myeloid stem cells, that later differentiate into the various mature blood cells. Pancytopenia results from a reduction in the ability of the red bone marrow to produce adequate numbers of these mature blood cells.

Aplastic anemia is a more severe effect of benzene and occurs when the bone marrow ceases to function and the stem cells never reach maturity. Depression in bone marrow function occurs in two stages-hyperplasia (increased synthesis of blood cell elements), followed by hypoplasia (decreased synthesis). As the disease progresses, bone marrow function decreases and the bone marrow becomes necrotic and filled with fatty tissue. This myeloblastic dysplasia without acute leukemia has been seen in persons exposed to benzene. Aplastic anemia can progress to a type of leukemia known as acute myelogenous leukemia (Aksoy 1980), which is discussed in Section 2.2.1.8.

At least one study revealed that very low levels of benzene exposure produce no adverse hematological effects. This study of workers in a Texas refinery showed no changes in erythrocytes, leukocytes, thrombocytes, hemoglobin (Hgb), or hematocrit in workers who were exposed to benzene at 0.53 ppm for 1-21 years (Tsai et al. 1983).

Two studies of workers exposed to low levels of benzene in the workplace showed slight decreases in erythrocyte counts. A morbidity study of 282 workers in a chemical factory reported that 10 persons who were exposed to over 25 ppm of benzene in the workplace for an average of 9 years (range 3-29 years) had an increased mean corpuscular volume at the end of the high exposure period (1963), but normal values 11 years later (1974) (Fishbeck et al. 1978). Further study of these 282 workers revealed slight decreases in erythrocyte counts that were not correlated with levels of benzene exposure (2-35 ppm) or with duration of employment (1 month to over 20 years) (Townsend et al. 1978).

Persons employed in the rubber industry have also shown decreased blood cell counts that did not persist when benzene exposure decreased. Individual hematological records, demographic data, and chronological work histories of 459 workers employed between 1940 and 1975 at a rubber products manufacturing plant in Ohio were assessed (Kipen et al. 1989). Significant decreases in white and red

cell counts and hemoglobin were recorded for the period of 1940-48 when the exposure was 75 ppm. The trend was not apparent during later years (1949-75) when the exposure was decreased to 15-20 ppm. In a more in-depth study of the same workers, Cody et al. (1993) examined the blood counts of 161 workers for whom pre-employment counts were done prior to exposure in the rubber factory. The results indicated that during the first year of employment in the rubber factory, employees exposed to benzene levels higher than the median exposure (estimated at 40-54 ppm) had significantly lower white and red blood cell counts than employees exposed to benzene levels below the median exposure.

A series of studies was conducted on Turkish workers exposed to benzene-containing adhesives in various occupations. These studies showed increased severity of effects with increased levels or duration of exposure. The initial hematological study examined 217 male workers who were exposed for between 4 months and 17 years to benzene-containing solvents in small, shoe-manufacturing shops (Aksoy et al. 1971). The concentration of benzene in the work area ranged from 15 to 30 ppm outside work hours and reached a maximum of 210 ppm when benzene-containing adhesives were being used. Fifty-one of the workers showed clinical hematological abnormalities. Of these, 46 showed loss of leukocytes. The most common findings were leukopenia (21 cases, 9.7%), thrombocytopenia (4 cases, 1.8%), leukopenia associated with thrombocytopenia (10 cases, 4.6%), pancytopenia (6 cases, 2.8%), and eosinophilia (5 cases, 2.3%). An additional cohort was identified that included 32 shoe manufacturers who had worked with benzene for 4 months to 15 years at concentrations of 15-30 ppm outside work hours and 210-640 ppm during the use of benzene, and who showed pancytopenia (Aksoy et al. 1972). Examination of these people revealed disruptions in bone marrow function, including cases of hypoplastic, acellular, hyperplastic, or normoblastic bone marrow. The continuing assessment further identified that ineffective erythropoiesis or increased hemolysis may have been responsible for the reticulocytosis, hyperbilirubinemia, erythroblastemia, increase in quantitative osmotic fragility, and elevated serum lactate dehydrogenase levels observed in some patients.

More severe effects, including preleukemia or acute leukemia, were observed in 26 out ef 28,500 benzene workers exposed to 210-650 ppm for l-15 years (Aksoy et al. 1974). Clinical features of the preleukemia included one or more of the following: anemia, leukopenia, pancytopenia, bone marrow hyperplasia, pseudo-Pelger-Huet anomaly, and splenomegaly. A study was conducted 2-17 years following the last exposure of 44 pancytopenic patients exposed to benzene (150-650 ppm) in adhesives for 4 months to 15 years (Aksoy and Erdem 1978). Of these patients, complete

remission was seen in 23, death due to complications of pancytopenia in 14, death due to myeloid metaplasia in 1, and leukemia in 6. When benzene concentrations in factories decreased in later years, less severe effects were seen. At 40 tire manufacturing plants, 231 workers were exposed for 28 months to 40 years (mean 8.8 years) to benzene-containing solvents and thinners (Aksoy et al. 1987). The decrease in benzene content of materials used in these workshops and the corresponding reduction in air concentration (most samples <l ppm) paralleled the decrease in the number of hematological abnormalities reported for benzene-exposed workers. Mild hematological abnormalities were noted in 14 workers including leukopenia in 9, thrombocytopenia in 4, and pancytopenia in 1. No LOAEL for the exposure to benzene alone could be defined in this study.

Another study revealed effects, ranging from mild to severe, of benzene exposure in factory workers in China (Yin et al. 1987c). The primary activities in these factories were the manufacture of paints, shoes, rubber, leather, and/or adhesives (Yin et al. 1987c). Of the 528,729 workers, 95% were exposed to mixtures of benzene, toluene, and xylene, while 5% (26,319 workers) were exposed to benzene alone at 0.02-264 ppm in air in 95% of the work stations. Over half of the work stations had levels of benzene in the air of less than 13 ppm; about 1% had levels of 13-264 ppm. Benzene toxicity, as indicated by leukopenia (leukocyte <4,000/mm^3), aplastic anemia, and leukemia, was seen in 0.94% of the workers exposed to benzene and 0.44% of the workers exposed to the mixtures. Similar toxicity was found in employees of 28 of the 141 shoe factories studied (124 cases in 2,740 employees) (Yin et al. 1987c): A positive correlation was observed for prevalence of adverse benzene effects and benzene concentration in data from these 28 shoe factories. The authors determined that the affected people were exposed to benzene concentrations ≥29 ppm. In one workshop, there were 4 cases of aplastic anemia in 211 workers. These workers were exposed to benzene at a mean concentration of 324 ppm during an 8-month period of employment. The prevalence of aplastic anemia in the shoe-making industry was about 5.8 times that in the general population. The main limitation of this study is the lack of information on the duration of exposure.

A study conducted by Li et al. (1994) during 1972-87 examined 74,828 benzene-exposed workers employed in 672 factories and 35,805 unexposed workers from 109 factories located in 12 cities in China. Estimates of gender-specific rate ratios and a comparison of the rate ratios for females to the rate ratios for males were calculated for the incidence of hematopoietic and lymphoproliferative (HLP) disorders, comparing all exposed workers in each of the occupational groups to unexposed workers. Small increases in relative risks for all HLP disorders for both genders were observed among chemical

and rubber manufacturing workers, painters, and paint manufacturers. There were no gender differences in relative risks for mortality and cancer mortality, although risks tended to be somewhat higher in male workers than in female workers.

Another study suggested a correlation for hematotoxicity in workers exposed to benzene for l-3 years at 3.2-12.8 ppm levels, which are 2-8 times the Russian standard (Doskin 1971). Included in the hematological findings were lymphocytosis, a biphasic leukocyte response (leukocytosis followed by leukopenia), or bone marrow hypercellularity for individuals that presented hematological abnormalities. Benzene levels and monitoring procedures were not clearly defined in this study. Leukopenia was observed by Xia et al. (1995) in Chinese workers exposed to 0.69-140 ppm (mean=6 ppm) benzene for more than 1 year.

People who were exposed to high levels of benzene vapors in the printing industry also showed severe hematological effects. One study evaluated 332 workers who were exposed to 1l-1,060 ppm of benzene for 6 months to 5 years. Detailed blood studies performed on 102 of these workers revealed benzene poisoning in 22 workers characterized by pancytopenia or other clinical signs (Goldwater 1941; Greenburg et al. 1939). Another study reported 6 cases of pancytopenia and bone marrow dysplasia in printers exposed to 24-1,060 ppm of benzene (Erf and Rhoads 1939).

In summary, benzene poisoning results in the development of pancytopenia, a condition characterized by decreased numbers of circulating erythrocytes, leukocytes, and thrombocytes. The potential mechanisms for the development of pancytopenia in humans include the destruction of bone marrow stem cells, the impairment of the differentiation of these cells, and/or the destruction of more mature hematopoietic cell precursors and circulating cells. Furthermore, pancytopenia can result from the combined destruction of the peripheral blood and bone marrow elements. In effect, individuals that develop pancytopenia and have continued exposure to benzene may develop aplastic anemia (i.e., pancytopenia associated with fatty replacement of functional bone marrow); others may exhibit both pancytopenia and bone marrow hyperplasia, a condition that suggests a preleukemic state. The data suggest that individual workers vary in their reactions to benzene.

More animal than human data are available from which to determine LOAEL or NOAEL values of benzene hematotoxicity. The data show that animal responses to benzene exposure are variable and may depend on factors such as species, strain, duration of exposure, and whether exposure is

intermittent or continuous. Wide variations have also been observed in normal hematological parameters, complicating statistical evaluation. The studies show that benzene exerts toxic effects at all phases of the hematological system, from stem cell depression in the bone marrow, to pancytopenia, to histopathological changes in the bone marrow. The following studies demonstrate these adverse hematological effects in animals. Effects on leukocytes, lymphocytes, and bone marrow are also discussed in Section 2.2.1.3.

Acute studies have shown that benzene exposure leads to anemia (any condition in which the number of erythrocytes in 1 mm^3 of blood, or the amount of hemoglobin in 100 mL of blood, or the volume of packed erythrocytes per 100 mL of blood is below normal levels). One study found changes in the levels of peripheral erythrocytes in C57BL/6 male mice that were acutely exposed to benzene by inhalation. Erythrocytes were elevated at 10 ppm, equal to levels in control animals at 31 ppm, and depressed at 100 and 301 ppm (Rozen et al. 1984). Decreased erythrocytes and lymphocytes were observed in mice exposed to 300 ppm intermittently for 6 hours a day, 5 days a week, over a period of 6 days to 23 weeks (Rozen and Snyder 1985; Ward et al. 1985). Ward et al. (1985) also noted decreased hemoglobin content, leukopenia, and decreased hematocrit after 2 weeks exposure at 300 ppm, progressing to pancytopenia. Decreased levels of leukocyte counts have also been noted in the blood and spleen of mice exposed to 211 ppm of benzene for 6 hours per day for 7 days or 48 ppm for 14 days (Aoyama 1986). Male Swiss-Webster mice were exposed via inhalation to benzene (3-2,290 ppm) for 6 hours per day for 5 days (Wells and Nerland 1991). Decreased leukocytes were observed at doses of 25 ppm and above. Green and colleagues examined the effect of benzene inhalation on the peripheral blood (Green et al. 1981b), and bone marrow and spleen cells (Green et al. 1981a, 1981b) of CD-l mice following exposures to a number of regimens. In the acute studies, individual test groups were exposed by inhalation to mean benzene concentrations of 0, 1.1, 9.9, 103, 306, 603, 1,276, 2,416, or 4,862 ppm, 6 hours per day for 5 days. Green et al. (1981b) observed granulocytopenia and lymphocytopenia at ≥103 ppm, with no change in leukocytes differential; leukocytes decreased 43% compared to controls. Exposures at 306 to 4,862 ppm benzene decreased peripheral leukocytes to levels that were from 36 to 27% of control values. Lymphocytes and polymorphonucleocytes were also significantly depressed at doses of 103 ppm and above. Erythrocyte counts were depressed only at 2,416 and 4,862 ppm concentrations. Hematocrits of test animals were equivalent to control values at 1.l, 9.9, 603, and 1,276 ppm and were depressed at 103, 306, and 2,416 ppm showing no clear dose/response effect. Marrow cellularities were reduced at doses of 103 ppm and above (Green et al. 1981a, 1981b).

NMRI male mice exposed to benzene at concentrations ranging from 1 to 200 ppm, exhibited a reduction in bone marrow cellularity (nucleated cells per tibia), a reduction in the number of colonyforming granulopoietic stem cells (CFU-C) per tibia, and an increase in frequency of micronucleated polychromatic erythrocytes (MN-PCE) that varied with dose and duration of exposure as explained below (Toft et al. 1982). Mice exposed continually (24 hours/day) to 21 ppm or more benzene in air for 4-10 days showed significant changes in all of the parameters. No adverse effects on hematological parameters were noted in mice exposed to 14 ppm benzene continuously for l-8 weeks (Toft et al. 1982). Intermittent exposure (8 hours a day, 5 days a week for 2 weeks) to 21 ppm, but not to 10.5 ppm, significantly reduced the number of CFU-C per tibia and elevated the frequency of MN-PCE, but did not affect bone marrow cellularity. Intermittent exposure to 50 ppm or more caused significant changes in all 3 parameters and decreased the ability of the spleen to form mature cells (as measured by numbers of colony-forming units of stem cells). When mice were intermittently exposed to 95 or 201 ppm benzene for 2-8 hours per day, 5 days per week for 2 weeks, decreased cellularity and CFU-C per tibia were observed at 95 ppm after 6-8 hours per day exposure, whereas at 201 ppm benzene, decreased cellularity but no effect on CFU-C was noted after 2 hours per day exposure. A decrease in cellularity and CFU-C was observed after 4-8 hours per day exposure to 201 ppm benzene (Toft et al. 1982). DBA/2 mice exposed to 10 ppm benzene for 6 hours per day for 5 days exhibited a 50% reduction in CFU-E (erythroid progenitor cells) per tibia (Dempster and Snyder 1991). Decreases in bone marrow cellularity in the femur, hematocrit, and leukocytes were seen in DBA/2 mice exposed to 300 ppm benzene in air for 6 hours per day, 5 days per week for 2 weeks (Chertkov et al. 1992). Similar effects on bone marrow cellularity and CFU-E development were observed after a similar exposure of Swiss Webster and C57BL/6J mice (Neun et al. 1992, 1994). Neun et al. (1994) also extended these experiments, in which the bone marrow of Swiss Webster mice and C57BL/6J mice exposed to benzene under the same regimen (300 ppm, 6 hours per day, 4 days per week for 2 weeks) were cultured with benzene metabolites to assess bone marrow viability (see Section 2.5). Wistar rats exposed to doses of benzene up to 300 ppm for 8 hours per day for 7 days exhibited leukopenia at 100 ppm and greater, but not at 50 ppm (Li et al. 1986). Leukopenia was also observed in C57BL/6J mice exposed continuously to 100-1,000 ppm for 2-8 days (Gill et al. 1980). Sprague-Dawley SD/Tex rats were exposed to benzene vapor at 0 or 500 ppm for 5 day per week, 6 hours per day for 3 weeks (Dow 1992). Blood from the animals was evaluated for hematologic changes and the bone marrow for the presence of multinucleated erythroblasts. Animals exposed to 500 ppm showed decreased lymphocyte and leukocyte counts. Erythrocytes and hemoglobin values increased. In the bone marrow differential counts, rats showed a

relative decrease in lymphoid and myeloid cells at the 500 ppm dose level and an increase in erythroic cells. In a companion study, purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours per day, 5 days per week for 3 weeks (Dow 1992). Decreased peripheral leukocytes, total lymphocytes and T-cells, and increased erythroid cells were observed at 100 ppm. Exposure to 300 ppm for 6-9 weeks caused a reduction in erythroid progenitor cells in the femurs of C57BL/6 or hybrid mice (Baarson and Snyder 1991; Vacha et al. 1990). Depression of CFU-E was also noted in C57BL/6 mice from exposure to as little as 10 ppm of benzene for 9 weeks (Baarson et al. 1984).

Female BDFl mice (C57BL/6xDBA/2F$_1$ hybrids) were exposed to 100, 300, and 900 ppm benzene 6 hours per day, 5 days per week for up to 8 weeks (Plappert et al. 1994a). Benzene concentrations in the chamber were monitored 2 times per day by gas chromatography (GC). Hematological studies included peripheral blood data, T4 and T8 lymphocyte counts in the blood and spleen, and hemopoietic stem and progenitor cell assays in the marrow (spleen colony-forming units [CFU-S], colony-forming unit-culture [CFU-C], and erythroid burst-forming unit [BFU-E], erythroid colonyforming unit [CFU-El]). No significant changes were observed in the peripheral blood data of mice exposed to 900 ppm benzene. Some perturbation of the reticulocyte numbers was observed, but values at days 3 and 5 did not differ from controls. Absolute numbers of lymphocytes and neutrophils did not differ from controls (no data shown). A slight anemia was observed at 4 and 8 weeks of treatment with 300 and 900 ppm benzene. Results showed minor changes in the stem and progenitor cells. CFU-E depression after 4 days of exposure was significant. There was a dose-dependent depression of colony forming cell numbers present at 4 and 8 weeks of exposure with a maximal effect at the level of CFU-E. Decreased CFU-E and BFU-E were observed in a companion study in which hybrid mice were exposed to 300 or 900 ppm benzene for the same period of time (Plappert et al. 1994b).

Granulocytic hyperplasia has been detected in the bone marrow of mice exposed to 300 ppm benzene in air for 6 hours per day, 5 days per week for 16 weeks, and held 18 months after the last exposure (Farris et al. 1993). Sprague-Dawley rats received 100 or 300 ppm benzene vapor for 6-hours per day, 5 days per week for 691 days (Snyder et al. 1978a, 1984). Anemia and leukopenia were observed at both concentrations. In addition, at both concentrations, hemosiderin was found in the spleen, which could be due to erythrocyte hemolysis. AKR mice were exposed to 100 or 300 ppm benzene vapor for 6 hours per day, 5 days per week for life (Snyder et al. 1978a, 1980). C57BL mice were exposed to 300 ppm benzene vapor for 6 hours per day, 5 days a week for life (Snyder et al. 1980). At

100 ppm, anemia, lymphocytopenia, and bone marrow hypoplasia were observed in AKR mice; the same effects at greater severity were observed in both strains at 300 ppm. Stem cell depression in the bone marrow was noted in several studies in mice exposed intermittently to benzene for 2 days to 16 weeks (Cronkite et al. 1982, 1985, 1989). Prolonged exposure to lower levels had a greater hematotoxic effect than exposure to higher levels for a shorter period of time (Cronkite et al. 1989). These studies also revealed that recovery could occur within a few weeks after cessation of exposure. Intermittent exposure of Hale-Stoner mice to 400 ppm benzene over 11 days caused decreased erythrocytes and leukocytes (Cronkite et al. 1982). Lymphopenia (at 25 ppm), anemia, and stem cell depression in the bone marrow (at 100 ppm) were reported in C57BL/6 mice after 2 weeks of exposure (Cronkite et al. 1985). Recovery of the bone marrow occurred following cessation of exposure. In addition, C57BL/6 mice or Hale-Stoner mice were exposed to 10, 25, 100, 300, or 400 ppm benzene for up to 16 weeks (Cronkite et al. 1982, 1985). At 2 weeks, exposure to 10 or 25 ppm did not result in adverse effects, but exposure to 100 ppm or higher concentrations for two or more weeks significantly reduced overall cellularity and the number of pluripotent stem cells of the bone marrow. Stem cell recovery was studied in the 300 ppm exposure group (Cronkite et al. 1985). In the animals exposed for 2 and 4 weeks, the stem cell numbers had returned to control levels by 2 weeks after exposure. Those exposed for 8 weeks had recovered by 16 weeks, and those exposed for 16 weeks had recovered incompletely to 92% of control values by 25 weeks. In CBA/Ca male mice, inhalation of 100, 300, or 400 ppm benzene for 6 hours per day, 5 days per week for up to 16 weeks produced dose-dependent decreases in bone marrow cellularity and marrow content of spleen colony-forming units (CFU-S) (Cronkite et al. 1989). In addition, a comparative study on effect and recovery of bone marrow cellularity and CFU-S was conducted on CBA/Ca mice exposed to the same total amount of benzene over different exposure regimens. Although the decrease in marrow cellularity of mice exposed to 3,000 ppm for 8 sessions (Regimen 1) was similar to that of mice exposed to 300 ppm for 80 sessions (Regimen 2), bone marrow cellularity in mice exposed for only 8 sessions recovered by 30 days after exposure, whereas recovery took longer (60 days) for mice exposed for the longer period of time (80 sessions). The number of CFU-S decreased to 62% and 38% of controls for Regimens 1 and 2, respectively. The number of CFU-S had recovered by 30 days post exposure for mice in Regimen 1; recovery was slower and incomplete by 178 days post exposure for mice in Regimen 2. Thus, prolonged exposure to lower levels of benzene had a greater hematotoxic effect than exposure to higher levels of benzene over a shorter duration. These findings were further confirmed in separate experiments on two groups of CBA/Ca mice following benzene inhalation exposure. One group was exposed 19 times to 316 ppm of benzene, 6 hours per day,

5 days per week and the second group 2 times to 3,000 ppm, 6 hours per day (Cronkite et al. 1989). Although the total amount of inhaled benzene was the same for both groups (6,000 ppm), the effects on lymphocytes, neutrophils, eosinophils, and the bone marrow content of CFU-S were much more pronounced and persisted for up to 214 days in the lower exposure group (316 ppm). These results suggest that longer exposure to 316 ppm of benzene may cause irreparable hematologic injury, while the hematotoxic effects caused by short exposure to 10 times higher benzene concentrations are reversible.

Green et al. (1981b) examined the effect of benzene inhalation on the peripheral blood and bone marrow of CD-l mice following exposures to 0 or 9.6 ppm for 6 hours per day, 5 days per week for 10 weeks (50 days), or to 0 or 302 ppm for 6 hours per day, 5 days per week for 26 weeks. Animals exposed to 9.6 ppm for 50 days showed no detectable changes in the peripheral blood or bone marrow. Exposure to 302 ppm benzene for 26 weeks resulted in lymphocytopenia and anemia. Total leukocytes decreased to 48% of control values. Differentials revealed that lymphocytes were reduced to 13% of control values while the number of circulating polymorphonuclear leukocytes (PMN) appeared to be elevated. Circulating erythrocyte levels of test mice were reduced to 63% of control values. Red cell morphology was characterized by polychromasia, anisocytosis, poikilocytosis, stippling, and numerous Howell-Jolly bodies. Marrow cellularity was reduced to 32% of control, which was due primarily to a reduction in lymphocytes and granulocytes. Morphologically, nucleated marrow cells displayed a variety of nuclear/cytoplasmic blebbing, vacuolization, and atypical mitotic figures. In addition, asynchronous nuclear/cytoplasmic maturation was observed in myeloid precursors.

One study revealed damaged erythrocytes in the peripheral blood of mice (Luke et al. 1988b). Cytotoxic damage in the bone marrow was dependent on strain and exposure duration. Peripheral blood smears were analyzed weekly from three strains of mice (DBA/2, B6C3F$_1$, and C57BL/6) exposed to 300 ppm benzene for 13 weeks (6 hours per day) for either 5 days per week (Regimen 1) or 3 days per week (Regimen 2). In all three strains, an initial severe depression in rate-of erythropoiesis was observed. The return to normal was dependent on strain (Luke et al. 1988b) and regimen (Cronkite et al. 1989; Luke et al. 1988b). An increase in frequency of micronucleated normochromatic erythrocytes (MN-NCE) was observed to be dependent on strain (C57BL/6 = B6C3F$_1$ > DBA/2) and regimen (Regimen 1 > Regimen 2), whereas the increase in

frequency of MN-PCE was dependent on strain (DBA/2 > C57BL/6 = B6C3F$_1$) but, for the most part, was not dependent on exposure regimen.

Damaged erythroblast-forming cells were also noted in bone marrow (Seidel et al. 1989b). A substantial decrease in erythroid colony-forming units and smaller decreases in erythroid burst-forming units occurred in BDFl mice intermittently exposed to 100 ppm of benzene for 8 weeks (6 hours/day, 5 days per week). Although the effects in the 100 ppm group were not apparent at 8 weeks (the end of experiment), they did occur, and it took over 3 weeks for the erythroid burst-forming units and erythroid colony-forming units to return to their initial values. This reduction in the number of erythroid precursors was reflected by a slight reduction in the number of erythrocytes.

The peripheral blood and bone marrow were altered in CD-l mice and Sprague-Dawley rats exposed for 91 days (13 weeks) to up to 300 ppm benzene (Ward et al. 1985). Interim sacrifices were performed on days 7, 14, 28, and 56 of exposure. No hematological effects were observed at 1, 10, or 30 ppm. At 300 ppm, however, mice exhibited significant increases in mean cell volumes and mean cell hemoglobin values on day 14. However, on days 28, 56, and 91, decreases in hematocrit, hemoglobin, erythrocyte count, leukocyte count, and platelet count were observed. The most common compound-related histological findings in mice occurred at 300 ppm and included myeloid hypoplasia of the bone marrow and increased extramedullary hematopoiesis in the spleen. Lesions were present at early sacrifice time points and were detected throughout the study, increasing in severity and incidence with time. The effects were present more often in males than in females and were more severe in males. The only significant change in rats exposed to 300 ppm was a decreased leukocyte count that was first noted in males at day 14 and in females at day 91. There was also a significant decrease in the percentage of lymphocytes in both male and female rats exposed to 300 ppm of benzene vapors throughout the whole study up to day 91.

Decreased levels of circulating leukocytes were reported following intermediate-duration exposure to benzene at levels of 80 ppm in the rabbit and 88 ppm in the rat and guinea pig (Wells and Nerland 1991; Wolf et al. 1956). Depression of lymphocytes and numbers of nucleated red cells formed in the spleen was also noted in C57BL/6 mice from exposure to as little as 10 ppm of benzene for 24 weeks (Baarson et al. 1984).

Musculoskeletal Effects. A case of myelofibrosis was diagnosed in a 46-year-old man in October 1992 (Tondel et al. 1995). The patient worked from 1962 to 1979 as a gasoline station attendant. The patient was referred to the Department of Hematology, University Hospital in Linkoping, Sweden, where a bone marrow biopsy was performed. The patient described symptoms of increasing muscle pain for 1 year, fatigue for 3 weeks, night sweats, and weight loss. A bone marrow biopsy showed myelofibrosis. The time-weighted average (TWA) concentration for gasoline station attendants was estimated to be <0.2 ppm. The occupational standard for benzene in Sweden was 0.5 ppm (TWA) and the Swedish short-term exposure limit was 3 ppm. Ruiz et al. (1994) reported musculoskeletal effects in employees from a steel plant of Cubatao, S. Paulo, Brazil, who presented with neutropenia due to benzene exposure. Patients either were employed at the steel plant (mean time of 7 years and 4 months), or were employees of a building construction company working at repairs in the steel plant (mean time of 5 years and 5 months). Sixty percent of the workers had nonspecific clinical complaints such as myalgia.

Hepatic Effects. No specific reports of adverse hepatic effects of inhalation exposure to benzene in humans were found, although Aksoy et al. (1972) reported enlarged livers in workers chronically exposed to benzene.

CFY rats (20/group) were exposed to pure air, benzene (125 ppm) or benzene (400 ppm) and toluene (265 ppm) for 24 hours per day from gestational day (Gd) 7 through 14 (Tatrai et al. 1980a). The rats were then sacrificed on day 21 of pregnancy. Exposure to 125 ppm benzene caused a slight increase in relative liver weight of 4.67% compared to 4.25% in controls, which was not considered adverse. In a companion study, CFY rats were exposed to continuous benzene inhalation 24 hours per day from day 7 to day 14 of gestation at 0, 47, 141, 470, or 939 ppm atmospheric concentrations (Tatrai et al. 1980b). At 141 ppm benzene, there was significant increase in relative maternal liver weight.

No treatment-related non-neoplastic histopathological effects on hepatic tissue were found in male Sprague-Dawley rats exposed to 0, 100, or 300 ppm benzene 5 days per week, 6 hours per day for life (Snyder et al. 1978a, 1984) or in AKR/J or C57B1/6J mice similarly exposed to 300 ppm for life (Snyder et al. 1978a, 1980).

Renal Effects. Very little data is available describing renal effects in humans after inhalation exposure to benzene. In a case study involving the death of an 18-year-old boy who intentionally

inhaled benzene, the autopsy revealed acute kidney congestion (Winek and Collom 1971). No other details or data were given.

No treatment-related histopathological effects on kidney tissue were found in male Sprague-Dawley rats were exposed to 0, 100, or 300 ppm benzene 5 days per week, 6 hours per day for life (Snyder et al. 1978a, 1984) or in AKR/J or C57BU6J mice similarly exposed to 300 ppm (Snyder et al. 1978a, 1980).

Dermal Effects. Dermal effects in humans have been reported after acute exposure to benzene vapors (Avis and Hutton 1993). After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic respiratory tissues, and second degree bums on the face, trunk, and limbs (Avis and Hutton 1993). Skin irritation has been noted at occupational exposures of greater than 60 ppm for up to three weeks (Midzenski et al. 1992). These effects are due to direct contact of the skin with the vapor, and other dermal effects L resulting from direct contact of the skin are discussed in Section 2.2.3.2.

Ocular Effects. Three hundred solvent workers who had inhalation exposures for more than 1 year to benzene at 33 and 59 ppm for men and women, respectively, complained of eye irritation (Yin et al. 1987b).

Male Charles River CD rats exposed to 0, 1, 10, 30, or 300 ppm benzene 6 hours per day, 5 days per week for 10 weeks exhibited lacrimation at concentrations ≥10 ppm during the first 3 weeks of treatment (Shell 1980).

These effects are due to direct contact of the eyes with the vapor, and other ocular effects resulting from direct contact of the eyes are discussed in Section 2.2.3.2

Body Weight Effects. Relatively few studies in animals report changes in body weight after inhalation exposure to benzene. No change in body weight was noted in Sprague-Dawley rats or CD-l mice exposed to 300 ppm benzene for 13 weeks (Ward et al. 1985). No significant decrease in body weight was observed in CD-l mice exposed to doses up to 4,862 ppm for 6 hours per day, for 5 days, or at lower doses of 9.6 ppm for 50 days (Green et al. 1981b). Decreases in body weight (15%) were seen in DBA/2 mice after exposure to 300 ppm benzene in air for 6 hours per day, 5 days

per week for 2 weeks (Chertkov et al. 1992). Decreased body weight (16-18%) has also been noted in mice exposed to doses of approximately 200 ppm of benzene for 6 hours per day for 7 or 14 days (Aoyama 1986). C57BL mice exhibited a 59% decrease in body weight gain after exposure to 300 ppm benzene, 5 hours per day, 6 days per week over their lifetime (Snyder et al. 1980). Decreased maternal body weight and weight gain have been observed in Sprague-Dawley rats exposed to 50 ppm benzene during Gds 6-15 (Kuna and Kapp 1981), but not in rats exposed to doses up to 300 ppm during premating, mating, gestation, and lactation (Kuna et al. 1992). CFY rats (20/group) were exposed to pure air, benzene (125 ppm) or benzene (400 ppm) plus toluene (265 ppm) for 24 hours per day from Gd 7 through 14 (Tatrai et al. 1980a). The rats were then sacrificed on day 21 of pregnancy. Exposure to 125 ppm benzene caused decreased maternal weight gain (46.74% of starting weight as opposed to 68.82% of starting weight in controls). Decreased maternal weight gain was also observed in a companion experiment in which the rats were exposed to doses as low as 47 ppm using the same study design (Tatrai et al. 1980b). Decreased maternal body weight was also observed in rats exposed to 2,200 ppm benzene during gestation (Green et al. 1978). Rabbits exposed to 313 ppm benzene on Gd 7-20 exhibited decreased maternal weight gain (Ungvary and Tatrai 1985). Kunming mice exposed to 12.52 ppm benzene for 2 hours per day, 6 days per week for 30 days exhibited no adverse effect on body weight (Li et al. 1992). Sprague-Dawley rats received 100 or 300 ppm benzene vapor for 6 hours per day, 5 days per week for life (Snyder et al. 1978a, 1984). Decreased weight gain, which continued throughout the study, was observed at 30 weeks at 300 ppm, but not at 100 ppm. AKR mice exposed to 100 or 300 ppm and C57BL mice exposed to 300 ppm benzene vapor for 6 hours per day, 5 days per week for life had decreased weight gain at 300 ppm (Snyder et al. 1978a, 1980).

2.2.1.3 Immunological and Lymphoreticular Effects

Immunological effects have been reported in humans with occupational exposure to benzene. There are two types of acquired immunity, humoral and cellular, and benzene damages both. First, benzene has been shown to alter humoral immunity (i.e., to produce changes in levels of antibodies in the blood). Painters who were exposed to benzene (3-7 ppm), toluene, and xylene in the workplace for 1-21 years showed increased serum immunoglobulin values for IgM and decreased values for IgG and IgA (Lange et al. 1973b). The decreased levels of immunoglobulins may represent suppression of immunoglobulin-producing cells by benzene. Other adverse reactions, characterized by a reaction between leukocytes and agglutinins, occurred in 10 of 35 of these workers (Lange et al. 1973a).

These results suggest the occurrence of allergic blood dyscrasia in some persons exposed to benzene. One of the problems with these studies is that the workers were exposed to multiple solvents; therefore, benzene alone may not be responsible for the noted effects.

The second type of immunity, cellular immunity, is affected by changes in circulating leukocytes and a subcategory of leukocytes, called lymphocytes. Leukopenia was found in a series of studies of workers exposed to benzene at levels ranging from 15 to 210 ppm in various manufacturing processes in Turkey (Aksoy et al. 1971, 1987). Another study also noted signs of preleukemia that included loss of leukocytes and other blood elements, bone marrow histopathology, and enlarged spleens (Aksoy et al. 1972, 1974). Other studies in chronically exposed workers also showed losses of lymphocytes and other blood elements (Cody et al. 1993; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Ruiz et al. 1994; Yin et al. 1987c). The levels of benzene exposure ranged from 1 to 1,060 ppm. These studies are more fully described in Section 2.2.1.2. In one study, routine leukocyte counts conducted every 3 months on employees of a small-scale industry in China revealed leukopenia in workers exposed to as little as 0.69 -140 ppm (mean = 6 ppm) for an average period of 5-6 years (Xia et al. 1995). Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period and is associated with both decreased white blood cell counts and with changes in bone marrow activity (Yin et al. 1982). The change in LAP activity could be used in the diagnosis of benzene poisoning since it was more sensitive than the change in the leukocyte count, although it is not a biomarker that is specific for benzene exposure. A study conducted by Li et al. (1994) during 1972-87 examined 74,828 benzene-exposed workers employed in 672 factories and 35,805 unexposed workers from 109 factories located in 12 cities in China. Estimates of gender-specific rate ratios and a comparison of the rate ratios for females to the rate ratios for males were calculated for the incidence of hematopoietic and lymphoproliferative (HLP) disorders, comparing all exposed workers in each of the occupational groups to unexposed workers. Small increases in relative risks for all HLP disorders for both genders were observed among chemical and rubber manufacturing workers, painters, and paint manufacturers. In another study, an increase in leukocyte count and alkaline phosphatase score was observed in a pipe-fitter who was chronically exposed to 0.9 ppm benzene in addition to other solvents (Froom et al. 1994).

Animal studies support the observations made in humans and show that benzene affects humoral and cellular immunity. A decrease in spleen weight was observed in mice after acute-duration exposure to benzene at 25 ppm, 6 hours per day for 5 days, the same dose levels at which a decrease in circulating

leukocytes was observed (Wells and Nerland 1991). Benzene decreases the formation of the
B-lymphocytes that produce the serum immunoglobulins or antibodies. Exposure to benzene at
10 ppm and above for 6 days decreased the ability of bone marrow cells to produce mature
B-lymphocytes in C57BL/6 mice (Rozen et al. 1984). The spleen was also inhibited from forming
mature T-lymphocytes at exposure levels of 31 ppm and above. Mitogen-induced blastogenesis of Band
T-lymphocytes was depressed at 10 ppm and above. Peripheral lymphocyte counts were
depressed at all levels, whereas erythrocyte counts were depressed only at 100 and 300 ppm. This
study is the basis for the acute inhalation MRL of 0.05 ppm (see footnote to Table 2-l and
Appendix A).

A continuation of this line of studies for 6 days to 23 weeks at 300 ppm showed continued decreases
in numbers of mature B- and T-lymphocytes produced in the bone marrow, spleen, and thymus (Rozen
and Snyder 1985). Abnormalities of humoral and cell-mediated immune responses following benzene
exposure are presumably caused by a defect in the lymphoid stem cell precursors of both T- and
B-lymphocytes. Bone marrow cellularity increased 3-fold, and the number of thymic T-cells increased
15-fold in benzene-exposed mice between the 6th and the 30th exposure. No corresponding increase
in splenic cells was noted. The marked increase in the numbers of cells in bone marrow and thymus
was interpreted by the authors to indicate a compensatory proliferation in these cell lines in response
to benzene exposure, which may play a role in the carcinogenic response of C57BL mice to inhaled
benzene. The lack of response in the spleen suggests a lack of lymphoid restorative capacity in that
organ.

Other studies have also shown similar effects on immune functions following acute-duration exposure
to benzene. These include decreased numbers of circulating leukocytes and decreases in bone marrow
cellularity in mice exposed to 100 ppm and higher, 24 hours per day for up to 8 days (Gill et al.
1980); decreased leukocytes and increased leukocyte alkaline phosphatase in rats exposed to 100 ppm
for 7 days (Li et al. 1986); decreased leukocytes and bone marrow cellularity in DBA/2 mice exposed
to 300 ppm benzene for 2 weeks (Chertkov et al. 1992); and decreased leukocytes in mice exposed to
300 ppm for 10 days (Ward et al. 1985). These studies are more fully described in Section 2.2.1.2.
Decreased spleen weight and levels of B- and T-lymphocytes have also been noted in the blood and
spleen of mice exposed 6 hours per day to 47-48 ppm of benzene for 7 or 14 days (Aoyama 1986).
Decreased thymus weights were also noted at the 48 ppm dose after 14-day exposure (Aoyama 1986).

Reduced bone marrow cellularity was observed in Swiss Webster and C57BL/6J mice after exposure to 300 ppm benzene for 2 weeks (Neun et al. 1992). Decreased granulopoietic stem cells were observed at 21 ppm in NMRI mice after exposures of 10 days to 2 weeks (Toft et al. 1982). Green and colleagues examined the effect of benzene inhalation on bone marrow and spleen cells (Green et al. 1981a, 1981b) of CD-1 mice following exposures to a number of regimens. In the acute studies, individual test groups were exposed by inhalation to mean benzene concentrations of 0, 1.1, 9.9, 103, 306, 603, 1,276, 2,416, or 4,862 ppm, 6 hours per day for 5 days. Intermediate studies consisted of two different exposure regimens and concentrations: 6 hours per day, 5 days per week for 10 weeks (50 days) to a mean concentration of 0 or 9.6 ppm benzene; or 6 hours per day, 5 days per week for 26 weeks to a mean concentration of 0 or 302 ppm benzene. In the acute studies, marrow and splenic cellularities were reduced at 103 ppm. Splenic granulocytes were reduced at all exposure levels except at 9.9 ppm (Green et al. 1981b). The decrease in spleen cellularity correlated with a reduction in spleen weight at all concentrations $\geq$103 ppm. Mean spleen weights were significantly depressed at 1.1 ppm and at doses above 9.9 ppm, but not at 9.9 ppm. In Green et al. (1981a), marrow concentration of GM-CFU-C was equivalent to or greater than control values at all levels; however, splenic GM-CFU-C concentration was decreased at 103 ppm. Femoral and splenic CFU-S and GM-CFU-C per organ were depressed at 103 ppm. Absolute numbers of GM-CFU-C/femur or spleen were significantly reduced at all higher concentrations. There was no change in the colony/cluster ratio observed in the 5-day experiment. In the 50-day experiments, increased spleen weight, splenic nucleated cellularity, splenic nucleated erythrocytes, and CFU-S were seen at 9.6 ppm (Green 1981a, 1981b). Exposure to 302 ppm for 26 weeks resulted in reduced marrow and spleen cellularity and decreased spleen weight (Green et al. 1981a, 1981b).

Sprague-Dawley SD/Tex rats were exposed to benzene vapor at 0 or 500 ppm for 5 days per week, 6 hours per day for 3 weeks (Dow 1992). In the bone marrow differential counts, rats showed a relative decrease in lymphoid cells at the 500 ppm dose level. There were also decreases in myeloid cells of animals exposed to 500 ppm benzene. In a companion study, purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours per day, 5 days per week for 3 weeks (Dow 1992). Exposure to 500 ppm showed a statistically significant T-cell depression and leukopenic and erythropathic effects in cells of the pigs.

Another series of experiments revealed that exposures as low as 25 ppm for 2 weeks caused decreases in the numbers of circulating lymphocytes but did not affect the bone marrow cellularity in C57BL/6

or CBA/Ca mice (Cronkite et al. 1985, 1986). Increasing the duration (up to 16 weeks) or concentration (up to 300-400 ppm) produced the same effect of reduced peripheral blood lymphocytes as well as decreased bone marrow cellularity that persisted for up to 8 weeks after exposure (Cronkite et al. 1982, 1985). Similar observations were made in 2 groups of male CBA/Ca mice exposed to a total of 6,000 ppm of benzene by inhalation using 2 different regimens (Cronkite et al. 1989). One group was exposed to 316 ppm for a total of 19 times, while the second group was exposed to 3,000 ppm twice. Although both groups had significantly decreased lymphocyte counts, the lymphocyte numbers were much more depressed in the group of mice exposed to 316 ppm of benzene over 19 days, indicating the importance of the exposure regimen. In both groups, the lymphocyte numbers had not returned to normal values by 214 days post exposure. The femoral bone marrow cellularity returned to normal levels 32 days after exposure in both groups of mice.

In female BDFl mice (C57BL/6xDBA/2F$_1$ hybrids) exposed to 0, 300, or 900 ppm benzene 6 hours per day for 5 days, there was a relative increase in helper lymphocytes (CD4) at days 3-5, leading to an increase of the T4/T8 (CD4-CD8) ratio from 2 in controls to higher values at 8 weeks (Plappert et al. 1994). No concentration dependency was observed. Granulcytic hyperplasia of the spleen was observed in CBA/Ca mice exposed to 300 ppm benzene for 16 weeks (Farris et al. 1993). For further information, see Section 2.2.1.2.

Intermediate-duration exposure to benzene also decreased leukocyte counts in Sprague-Dawley rats and CD-l mice exposed to 300 ppm for 14-91 days (2-13 weeks) (Ward et al. 1985). Leukopenia, granulocytopenia, and lymphocytopenia were also observed in C57BL/6 mice exposed intermittently to 1,000 ppm for 16 weeks (Gill et al. 1980). In mice, the most common compound-related histopathological findings were splenic periarteriolar lymphoid sheath depletion, lymphoid depletion in the mesenteric lymph node, and plasma cell infiltration of the mandibular lymph node. Exposure to the highest concentration caused a decrease in leukocyte and lymphocyte counts in Sprague-Dawley rats. Hematological changes at 300 ppm were accounted for by decreased leukocyte counts in males on day 14 and in females on day 91. Decreases in percentage of lymphocytes in males and females started on day 14 and lasted through day 91. Rats exhibited decreased femoral marrow cellularity as the only histological change. Treatment-related changes were not observed at lower concentrations. Rabbits, rats, and guinea pigs exposed to 80-88 ppm for 32-269 days also had decreased leukocyte counts (Wolf et al. 1956). Leukocyte alkaline phosphatase values were increased and leukocyte counts were decreased in rats exposed to 4,570 ppm for 20 weeks (Yin et al. 1982). Exposure of C57BL

mice to 10 ppm benzene for 6 hours per day, 5 days per week, for 24 weeks causes depressions in the numbers of splenic nucleated red cells and lymphocytes (Baarson et al. 1984). Leukopenia, granulocytopenia, and lymphocytopenia were observed in C57BL/6 mice exposed to 1,000 ppm benzene for 6 hours per day, 5 days per week for 6 weeks (Gill et al. 1980).

Li et al. (1992) observed a 26% decrease in spleen weight in male Kunming mice exposed to 12.52 ppm benzene 2 hours per day, 6 days per week for 30 days. Examination of the bone marrow showed decreases in myelocytes, premyelocytes, myeloblasts, and metamyeloblasts at the same dose level.

Benzene also affects functional immune responses, as indicated by decreased resistance to infectious agents. Pre-exposure to benzene at ≥30 ppm for 5-12 days increased the bacterial counts in mice on day 4 of infection with *Listeria monocytogenes* (Rosenthal and Snyder 1985). Recovery of the immune system was noted on day 7. The effects did not occur at 10 ppm. In addition, a concentration-dependent statistically significant depression was noted in T- and B-lymphocyte populations from day 1 through day 7 at 30 ppm and above. B-cells were more sensitive to benzene than were T-cells on a percentage-of-control basis. This indicates a benzene-induced delay in immune response to *L. monocytogenes.* Concentrations of 200 or 400 ppm for 4-5 weeks (5 days per week) suppressed the primary antibody response to tetanus toxin in mice, but there was no effect at 50 ppm (Stoner et al. 1981). In another intermediate-duration exposure study, no changes were noted in the numbers of splenic B-cells, T-cells, or T-cell subsets in C57BL/6 mice exposed to 100 ppm of benzene 5 days per week for 20 days (Rosenthal and Snyder 1987). However, when splenic T-cells from mice treated with 10 ppm and 100 ppm were tested *in vitro* for their capacity to respond to foreign antigens (alloantigens) in the mixed lymphocyte reaction (MLR), the MLR response was delayed. Further analysis showing that this delayed MLR response was not due to the presence of benzene-induced suppressor cells and indicated that benzene impaired the functional abilities of alloreactive T-cells. A similar *in vitro* observation was made using T-cells from mice exposed to 100 ppm of benzene 5 days per week for 3 weeks. These cells had a reduced tumor cytolytic activity, suggesting a benzene-induced impairment of cell-mediated immunity. The impaired cell-mediated immune function was also apparent *in vivo.* Mice exposed to 100 ppm for a total of 100 days were challenged with 10,000 polyoma virus-induced tumor cells (PYB6), and 9 of 10 mice had reduced tumor resistance and developed tumors that were lethal (Rosenthal and Snyder 1987).

Chronic exposure to benzene caused bone marrow hypoplasia, lymphocytopenia, and anemia in mice
exposed to 100 ppm for a lifetime (Snyder et al. 1980). Sprague-Dawley rats received 100 or
300 ppm benzene vapor for 6 hours per day, 5 days per week for life (Snyder et al. 1978a, 1984).
Leukopenia and lymphopenia were observed starting during the first year at 100 ppm. AKR mice
were exposed to 100 or 300 ppm benzene vapor for 6 hours per day, 5 days per week for life and
C57BL mice were exposed to 300 ppm benzene vapor using the same exposure regimen (Snyder et al.
1978a, 1980). At 100 ppm, anemia, lymphocytopenia, and bone marrow hypoplasia were observed in
AKR mice; the same effects were observed to a greater extent in both strains at 300 ppm.

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

2.2.1.4   Neurological Effects

Following acute inhalation of benzene, humans exhibited symptoms indicative of central nervous
system effects (Cronin 1924; Flury 1928; Greenburg 1926; Midzenski et al. 1992). These symptoms,
reported to occur at levels ranging from 300 to 3,000 ppm, included drowsiness, dizziness, headache,
vertigo, tremor, delirium, and loss of consciousness. Acute exposure (5-10 minutes) to higher
concentrations of benzene (approximately 20,000 ppm) can result in death, which has been associated
with vascular congestion in the brain (Avis and Hutton 1993; Flury et al. 1928). Lethal exposures are
also associated with neurological symptoms similar to those reported for nonlethal exposures. These
symptoms are similar to the consequences of exposure to multiple organic solvents and are reversible
when symptomatic workers are transferred from the problem area (Kraut et al. 1988; Yin et al. 1987b).
In reports of cases of benzene poisoning, subjects exhibited headaches, nausea, tremor, convulsions,
and unconsciousness, among other neurological effects (Cronin 1924; Davies 1929; Greenburg 1926;
Midzenski et al. 1992; Tauber 1970).

Chronic exposure to benzene has been reported to produce neurological abnormalities in humans. Of
eight patients (6 with aplastic anemia and 2 with preleukemia) with previous occupational exposure to
adhesives and solutions containing 9-88% benzene, 4 of the 6 patients with aplastic anemia showed
neurological abnormalities (global atrophy of lower extremities and distal neuropathy of upper
extremities) (Baslo and Aksoy 1982). Air concentrations in the workplace were reported to have
reached levels of 210 ppm or higher. These findings suggest that benzene may induce toxic effects on

the nervous system involving peripheral nerves and/or spinal cord. The limitations of this study are that benzene exposure levels were not monitored and that there was a possibility of an additional exposure to toluene (6.37-9.25%).

Chronic exposure to benzene and toluene was studied in 121 workers exposed to benzene for 2-9 years (Kahn and Muzyka 1973). The air concentration of benzene between 1962 and 1965 was 6-1.5.6 ppm (20-50 mg/m^3), while the toluene vapors did not exceed the 5 mg/m^3 level. Subsequently (the authors do not specify when), the air levels of both benzene and toluene have not exceeded the 5 mg/m^3 level. Seventy-four of the examined workers complained of frequent headaches (usually at the end of the work day), became tired easily, had difficulties sleeping, and complained of memory loss. The limitations of this study are that workers were exposed to both benzene and toluene and that the precise dose and duration of exposure are not known.

Tondel et al. (1995) reported the case of a gasoline station attendant who had worked from 1962 to 1979. The patient described symptoms of fatigue for 3 weeks and night sweats, among other symptoms.

The neurotoxicity of benzene has not been studied extensively in animals. Female Sprague-Dawley rats exhibited lethargy after exposure to 2,200 ppm benzene, but not 300 ppm, on Gd 6-15 (Green et al. 1978). Male albino SPF rats from a Wistar-derived strain exposed to benzene for 4 hours in glass chambers (dose not specified) exhibited depression of evoked electrical activity in the brain; the authors calculated the 30% effect level (depressed activity) as 929 ppm (Frantik et al. 1994). When female H strain mice were exposed to benzene for 2 hours, the 30% effect level for depression of evoked electrical activity in the brain was 856 ppm (Frantik et al. 1994). In rabbits, symptoms that occurred 3.7 minutes following acute exposure to benzene at 45,000 ppm were relaxation and light narcosis (Carpenter et al. 1944). As the time after exposure progressed, so did the symptoms to include excitation, chewing, and tremors (after 5 minutes), loss of pupillary reflex to strong light (after 6.5 minutes), loss of blinking reflex (after 11.4 minutes), pupillary contraction (after 12 minutes), and involuntary blinking (after 15.6 minutes). Behavioral tests of C57BL/6 mice showed significant increase in licking of sweetened milk after 1 week of exposure to 300 ppm; a 90% decrease in hind limb grip strength after one exposure to 1,000 or 3,000 ppm (data for 100 ppm were not reported); and tremors after one exposure to 3,000 ppm that subsided 30 minutes after the exposure (Dempster et al. 1984). In another study, designed to reflect occupational exposure, male CD-l and C57BL/6 mice

were exposed to 300 or 900 ppm of benzene 6 hours per day for 5 days followed by 2 weeks of no exposure after which the exposure regimen was repeated for an unspecified amount of time (Evans et al. 1981). The following seven categories of behavioral activities were monitored in exposed and control animals: stereotypic behavior, sleeping, resting, grooming, eating, locomotion, and fighting. Only minimal and insignificant differences were observed between the two strains of mice. Increased behavioral activity was observed after exposure to benzene in both strains of mice. Mice exposed to 300 ppm of benzene had a greater increase than those exposed to 900 ppm, probably because of narcosis-like effects induced at the higher exposure level (Evans et al. 1981). It is not known if benzene induces behavioral changes by directly acting upon the central nervous system. It is also not known whether these changes occur before or after hematological changes.

Li et al. (1992) exposed male Kunming mice to 0, 0.78, 3.13, or 12.52 ppm benzene for 2 hours per day, 6 days per week for 30 days, and then monitored brain and blood acetylcholinesterase, forelimb grip strength, locomotor activity, and rapid response. Significantly increased grip strength was observed at 0.78 ppm, whereas at the higher doses, grip strength decreased significantly. Rapid response showed a significant increase at the low dose; the two higher doses showed a significant depressed rapid response. Locomotor activity increased at the low dose, was similar to control values at the middle dose, and decreased at the high dose. However, these changes were not significantly different from the control values. A significant decrease in acetylcholinesterase activity was noted in the brain, but it was not large enough to be considered adverse; no change in acetylcholinesterase levels in the blood were observed. This study is the basis for the intermediate-duration inhalation MRL of 0.001 ppm (see the footnote to Table 2-l and Appendix A).

All reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.5   Reproductive Effects

Data on the reproductive effects of occupational exposure to benzene suggest that benzene may impair fertility in women (Mukhametova and Vozovaya 1972; Vara and Kinnunen 1946). However, the findings are inconclusive because the studies are limited. In one study, 30 women with symptoms of benzene toxicity were examined (Vara and Kinnunen 1946). The levels of benzene in air were not specified, but are assumed to have been much greater than the 1 ppm permitted in today's working

environment. Twelve of these women had menstrual disorders (profuse or scanty blood flow and dysmenorrhea). Ten of the 12 women provided information on fertility. Of these 10 women, 2 had spontaneous abortions, and no births occurred during their employment even though no contraceptive measures had been taken. This led the investigators to suggest that benzene has a detrimental effect on fertility at high levels of exposure. However, the study failed to provide verification that the absence of birth was due to infertility. Gynecological examinations revealed that the scanty menstruations of five of the patients were due to ovarian atrophy. This study is limited in that an appropriate comparison population was not identified. Additionally, little follow-up was conducted on the 30 women with regard to their continued work history and possible symptoms of benzene toxicity. In another study, disturbances of the menstrual cycle were found in women workers exposed to aromatic hydrocarbons (benzene, toluene, xylene) (Michon 1965). The exposure levels of benzene and toluene were below 0.25 ppm. The observed group consisted of 500 women, 20-40 years old. One hundred controls were included in the study. The results showed that 21% of exposed women whose work involved sitting or standing had irregular menstrual cycles compared to 12% in the control group. Brief (up to 2 days), long (6-9 days), and prolonged (over 9 days) menstrual cycles were present in 26% of women who performed lifting during their work as compared to 13% in the control group. Irregular amounts of menstrual flow and pain were also observed in female workers exposed to aromatic hydrocarbons. The major limitations of this study are that the exposure occurred from a mixture of chemicals, levels of exposure were not well defined, duration of exposure was not stated, and activities of the controls were not provided.

Another study examined the reproductive function and incidence of gynecological effects in 360 women exposed to petroleum (a major source of benzene) and chlorinated hydrocarbons both dermally and by inhalation (Mukhametova and Vozovaya 1972). However, dermal exposure was considered to be negligible. The concentrations of benzene in the air were not well documented. When compared to female workers with no chemical exposure, female gluers had developed functional disturbances of the menstrual cycle. Additionally, as chemical exposure time increased, there were increases in the number of premature interruptions of pregnancy, the percentage of casesin which the membranes ruptured late, and the number of cases of intrauterine asphyxia of the fetus. The study limitations (including lack of exposure history, simultaneous exposure to other substances, and lack of follow-up) make it difficult to assess the effects of benzene on reproduction. In a more recently published study, reproductive competence of male workers in two organic chemical factories in France was evaluated (Stucker et al. 1994). Analysis of 1,739 pregnancies that ended in spontaneous abortion

or birth was presented. Paternal exposure to benzene for each pregnancy was described as exposure in the 3 months immediately before conception, and as previous job exposures. Benzene exposure was graded at two levels: less than 5 ppm (low) and greater than or equal to 5 ppm (moderate). Of the 1,739 pregnancies described, 171 ended in a spontaneous abortion (rate = 9.8%). According to exposure categories, 1,277 pregnancies were defined as non-exposed (mate not exposed) and 270 whose mates were exposed at some time before conception. For the 270 pregnant women, 145 of their mates were exposed during the 3 months immediately preceding conception. The frequency of spontaneous abortion was not significantly higher for the paternal group exposed at any time before conception than in the non-exposed group; nor was it higher for the group exposed during the 3 months immediately before conception.

In CFY rats exposed to pure air, or benzene (125 ppm) for 24 hours per day from Gd 7 through 14, no effect on implantation number was observed (Tatrai et al. 1980a). No changes in maternal body weight were observed in Sprague-Dawley rats exposed to 100 ppm benzene for 6 hours per day on Gd 6-15 (Coate et al. 1984). Pregnant rabbits exposed 12 hours per day to 156.5 or 313 ppm benzene on Gd 7-20 showed an increase in the number of abortions and resorptions at 312 ppm (Ungvary and Tatrai 1985). However, in other developmental toxicity studies, no effect on the number of resorptions was seen in rats at doses as high as 2,200 ppm (Green et al. 1978), in mice at 500 ppm (Murray et al. 1979), or in rabbits at doses of 500 ppm (Murray et al. 1979).

Reproductive effects have been noted in experimental animals exposed for intermediate durations, but the levels of exposure were higher (80-6,600 ppm) than those to which humans are exposed in the modem industrial environment (Ward et al. 1985; Wolf et al. 1956). In an intermediate-duration inhalation study, male and female CD-l mice were exposed 5 days per week, 6 hours per day to benzene vapor for 13 weeks (Ward et al. 1985). Histopathological changes were observed in ovaries (bilateral cysts) and testes (atrophy/degeneration, decrease in spermatozoa, moderate increase in abnormal sperm forms) of mice exposed to 300 ppm benzene; the severity of gonadal lesions was greater in the males. An inhalation study was conducted exposing rats (6,600 ppm), rabbits (80 ppm), and guinea pigs (88 ppm) to benzene for 7-8 hours a day, 5 days a week for 93, 243, and 32 or 269 days, respectively (Wolf et al. 1956). Male rats showed an increase in testicular weight after 93 days at the 6,600 ppm level. The guinea pigs showed a slight increase in average testicular weight at the 88 ppm level. Rabbits showed slight histopathological testicular changes (degeneration of the germinal epithelium) when exposed to 80 ppm. Since only 1 or 2 rabbits were used in this study, it

was not possible to draw any conclusions regarding benzene's ability to induce testicular damage in the rabbits. Continuous exposure of female rats to 210 ppm benzene for l0-15 days before cohabitation with males and 3 weeks after cohabitation resulted in a complete absence of litters (Gofmekler 1968). It is not known whether this was due to failure to mate, infertility, or early preimplantation losses of fertilized ova. In a fertility study, female rats exposed up to 300 ppm benzene for 10 weeks during premating, mating, gestation, and lactation showed no effect on indices of fertility, reproduction, and lactation (Kuna et al. 1992).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2-l.

2.2.1.6 Developmental Effects

The available human data on the developmental effects of benzene after inhalation exposure are inconclusive. The studies designed specifically to investigate developmental effects are limited, primarily because of concomitant exposure to other chemicals, inadequate sample size, and lack of quantification of exposure levels (Budnick et al. 1984; Goldman et al. 1985; Heath 1983; Olsen 1983). Benzene crosses the human placenta and is present in the cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976). In a study of subjects with known benzene poisoning in Italy, Forni et al. (1971a) reported the case of one pregnant worker exposed to benzene in the air during her entire pregnancy. Although she had severe pancytopenia and increased chromosomal aberrations, she delivered a healthy son with no evidence of chromosomal alterations. The following year she delivered a healthy daughter. However, increased frequency of chromatid and isochromatid breaks and sister chromatid exchange was found in lymphocytes from 14 children of female workers exposed by inhalation to benzene (dose not specified) and other organic solvents during pregnancy (Funes-Cravioto et al. 1977). No mention was made of whether the mothers showed signs of toxicity or whether physical abnormalities occurred among their offspring.

There are numerous inhalation studies in which animals have been exposed to benzene during pregnancy (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). None of these studies demonstrated that benzene was teratogenic even at levels that induced maternal and fetal toxicity. Fetotoxicity was evidenced by decreased body weight and by increased skeletal variants such as missing stemebrae and extra ribs,

which were not considered to be malformations. Alterations in hematopoiesis have also been observed in the fetuses and offspring of pregnant mice exposed to low levels of benzene (Keller and Snyder 1986, 1988). These studies are discussed below.

Mice exposed to 500 ppm benzene for 7 hours per day on days 6-15 of pregnancy exhibited fetal growth retardation (i.e., decreased fetal body weight) and increased minor skeletal variants (i.e., delayed ossification) (Murray et al. 1979). There were no fetal malformations and no significant effect on the incidence of pregnancy, average number of live fetuses, resorptions per litter, or maternal weight gain. No malformations in fetuses and no significant effects on incidence of pregnancy, average number of live fetuses, or resorptions per litter were observed when rabbits were exposed to benzene 7 hours per day at 500 ppm during gestation for 9 days, although an increase in minor skeletal variations was observed (Murray et al. 1979).

Pregnant mice exposed 12 hours per day to 156.5 or 313 ppm benzene on Gd 6-15 had pups with significant weight retardation and retardation of skeletal development, but no malformations (Ungvary and Tatrai 1985). A parallel study in rabbits showed that inhalation of benzene at 313 ppm caused fetal weight reduction, and an increase in minor fetal anomalies (Ungvary and Tatrai 1985).

As was the case with mice and rabbits, the fetotoxicity of benzene in rats is also demonstrated by retarded fetal weight and/or minor skeletal variants (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Tatrai et al. 1980b). In an experiment conducted by Green et al. (1978), pregnant Sprague-Dawley rats were exposed to 100, 300, or 2,200 ppm benzene for 6 hours per day on Gd 6-15. Exposure to high levels of benzene (2,200 ppm) during gestation resulted in a significant decrease in fetal weight, whereas dams breathing air containing lower levels of benzene (100 or 300 ppm) during gestation bore young that were similar in weight and crown-rump length to control pups. Statistically significant numbers of fetuses with delayed ossification were found in groups exposed to concentrations of 300 and 2,200 ppm. The litter incidence of missing stemebrae was significantly increased in the 100 ppm and 2,200 ppm exposure groups. Maternal toxicity, as indicated by a decrease in maternal weight gain, was evident only at the 2,200 ppm level. The female offspring appeared to be affected to a greater extent than males with respect to delayed ossification and missing stemebrae.

Kuna and Kapp (1981) found decreased fetal weight after exposure to 50 ppm and demonstrated that there was only 1 exencephalic rat in a group of 151 pups examined after *in utero* exposure to 500 ppm benzene. In the same study, of 98 pups examined for skeletal effects after *in utero* exposures of 500 ppm, only 1 pup had angulated ribs and 2 others had nonsequential ossification of the forefeet. These anomalies were not statistically significant and may have resulted from maternal nutritional stress.

No significant skeletal malformations occurred in pups of rats exposed during gestation to 47 ppm for 8 days, 24 hours per day (Tatrai et al. 1980b) or 100 ppm for 10 days, 6 hours per day (Coate et al. 1984). Decreased fetal weights were seen at 47 ppm (Tatrai et al. 1980b) and 100 ppm (Coate et al. 1984), and increased fetal mortality was observed at 141 ppm (Tatrai et al. 1980b). In CFY rats exposed to pure air or 125 ppm benzene on Gd 7-14, there was a 17% decrease in placental weight, a decrease in mean fetal weight, and evidence of skeletal retardation (Tatrai et al. 1980a). Continuous exposure of female rats to 6 concentrations of benzene ranging from 0.3 to 210 ppm for 10-15 days before cohabitation with males and 3 weeks after did not affect newborn weight or induce malformations, but there were differences in the weights of individual organs of the dams at all exposure levels (Gofmekler 1968). There was a slight tendency toward decreased litter sizes at 20 ppm of benzene. A complete absence of litters resulted from exposure to 210 ppm. It is not known whether this was due to failure to mate, infertility, or early preimplantation losses of fertilized ova.

Alterations in hematopoiesis have also been observed in the fetuses and offspring of pregnant mice exposed to benzene (Keller and Snyder 1986). Administration of 20 ppm benzene to pregnant Swiss Webster mice for 6 hours per day on Gd 6-15 caused reductions in the levels of the CFU-E of the fetuses, whereas 5 and 10 ppm benzene caused enhancement of these colony-forming cells. In 2-day-old neonates, CFU-E numbers in the 5 ppm group returned to control values, but the 10 ppm neonates showed a bimodal response by litter. Granulocytic colony-forming cells were enhanced in neonates exposed *in utero* to 20 ppm benzene. Some of the mice exposed to 10 ppm prenatally were re-exposed to 10 ppm as adults. Their hematopoietic progenitor cell numbers were depressed compared with controls exposed for the first time as adults. No tests were conducted on the dams after benzene exposure.

In a follow-up study, pregnant Swiss Webster mice were exposed 6 hours per day on Gd 6-15 to 5, 10, or 20 ppm benzene (Keller and Snyder 1988). The results indicated that 16-day fetuses, when checked for erythrocyte and leukocyte counts, hemoglobin analysis, and the proliferating pool of differentiating hematopoietic cells, had no noteworthy change at any of the exposure levels. In contrast, 2-day neonates exposed *in utero* to all concentrations of benzene exhibited a reduced number of circulating erythroid precursor cells and, at 20 ppm, had increased numbers of hepatic hematopoietic blast cells and granulopoietic precursor cells accompanied by decreased numbers of erythropoietic precursor cells. Six-week-old adult mice exposed *in utero* to 20 ppm of benzene had a similar pattern of enhanced granulopoiesis. However, this effect was not clearly evident in 6-week-old adult mice exposed to 5 or 10 ppm.

The results of inhalation studies conducted in experimental animals have been fairly consistent across species. It has been suggested that benzene fetotoxicity in animals is a function of maternal toxicity because the joint occurrence of a decrease in fetal weight and an increase in skeletal variants usually occurs when there is a decrease in maternal weight (Tatrai et al. 1980b). However, the mechanism underlying developmental toxicity has not been fully elucidated, and there are few data on the effect of benzene on maternal food consumption and on blood levels of benzene and its metabolites in the dams and their fetuses. There are apparently none of the usual fetotoxic findings after exposure *in utero* to low concentrations of benzene (≈10 ppm) (Coate et al. 1984; Kuna and Kapp 1981). As stated above, there is evidence for persistent hematopoietic anomalies in animals exposed *in utero* to benzene at 20 ppm (Keller and Snyder 1988). They may also exist at lower concentrations, but adequate testing has not been performed.

The highest NOAEL value and all reliable LOAEL values for developmental effects in each species following acute exposure are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.7 Genotoxic Effects

The available evidence for benzene-induced genetic toxicity in humans comes from studies of occupational exposure to benzene. In most of these studies, chromosome abnormalities were detected at exposure levels sufficient to produce blood dyscrasias. Exposures reported in these studies were primarily via inhalation, although dermal exposure may have occurred in some cases. The limitations of occupational studies include lack of accurate exposure data, possible coexposure to other chemicals,

and lack of appropriate control groups. The majority of these studies suggest that benzene and its metabolites are genotoxic, specifically clastogenic (i.e., capable of breaking the deoxyribonucleic (DNA) molecule in a manner observable at the chromosome level).

The evidence that benzene is clastogenic in humans comes from observed chromosomal aberrations in peripheral lymphocytes (Ding et al. 1983; Forni et al. 1971a; Karacic et al. 1995; Major et al. 1994; Picciano 1979; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Tompa et al. 1994; Tough and Court Brown 1965; Tough et al. 1970; Turkel and Egeli 1994). In one study, 21 people with hematological signs of chronic benzene poisoning exhibited significantly more chromosomal abnormalities (including hypo- and hyperdiploidy, deletions, gaps, and breaks) than the controls (Ding et al. 1983). The levels of benzene in air were not reported. Another study investigated benzene's effects in 33 workers exposed to air concentrations below 0.1 mg/L (31 ppm) for l0-26 years. Thirtyone of the workers did not show any clinical or hematological symptoms of chronic benzene intoxication. Two cases of pancytopenia were observed. The researchers reported nonrandom breaks and gaps in the chromosomes of the exposed group: chromosomes two, four, and nine were nearly twice as prone to breaks, while chromosomes one and two were nearly twice as prone to gaps (Sasiadek et al. 1989). However, this study is limited because relatively few controls were used, and all participants smoked which pre-exposed them to toxic compounds, including various levels of benzene, a component of tobacco smoke. The incidence of chromosome damage was much lower among the controls, and in addition, the distribution of damage in the controls was found to be random. The implication is that benzene and/or its metabolites may preferentially attack certain chromosomes (Sasiadek et al. 1989).

The peripheral lymphocytes of 20 male factory workers exposed to ambient benzene (concentrations not specified) for l-20 years were tested for chromosomal aberrations; the numbers of aberrant chromosomes were compared to those of 43 controls (Tough and Court Brown 1965). The researchers found a significantly higher number of unstable chromosomal aberrations, including acentric, dicentric, tricentric, and ring chromosomes, among the 20 exposed workers. Fourteen of the men had neutropenia. Inquiries were made regarding past exposure to radiation since radiation can cause similar effects. The researchers concluded that radiation exposure was not a causal factor (Tough and Court Brown 1965). In an update of this study (Tough et al. 1970), chromosomal aberrations in peripheral lymphocytes of groups of individuals from three different factories were examined. Group 1 workers from factory 1 consisted of 20 men exposed to benzene from l-20 years and 5 men

not exposed to benzene and employed for similar periods as previously described (Tough and Court Brown 1965). Exposure ceased 2-3 years before the blood samples were obtained. Group 2 workers from factory 2 consisted of 18 men employed for 6-25 years (12 exposed and 6 not exposed to benzene) with intermittent exposure to benzene, ending 4 years before the blood samples were obtained. Group 3 workers from factory 3 consisted of 20 men employed in a closed distillation plant for 2-26 years where benzene exposure continued until the time of the study. Five additional men in Group 3 who worked in factory 3 but were never employed in the distillation plant were used as controls. Blood samples were obtained and analyzed. The number of cells were counted and those containing unstable aberrations (Cu cells) and stable aberrations (C8 cells) were scored. Results showed significant increases in the percentage of cells with unstable chromosome aberrations (Cu cells) in groups of men exposed to benzene as compared with their corresponding controls and controls randomly selected from the general population. There were significant increases in the percentage of Cu cells in the exposed workers in Groups 1 and 2. In Group 2, the frequency of aberrations observed in the exposed workers was higher than the controls from the general population but did not differ from their on-site controls. In Group 3, the level of aberrations was approximately the same in the exposed workers and their on-site controls and neither differed significantly from the general population. Although the results suggest a relationship between benzene exposure and chromosomal aberrations in peripheral lymphocytes, the relationship does not appear to be a simple one.

In a study evaluating 52 workers exposed to benzene for 1 month to 26 years, chromatid breaks, chromosome breaks, marker chromosomes (rings, dicentrics, translocations, and exchange figures), and abnormal cells were evaluated in 200 peripheral lymphocytes from each worker, and the results compared to those from 44 workers with no recent history of benzene exposure (Picciano 1979). Monitoring of workroom air level of benzene during a 4-year period prior to the study indicated TWA levels of 2.1 ppm. However, some of the workers who were employed for up to 26 years may have been previously exposed to much higher levels. The percentage of aberrant findings was 3.5% for the exposed workers, and 2.9% for the unexposed workers. With regard to individual cytogenetic findings, there was no difference in the percentage of chromatid breaks or abnormal cells, but significant increases in frequencies of chromosome breaks (2-fold), and marker chromosomes (3-fold) were found in the peripheral lymphocytes of the exposed workers as compared to 44 control subjects. A significantly higher proportion of exposed workers with either chromosome breaks, or chromosome breaks and marker chromosomes was found. The distribution of individuals with these cytogenetic

aberrations was not associated with age. The lack of blood work-ups precluded the diagnoses of any hematological disorders in these workers. Exposure to ionizing radiation and/or other chemicals may have contributed to the chromosomal aberrations observed in the exposed group.

Case reports involving individuals with long-term occupational exposure to benzene also suggest that benzene damages chromosomes in hematopoietic cells and lymphocytes (Forni and Moreo 1967, 1969; Hartwich et al. 1969; Sellyei and Kelemen 1971; Van den Berghe et al. 1979). One of these case studies reported benzene exposure levels of 200-1,640 mg/m^3 (62.6-513.4 ppm) for a worker employed for 18 months who developed severe anemia, neutropenia, and thrombocytopenia. The patient was treated with transfusions and corticosteroids and showed improvement; however, the benzene-induced hemopathy evolved into leukemia (Sellyei and Kelemen 1971). At the same time, bone marrow specimens taken from this worker revealed the emergence of a new cell line characterized by supernumerary D-group chromosomes.

In another study, metaphase chromosome spreads were evaluated from 48 of 66 benzene-exposed individuals and 29 of 33 controls. The incidences of chromatid deletions and gaps were slightly increased in workers exposed to benzene levels of l0-100 ppm compared with control groups (Yardley-Jones et al. 1990). No concurrent hematological disorders were reported. However, these results were not statistically significant because of the heterogeneity of those examined. A small fraction of the exposed individuals had a higher frequency of chromosomal aberrations. Benzene exposure in these individuals was >5 years, and most of the time, air concentrations were <l ppm with peaks of 100 ppm. Eliminating the individual with the greatest number of aberrations from the study markedly reduced the significance of the comparison (Yardley-Jones et al. 1990). No mention was made of the presence of hematological disorders in these individuals.

Both numerical and structural aberrations were reported in 9 of 13 patients that worked in an environment with benzene concentrations ranging from 150 to 210 ppm (Erdogan and Aksoy 1973). All 13 patients had abnormalities of the blood (10 with pancytopenia, one with acute myeloblastic leukemia, one with acute monocytic leukemia, and one with preleukemia). Thirty-four workers exposed to high concentrations of benzene showed chromosome damage in peripheral lymphocytes and bone marrow cells that persisted after cessation of exposure (Forni et al. 1971a). Of the 34, 16 had severe hemopathy. Although persistent effects in peripheral lymphocytes were considered to be an indicator that damage had occurred, they provide no conclusive evidence linking chromosomal damage

to the development of leukemic changes. However, stable chromosome aberrations in bone marrow cells could give rise to leukemic clones (Forni and Moreo 1967). For example, a 38 year-old female with benzene exposure for approximately 22 years (level not reported) presented with anemia that evolved, after a long latent period, into acute myeloblastic leukemia. During the latent period, multiple cytogenic studies showed a high rate of unstable and stable chromosome aberrations in cultured peripheral blood lymphocytes (Forni and Moreo 1967).

Several studies looked for benzene's effects on sister chromatid exchange in humans. One of these reports involved workers exposed to benzene levels ranging from 10 to 100 ppm in excess of 5 years (Yardley-Jones et al. 1988). The average benzene concentration from another report was determined to be around 50 ppm (Seiji et al. 1990). The findings were negative in both studies. However, in a third study of occupational exposure, a slight but statistically significant (6.55 compared to 6.05) increase in sister chromatid exchanges was observed in peripheral lymphocytes from workers exposed for an 8-hour TWA of 1.3 ppm benzene (range 0.3-5 ppm), in combination with toluene (Popp et al. 1992).

Data from *in vivo* animal studies strongly support the genotoxicity of benzene. Analyses of chromosomal aberrations, sister chromatid exchange, and micronuclei in the bone marrow and lymphocytes of both mice and rats consistently showed positive results. Acute exposure of male rats to 750 and 7,500 ppm benzene for both single and multiple exposure periods resulted in a significantly increased frequency of chromosomal aberrations in bone marrow cells (Anderson and Richardson 1981). Similarly, male Wistar rats exposed to 100 and 1,000 ppm benzene for 6 hours had elevated frequencies of chromosomal aberrations in bone marrow cells (Styles and Richardson 1984). Male and female DBA/2 mice acutely exposed to 3,100 ppm showed significant increases in sister chromatid exchange among bone marrow cells, but the rise in chromosomal aberrations was not significant (Tice et al. 1980, 1982). Both DBA/2 and C57BL/6 mice exposed to benzene concentrations ranging from 28 to 5,000 ppm exhibited a significant linear increase in sister chromatid exchange frequencies starting at the lowest concentration. The response leveled at 3,000 ppm for the DBA/2 mice and at 2,000 ppm for the C57BL/6 mice (Tice et al. 1982).

The evaluation of micronuclei frequencies in peripheral blood polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) permits an assessment of both recently induced and chronically accumulated bone marrow damage. PCEs have a life span of about 24 hours and are good indicators

of acute damage. NCEs have a life span of about 30 days and are good indicators of chronic damage. A study involving male DBA/2 mice reported a significant dose-dependent increase in lymphocyte sister chromatid exchange frequency and in micronuclei of bone marrow PCEs following a 6-hour exposure to 10, 100, and 1,000 ppm benzene (Erexson et al. 1986). The same experiment also involved Sprague-Dawley rats; there were significant increases in sister chromatid exchange frequencies in peripheral blood lymphocytes following exposures to 3, 10, and 30 ppm and dose-dependent increases in micronuclei of bone marrow PCEs after exposures to 1, 3, 10, and 30 ppm benzene (Erexson et al. 1986). Dose-dependent increases in micronuclei frequencies in bone marrow PCEs were observed in male NMRI mice following both continuous and intermittent acute exposure to benzene; significant increases began at 21 ppm (Toft et al. 1982). One study compared the effects of the duration of benzene inhalation on peripheral blood PCEs and NCEs in male and female DBA/2 mice (Luke et al. 1988a). Experimental DBA/2 mice were exposed to 300 ppm benzene for 6 hours per day for 5 days per week (Regimen 1) or 3 days per week (Regimen 2) for a duration of 1-13 weeks. PCEs were affected by benzene inhalation independent of exposure duration and regimen, while NCEs were affected only following Regimen 1 exposure. Males were more sensitive to benzene inhalation than females (Luke et al. 1988a). In a similar experiment, the effects of benzene on three different strains of male mice (DBA/2, C57BL/6, and B6C3F$_1$) were compared (Luke et al. 1988b). The male mice were exposed to 300 ppm benzene for 6 hours per day for 5 days per week (Regimen 1) or 3 days per week (Regimen 2) for a duration of 13 weeks. Micronuclei increases in PCEs were found to be strain specific (DBA/2 > C57BL/6 = B6C3F$_1$). Again, the effects on the PCEs were independent of exposure and regimen. Micronuclei increases in NCEs were observed to be both strain and regimen dependent with C57BL/6 = B6C3F$_1$ > DBA/2 and Regimen 1 > Regimen 2. The authors concluded that genotoxic damage by benzene inhalation can be dependent upon sex, strain, exposure duration, and exposure regimen (Luke et al. 1988a, 1988b).

CD-l mice were exposed to purified air or benzene by inhalation at 0.04, 0.1, or 1.0 ppm for 22 hours per day, 7 days per week for 6 weeks (Ward et al. 1992). The effects of *in vivo* exposure to benzene were evaluated by using an autoradiographic assay to determine the frequency of mutants which represent mutations at the hypoxanthine-guanine phosphoribosyl transferase (hprt) locus in spleen lymphocytes. At the end of the six weeks exposure period, lymphocytes were recovered from the spleens of the mice and cryopreserved prior to assay. Mutant cells were selected on the basis of their ability to incorporate tritiated thymidine in the presence of 6-thioguanine. The increased frequencies of mutant spleen lymphocytes were significant at the low and mid, but not the high dose, and the

method does not take into account possible clonal expansion. Further evaluation of the induction of gene mutation at these dose levels seems warranted.

Mutations have also been detected in tissue cells after inhalation exposure of animals to benzene. Male C57BL/6 mice were exposed to a target dose of 300 ppm benzene 6 hours per day, 5 days per week for 12 weeks (Mullin et al. 1995). Control animals were exposed to filtered conditioned air. Animals were sacrificed and genomic DNA was purified from the lungs, spleen, and liver tissues. . Increased frequencies of mutations in the lacI transgene were detected in lung and spleen, but not in the liver. In another study, female BDFI mice (C57BL/6xDBA/2F$_1$ hybrids) were exposed to 0, 100, 300, and 900 ppm benzene 6 hours per day, 5 days per week for up to 8 weeks (Plappert et al. 1994a). An increase in DNA damage was noted in peripheral blood ceils after exposure to 300 or 900 ppm benzene for 3 days up to 4 weeks. In the bone marrow, 100 and 300 ppm benzene produced an effect after 5 days of exposure. No accumulation of DNA damage was seen after 4 weeks. At 300 ppm benzene, the repair was not complete even after 48 hours. At 100 ppm benzene, significant changes were seen in the liver after 5 days, but not in the blood. The repair, which was measured 24 and 48 hours after the end of the exposure, was almost complete in the blood and the liver after the 5-day exposure to both concentrations. However, the repair was not complete in the blood after 4 weeks of exposure to 300 ppm in the liver after 4 weeks of exposure to 100 and 300 ppm. In a companion study, female BDFl mice (C57BL/6xDBA/2F$_1$ hybrids) were exposed in inhalation chambers to benzene (300 or 900 ppm) and/or toluene (250 or 500 ppm) 6 hours per day, 5 days per week for up to 8 weeks (Plappert et al. 1994b). DNA damage was studied in peripheral blood cells, bone marrow, and liver using the single cell gel assay and pooled blood from 3 animals/group. At 300 or 900 ppm benzene exposure, significant increases in DNA damage were detected in cells sampled from blood, liver and bone marrow. Combined exposures of benzene and toluene did not induce significant increases in DNA damage.

Other evidence of genotoxicity induced by benzene includes inhibition of DNA synthesis in certain cell types, although inhibition of DNA synthesis is not always indicative of genotoxic damage. Acute 4-hour inhalation exposure of female ICR mice to 2,000 ppm benzene produced a 15% inhibition of DNA synthesis in bone marrow cells, and exposure to 3,000 ppm inhibited DNA synthesis by 33% (Lee et al. 1988). Delayed cell cycle in mouse bone marrow and DNA adducts with benzene metabolites in mouse and rat hemoglobin or rat liver cells were also evidence of benzene inhalation

exposure (Lutz and Schlatter 1977; Sabourin et al. 1990; Tice et al. 1980, 1982). Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

Neoplastic diseases of the hematopoietic system differ with respect to morphologic and clinical manifestations and often in their response to therapy. Leukemia may be divided into granulocytic leukemias (which include myelocytic, monocytic, and erythroblastic cell types) and lymphocytic leukemias. Both granulocytic and lymphocytic leukemias may be separated into acute and chronic forms. In acute myeloid leukemia (AML), there is diminished production of normal erythrocytes, granulocytes, and platelets, which leads to death by anemia, infection, or hemorrhage. These events can be rapid. In chronic myeloid leukemia (CML), the leukemic cells retain the ability to differentiate and perform function; later there is a loss of ability to differentiate. Case reports and epidemiological studies of workers have established a causal relationship between benzene exposure and AML. While some studies have implicated other types of leukemia or even lymphomas, only the incidence of AML and its variants has consistently been increased in groups of workers with excess benzene exposure (Goldstein 1988).

The clinical and epidemiological evidence is summarized below. The deficiencies in the studies include the lack of appropriate sampling techniques, exposure determinations, mortality standards, and other aspects of experimental design or methodology. Additionally, intermittent exposures to benzene made it difficult to assume that the average concentrations of benzene measured in a workplace actually indicated the true exposure experienced by each worker (Goldstein 1985). A cause-effect relationship between benzene and leukemia is sufficiently clear; however, there are few data from which dose-response relationships can be established.

Aksoy et al. (1974) reported the crude incidence of leukemia between 1967 and 1973 among 28,500 shoe, slipper, and handbag workers exposed to benzene in Istanbul, Turkey, to be approximately 13.5 per 100,000, compared to an estimated annual leukemia incidence of 6 per 100,000 for the general population of Turkey, resulting in a relative risk of 2 for all cell types of leukemia combined. This risk estimate was based on the diagnosis of leukemia or preleukemia (acute erythroleukemia) in 26 shoe workers in Istanbul and did not take into consideration differences in the age structure of the population of shoe workers and the general population. Average air concentrations

of benzene in the factories were reported to be between 15 and 30 ppm during non-work hours and about 150 ppm during work hours, and reached a maximum of 210 ppm (and in rare instances, 640 ppm) when adhesives containing benzene were used (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974). The duration for exposure of these workers with leukemia ranged from 3 months to 17 years. The clear association between exposure to benzene and the development of pancytopenia, which may progress to leukemia, alerted officials of these industries in Turkey that worker exposure to benzene needed to be reduced. The number of cases of pancytopenia in Turkey was reduced following the prohibition of benzene in 1969; the persistence of aplastic anemia associated with exposure to benzene is due to the use of benzene as a solvent for rubber and dyes, or for cleaning in some workplaces; it also may be due to the exposure to this agent before its use as a solvent was regulated (Aksoy et al. 1984). In a more recent study of 231 workers in 40 workplaces in Turkey, Aksoy et al. (1987) reported that 76.4% of the solvents and 19.1% of the thinners contained benzene concentrations of more than l%, ranging between 0.7% and 7.6% (mean, 2.88%) for the solvents and between 0% and 6.6% (mean, 0.73%) for the thinners. The air concentration of benzene bracketed around 1 ppm. However, the concentration of benzene in one part of the tire cord plant was 110 ppm. Five cases of cancer, including leukemia and malignant lymphoma, were found among the exposed workers. Results of the hematological analysis also showed that 14 of the 231 workers had mild abnormalities, including leukopenia, thrombocytopenia, and pancytopenia (Aksoy et al. 1987).

Subjects with benzene-induced hyporegenerative anemia or pancytopenia of a long-standing duration subsequently developed acute leukemia. Between 1942 and 1975, 66 cases of benzene hemopathy were seen at the Institute of Occupational Health in Milan, Italy (11 of these were leukemia) (Vigliani and Forni 1976). The affected individuals worked in rotogravure plants, shoe factories, and other industries where benzene was used as a solvent. Benzene concentrations in the air near rotogravure machines were calculated to range between 200 and 400 ppm, with peaks up to 1,500 ppm.

A cohort of chemical workers exposed to benzene from 1946 to 1975 was identified. A dose-response relationship between cumulative exposure to benzene and mortality (from all lymphopoietic cancer combined and from leukemia) was reported for this population of 3,536 male chemical workers who had cumulative benzene exposures of <180 ppm-months (1,809 workers), 180-719 ppm-months (1,047 workers), or ≥720 ppm-months (680 workers) (Wong 1987b; Wong et al. 1983). Workers exposed to a cumulative benzene dose of ≥720 ppm-months were 4 times more likely (relative risk, 3.93) to develop leukemia or other lymphopoietic cancers. Of the 3,536 workers that had continual

exposure to benzene, 7 died of leukemia. When compared to nonexposed workers, the excess lymphopoietic cancer was significant for all males (relative risk, 3.2). None of the 7 leukemias were of the acute myelogenous cell type. A dose-response relationship between cumulative exposure and non-Hodgkin's lymphopoietic cancer was of borderline statistical significance (p=0.06). Although the dose-response relationship adds strength to the association between exposure to benzene and leukemia and lymphatic and hematopoietic cancer, the estimated historical exposure data were not precise enough for quantitative risk assessment. Additionally, when exposure to benzene is expressed as cumulative exposure (in ppm-months exposure), it is not possible to distinguish between concentration and duration of exposure.

A retrospective cohort study was done in 1982-83 on 28,460 benzene workers in China, all of whom had worked in various factories for at least half a year between 1972 and 1981; however, exposure and employment duration were not necessarily limited to those years (Yin et al. 1987a). Thirty cases of leukemia (23 AML, 7 CML) with a mean latency of 11.4 years (0.8-49.5 years) were found in the benzene cohort, as opposed to 4 cases in a matched control cohort. Twenty-five of the leukemic workers had already died. Information on exposure levels was collected from company records, but there is no indication of the extent of these records with the exception of a note that three of the reported levels were based on only one measurement. Mean benzene levels to which workers with leukemia were exposed ranged from 3 to 313 ppm, with the majority falling between 16 and 157 ppm. Exposure ranges from which the means were derived were rather wide, indicating the possibility of at least occasional high exposures. Only 4 upper-level measurements were less than 10 ppm, while half of the remaining measurements were between 10 and 100 ppm, and the other half were between 100 and 2,000 ppm. The authors observed that the cumulative mortality due to leukemia was proportional to the duration of the exposure to benzene for exposure of up to 20 years and then leveled off.

The retrospective cohort study was also used to examine all causes of mortality. The authors (Yin et al. 1989) reported that mortality from all causes was significantly higher in the exposed - group-265.46 deaths per 100,000 person-years for the exposed group as compared to 139.06 deaths per 100,000 person-years for the unexposed group. Additionally, incidences of malignant neoplasms for the exposed group were significantly higher than for unexposed workers-123.21 cases per 100,000 person-years for the exposed group compared to 54.7 cases per 100,000 person-years for the unexposed group. Increased mortality was also noted in benzene-exposed men as indicated by

standardized mortality ratios (SMRs), and SMRs were significantly elevated for leukemia (574) and lung cancer (231) and nonsignificantly elevated for primary hepatocarcinoma (112) and stomach cancer (122). (An SMR of 100 is the normal value if an excess is not observed. An SMR of 200 represents 100% excess risk over normal.) The authors emphasized that the estimated average and cumulative lifetime benzene exposure levels were based on relatively few measurements; therefore, their findings are to be interpreted cautiously. They do emphasize, however, that the study supports the conclusion of an excess of acute nonlymphocytic leukemia among benzene-exposed subjects.

The same group reported that between 1979 and 1981, Chinese workers using benzene or benzene-containing mixtures were examined. Nine cases of leukemia were found out of 4,602 exposed workers. Presumably, some of these may have been included in the study discussed above. Although one worker was exposed for only 2 years, the others were exposed for between 7 and 25 years. No estimate of exposure levels was given for the leukemia cases, but exposure estimates for aplastic anemia cases found in the same study were 29-362 ppm (Yin et al. 1987c).

A retrospective cohort study of incident cases of hematopoietic neoplasms and related disorders among 74,828 benzene-exposed workers employed between January 1, 1972 to December 31, 1987 in 672 factories in 12 Chinese cities was conducted (Travis et al. 1994). Workers (35,805) not occupationally exposed to benzene employed in 109 factories during the same period were used for comparison. Follow-up of both exposed and nonexposed workers was carried out using occupational and medical records, and histopathologic material were reviewed for all patients with hematopoietic malignancies to ensure correct classification. Among benzene-exposed workers, 82 patients with hematopoietic neoplasms and related disorders were diagnosed: 32 (39%) cases of acute leukemia, 9 (11%) aplastic anemia, 7 (9%) myelodysplastic syndrome (MDS), 9 (11%) chronic granulocytic leukemia (CGL), 20 (24%) malignant lymphoma (ML) and related disorders, and 5 (6%) others. Among the nonexposed group, 13 hematologic malignancies were diagnosed: 6 (46%) patients with acute leukemia, 2 (15%) CGL, 3 (23%) ML, and 2 (15%) others. The hematopathologic features of acute nonlymphocytic leukemia (ANLL) associated with benzene exposure resembled the hematological features following chemotherapy or radiotherapy. In addition, this study documented MDS in association with benzene exposure. ANLL in workers exposed to benzene may represent a distinct clinicopathologic entity and may display a preleukemic phase in some patients, as does therapy-related ANLL. This study also showed greater diversity of hematologic malignancies in benzene exposed workers than previously reported. No significant differences were found in the

relative risks for cancer mortality between male and female workers in this cohort as reported by Li et al. (1994).

A cohort of 748 white male workers occupationally exposed to benzene for varying time periods between 1940 and 1949 in 2 manufacturing facilities producing rubber hydrochloride (Pliofilm®) has been studied (Infante 1978; Infante et al. 1977). The initial report (Infante et al. 1977) included evaluation of mortality statistics up to 1975, and the follow-up study (Infante 1978) included evaluation up to 1977. A summary of the overall results is presented here. Included for the study were all white males assigned to Pliofilm ® production who were hourly employees at any time between January 1, 1940 and December 31, 1949. Personnel employed in Pliofilm ® operations but who were not assigned to production jobs were not studied. All study cohort members were followed from the time of termination of employment to June 30, 1975. The risk of lymphatic and hematopoietic malignancies was calculated. The vital status of approximately 75% of the 748 cohort members who were originally in the study was verified. Persons for whom vital status could not be verified were assumed alive. In order not to overestimate the true risk of hematopoietic and lymphatic malignancies which may have been associated with benzene exposure, causes of death were determined from death certificate reporting, and coded according to an international standard list. A modified life table technique was used to generate person-years at risk of dying according to 5-year age grouping and 5-year calendar time periods. Two populations were chosen as control groups for generating the number of expected deaths in the study population. The first group was U.S. white males, and the second group was made up of employees at a fibrous-glass-construction products facility over the same period of time as the test population (January 1, 1940 to December 31, 1949) and who had achieved 5 or more years of employment by June 1972, which was the cutoff date for that study. In the test population, 140 total deaths were observed in the time period ending April 13, 1977, compared to 187 expected on the basis of the U.S. white male control group. The deficit was probably due to lack of follow-up for the test population. For total lymphatic and hematopoietic cancer, there were 9 observed versus 3.4 expected deaths when compared to U.S. white males. This difference was statistically significant, and is attributed to an excess in the incidence of leukemia. A mortality study of rubber workers exposed to benzene between 1940 and 1949 revealed lymphatic hematopoietic cancer, myeloid leukemia, and monocytic leukemia with a Standard Mortality Ratio (SMR) of 260. There were 7 observed compared to 1.38 expected deaths from leukemia. The SMR was 506, indicating a 5-fold excessive risk. When compared to the fibrous glass factory population, there were 7 observed compared to 1.47 expected deaths from leukemia (SMR=474), essentially the

same risk. The authors indicated that this estimate was conservative. When the population was followed for an additional 3 months, the SMR for leukemia was 560. Identification of type of leukemia revealed 4 cases of acute myelogenous leukemia, 2 cases of monocytic leukemia, and 1 case of chronic myelogenous leukemia. An additional case of myelogenous leukemia was subsequently identified that did not fit into the definition of the cohort, since the person started employment at the plant in 1950. This person was not included in the cohort or in the statistical analysis. With regard to the specific cell type of leukemia, the increased risk of myelogenous leukemia is between 5- and 10-fold, depending on the group used for comparison. Comparison with the National Cancer Institute age-specific leukemia mortality rate or the Connecticut Tumor Registry yields an 8.5-fold or l0-fold increased risk, respectively. The average benzene exposure for workers between 1941 and 1949 ranged from 10 to 100 ppm. Furthermore, the industrial hygiene assessments led to the conclusion that the environment of the workers in Pliofilm ® production was not contaminated with other materials known to be associated with the induction of blood disorders.

Rinsky et al. (1981) reexamined the population at the Pliofilm ® factories used by Infante (Infante 1978; Infante et al. 1977). In another retrospective cohort mortality study, workers exposed to benzene in the manufacture of rubber hydrochloride at these two locations in Ohio were evaluated for possible association between exposure to benzene and subsequent death from leukemia. The study population was in two groups. Group 1 was the same as in the Infante et al. (1977) study. In this later follow-up study, vital status of 98% of the 748 men in the cohort was ascertained (Rinsky et al. 1981), compared to 75% by Infante (Infante 1978; Infante et al. 1977). The remaining 2% were considered alive as of the study end date. Group 2 consisted of 258 individuals who first worked in a department with benzene exposure between January 1, 1950 and December 31, 1959. No examination was attempted of persons whose first exposure to benzene occurred after 1959 because the number of individuals was small and because one of the locations ceased production of rubber hydrochloride in 1965. Vital status follow-up was conducted to June 30, 1975. In Group 1, there were 180 deaths from all causes, compared with an expected number of 161 (SMR=lll). Ten cases of malignancies of the hematopoietic and lymphatic systems were observed, versus 3.03 expected, which was a significant increase. With regard to leukemia, 7 cases were observed compared 1.25 expected (SMR=560). All 7 cases were of the myeloid or monocytic cell types. The 7 cases of leukemia were described. The ages of the men ranged from 29 to 65 at the time of death. Length of exposure ranged from 1 month to 20 years. Average exposures for these men ranged from 35 to 100 ppm benzene over the course of their employment. The time interval between first exposure and death ranged from 2 years to

22 years. In Group 2, there were 49 observed deaths from all causes compared to 56 expected (SMR=87). One case of leukemia was observed (as described as case 8 in Infante et al. 1977). Four additional cases of leukemia were discovered, but were not included in the later study since they did not comply with the cohort description of Group 2. The major findings of the later report confirm those of the previous report. Forty more deaths from all causes were reported in the Rinsky study (Rinsky et al. 1981). No additional cases of leukemia were noted among persons fitting the cohort description. There is a suggestion from the data that persons exposed at levels slightly higher than 10 ppm may exhibit a longer latency interval between exposure and the development of leukemia.

Rinsky et al. (1987) later published an epidemiological risk assessment in an update of the earlier studies (Infante 1978; Infante et al. 1977; Rinsky et al. 1981). In this update, cumulative benzene exposures were calculated for each member of the cohort in ppm-years. A total of 1,165 white males with at least 1 ppm-day of cumulative exposure to benzene through December 31, 1965, were included in the cohort. Causes of death were ascertained from death certificates up to December 31, 1981. SMRs were determined for leukemia by four cumulative exposure categories: 1 ppm-day to 40 ppm-year, 40-199.99 ppm-year, 200-399.99 ppm-year, and more than 400 ppm-year. A total of 1,165 white men included in the cohort contributed 31,612 person-years at risk. On December 31, 1981, a total of 819 (70.3%) were alive, 330 (28.3%) were dead, and 16 (1.4%) were lost to follow-up (total 98.6 accountability). Neither the mortality from all causes or the mortality from all malignant neoplasms combined was above the expected rate. There was a significant increase in deaths from all lymphatic and hematopoietic neoplasms (15 observed compared to 6.6 expected; SMR=227). This increase was due to an increase in the incidence of leukemia (9 cases observed versus 2.7 expected; SMR=337), and from multiple myeloma (4 observed versus 1 expected; SMR 409). In one location, 2 cases of leukemia were observed versus 0.58 expected (SMR=345); and in the other location, 5 cases were observed versus 0.67 expected (SMR=746). This follow-up study by Rinsky et al. (1987) revealed malignant neoplasias of the lymphatic and hematopoietic tissue (SMR=330) and both myelogenous and monocytic leukemias (SMR=560) following chronic benzene exposure (cumulative exposure levels were from <40 to >400 ppm over a 40-year working lifetime). The principle findings of this study were that there is a strongly positive exposure-response relation between benzene and leukemia. A question remains concerning the precise levels of benzene to which workers were exposed. Nevertheless, cumulative exposures categorized as <40, 40-200, 200-400, and >400 ppm-years had SMRs for leukemia of 109, 322, 1,186, and 6,637, respectively (a progressive increase in SMR with increasing cumulative dose).

The majority of benzene workers with hematologic disorders in the Rinsky studies (1981, 1987)were diagnosed before 1955. Thus, it is likely that diagnoses in the Rinsky studies of aplastic anemia, acute myelocytic anemia, and blood dyscrasia with secondary anemia and leukemoid reaction may actually represent myelodysplastic syndromes (i.e., patients whose stem cells had undergone malignant transformation but who died of anemia plus complications before frank acute leukemia became manifest). The myelodysplastic syndromes are disorders of stem cells and are characterized by maturation defects. Patients with myelodysplastic syndromes present with anemia and may die of infection or hemorrhage (due to platelet/clotting abnormalities), or their condition may progress to acute leukemia. Recently, the syndromes have been recognized in increasing numbers of younger patients who had received cytotoxic chemotherapy, radiation therapy, or both forms of treatment for a variety of malignancies. Diagnosis of myelodysplastic syndromes requires thorough examination of peripheral blood films and bone marrow preparations with precise application of diagnostic criteria. These diagnostic criteria changed between 1976 and 1982. Individuals with myelodysplastic syndromes should be included in the population at risk of developing leukemia following benzene exposure.

A more recent update and risk analysis of this cohort has been published (Paxton et al. 1994a, 1994b). The Pliofilm ® cohort consisted of 1,868 individuals; 1,044 worked at the St. Marys plant location from 1940 to 1975 and 824 at the Akron plant site from 1936 to 1965. This cohort was classified into two groups: the wetside workers (1,291 workers) with direct benzene exposure and the dryside workers (577) with no direct benzene exposure. In the most recent update of these, NIOSH followed up the vital status of only the wetside workers through 1987. The updated vital status of the wetside workers from 1970 to 1981 resulted in the identification of an additional 123 deaths for a total of 481 deaths in the wetside subgroup. No new cases of multiple myeloma were reported. The results of this analysis support previous studies of this cohort.

A historical prospective study of mortality was conducted among 594 white males occupationally exposed to benzene in a chemical manufacturing facility and employed for varying time periods between 1940 and 1970 (Ott et al. 1978). The TWA exposures to benzene ranged from 0.3 to 35 ppm for various job categories. Three cases of myelogenous leukemia were identified (as compared to 0.8 expected cases); this is statistically significant based on incidence data from the Third National Cancer Survey (NOES 1990). Of the three cases, two were categorized as myelogenous leukemia, and one was categorized as bilateral bronchopneumonia, but myeloblastic leukemia was noted as an "other

significant condition." A fourth cohort member died from aplastic anemia, while a fifth died from pernicious anemia. Bond et al. (1986b) performed an update of the Ott et al. (1978) study. This follow-up studied an additional 362 chemical workers potentially exposed between 1976 and 1982. Two additional deaths from myelogenous leukemia were identified, bringing the total number of deaths primarily from myelogenous leukemia to four in this cohort. Four deaths due to myelogenous leukemia represented a nonsignificant increase compared to the expected number of leukemia deaths (all types, 2.1 deaths expected, SMR=194), but it did represent a significant increase in the number of myelogenous leukemia deaths (versus 0.9 expected myelogenous leukemia deaths, SMR=444). The workers who died of leukemia had the potential for unquantified, brief, but potentially high, exposures to benzene (Bond et al. 1986b). The authors note that although the study provides support for the association between exposure to benzene and acute myelogenous leukemia, the data are complicated by small numbers of affected workers, concomitant exposure to other toxic compounds, and the uncertainty of the actual exposure level.

A similar study detected 3 cases of death from leukemia out of 6,520 workers in the fuel and steel industry who were followed for 20 years (Hurley et al. 1991). This epidemiologic evidence leads to the conclusion that leukemia risk exists at low benzene concentrations.

Another epidemiological study showed no leukemia deaths following benzene exposures to concentrations less than 1 ppm (Tsai et al. 1983). In this study, the cohort consisted of 454 employees at a Texas refinery who worked at aromatic distillate hydrogenation or cumene unit work stations between 1952 and 1978. The median benzene exposures were 0.14 ppm for refinery workers and 0.53 ppm for those in benzene-related units. Not all of the median exposures were TWAs. Within this cohort, the relative risk for all cancer was not significant as compared to case referents or to the general population of the United States. Furthermore, the evaluation of medical records of this cohort showed no significant changes in blood indices (leukocyte, erythrocyte, hemoglobin, hematocrit, platelet, clotting and bleeding time in minutes).

An evaluation of occupational chemical exposure, histological subtype, and cytogenetics was conducted on case studies of newly diagnosed AML or CML, or myelodysplastic syndromes (MDS) treated in the Main Hospital of Torino, Northern Italy, between October 1, 1989 and December 31, 1990 (Ciccone et al. 1993). There was a nonstatistically significant increased relative risk for exposure

to benzene. Data show 3 AML, 2 CML, and 4 MDS cases with regard to benzene exposures. No excess of clonal chromosome abnormalities was detected among occupationally exposed AML patients. Other studies also suggest increased risk of kidney, brain, bladder cancer, and soft-tissue sarcoma in addition to hematopoietic cancers after chronic-duration inhalation exposure to benzene (Greenland et al. 1994; Hunting et al. 1995; Lagorio et al. 1994b; Serraino et al. 1992; Steineck et al. 1990).

EPA (IRIS 1996) has reviewed the human and animal carcinogenicity data on benzene. A summary of the EPA calculations of unit risk values for leukemia based on human epidemiological studies is given below. It should be noted that these values are estimates of human risk since the true human risk at low doses cannot be accurately identified. The unit risk should not be used if the air concentration exceeds 100 $\mu g/m^3$ (0.031 ppm) since above this concentration the unit risk may not be appropriate. One ppm of benzene (3,190 $\mu g/m^3$) exceeds the upper limit of 100 $\mu g/m^3$.

The potency of benzene has been estimated by EPA based on three separate epidemiological studies (Ott et al. 1978; Rinsky et al. 1981, 1987; Wong et al. 1983). Equal weight was given to cumulative dose and weighted cumulative dose as well as to relative and absolute risk model forms. EPA estimated the lifetime probability of cancer to be 2.9×10^{-2} $(mg/kg/day)^{-1}$ or 2.7×10^{-2} $(ppm)^{-1}$ (IRIS 1996). Based on this value, the exposure levels associated with individual lifetime upper-bound risks of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} have been calculated to be 4×10^{-3}, 4×10^{-4}, 4×10^{-5}, and 4×10^{-6} ppm, respectively. These exposure level are indicated in Figure 2-l.

Crump (1994) published a risk estimate for benzene, using data from the Pliofilm ® cohort through 1987, and applying a sensitivity analysis. The sensitivity of the risk estimates to model the assumptions was evaluated by applying dose-response models, and by investigating the types of cancer that were linked to benzene exposure. The analyses conducted by Crump (1994) were based on the incidence of acute myelogenous leukemia, and predicted a latency from benzene exposure to death of 7-20 years, compared to death from leukemia after radiation in 3-4 years. Multiplicative risk models were found to be better than additive risk models. Lifetime risks from 45 years of occupational exposure to 1 ppm benzene were between 2×10^{-5} and 3.6×10^{-4}. Unit risk (continuous lifetime exposure to 1 ppm benzene) ranged from 9.7×10^{-5} to 1.7×10^{-4}. Upper-bound risks for 45 years of occupational exposure were 1.6×10^{-3} to 3.1×10^{-3}, and from 7.7×10^{-3} to 1.5×10^{-2} for continuous lifetime exposure (unit risk). Wong (1995) also published an analysis of risk of acute myeloid

leukemia (AML) and multiple myeloma using the Pliofilm ® worker data. No increased risk of AML was detected for cumulative exposure to benzene below 200 ppm-years. Above 200 ppm-years, risk of AML rose reaching an SMR of 98.37 for exposures of >400 ppm-years. No relation between multiple myeloma and benzene exposure was found. Wong and Raabe (1995) looked at the risk of cell-type specific leukemia in workers in both the United States and the United Kingdom. No increase was found in the risk for AML, chronic myelogenous, acute lymphocytic, or chronic lymphocytic leukemias.

Recent studies have shown benzene to be carcinogenic in animals by inhalation. However, all results are not universally accepted, primarily because of the difficulty in developing reliable animal models for benzene-induced leukemia. For example, inhalation of air concentrations of 300 ppm benzene for 4 weeks, 5 days per week, 6 hours per day by HRS/J mice did not alter the time of onset or the incidence of leukemia expected in HRS/J mice (Stoner et al. 1981). Stoner et al. (1981) suggested that perhaps a genetic shift has occurred in the HRS/J mouse during the past 10 years so that the (hr) gene locus no longer influences the previously observed greater susceptibility to leukemia in homozygous (hr/hr) animals.

Results from a series of ongoing studies in which Sprague-Dawley rats (13-week-old adults and 12-day-old embryos) were exposed 5 days per week, 4-7 hours per day to 200-300 ppm benzene via inhalation (for 15 or 104 weeks) and observed until spontaneous death suggest that benzene is a multipotential carcinogen (Maltoni et al. 1982a, 1982b, 1983, 1985, 1989). Exposure to benzene developed an increased incidence of Zymbal gland carcinomas, carcinomas of the oral and nasal cavities, hepatomas, and other malignant tumors (Maltoni et al. 1982b, 1983). An overall increase in incidence of total malignant tumors in benzene-exposed animals was observed, but there was no information on the significance of these findings. The incidence of certain carcinomas appeared to be dependent, to some extent, on exposure duration and the age of the treated animals (embryos were more susceptible than adults). The EPA Gene-Tox Carcinogenesis Panel (Nesnow et al. 1986), in an evaluation of the Maltoni et al. (1982b) study in which Zymbal gland carcinoma was induced, called results of the inhalation experiments inconclusive. In effect, the panel (after reevaluating the raw data, including the slides used for histopathology determinations) would not support a direct association between benzene and the malignant tumors reported by Maltoni et al. (1982b). However, in a study by Snyder et al. (1984), Sprague-Dawley rats exposed to 100 ppm benzene for 6 hours per day, 5 days per week for a lifetime exhibited not only Zymbal gland carcinoma, but also increased incidence of

myelogenous leukemia and liver tumors. It should be noted that Snyder et al. (1978a) found no indications of leukemia or preleukemic responses in Sprague-Dawley rats similarly exposed to 300 ppm.

A major study (Cronkite 1986; Cronkite et al. 1984, 1985) provides a basis for a reproducible animal model. In this study, mice were exposed to 300 ppm benzene by inhalation 6 hours per day, 5 days per week for 16 weeks. This exposure regimen was selected because the authors thought it most closely paralleled likely human exposure. Human epidemiological studies in the literature reported that workers were exposed for about 15% of their life span; 16 weeks represent $\approx$15% of the life span of mice. Although precise occupational exposures were not known, most studies indicated that workers had been exposed to concentrations up to 250-300 ppm during at least part of a working day; thus, 300 ppm was chosen. The C57BL/6 and CBA/Ca mouse strains were chosen for these studies because of their susceptibilities to ionizing radiation-induced thymic lymphoma. The strains are also recognized for their low spontaneous rates of AML, the disease most frequently associated with benzene exposure in humans.

Cronkite et al. (1984) reported a highly significant increase in thymic and nonthymic lymphomas in C57BL/6 mice exposed to 300 ppm of benzene by inhalation 6 hours per day, 5 days per week for 16 weeks. In a continuation of that study (Cronkite et al. 1985), a definite pattern for thymic and nonthymic lymphoma appearance and mortality was observed. Lymphoma was first observed at about 150 days after exposure. The mice began to die at 330 days, and mortality increased through 390 days, at which time it leveled off. An increased incidence of lymphoma and solid tumors was again observed 420 days after exposure, and mortality did not increase again until 570 days after exposure. Increased incidences of Zymbal gland and ovarian tumors were also found. In a subsequent study, CBA/Ca male mice that breathed 100 ppm of benzene 6 hours per day, 5 days per week for 16 weeks developed leukemia, while mice similarly exposed to 300 ppm had a significant increase in the incidence of myelogenous neoplasms (Cronkite 1986; Cronkite et al. 1989). Increased incidences of harderian and Zymbal gland, squamous cell and mammary carcinoma, papilloma, andadenocarcinoma of lungs were also seen. Lymphoma was observed in 12% (significant increase) of CBA/Ca mice exposed to 300 ppm benzene for 16 weeks (Farris et al. 1993). Acute granulocytic leukemia was not observed by Cronkite et al. (1989) or by Farris et al. (1993).

AKR mice were exposed to 100 or 300 ppm benzene vapor for 6 hours per day, 5 days a week for life (Snyder et al. 1978a, 1980). C57BL mice were exposed to 300 ppm benzene vapor for 6 hours per day, 5 days a week for life. No significant increase in leukemia or malignant lymphoma was found in AKR mice, whereas C57BL mice exhibited a significant increase (8/40) compared with 2/40 in controls in the incidence of hematopoietic neoplasms including 6 cases (15%) of thymic lymphoma. Thymic lymphoma is rare in C57BL mice. In a later study, CD-l mice given an intermediate exposure (6 hours per day, 5 days per week for 10 weeks) to 1,200 ppm benzene and observed until death showed an elevated incidence of lung adenoma (Snyder et al. 1988). No increased incidence of lung adenomas was observed in C57BL/6 mice similarly exposed. Both CD-l and C57BL/6 mice responded to intermittent lifetime exposures to benzene with elevated incidences of Zymbal gland tumors (Snyder et al. 1988). Neither of the protocols induced elevated incidences of leukemia/lymphomas in either strain (Snyder et al. 1988). The authors concluded that lifetime exposure to benzene, even when intermittent, is a more potent carcinogenic pattern of exposure than a short-term intense exposure pattern.

The cancer effect levels (CELs) for each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2 Oral Exposure

2.2.2.1 Death

Individual case reports of death from acute oral exposure to benzene have appeared in the literature since the early 1900s. The benzene concentrations encountered by the victims were often not known. However, lethal oral doses for humans have been estimated at 10 mL (8.8 g or ≈125 mg/kg for a 70-kg person) (Thienes and Haley 1972). Lethality in humans has been attributed to respiratory arrest, central nervous system depression, or cardiac collapse (Greenburg 1926). Accidental ingestion and/or attempted suicide with lethal oral doses of benzene have produced the following signs and symptoms: staggering gait; vomiting; shallow and rapid pulse; somnolence; and loss of consciousness, followed by delirium, pneumonitis, collapse, and then central nervous system depression, coma, and death (Thienes and Haley 1972). Ingestion of lethal doses may also result in visual disturbances and/or feelings of excitement and euphoria, which may quite suddenly change to weariness, fatigue, sleepiness, convulsion, coma, and death (Von Oettingen 1940).

Animal lethality data indicate that benzene is of low toxicity following acute oral exposure (O'Bryan and Ross 1988). Oral LD_{50} values for rats ranged from 930 to 5,600 mg/kg; the values varied with age and strain of the animals (Cornish and Ryan 1965; Wolf et al. 1956). Male Sprague-Dawley rats were given various doses of benzene to determine the LD_{50} (Cornish and Ryan 1965). The LD_{50} for nonfasted rats was found to be 930 mg/kg. In 24-hour fasted rats, the LD_{50} was 810 mg/kg. No increase in mortality was reported in Fischer 344 rats or $B6C3F_1$ mice treated with 600 mg/kg/day for up to 17 weeks (Huff et al. 1989; NTP 1986).

Sprague-Dawley rats (30-35 males, 3&35 females) were exposed to benzene by ingestion (stomach tube), in olive oil, at 0, 50, or 250 mg/kg/day for 4-5 days weekly for 52 weeks and then kept under supervision until spontaneous death (Maltoni et al. 1983). Exposure to 50 mg/kg/day benzene after 52 weeks resulted in deaths in 9 of 30 male (same as controls) and 2 of 30 female rats. At 250 mg/kg/day exposure, 13 of 35 males and 9 of 35 females died. For rats receiving only olive oil, 9 of 30 males and 0 of 30 females died. In a companion study, Sprague-Dawley rats were exposed to 500 mg/kg/day benzene by ingestion (stomach tube), in olive oil, 4-5 days per week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Mortality rates were the same as the controls.

In a chronic-duration oral study conducted by the NTP (1986), increased mortality was observed in male Fischer 344 rats exposed to 200 mg/kg/day benzene in corn oil, and in female Fischer 344 rats exposed to 50 mg/kg/day benzene. $B6C3F_1$ mice given 100 mg/kg/day also had increased mortality compared to control mice.

The LD_{50} values and ail reliable LOAEL values for death in each species following acute and chronic exposure are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2   Systemic Effects

No studies were located regarding respiratory, cardiovascular, musculoskeletal, hepatic, renal, endocrine, ocular, or body weight effects in humans. Human and animal data pertaining to other systemic effects are presented below.

Table 2-2. Levels of Significant Exposure to Benzene - Oral

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Human	once				126 (death)	Thienes and Haley 1972
2	Rat (Sprague-Dawley)	once (G)				930 M (LD$_{50}$)	Cornish and Ryan 1965
3	Rat (Wistar)	once (GO)				5600 M (LD$_{50}$)	Wolf et al. 1956
Systemic							
4	Rat (Sprague-Dawley)	Gd 6-15 daily (G)	Renal	1000 F			Exxon 1986
			Dermal		50 F (alopecia of hindlimbs & trunk)		
			Bd Wt	500 F	1000 F (body weight decreased 11%)		
			Other	50 F	250 F (decreased food consumption)		
5	Rat (CF)	1-3 d	Hepatic		1402 M (increased liver weight, biochemical changes)		Pawar and Mungikar 1975
Neurological							
6	Human	once				126 (muscular incoordination, unconsciousness)	Thienes and Haley 1972

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
7	Rat (Sprague-Dawley)	1 d			88 M (slight CNS depression)	1870 M (tremors)	Cornish and Ryan 1965
8	Rat (Sprague-Dawley)	once (G)			950 M (altered neurotransmitter concentrations)		Kanada et al. 1994
Reproductive							
9	Rat (Sprague-Dawley)	Gd 6-15 daily (G)		1000 F			Exxon 1986
Developmental							
10	Rat (Sprague-Dawley)	Gd 6-15 daily (G)		1000 F			Exxon 1986
11	Mouse (ICR/SIM)	Gd 8-12 (GO)			1300 F (decreased pup weight on neonatal days 1-3)		Seidenberg et al. 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
INTERMEDIATE EXPOSURE							
Death							
12	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1x/d (GO)				250 F (9/35 died)	Maltoni et al. 1983, 1985
Systemic							
13	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1x/d (GO)	Bd Wt	50	250 (body weight decreased 19%)		Maltoni et al. 1983, 1985
14	Rat (F-344/N)	60-120 d 5 d/wk (GO)	Resp	600			NTP 1986
			Cardio	600			
			Gastro	600			
			Hemato		200 (dose-related leukopenia at 60 days) 25 F (dose-related leukopenia at 120 days)		
			Musc/skel	600			
			Hepatic	600			
			Renal	600			
			Endocr	600			
			Bd Wt		200 (body weight decreased 14% in males & 16% in females)		

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
15	Rat (F-344/N)	<1 yr 5 d/wk (GO)	Hemato		50M (lymphocytopenia and leukocytopenia) 25 F (lymphocytopenia and leukocytopenia)		NTP 1986
			Bd Wt	100	200M (body weight decreased 11% or more in 25 weeks)		
16	Rat (Fischer- 344)	6 wk 5 d/wk (GO)	Hepatic	400 M			Taningher et al. 1995
			Bd Wt	400 M			
17	Rat (Wistar)	6 mo 5 d/wk (GO)	Hemato	1 F		50 F (leukopenia, erythrocytopenia)	Wolf et al. 1956
18	Mouse (C57BL/6)	28 d (W)	Bd Wt	1000 M			Fan 1992
19	Mouse (CD-1)	4 wk ad lib (W)	Hemato			8 M (erythrocytopenia, increased mean corpuscular volume; leukopenia)	Hsieh et al. 1988b
20	Mouse (CD-1)	4 wk (W)	Hemato			31.5 M (decreased erythrocyte, leukocyte counts)	Hsieh et al. 1990
			Hepatic	31.5 M			
			Renal	31.5 M			
			Bd Wt	31.5 M			

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
21	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)	Resp	600			NTP 1986
			Cardio	600			
			Gastro	600			
			Hemato	25 M	50 M (dose-related leukopenia)		
				200 F	400 F (dose-related lymphopenia)		
			Musc/skel	600			
			Hepatic	600			
			Renal	600			
			Endocr	600			
			Bd Wt	600			
22	Mouse (B6C3F1)	<1 yr 5 d/wk (GO)	Hemato		25 (lymphocytopenia)		NTP 1986
			Bd Wt	100			
23	Mouse (B6C3F1)	30 d ad lib (W)	Hemato	12 F	195 F (decreased leukocytes)	350 F (decreased hemoglobin, hematocrit, leukocytes, MCV, and MCH)	Shell 1992
			Hepatic	350 F			
			Renal	350 F			
			Bd Wt	350 F			
			Other		12 F (decreased fluid consumption)		

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	

Immunological/Lymphoreticular

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	Less Serious (mg/kg/day)	Serious (mg/kg/day)	Reference
24	Rat (F-344/N)	60-120 d 5 d/wk (GO)			200 (lymphopenia at 60 days, lymphoid depletion in B-cell of the spleen) 25 F dose-related lymphopenia at 120 days)		Huff et al. 1989; NTP 1986
25	Rat (Wistar)	6 mo 5 d/wk (GO)		1 F	50 F (leukopenia)		Wolf et al. 1956
26	Mouse (C57BL/6)	28 d (W)			27M (decreased number of splenocytes & IL-2 production)		Fan 1992
27	Mouse (CD-1)	4 wk ad lib (W)				8 M (leukopenia and lymphopenia; enhanced splenic lymphocyte proliferation)	Hsieh et al. 1988b
28	Mouse (CD-1)	4 wk (W)				31.5 M (reduction in thymus mass; suppresion of both B- and T-cell mitogeneses; suppressed IL-2 secretions; leukopenia, lymphopenia)	Hsieh et al. 1990
29	Mouse (CD-1)	4 wk ad lib (W)				40 M (elevated corticosterone levels; T-lymphocyte suppression)	Hsieh et al. 1991
30	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)		25 M 200 F	50 M (dose-related 400 F lymphopenia)		Huff et al. 1989; NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
31	Mouse (B6C3F1)	30 d ad lib (W)			12 F (decreased leukocytes)		Shell 1992
Neurological							
32	Rat (F-344/N)	60-120 d 5 d/wk (GO)		600			NTP 1986
33	Mouse (CD-1)	4 wk ad lib (W)			8 M (fluctuation of neurotransmitter levels)		Hsieh et al. 1988a
34	Mouse (CD-1)	4 wk (W)			31.5 M (decreased NE, DA, 5-HT)		Hsieh et al. 1990
35	Mouse (CD-1)	4 wk ad lib (W)		8 M	40 M (increased hypothalmic NE and VMA)		Hsieh et al. 1991
36	Mouse (B6C3F1)	60-120 d 5 d/wk (GO)		200	400 (intermittent tremors)		NTP 1986
37	Mouse (B6C3F1)	30 d ad lib (W)		195 F	350 F (decreased brain weight)		Shell 1992
Reproductive							
38	Rat (F344/N)	17 wk 5 d/wk (GO)		600			NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
39	Mouse (B6C3F1)	17 wk 5 d/wk (GO)		600			NTP 1986
Cancer							
40	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1x/d (GO)				50 F (CEL: Zymbal gland carcinoma in 2/30; oral cavity carcinoma)	Maltoni et al. 1983, 1985
41	Rat (Sprague-Dawley)	52 wk 4-5 d/wk 1x/d (GO)				50 (CEL: Zymbal gland carcinoma)	Maltoni et al. 1989
						250 (CEL: nasal cavity carcinoma, fore-stomach liver angiosarcoma; Zymbal gland carcinoma)	
42	Mouse (RF/J)	52 wk 4-5 d/wk (GO)				500 (CEL: mammary pulmonary leukemias)	Maltoni et al. 1989

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure [a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
CHRONIC EXPOSURE							
Death							
43	Rat (F-344/N)	2 yr 5 d/wk (GO)				200 M (30/50 died) 50 F (14/50 died)	NTP 1986
44	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				100 (41/50 died M, 35/50 died F)	NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
	Systemic						
45	Rat (F-344/N)	2 yr 5 d/wk (GO)	Resp	200 M 100 F			Huff et al. 1989; NTP 1986
			Cardio	200 M 100 F			
			Gastro	100	200M (hyperkeratosis & acanthosis in nonglandular forestomach)		
			Hemato		50 M (lymphocytopenia and leukocytopenia) 25 F (lymphocytopenia and leukocytopenia)		
			Musc/skel	200 M 100 F			
			Hepatic	200 M 100 F			
			Renal	200 M 100 F			
			Endocr	200 M 100 F			
			Dermal	200 M 100 F			
			Ocular	200 M 100 F			
			Bd Wt	100		200 M (body weights decreased 23% in 103 wks)	
46	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1 x/d (GO)	Hemato			500 (decreased RBCs and WBCs after 84 weeks)	Maltoni et al. 1983, 1985
			Bd Wt		500 (decreased body weights in 92 weeks)		

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
47	Mouse (B6C3F1)	2 yr 5 d/wk (GO)	Resp	50 M 25 F	100 50	(alveolar hyperplasia)		NTP 1986
			Cardio	100			
			Gastro		25	(epithelial hyperplasia and hyperkeratosis of forestomach)·	
			Hemato		25	(lymphocytopenia; increased frequency of micronucleated normochromatic peripheral erythrocytes)	
			Musc/skel	100			
			Hepatic	100			
			Renal	100			
			Endocr		25	(hyperplasia of adrenal gland and harderian gland)	
			Dermal	100			
			Bd Wt	50	100	(mean body weight decreased 10% in 47 wks to 19% in 103 wk in males) (mean body weight decreased 14-15% in week 99-103 in females)	

Immunological/Lymphoreticular

| 48 | Rat (F-344/N) | 2 yr 5 d/wk (GO)[i] | | | 50M 25 F | (lymphoid depletion of spleen & thymus) (lymphoid depletion of spleen & thymus) | | Huff et al. 1989; NTP 1986 |

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
49	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1x/d (GO)			500 (decreased WBCs after 84 weeks)		Maltoni et al. 1983, 1985
50	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			25 (lymphopenia, hematopoietic hyperplasia in the bone marrow, splenic hematopoiesis)		Huff et al. 1989; NTP 1986
Neurological							
51	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M 100 F			NTP 1986
52	Mouse (B6C3F1)	2 yr 5 d/wk (GO)		100			NTP 1986
Reproductive							
53	Rat (F-344/N)	2 yr 5 d/wk (GO)		200 M 50 F		100 F (endometrial polyps)	NTP 1986
54	Mouse (B6C3F1)	2 yr 5 d/wk (GO)			25M (preputial gland hyperplasia) 25 F (ovarian hyperplasia and senile atrophy)		NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
Cancer							
55	Rat (F-344/N)	2 yr 5 d/wk (GO)				50 M (CEL: squamous cell papillomas & carcinomas of the oral cavity) 25 F (CEL: Zymbal gland carcinomas)	Huff et al. 1989; NTP 1986
56	Rat (Sprague-Dawley)	92 wk 4-5 d/wk 1x/d (GO)				500 (CEL: Zymbal gland carcinoma; oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1983, 1985
57	Rat (Wistar)	104 wk 4-5 d/wk 1x/d (GO)				500 (CEL: Zymbal gland and oral and nasal cavity carcinoma; angiosarcoma of the liver)	Maltoni et al. 1989
58	Rat (Sprague-Dawley)	104 wk 5 d/wk (GO)				50 (CEL: Zymbal gland carcinoma)	Maltoni et al. 1989
59	Mouse (B6C3F1)	2 yr 5 d/wk (GO)				25 M (CEL: harderian gland adenoma, lymphoma 25 F (CEL: lymphoma)	Huff et al. 1989; NTP 1986

Table 2-2. Levels of Significant Exposure to Benzene - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
60	Mouse (Swiss)	78 wk 4-5 d/wk 1x/d (GO)				500 (CEL: mammary tumors, lung tumors, Zymbal gland)	Maltoni et al. 1989

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

ad lib = ad libitum; Bd Wt = body weight; CEL = cancer effect level; CNS = central nervous system; d = day(s); DA = dopamine; F = female; (F) = feed; (G) = gavage; Gd = gestational day; (GO) = gavage in water; Hemato = hematological; 5-HT = 5-hydroxystryptamine; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; mo = month(s); NE = norepinephrine; NOAEL = no-observed-adverse-effect level; NS = not specified; RBC = red blood cells: VMA = vanillyn mandelic acid; (W) = water; WBC = white blood cells; wk = week(s; yr = year(s); x = times

Figure 2-2. Levels of Significant Exposure to Benzene - Oral
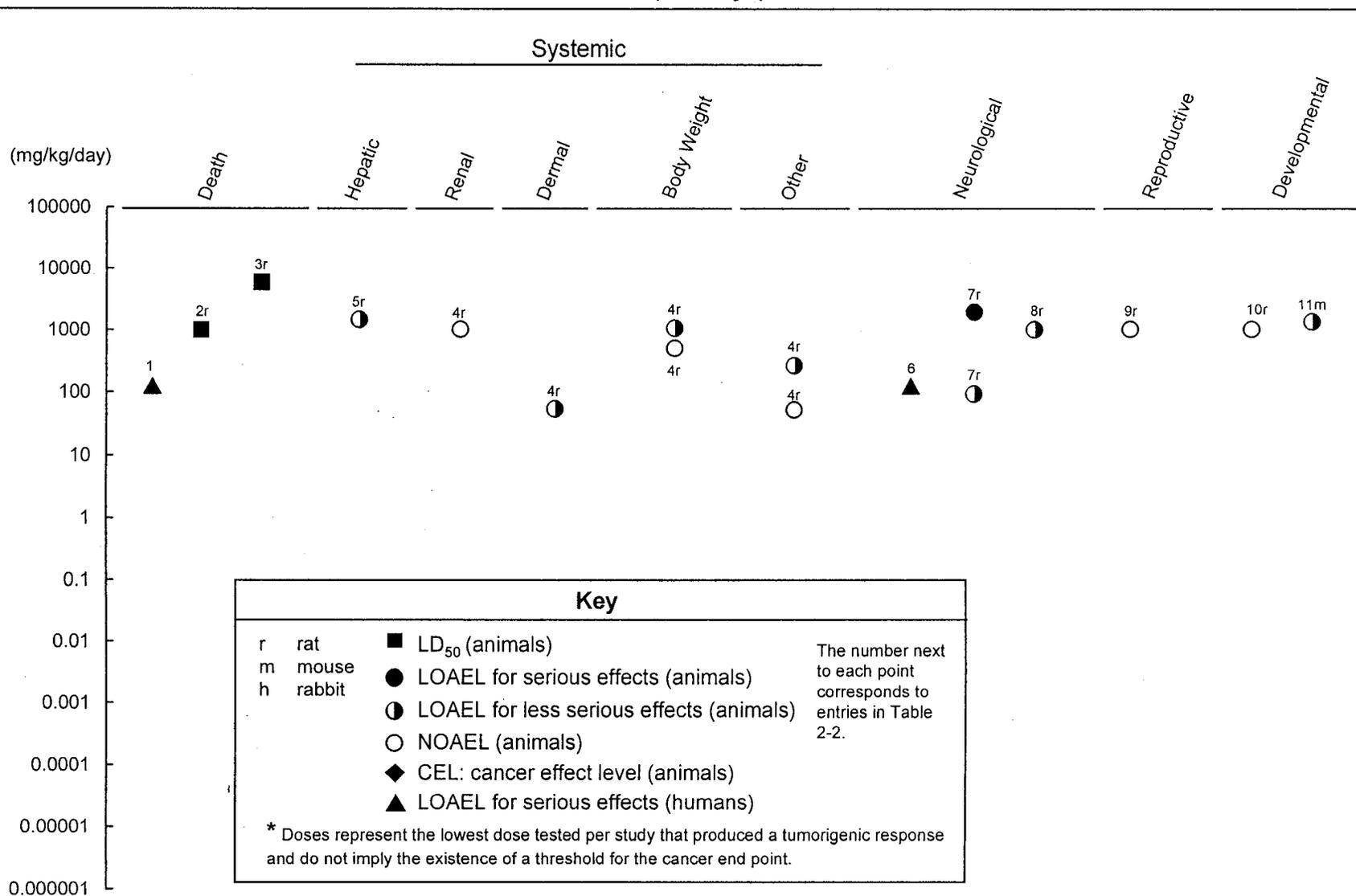
Acute (≤14 days)

Systemic

(mg/kg/day)

Death   Hepatic   Renal   Dermal   Body Weight   Other   Neurological   Reproductive   Developmental

100000

10000 — 3r

1000 — 2r   5r   4r   4r   7r   8r   9r   10r   11m

100 — 1   4r   4r   6   7r

10

1

0.1

Key

r rat	■ LD$_{50}$ (animals)	The number next
m mouse	● LOAEL for serious effects (animals)	to each point
h rabbit	◐ LOAEL for less serious effects (animals)	corresponds to entries in Table 2-2.
	○ NOAEL (animals)	
	◆ CEL: cancer effect level (animals)	
	▲ LOAEL for serious effects (humans)	

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

0.01

0.001

0.0001

0.00001

0.000001

Figure 2-2. Levels of Significant Exposure to Benzene - Oral (cont.)

Intermediate (15-364 days)

Systemic

(mg/kg/day)

Death Respiratory Cardiovascular Gastrointestinal Hematological Musculoskeletal Hepatic Renal

10000

1000   12r   14r 21m   14r 21m   14r 21m   23m   14r 21m   14r 16r 21m 23m   14r 21m 23m

100   14r 15r 17r   20m 21m 22m 23m   20m   20m
         19m 21m 23m

10

1   17r

0.1

0.01

0.001

0.0001

0.00001

0.000001

Key		
r rat m mouse h rabbit	■ LD₅₀ (animals) ● LOAEL for serious effects (animals) ◐ LOAEL for less serious effects (animals) ○ NOAEL (animals) ◆ CEL: cancer effect level (animals) ▲ LOAEL for serious effects (humans)	The number next to each point corresponds to entries in Table 2-2.

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

Figure 2-2. Levels of Significant Exposure to Benzene - Oral (cont.)

Intermediate (15-364 days)
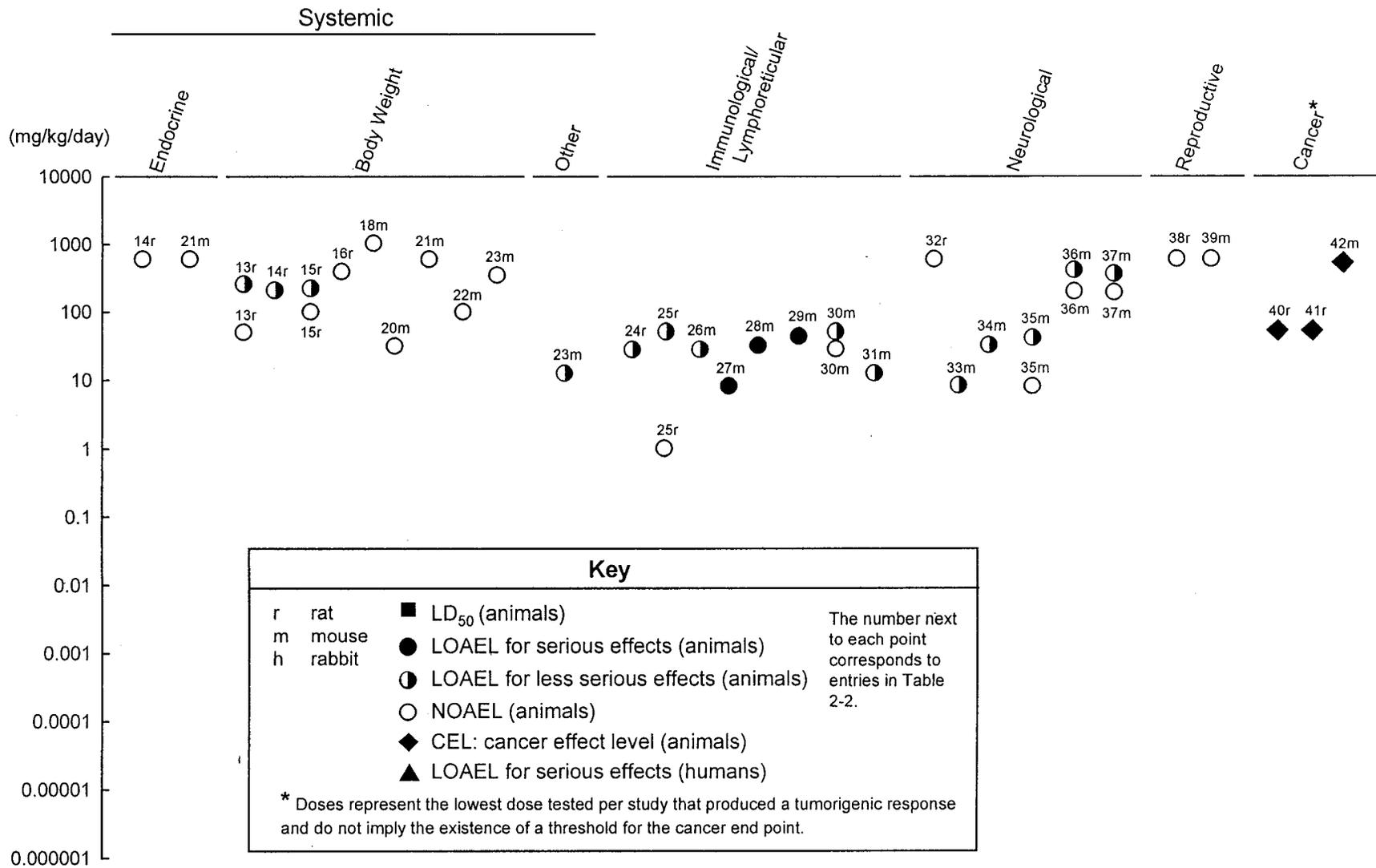
Figure 2-2. Levels of Significant Exposure to Benzene - Oral (cont.)

Chronic (≥365 days)

Systemic

(mg/kg/day)

Death — Respiratory — Cardiovascular — Gastrointestinal — Hematological — Musculoskeletal — Hepatic — Renal — Endocrine — Dermal — Ocular — Body Weight — Immunological/ Lymphoreticular — Neurological — Reproductive — Cancer*

10000

1000 — 46r (●); 56r ◆ 57r ◆ 60m ◆

100 — 45r, 45r, 45r, 47m, 44m ●, 43r ■, 47m, 45r, 45r 47m, 45r 47m, 45r, 45r 47m, 45r, 45r ●47m, 49r, 51r 52m, 53r ●, 58r ◆

(various points: 45r, 47m, 45r 47m, 47m, 47m, 45r, 47m, 48r 50m, 53r, 54m, 55r ◆, 59m ◆)

10

1

0.1

0.01

0.001

0.0001

0.00001

0.000001

Key

r   rat
m   mouse
h   rabbit

■ LD₅₀ (animals)
● LOAEL for serious effects (animals)
◑ LOAEL for less serious effects (animals)
○ NOAEL (animals)
◆ CEL: cancer effect level (animals)
▲ LOAEL for serious effects (humans)

The number next to each point corresponds to entries in Table 2-2.

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer end point.

10⁻⁴
10⁻⁵
10⁻⁶
10⁻⁷

Estimated Upper-Bound Human Cancer Risk Levels

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

Respiratory Effects. Male and female Fischer 344 rats and B6C3F$_1$ mice were given oral doses
of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). No
histopathological lesions were observed in lungs, trachea, or mainstream bronchi. After chronicduration
exposure to 50, 100, or 200 mg/kg/day (male rats) or 25, 50, or 100 mg/kg/day (female rats,
male and female mice), no histopathological lesions were observed in trachea, lungs, or mainstream
bronchi in rats (NTP 1986). In mice, a significantly increased incidence of alveolar hyperplasia was
observed at 50 and 100 mg/kg/day in females and at 100 mg/kg/day in males.

Cardiovascular Effects. No histopathological lesions were observed in cardiac tissue from male
and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or
600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). After chronic-duration exposure to
$\leq$200 mg/kg/day (male rats) or $\leq$100 mg/kg/day (female rats, male and female mice) no
histopathological lesions were observed in the heart (NTP 1986).

Gastrointestinal Effects. A man swallowed an unspecified amount of benzene and survived, but
developed an intense toxic gastritis and later pyloric stenosis (Greenburg 1926).

No histopathological lesions were observed in esophageal and stomach tissue or in the small intestine
and colon from male and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100,
200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). After chronic-duration
exposure to 50-200 mg/kg/day (male rats) or 25-100 mg/kg/day (female rats, male and female mice),
male rats exhibited hyperkeratosis and acanthosis in the nonglandualar forestomach at 200 mg/kg/day,
and mice exhibited epithelial hyperplasia and hyperkeratosis in the forestomach at $\geq$25 mgfkglday
(NTP 1986).

Hematological Effects. Prior to 1913, benzene was used as a treatment for leukemia. Benzene
was given in gelatin capsules starting with 43 mg/kg/day and increasing to 71 mg/kg/day for
unspecified durations (Selling 1916). Leukemia patients showed a great reduction in leukocyte count
and multiple hemorrhages with advanced anemia. However, it is difficult to determine which effects
were due to the leukemia and which were due to the benzene treatment.

Intermediate-duration studies in animals have revealed decreases in numbers of erythrocytes and leukocytes following exposure to benzene. Male and female Fischer 344 rats and B6C3F$_1$ mice were given oral doses of 0, 25, 50, 100, 200, 400, and 600 mg/kg/day benzene in corn oil for 120 days. Dose-related leukopenia and lymphopenia were observed at 200 and 600 mg/kg/day for both male and female rats killed on day 60, and at all doses in female rats killed on day 120. Dose-related leukopenia and lymphopenia were observed in male mice at ≥50 mg/kg/day and female mice at ≥400 mg/kg/day for 120 days, but not for 60 days. Mice exposed to ≥8 mg/kg/day in the drinking water for 4 weeks had decreased numbers of erythrocytes, increased mean corpuscular volumes, and decreased numbers of lymphocytes (Hsieh et al. 1988b, 1990). Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased hemoglobin, hematocrit, leukocytes, MCV, and MCH were observed at 350 mg/kg/day. Decreased leukocytes also occurred at 195 mg/kg/day. Decreased leukocytes also occurred at 195 mg/kg/day. Rats gavaged with 50 mg/kg/day of benzene 5 days per week for 6 months also had decreased numbers of erythrocytes and leukocytes (Wolf et al. 1956). One chronic-duration study showed that gavage doses of ≥25 mg/kg/day resulted in leukopenia and/or lymphocytopenia in both rats and mice, both at the interim sacrifices at 3-l 8 months, and at the end of 2 years (Huff et al. 1989; NTP 1986). Increased frequency of micronucleated normochromatic peripheral erythrocytes was observed in mice at ≥25 mg/kg/day after 2 years. Sprague-Dawley rats were exposed to 500 mg/kg benzene by ingestion (stomach tube), in olive oil, once daily, 4-5 days per week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased erythrocytes and leukocytes after were observed after 84 weeks.

Musculoskeletal Effects. No histopathological lesions were observed in femoral tissue from male and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in the sternebrae, femur, or vertebrae from Fischer 344 rats and mice exposed to 50-200 mg/kg/day (male rats) or 25-100 mg/kg/day (female rats, male and female mice) for 2 years (NTP 1986).

Hepatic Effects. Acute oral administration of 1,402 mg/kg/day benzene for 3 days induced hepatic changes in rats evidenced by increased liver weight, decreased protein in the postmitochondrial supematant fractions (9,000 times specific gravity), and changes in hepatic drug metabolism and lipid peroxidation (Pawar and Mungikar 1975). The initiation-promotion-progression (IPP) model for the induction of malignant neoplasms in the liver was evaluated for benzene in male and female Sprague-

Dawley rats (Dragan et al. 1993). Initiation was begun in 5-day-old rats with administration of a single intraperitoneal injection of diethylnitrosamine during the time when the liver is undergoing rapid growth. Promotion began at 6 months of age with phenobarbital in the feed, and continued into young adulthood. Partial hepatectomy was performed, and at the height of the regenerative proliferation phase following the hepatectomy, benzene (1 g/kg) was administered by gavage; phenobarbital treatment was maintained after the administration of benzene. A slight increase in the incidence of altered hepatic foci was observed after initiation and promotion. Few hepatic foci were observed in the livers of male Fischer 344 rats treated by gavage with 400 mg/kg/day benzene in corn oil for 5 days per week for 6 weeks (Taningher et al. 1995). No histopathological non-neoplastic lesions were observed in hepatic tissue from male and female Fischer 344 rats given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days or in male rats exposed to 50-200 mg/kg/day and female rats exposed to 25-100 mg/kg/day for 2 years (NTP 1986).

Female $B6C3F_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). No adverse liver effects, as evidenced by gross necropsy, liver weight determination, and serum levels of hepatic enzymes, were observed. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect liver weight in CD-l mice (Hsieh et al. 1990). No histopathological non-neoplastic lesions effects were observed in hepatic tissue from male and female $B6C3F_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in male and female mice exposed to 25-100 mg/kg/day for 2 years (NTP 1986).

Renal Effects. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene on Gd 6-15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted in the kidneys based on gross necropsy. No adverse effects based on histological examination were observed on renal tissue or the urinary bladder from male and female Fischer 344 rats given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days or in male rats exposed to 50-200 mg/kg/day and female rats exposed to 25-100 mg/kg/day for 2 years(NTP 1986). Female $B6C3F_1$ mice were exposed to 0, 12, 195, or 350 mg/kg benzene in drinking water for 30 days (Shell 1992). No adverse effects were observed in the kidneys, based on kidney weights, gross examination, and blood urea nitrogen and creatinine determinations. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect kidney weight in CD-l mice (Hsieh et al. 1990). No adverse effect based on histological examination was observed on

renal tissue or the urinary bladder from male and female B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days, or in male and female mice exposed to 25-100 mg/kg/day for 2 years (NTP 1986).

Endocrine Effects. No histopathological lesions were observed in salivary, thyroid, parathyroid, pancreas, adrenal, or pituitary glands from male and female Fischer 344 rats or B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986). In the companion chronic-duration oral study, male Fischer 344 rats were exposed to 25, 50, 100, or 200 mg/kg/day benzene, while female rats received 25, 50, or 100 mg/kg/day benzene. Hyperplasia of the Zymbal gland was increased in low-dose males and in mid-dose females. In the adrenal gland, hyperplasia was observed in both sexes (males: 27 and 4% at 50 and 200 mg/kg, respectively; females: 34% at 25 mg/kg). In the thyroid gland, incidences of C-cell hyperplasia were 14, 26, 15, and 15% in males treated with 0, 50, 100, and 200 mg/kg, respectively. Analysis of the pituitary gland showed incidence of hyperplasia in males treated with 0, 50, 100, and 200 mg/kg at 6, 16, 20, and 10%, respectively; in females treated with 0, 25, 50, and 100 mg/kg at 11, 20, 10, and 14%, respectively. None of the increased incidences of hyperplasia in these glands were considered to be treatment-related by NTP. The non-dose-related increase of hyperplasia of the Zymbal gland could represent a progression to the neoplasms (see Section 2.2.2.8). In mice, Zymbal gland lesions showed epithelial hyperplasia in males (0, 9, 30, and 26%) and in females (2, 3, 5, and 19%) exposed to 0, 25, 50, or 100 mg/kg, respectively. Hyperplasia of the adrenal cortex occurred at incidences of 4, 67, 29, and 9% in males and 10, 43, 68, and 13% in females, respectively. Hyperplasia of the harderian gland occurred at incidences of 0, 11, 22, and 15% in males and 13, 23, 22, and 21% in females, respectively (NTP 1986).

Dermal Effects. A case of accidental poisoning in which the patient survived but developed an odd skin condition consisting of swelling and edema has been reported (Greenburg 1926).

Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day benzene daily on Gd 6-15 and killed on Gd 20 (Exxon 1986). Alopecia of the hind limbs and trunk was noted in all dose groups.

No histopathological lesions were observed in the skin of male and female Fischer 344 rats and B6C3F$_1$ mice after chronic oral exposure to 50-200 mg/kg/day (male rats) or 25-100 mg/kg/day (female rats and male and female mice) (NTP 1986).

Ocular Effects. No histopathological lesions were noted in the eyes of male and female Fischer 344 rats and B6C3F$_1$ mice after chronic-duration oral exposure to 50-200 mg/kg/day (male rats) or 25-100 mg/kg/day (female rats and male and female mice) (NTP 1986).

Body Weight Effects. No significant change in body weight was observed in male Fischer 344 rats treated by gavage with 400 mg/kg/day benzene in corn oil for 5 days per week for 6 weeks (Taningher et al. 1995). Body weight was unaffected in male and female Fischer 344 rats given oral doses of 0, 25, 50, or 100 mg/kg/day benzene in corn oil for 120 days (NTP 1986). However, animals receiving 200, 400, or 600 mg/kg/day benzene exhibited a 14-22% decrease in body weight after 120 days. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg benzene daily on Gd 6-15 and killed on Gd 20 (Exxon 1986). Maternal body weight decreased 11% at the high dose. C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Groups of mice were killed on day 7, 14, 21, and 28 of administration, and on day 7, 14, 21, and 28 after the last administration of benzene at 200 mg/L. There was no effect of treatment on body weight. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). There was no significant effect on body weight at the highest treatment level. Oral administration of 31.5 mg/kg/day benzene continuously in drinking water for 4 weeks did not affect body weight in CD-l mice (Hsieh et al. 1990). No adverse effect was observed on body weight of male and female B6C3F$_1$ mice given oral doses of 0, 25, 50, 100, 200, 400, or 600 mg/kg/day benzene in corn oil for 120 days (NTP 1986).

Sprague-Dawley rats were exposed to benzene by gavage in olive oil, at 0, 50, or 250 mg/kg/day body weight for 4-5 days per week for 52 weeks, and then kept under supervision until spontaneous death (Maltoni et al. 1983, 1985). A 19% decrease in body weight was reported in animals exposed to 250 mg/kg benzene for 52 weeks. In a companion study, Sprague-Dawley rats were exposed to 500 mg/kg benzene by ingestion (stomach tube), in olive oil, once daily, 4-5 days per week for 92 weeks, and then kept under observation until spontaneous death (Maltoni et al. 1983). Decreased

body weight was observed after 92 weeks. In a chronic-duration oral study, male Fischer 344 rats exhibited a decrease in body weight of 11% or more after 25 weeks exposure to doses of 200 mg/kg/day benzene in corn oil (NTP 1986). Females rats and male and female B6C3F$_1$ mice in the same study exposed to doses up to 100 mg/kg/day benzene did not show any change in body weight after 12 months of exposure, or after 2 years exposure (female rats). Male and female mice exhibited body weight effects after chronic exposure. In male mice given 100 mg/kg, mean body weights decreased from 10% after 47 weeks to 19% in 103 weeks of exposure relative to controls. In female mice given 100 mg/kg, mean body weights decreased 14-15% in weeks 99-103 of exposure (NTP 1986).

Other Systemic Effects. C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Groups of mice were killed on day 7, 14, 21, and 28 of administration, and on day 7, 14, 21, and 28 after the last administration of benzene at 200 mg/L. There was no effect of treatment on food or water consumption. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg/day on Gd 6-15 and killed on Gd 20 (Exxon 1986). Decreased feed consumption was noted at doses of 250 mg/kg and above, and body weight decreased 11% at the high dose. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased fluid consumption was observed at ≥12 mg/kg.

2.2.2.3 Immunological and Lymphoreticular Effects

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

Oral administration of benzene to CD-l mice produced an immunotoxic effect on both the humoral and cellular immune responses (Hsieh et al. 1988b). Exposure to benzene at 8, 40, or 180 mg/kg/day for 4 weeks caused a significant dose-response reduction of total peripheral blood leukocytes and erythrocytes. Lymphocytes, but not neutrophils or other leukocytes, were decreased in number. Splenic lymphocyte proliferative response to B- and T-cell mitogens was biphasic-enhanced in the 8 mg/kg/day dosage group and depressed in the 40 and 180 mg/kg/day dosage groups. Cell-mediated immunity evaluated in mixed-lymphocyte reaction and in the ^{51}Cr-release assay showed a similar biphasic response. Antibody production was significantly suppressed in mice dosed at 40 and

180 mg/kg/day. The results indicate that administration of 40 mg/kg/day benzene has an immunosuppressive effect evident in decreased immune functions evaluated in *in vitro* assays for cell-mediated immunity and antibody production. A dose-related decrease in spleen weight was observed, which was significant only in the 180 mg/kg/day group.

C57BL/6 male mice were given benzene at concentration levels of 200 and 1,000 mg/L (assumed benzene intake of 27 and 154 mg/kg/day) in drinking water for 28 days (Fan 1992). Control groups were given untreated tap water. Mice were sacrificed on days 7, 14, 21, and 28 of administration. For tracing gradual disappearance or delayed appearance of the toxicity after ending benzene administration, groups of mice were killed on day 7, 14, and 21 after the last administration of 27 mg/kg/day benzene for 28 days. At 27 mg/kg/day, a decreased number of splenocytes was observed on day 21 and 28 of exposure. At 154 mg/kg/day, spleen cell numbers decreased significantly as a function of time in mice treated for 14, 21 and 28 days. Benzene treatment for 3 weeks raised natural killer (NK) cell activity significantly at both doses. However, after another week of benzene treatment, NK cell activity resumed to control levels. Significant depression of interleukin-2 (IL-2) production was detected in both levels for 28 days. The NK cell activity showed normal levels on day 7, 14, and 21 after the last administration of benzene exposure for 28 days at 27 mg/kg/day. IL-2 production decreased significantly on day 7 and 14 after cessation of benzene administration, but recovered with time (43, 71, and 79% of control on day 7, 14, and 21, respectively). Spleen cell number decreased significantly on the 7th day, but recovered on day 14 and 21. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased leukocytes were observed at 12 mg/kg/day, and decreased spleen cell number was observed at 195 mg/kg/day.

In the NTP-sponsored intermediate-duration oral study using Fischer 344 rats and B6C3F$_1$ mice, dose-related leukopenia and lymphopenia were observed for both male and female Fischer 344 rats at 200 and 600 mg/kg/day killed on day 60, and at all doses in female rats killed on day 120 (NTP 1986). Decreased leukocytes were observed in male and female rats exposed for 60 days to 200 and 600 mg/kg/day benzene. Lymphoid depletion in the B-cell of the spleen was observed in animals exposed to 200 mg/kg/day (3 of 5 males, 4 of 5 females) and 600 mg/kg/day (5 of 5 males, 5 of 5 females) benzene for 60 days and in animals that received 600 mg/kg/day (10 of 10 males, 10 of 10 females) benzene for 120 days. At 600 mg/kg/day benzene exposure, increased extramedullary hematopoiesis was observed in the spleen of 4 of 5 male and 3 of 5 female rats. Dose-related

leukopenia and lymphopenia were observed for both male and female mice exposed for 120 days, but not for 60 days. Leukocytes and lymphocytes were significantly decreased in male mice exposed for 120 days to 50, 100, 200, 400, and 600 mg/kg/day benzene. At 120 days of exposure, leukocytes significantly were decreased in female mice at 600 mg/kg/day and lymphocytes at 400 and 600 mg/kg/day. Histological examination revealed no adverse effects in mandibular lymph node or the thymus for either rats or mice (Huff et al. 1989; NTP 1986). Rats exposed to benzene at 50 and 100 mg/kg/day for 6 months had significant leukopenia (Wolf et al. 1956).

Leukopenia and lymphopenia were observed in mice at 31.5 mg/kg/day after 4 weeks of oral exposure (Hsieh et al. 1990). Reduction in thymus mass, suppression of B- and T-cell mitogenesis, and suppressed IL-2 secretions were also noted. Similar results were noted at 40 mg/kg/day (Hsieh et al. 1991).

Oral administration of benzene to B6C3F$_1$ mice and Fischer 344 rats at doses of 50-200 mg/kg/day (male rats) or 25-100 mg/kg/day (female rats and male and female mice), 5 days per week for 103 weeks resulted in significant leukocytopenia and lymphocytopenia in both species (Huff et al. 1989; NTP 1986). In the thymus, lymphoid depletion was observed at 0, 10, 20, or 28% in male rats treated with 0, 50, 100, or 200 mg/kg/day, respectively. Increased incidences of lymphoid depletion of the spleen were observed in male rats treated with 0 (0%), 50 (40%), 100 (17%), and 200 (49%) mg/kg/day and in female rats treated with 0 (0%), 25 (22%), 50 (16%), and 100 (20%) mg/kg/day. In mice, an increased incidence of hematopoietic hyperplasia was observed in the bone marrow of dosed animals in both sexes. Splenic hematopoiesis was increased in dosed animals of both sexes of mice (Huff et al. 1989; NTP 1986). Maltoni et al. (1983, 1985) observed decreased leukocytes in Sprague-Dawley rats dosed with 500 mg/kg/day benzene for 84 weeks or more.

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

2.2.2.4   Neurological Effects

In humans, symptoms of central nervous system toxicity (including giddiness, vertigo, muscular incoordination, and unconsciousness) have been reported following one-time ingestion of benzene at 125 mg/kg (Thienes and Haley 1972).

Neurochemical profiles were conducted on rats after oral exposure to benzene (Kanada et al. 1994). Sprague-Dawley rats received a single dose of 950 mg/kg benzene by gavage and were sacrificed 2 hours after treatment. The control group received nothing. Brains were dissected into small-brain areas and stored until analysis. Acetylcholine, 3,4-dihydroxyphenylalanine (DOPA), dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG), serotonin, and 5hydroxyindoleacetic acid (5HIAA) contents in the small-brain regions were measured. Results showed that benzene decreased acetylcholine content of rat hippocampus. DOPA and norepinephrine content decreased in the rat midbrain. Dopamine, serotonin and 5HIAA content increased in the rat midbrain. Dopamine, DOPAC, norepinephrine, and 5HIAA content increased and serotonin content decreased in the rat hypothalmus after oral administration of benzene. Increased dopamine, HVA, MHPG, and serotonin content of rat medulla oblongata was observed. Decreased norepinephrine and 5HIAA content of rat medulla oblongata by benzene treatment was observed.

Oral exposure to benzene induced both synthesis and catabolism of monoamine neurotransmitters in CD-l mice (Hsieh et al. 1988a). Mice given 8, 40, or 180 mg/kg/day of benzene for 4 weeks in drinking water exhibited changes in the levels of norepinephrine, dopamine, serotonin, and catecholamine metabolites in several brain regions but no treatment-related behavioral changes. Similar results were seen at 31.5-40 mg/kg/day (Hsieh et al. 1990, 1991). Because of the lack of association with behavioral changes, the effects on the neurotransmitters cannot be adequately assessed. Female B6C3F$_1$ mice were exposed to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days (Shell 1992). Decreased brain weight was observed at 350 mg/kg/day. Sprague-Dawley rats given one oral dose of 88 mg/kg benzene exhibited slight central nervous system depression, whereas at 1,870 mg/kg/day, tremors were observed (Cornish and Ryan 1965). Histological examination of the brain revealed no treatment-related lesions after gavage treatment of male and female Fischer 344 rats and B6C3F$_1$ mice with doses up to 600 mg/kg/day for 120 days (NTP 1986). In the same experiment, B6C3F$_1$ mice exhibited tremors intermittently at doses of 400 mg/kg/day, which were more pronounced in males during the last 3 weeks of the study. No adverse effects based on histological examination of brain or spinal cord were observed in male and female Fischer 344 rats and B6C3F$_1$ mice after chronic oral exposure to 50-200 mg/kg/day (male rats) or 25-100 mg/kg/day (female rats and male and female mice) (NTP 1986).

The highest NOAEL values and all reliable LOAEL values for neurological effects 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 benzene.

Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or 1,000 mg/kg benzene daily on Gd 6-15 and killed on Gd 20 (Exxon 1986). No adverse effects were noted on reproductive competency. No histological changes were reported in the prostate, testes, ovaries, mammary gland, or uterus of male and female Fischer 344 rats and B6C3F$_1$ mice dosed by gavage with up to 600 mg/kg/day benzene for 17 weeks (NTP 1986). In male and female Fischer 344 rats and B6C3F$_1$ mice after chronic oral exposure to 50-200 mg/kg/day (male rats) or 25-100 mg/kg/day (female rats and male and female mice), endometrial stromal polyps occurred with a significant positive trend in female rats (NTP 1986). The incidence in high dose group (14/50) was significantly greater than that in the control (7/50). In mice, analysis of preputial gland lesions in male mice dosed at 0, 25, 50, or 100 mg/kg showed increased incidences of focal, diffuse or epithelial hyperplasia (5, 65, 31, and 3%, respectively). The lower incidences of hyperplasia in the higher dose groups were probably due to the progression of the preputial gland lesions to neoplasias (see Section 2.2.2.8). Various non-neoplastic and neoplastic ovarian lesions were observed in dosed female mice, including epithelial hyperplasia and senile atrophy (NTP 1986).

The NOAEL and LOAEL values for reproductive effects in rats and mice 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 tc benzene.

Benzene was embryotoxic as evidenced by reduced pup body weights when mice were administered 1,300 mg/kg/day of benzene by gavage on Gd 8-12 (Seidenberg et al. 1986). No maternal toxicity was observed. Female Sprague-Dawley rats were dosed by gavage with 0, 50, 250, 500, or

1,000 mg/kg benzene daily on Gd 6-15 and killed on Gd 20 (Exxon 1986). No adverse effects were
noted on morphological development.

The NOAEL value for rats and the LOAEL value for mice for developmental effects following acute
oral exposure 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 benzene.

Oral *in vivo* animal studies provide convincing evidence that benzene is genotoxic. Tests on bone
marrow cells, lymphocytes, and erythrocytes in mice gave positive results for chromosomal aberrations
and micronuclei increases. Studies clearly demonstrate that, concerning micronuclei and chromosomal
aberrations, male mice are considerably more sensitive than females (Barale et al. 1985; Choy et al.
1985; Ciranni et al. 1988; Hatakeyama et al. 1992; Meyne and Legator 1980; Siou et al. 1981). Oral
administration of 440 and 879 mg/kg/day benzene for 3 days to male and female CD-l mice resulted
in significantly elevated frequencies of chromosomal aberrations and micronuclei in bone marrow
(Meyne and Legator 1980). A significant gain in chromosomal aberrations was observed at
440 mglkglday in male mice, while 879 mg/kg/day was needed to produce a similar effect in females.
Significant positive responses for micronuclei also occurred after administration of 440 and
879 mg/kg/day to both males and females (Meyne and Legator 1980). In another study, male ICR
mice given 36.6, 73.2, and 146.4 mgfkglday for 14 days exhibited significant dose-dependent increases
in chromatid aberrations in spleen lymphocytes and micronuclei in blood NCEs (Rithidech et al. 1987,
1988). Doses of 56.2, 141, 352, and 2,189 mg/kg/day for 2 days produced significant dose-dependent
increases in chromosomal aberrations and micronuclei in bone marrow of both male and female Swiss
mice, with males being again more sensitive (Siou et al. 1981). In the same study, Chinese hamsters
(male and female) were given 2 doses of benzene of either 2,198 or 8,790 mg/kg/day. Neither males
nor females showed significant positive responses for increased micronuclei, and only the males given
2 doses of 8,790 mg/kg/day exhibited a significant increase in chromosomal aberrations (Siou et al.
1981). Therefore, hamsters are less sensitive to these effects. In another study, male and female CD-1
mice were given benzene doses ranging from 26 to 440 mg/kg/day. An increase in MN-NCEs was
only observed after 21 days in mice exposed to 220 mg/kg/day or more (Barale et al. 1985). Male
B6C3F$_1$ mice exhibited dose-dependent increases of MN-NCEs for intermediate-duration exposures of

oral doses ranging from 25 to 600 mg/kg/day (Choy et al. 1985). Females were less sensitive, and significant dose-dependent increases were observed in male mice starting at 400 mg/kg/day. At each dose and sampling time, the frequency of MN-NCEs was higher in males than in females. Chronic administration of benzene to B6C3F$_1$ mice produced significant dose-dependent elevations in MN-NCEs at doses of 50 mg/kg/day or higher. At each dose and sampling time, the frequency was higher in males than in females (Choy et al. 1985). For bone marrow cells, increases in MN-PCEs were noted in mice after acute doses of 80 (Ciranni et al. 1988), up to 880 (Ciranni et al. 1991) 500 (Suzuki et al. 1989), 200 (Harper et al. 1984), 220 and 110 (Hite et al. 1980) mg/kg/day. A dose of 50 mg/kg/day for 40 weeks increased the frequency of MN-PCEs and MN-NCEs in both male and female hybrid mice (Armstrong et al. 1993). A comparison of the effects of benzene on MN-PCEs in bone marrow was also made between virgin females, pregnant mice, and their offspring (Ciranni et al. 1988). The researchers observed that virgin females had a higher frequency of micronuclei than pregnant females. Data from the fetuses suggest a benzene sensitivity comparable to that of adult female mice (Ciranni et al. 1988). Evaluation of spermatogonia from the testes of mice treated with doses of benzene up to 880 mg/kg revealed an increase in chromatid aberrations (Ciranni et al. 1991).

Male CD-l mice were administered benzene dissolved in olive oil at gavage doses of 220 and 440 mg/kg (Chen et al. 1994). Control mice received olive oil. Bone marrow preparations were made 24 hours following benzene treatment. The characterization of the origin of micronuclei formed in the bone marrow erythrocytes and splenic lymphocytes following the *in vivo* exposure to benzene to mice was determined using two molecular cytogenetic approaches: (a) fluorescence *in situ* hybridization with a centromeric DNA probe and (b) immunofluorescent labeling with calcinosis-Raynaud's phenomenon-esophageal dismobility-sclerodactyly-telangiectasia syndrome of sclerodenna (CREST) antibody. Significant increases in the frequency of micronucleated PCE were observed at both 220 and 420 mg/kg. The mean of the polychromatic erythrocytes to normochromatic erythrocytes ratios per animal (PCE:NCE) showed a significant increase at 220 and 440 mg/kg benzene as compared to controls, indicating an increase in the presence of newly formed erythrocytes. *In situ* hybridization with a centromeric DNA probe or immunofluorescent staining with the CREST antibody indicated that the induced micronuclei resulted from both chromosome breakage and chromosomal loss. The frequency of CREST-positive micronuclei increased from approximately 3/l ,000 binucleated cells in the controls to 1l/1,000 cells in animals treated with 440 mg/kg benzene. The frequency of CREST-negative micronuclei increased from 1.3 of 1,000 binucleated cells in controls to 3 of 1,000 in

mice treated with 440 mg/kg benzene. The nuclear division index (NDI) for treated animals showed significant increases at 220 and 440 mg/kg benzene as compared to controls.

In an attempt to elucidate the *in vivo* adduct formation by benzene metabolites, Reddy et al. (1989b) developed a highly sensitive nuclease P_1 -enhanced ^{32}P-postlabeling assay for adduct detection at a frequency of one modification per $10^{9\text{-}10}$ DNA nucleotides. When DNA was prepared from Zymbal glands of rats given 200 mg/kg of benzene, orally, 5 days per week for 10 weeks, 3 potential adducts were discovered. However, no adducts were detected in the DNA from the liver, kidney, or mammary gland of rats at any dose (Reddy et al. 1989a). The adduct levels corresponded to four lesions per 10^9 DNA nucleotides. The presence of adducts in the Zymbal DNA from benzene-treated rats may be related to the tumor formation in this organ following benzene treatment although this relationship is not clearly defined (see Section 2.2.2.8). Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8   Cancer

No studies were located regarding cancer effects in humans after primarily oral exposure to benzene.

Lymphomas are sometimes, but not always, a variety of leukemia, and the fact that they are seen as the predominant form of benzene-induced hematological neoplasia in rodents may represent species specificity. Leukemia of the myeloid cell line, most frequently associated with benzene exposure in humans, has a low spontaneous rate in rodents. Lymphatic and hematopoietic cancers were seen in vehicle maintenance workers who occasionally siphoned gasoline by mouth, in addition to inhaling gasoline vapors and using gasoline to wash their hands and clean engine parts (Hunting et al. 1995)

Doses of 50, 100, or 200 mg/kg/day benzene in corn oil were administered by gavage to male Fischer 344 rats, 5 days per week for 103 weeks (Huff et al. 1989; NTP 1986). Doses of 25, 50, or 100 mg/kg/day benzene in corn oil were administered to female Fischer 344 rats and to male and female B6C3F$_1$ mice according to the same regimen (Huff et al. 1989; NTP 1986). Male rats treated with benzene for 2 years developed increased incidences of Zymbal gland carcinomas at 100 and 200 mg/kg/day (24% and 43%, respectively, compared to 6% in controls), squamous cell papillomas and squamous cell carcinomas of the oral cavity at all doses (18%, 32%, and 38%, respectively, compared to 2% in controls) and squamous cell papillomas and squamous cell carcinomas of the skin at the high dose (24 compared to 2% for controls). For female rats, benzene caused increased

incidences of Zymbal gland carcinomas at all doses (13, 14, and 33% compared to 0% for controls) and squamous cell papillomas and squamous cell papillomas and squamous cell carcinomas of the oral cavity at the mid and high doses (24% and 18%, respectively, compared to 2% for controls). Male mice treated with benzene for 2 years developed increased incidences of Zymbal gland squamous cell carcinomas at the mid and high dose (10 and 54%, respectively, compared to 0% in the controls), malignant lymphomas at all doses (21, 20, and 30% compared to 8% for controls), alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined) at the mid and high doses (38 and 43%, respectively, compared to 20% for controls), harderian gland adenomas at all doses (22, 27, and 29% compared to 2% for controls), and squamous cell carcinomas of the preputial gland at the mid and high dose (66% and 89%, respectively, compared to 0% for controls). For female mice, benzene caused increased incidences of .malignant lymphomas, at all dose levels (56, 52, and 45% compared to 31% for controls), ovarian granulosa cell tumors and ovarian benign mixed tumors at the mid and high doses (49 and 40%, respectively, compared to 2% for controls), carcinomas of the mammary gland at the mid and high doses (12 and 28%, respectively, compared to 0% for controls), alveolar/bronchiolar adenomas and alveolar/bronchiolar carcinomas in the high dose (27% compared to 8% for controls) and Zymbal gland squamous carcinomas in the high-dose (10% compared to 0% for controls) group. The tumor incidence was dose-related for both rats and mice.

Maltoni et al. (1983, 1985, 1989) conducted a series of experiments on the carcinogenicity of benzene administered orally in olive oil to Sprague-Dawley rats, Wistar rats, RF/J mice, or Swiss mice. Sprague-Dawley rats were given 50, 250, or 500 mg/kg/day of benzene by stomach tube for up to 104 weeks (Maltoni et al. 1983, 1985, 1989). Animals were evaluated for carcinogenicity at different times during the experiment. At 52 weeks, carcinoma of the Zymbal gland was noted in 3% of the Sprague-Dawley rats exposed to 50 mg/kg/day and 11% of rats exposed to 250 mg/kg/day, while no carcinomas were present in controls fed olive oil (Maltoni et al. 1983, 1985, 1989). An increase in the incidence of carcinoma of the oral cavity (3%) was observed in animals receiving 250 mg/kg/day (3%). Carcinoma of the nasal cavities and forestomach, and liver angiosarcoma were also noted at 250 mg/kg/day. At 92 weeks, 15% of Sprague-Dawley rats exposed to 500 mglkglday benzene were found to have carcinomas of the Zymbal gland (Maltoni et al. 1983, 1985). Carcinoma of the oral (14%) and nasal (2.5%) cavities, and liver angiocarcinoma (2.5%) were also noted. At 104 weeks, Sprague-Dawley rats given 50-250 mg/kg/day exhibited a similar spectrum of tumors: carcinoma of the Zymbal gland at all doses, carcinoma of the oral cavity at 250 and 500 mg/kg/day, and carcinoma of the nasal cavity and forestomach, and angiosarcoma of the liver at 500 mg/kg/day. At the high

dose, incidence of oral carcinomas was 51%. A 7.5% incidence of forestomach carcinomas (compared to 0% in controls), 5% incidence of hepatomas (compared to 3% in the control group), and 6% incidence of angiosarcomas of the liver (compared to 0% in the control group) was also observed in these animals. Wistar rats exhibited a similar spectrum of tumor development. Sixteen percent of Wistar rats exposed to 500 mg/kg/day benzene for 104 weeks developed Zymbal gland carcinomas, but only 7.5% developed oral carcinomas, in contrast to the Sprague-Dawley rats (Maltoni et al. 1989). Nasal, forestomach, and hepatic tumors were also observed (Maltoni et al. 1989). Leukemia was also noted in the high-dose rats at 104 weeks. The incidences of leukemia in Sprague-Dawley and Wistar rats fed 500 mg/kg/day of benzene for 104 weeks were 5 and 7.5%, compared to 4 and 5% in the respective control groups. There were dose response relationships for the incidences of carcinomas of the Zymbal gland and carcinomas of the nasal and oral cavities. Maltoni also conducted oral carcinogenicity studies of benzene in mice (Maltoni et al. 1989). Carcinomas of mammary glands developed in 24% (compared to 3.7 for controls) and 11% (compared to 1.2% for controls) of Swiss and RF/J mice exposed to 500 mg/kg/day for 78 and 52 weeks, respectively (Maltoni et al. 1989). Increased incidence of tumors of the lung and Zymbal gland were also noted at 500 mg/kg/day in this study.

The IPP model for the induction of malignant neoplasms in the liver was evaluated for benzene in male and female Sprague-Dawley rats (Dragan et al. 1993). Initiation was begun in 5-day-old rats with administration of a single intraperitoneal injection of diethylnitrosamine during the time when the liver is undergoing rapid growth. Promotion began at 6 months of age with phenobarbital in the feed, and continued into young adulthood. Partial hepatectomy was performed, and at the height of the regenerative proliferation phase following the hepatectomy, benzene (1 g/kg) was administered by gavage; phenobarbital treatment was maintained after the administration of benzene. Tabulated data showed progression by benzene in rat hepatocarcinogenesis. Results indicated that benzene may have some initiating as well as progressor action at the dose tested; however, stereologic determination of the number of altered hepatic foci (AHF) detected with glutathione-S transferase and g-glutamyl-transpeptidase found very few AHF initiated with benzene and promoted by phenobarbital, which suggests that benzene is a poor initiator under these conditions. Since a single administration of benzene increased the incidence of hepatocellular carcinoma in both male and female rats compared with appropriate controls, benzene may possess progressor activity and increase the risk of cancer development through action at this stage of carcinogenesis. In a similar experiment, male Fischer 344 rats were divided into 4 groups (Taningher et al. 1995). Rats were given a 200 mg/kg

diethylnitrosamine (DENA) intraperitoneal injection in saline followed by gavage treatment with corn oil vehicle; a saline intraperitoneal injection without DENA and then daily doses of 400 mg/kg benzene in corn oil by gavage 5 days per week for 6 weeks; a single intraperitoneal injection of 200 mg/kg DENA in saline to initiate hepatocarcinogenesis, and then two weeks later, daily doses of 400 mg/kg benzene in corn oil 5 days per week for 6 weeks by gavage; or a single intraperitoneal injection of 200 mg/kg DENA in saline to initiate hepatocarcinogenesis then 2 weeks later followed by 500 ppm phenobarbital in their drinking water for 6 weeks. Benzene treatment did not significantly increase the foci number and area in rats treated with DENA alone compared to rats treated with DENA plus benzene. Practically no foci were observed in the liver of benzene treated rats. Thus, benzene exhibited no significant promoting activity.

The EPA Gene-Tox Carcinogenesis Panel categorizes benzene as having sufficient positive evidence for carcinogenicity in animal studies (IRIS 1996), and NTP (1986) concluded that there was evidence of carcinogenicity of benzene for the strains of rats and mice tested in their 2-year oral/gavage bioassay program.

EPA (IRIS 1996) reviewed human and animal carcinogenicity data on benzene. The following paragraph is a summary of the EPA calculations of unit risk values for leukemia based on human epidemiological studies. It should be noted that these values are estimates of human risk since the true human risk at low doses cannot be accurately identified. The unit risk for drinking water of 8.3×10^{-7} per μg/L should not be used if the water concentration exceeds 10,000 μg/L, since above this concentration the slope factor may no longer be accurate.

EPA has used cancer risk data from human epidemiological studies to derive risk factors associated with oral exposure to benzene. Oral dose levels associated with specific carcinogenic risks have been extrapolated: the risk value of 2.7×10^{-2} for lifetime inhalation exposure to 1 ppm was converted to a slope factor of 2.9×10^{-2} for oral exposure of 1 mg/kg/day, assuming identical levels of absorption of benzene following both routes of exposure. Using the method described by EPA (IRIS 1996), the drinking water levels associated with individual upper-bound estimates of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} have been calculated to be 1×10^{-1}, 1×10^{-2}, 1×10^{-3}, and 1×10^{-4} mg/L, respectively, which are equivalent to dose levels of 3×10^{-3}, 3×10^{-4}, 3×10^{-5}, and 3×10^{-6} mg/kg/day, respectively. These dose levels are indicated in Figure 2-2.

The CELs for each species and duration category are recorded in Table 2-2 and Figure 2-2.

2.2.3   Dermal Exposure

2.2.3.1   Death

No studies were located regarding deaths in animals after dermal exposure to benzene.

A cohort of 338 men was investigated as to causes of death among employees of the fleet maintenance division of Washington DC's Department of Public Works (Hunting et al. 1995). This mortality study was undertaken because of 3 cases of leukemia among car and mobile equipments mechanics. Preliminary evaluation showed that the garage mechanics regularly used gasoline to clean parts and wash their hands; these workers also experienced dermal and inhalation exposure to gasoline during maintenance of vehicles. The men were employed for at least 1 year between January 1, 1977, and December 31, 1989. Cause-specific SMRs were calculated. Increased risk of death was found in some categories.

2.2.3.2   Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, or body weight effects in humans or animals or musculoskeletal effects in animals after dermal exposure to benzene. Available data pertaining to musculoskeletal, dermal, and ocular effects are presented below. All reliable LOAEL values for systemic effects in humans and rabbits for acute- and chronic-duration dermal exposure are recorded in Table 2-3.

Musculoskeletal Effects. Tondel et al. (1995) reported a case of myelofibrosis that was diagnosed in a 46-year-old man who had worked from 1962 to 1979 as a gasoline station attendant. Although the exposure was primarily by inhalation, it is likely that dermal exposure also occurred.

Dermal Effects. In humans, benzene is a skin irritant. By defatting the keratin layer, it may cause erythema, vesiculation, and dry and scaly dermatitis (Sandmeyer 1981). Acute fatal exposure to benzene vapors caused second degree bums on the face, trunk, and limbs of the victims (Avis and Hutton 1993). Fifteen male workers were exposed to benzene vapors (>60 ppm) over several days

Table 2-3. Levels of Significant Exposure to Benzene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
ACUTE EXPOSURE						
Systemic						
Human	1-21 d 2.5 - 8 hr/d	Dermal		60 ppm M (mucous membrane and skin irritation)		Midzenski et al. 1992
Rabbit (NS)	once	Ocular		2 drops (moderate conjunctival irritations; light corneal injury)		Wolf et al 1956
INTERMEDIATE EXPOSURE						
Systemic						
Rat (CD)	10 wk 5 d/wk 6 hr/d	Ocular	1 ppm M	10 ppm M (lacrimation)		Shell 1980
CHRONIC EXPOSURE						
Systemic						
Human	>1 yr (occup)	Ocular		33 ppm M (eye irritation) 59 ppm F (eye irritation)		Yin et al. 1987b

d = day(s); F = female; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; yr = year(s)

during the removal of residual fuel from shipyard fuel tanks (Midzenski et al. 1992). Exposures to benzene range from 1 day to 3 weeks (mean of 5 days), 2.5-8 hours/day (mean of 5.5 hrs). Workers with more than 2 days (16 hours) exposure reported mucous membrane irritation (80%), and skin irritation (13%) after exposure to the vapor.

Benzene was slightly irritating to the skin of rabbits (Wolf et al. 1956). The skin showed moderate erythema, edema, and moderate necrosis following application one time per day for 4 weeks.

Ocular Effects. Solvent workers who were exposed to 33 (men) or 59 (women) ppm benzene exhibited eye irritation while being exposed to the vapors (Yin et al. 1987b).

A transient increase in lacrimation was observed in male rats exposed to l0-300 ppm benzene for 6 hours per day, 5 days per week (Shell 1980). Moderate conjunctival irritation and transient cornea1 damage were observed in rabbits subsequent to placement of 2 drops of benzene onto the eyeball (Wolf et al. 1956).

2.2.3.3   Immunological and Lymphoreticular Effects

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

2.2.3.4   Neurological Effects

Tondel et al. (1995) reported the case of a gasoline station attendant who had worked from 1962 to 1979. The patient described symptoms of fatigue for 3 weeks and night sweats, among other symptoms. Although the exposure was primarily by inhalation, it is probable that dermal exposure also occurred.

No studies were located regarding neurological effects in animals after dermal exposure to benzene.

2.2.3.5   Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to benzene.

2.2.3.6   Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to benzene.

2.2.3.7   Genotoxic Effects

A few case studies were found involving individuals whose job descriptions suggest dermal as well as inhalation exposure to benzene (Forni and Moreo 1967, 1969; Van den Berghe et al. 1979; Yardley-Jones et al. 1988, 1990). These cases are discussed under inhalation in Section 2.2.1.7.

No studies were located regarding genotoxic effects in animals after dermal exposure to benzene. Other genotoxicity studies are discussed in Section 2.5.

2.2.3.8   Cancer

A cohort of 338 men were investigated as to causes of death among employees of the fleet maintenance division of Washington DC's Department of Public Works (Hunting et al. 1995). This mortality study was undertaken because of 3 cases of leukemia among car and mobile equipments mechanics. Preliminary evaluation showed that the garage mechanics regularly used gasoline to clean parts and wash their hands; these workers also experienced dermal and inhalation exposure to gasoline during maintenance of vehicles and some workers would occasionally siphon gasoline by mouth. The men were employed for at least 1 year between January 1, 1977, and December 31, 1989. Cause specific SMRs were calculated and the relative risk of leukemia and other hematological cancers among all fleet maintenance workers and for the high exposure subgroup were estimated. The allcause SMR was 0.50 with 33 deaths. The all-cancer SMR was 0.55 with 9 total deaths. The underlying causes of death from cancers are as follows: 1 from cancer of the digestive organs and peritoneum (SMR=0.22); 2 from cancer of the respiratory system (SMR=0.33); 2 from cancer of the

male genitalia (SMR=1.91); 3 from lymphatic and hematopoietic cancer (SMR=3.63); 1 from other and unspecified cancer (SMR=0.49). A subgroup analysis (n=297) reported 3 observed deaths for lymphatic and hematopoietic cancers (SMR=4.22); 2 observed deaths for leukemia and aleukemia (SMR=9.26); and 1 observed death for other neoplasms of lymphatic and hematopoietic tissue (SMR=2.57. The SMR of 9.26 for leukemia and aleukemia was statistically significant ($p<0.05$).

Dermal exposure to benzene did not induce skin tumors in mice (Bull et al. 1986). No papillomas developed in mice that were given a 2-week, 800 mg/kg/day topical application of benzene as the initiator and a 1 μg topical application of 12-*o*-tetradecanoylphorbol-13-acetate 3 times a week for 20 weeks and observed for 52 weeks (Bull et al. 1986). The authors concluded that it is difficult to estimate benzene-induced tumor incidence after dermal exposure, and that mouse skin may not be the optimal study system. This is because of the high rate of false-negative responses to chemicals, like benzene, with recognized carcinogenic activity.

2.3   TOXICOKINETICS

The toxicokinetics of benzene has been extensively studied. Inhalation exposure is probably the major route of human exposure to benzene, although oral and dermal exposure are also important. Absorption, distribution, metabolism, and elimination have been studied in both humans and animals. Investigations of the metabolism of benzene have led to the identification of toxic metabolites, and to hypotheses about the mechanism of toxicity.

2.3.1 Absorption

2.3.1.l Inhalation Exposure

Inhalation exposure is probably the major route of human exposure to benzene, and numerous studies of absorption of benzene after inhalation exposure in different settings have been conducted (Ashley et al. 1994; Avis and Hutton 1993; Boogaard and van Sittert 1995; Brunnemann et al. 1989; Byrd et al. 1990; Etzel and Ashley 1994; Fustinoni et al. 1995; Ghittori et al. 1995; Gordian and Guay 1995; Hajimiragha et al. 1989; Hanzlick 1995; Karacic et al. 1995; Kok and Ong 1994; Lagorio et al. 1994a; Laitinen et al. 1994; Lauwerys et al. 1994; Lindstrom et al. 1993; Mannino et al. 1995; Nomiyama and Nomiyama 1974a; Ong et al. 1994, 1995; Pekari et al. 1992; Popp et al. 1994; Rauscher et al. 1994;

Rothman et al. 1995; Ruppert et al. 1995; Scherer et al. 1995; Shamy et al. 1994; Srbova et al. 1950). Existing evidence indicates that benzene is rapidly absorbed by humans following inhalation exposure. Results from a study of 23 subjects that inhaled 47-110 ppm benzene for 2-3 hours showed that absorption was highest in the first few minutes of exposure, but decreased rapidly thereafter (Srbova et al. 1950). In the first 5 minutes of exposure, absorption was 70-80%, but by 1 hour it was reduced to approximately 50% (range, 20-60%). Respiratory uptake (the amount of benzene absorbed from the lungs following inhalation of the vapors) in six volunteers including males and females exposed to 52-62 ppm benzene for 4 hours was determined to be approximately 47%. Respiratory retention (the amount of absorbed benzene that is not excreted via the lungs) was approximately 30% (Nomiyama and Nomiyama 1974a). In a similar study, 3 healthy non-smoking volunteers were exposed to benzene at levels of 1.6 or 9.4 ppm for 4 hours (Pekari et al. 1992). The amount of benzene absorbed was estimated from the difference between the concentration inhaled and the concentration exhaled. Estimates were 48% for the high dose and 52% for the .low dose, supporting the evidence of Nomiyama and Nomiyama (1974a).

Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different workphases were measured at 5 Finnish garages. Blood samples from car mechanics (8 nonsmokers) were taken 3-9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The average background concentration (stationary samples) of gasoline vapors was 6 ± 7 cm^3/m^3 (2 ± 2 ppm) and the concentration of benzene was under the detection limit of 0.2 cm^3/m^3 (0.1 ppm). The concentrations of benzene in the breathing zone varied from the detection limit of 0.2 cm^3/m^3 to 1.3 cm^3/m^3 (0.1-0.4 ppm) for unleaded gasoline and from the detection limit to 3.7 cm^3/m^3 (1.2 ppm) for leaded gasoline. The highest benzene exposure level (2.4-3.7 cm^3/m^3 or 0.8-1.2 ppm) was measured when changing the filter to the fuel pump. The mechanics worked without protective gloves, and the risk of contamination and penetration through the skin was significant. During carburetor renewal and gathering, benzene concentrations were 0.5-1.1 cm^3/m^3 (0.2-0.3 ppm). During changing of the fuel filter to electronic fuel-injection system, benzene concentration ranged from 0.9 to 3.4 cm^3/m^3 (0.3-1.1 ppm). The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according

to the corresponding air measurements (8-hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposure were present, to the levels at the time point of 16 hours after the exposure showed that the dermal route must be the source of about 68% of exposure (range 1.l-88.2%). Two of 8 workers had minimal exposure through the skin (0-1.1%). The other 6 workers showed high dermal exposure (79.4%).

Exposure to benzene-contaminated water can also provide an opportunity for both inhalation and dermal absorption. In a series of experiments conducted in a single-family residence from June 11 to 13, 1991, exposure to benzene through contaminated residential water was monitored (Lindstrom et al. 1993). The residential water was contaminated with benzene and other hydrocarbons in 1986. Periodic testing conducted from 1986 to 1991 showed benzene concentrations ranging from 33 to 673 µg/L (ppb). The experiment involved an individual taking a 20-minute shower with the bathroom door closed, followed by 5 minutes for drying and dressing; then the bathroom door was opened and this individual was allowed to leave the house. Integrated 60- and 240-minute whole-air samples were collected from the bathroom, an adjacent bedroom, living room, and in ambient air. Glass, gas-tight syringe grab samples were simultaneously collected from the shower, bathroom, bedroom, and living room at 0, 10, 18, 20, 25, 25.5, and 30 minutes. Two members of the monitoring team were measured for six hours using personal Tenax gas GC monitors. For the first 30 minutes of each experiment, one member was based in the bathroom and the other in the living room. Benzene concentrations in the shower head ranged from 185 to 367 µg/L (ppb) while drain level samples ranged from below the detectable limit (0.6 µg/L or ppb) to 198 µg/L (ppb). Analysis of the syringe samples suggested a pulse of benzene moving from the shower stall to the rest of the house over approximately 60 minutes. Peak benzene levels were measured in the shower stall at 18-20 minutes (758-1,670 µg/m^3), in the bathroom at 10-25 minutes (366-498 µg/m^3), in the bedroom at 25.5-30 minutes (81-146 µg/m^3), and in the living room at 36-70 minutes (40-62 µg/m^3). The individual who took the 20-minute shower had inhalation doses of 79.6, 105, and 103 µg (mean = 95.9 µg) for the 3 consecutive sampling days. These doses were calculated by taking the products of the concentration of benzene in water, the minute ventilation rate, the duration of exposure, and a 70% benzene absorption factor. This was 2.1-4.9 times higher than corresponding 20-minute bathroom exposures. Adding the average dose absorbed in the bathroom during the 5.5 minutes following the shower (using the overall 20-25 minutes mean syringe level of 318 µg/m^3) gave a total average shower-related inhalation dose of 113 µg. An average dermal dose of 168 µg was calculated for the 20-minute shower by multiplying the average concentration of benzene in water by the surface area of the male volunteer, an exposure

factor of 75% body surface area exposed, a dermal permeability constant for benzene of 0.11 cm/hr, an exposure duration of 0.33 hours, and a unit conversion factor of $1l/1,000$ cm^2. The total benzene dose resulting from the shower was estimated to be approximately 281 μg (40% via inhalation and 60% via dermal), suggesting a higher potential exposure to benzene via dermal contact from the water than through vaporization and inhalation. This exposure was 2-3.5 times higher than the mean 6-hour inhalation dose received by the sampling members.

Additional evidence of benzene absorption following inhalation exposure comes from data on cigarette smokers. Benzene levels were significantly higher in the venous blood of 14 smokers (median level of 493 ng/L) than in a control group of 13 nonsmokers (median level of 190 ng/L) (Hajimiragha et al. 1989). Cigarette smoke is known to contain benzene (Brunnemann et al. 1989; Byrd et al. 1990), and the subjects had no known exposure to other sources of benzene (Hajimiragha et al. 1989). Kok and Ong (1994) report blood and urine levels of benzene for nonsmokers as 110.9 and 116.4 ng/L, respectively, and for smokers, 328.8 and 405.4 ng/L, respectively. The National Association of Medical Examiners Pediatric Toxicology (PedTox) Registry reported blood benzene concentrations ranging from 0.2 to 4.9 mg/L in 8 children who died in fires and were dead at the scene, indicating absorption of benzene from burning materials (Hanzlick 1995). Blood benzene levels taken from U.S. engineers (Group I) and firefighters (Group II) working at burning oil wells in Kuwait were compared to blood benzene levels from non-exposed U.S. citizens (Etzel and Ashley 1994). The median concentrations of benzene in whole blood from Groups I, II, and U.S. reference group were 0.035 (range ND-0.055) μg/L, 0.18 (range 0.063-l.l) μg/L, and 0.066 (range ND-0.54) μg/L, respectively. The median concentration in group II was generally higher than the median concentrations in group I or the reference group. Statistically significant higher concentrations of benzene ($p<0.0001$) were found in group II smokers than in Group II nonsmokers.

Indoor and ambient air samples were measured from December 1992 to February 1993 by the Alaska Department of Environmental Conservation (Gordian and Guay 1995). Concentration levels for methyl tertiary butyl ether (MTBE), benzene, and formaldehyde were measured in ambient and indoor air samples. Blood samples were also taken from service station workers at the same time period. Blood samples from workers showed an increase of 300% in blood benzene concentrations after MTBE reformulated fuel was discontinued. Gasoline in Alaska has a benzene concentration of 5% (average U.S. national concentration is 1.5%).

Animal data confirm that benzene is rapidly absorbed through the lungs. Inhalation studies with laboratory dogs indicate that distribution of benzene throughout the animal's body is rapid, with tissue values dependent on blood supply. A linear relationship existed between the concentration of benzene in air (200-1,300 ppm) and the equilibrium concentration in blood (Schrenk et al. 1941). At these exposures, the concentrations of benzene in the blood of dogs exposed to benzene reached a steady state within 30 minutes.

In rodents, the extent of uptake increased linearly with concentration for exposures up to $\approx$200 ppm. At concentrations of >200 ppm, zero-order kinetics were observed (i.e., uptake became nonlinear, indicating saturation of the metabolic capacity). The percentage of inhaled benzene that was absorbed and retained during a 6-hour exposure period decreased from 33% to 15% in rats and from 50% to 10% in mice as the exposure concentration was increased from about 10 to 1,000 ppm (Sabourin et al. 1987). When rats and mice were exposed to approximately 300 ppm, mice had greater uptake than rats. Mice and rats had different absorption characteristics; the cumulative inhaled dose in mice was greater than that in rats (Eutermoser et al. 1986; Sabourin et al. 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours per day, 5 days per week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

2.3.1.2   Oral Exposure

Although definitive scientific data are not available on oral absorption of benzene in humans, case studies of accidental or intentional poisoning indicate that benzene is absorbed by the oral route (Thienes and Haley 1972).

Benzene appears to be efficiently absorbed following oral dosing in animals. Oral absorption of benzene was first demonstrated by Parke and Williams (1953a). After radiolabeled (^{14}C) benzene was administered orally to rabbits (340-500 mg/kg), the total radioactivity eliminated in exhaled air and urine accounted for approximately 90% of the administered dose, indicating that at least this much of the administered dose was absorbed. Studies in rats and mice showed that gastrointestinal absorption was greater than 97% in both species when the animals were administered benzene by gavage at doses of 0.5-150 mg/kg/day (Sabourin et al. 1987). In many animal studies, benzene is administered orally in oil to insure predictable solubility and dose concentration control. This is unlike the predicted human oral exposure, which is likely to be in drinking water. There are a number of studies in which

benzene has been administered to animals in the drinking water, which more closely resembles predicted human oral exposure (Lindstrom et al. 1993).

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of benzene alone, or adsorbed to clay or sandy soil. Plasma concentration, half-life, tissue distribution, respiratory excretion, and urinary excretion were monitored. Peak plasma concentration of radioactivity was increased in the presence of either soil as opposed to benzene alone, while sandy soil also decreased the time to peak plasma concentration as opposed to benzene alone. Soil increased the area under the plasma radioactivity-time curve as opposed to benzene alone, a difference that was significant with clay soil. The half-life in plasma was not affected by soil.

2.3.1.3 Dermal Exposure

Studies conducted *in vivo* in humans and *in vitro* using human skin indicate that benzene can be absorbed dermally. The data show that dermal absorption is not as substantial as absorption following inhalation exposure to benzene vapor or oral exposure. The movement of a substance through the skin to the blood occurs by passive diffusion and has been described mathematically by Fick's law. However, this is an oversimplification of the process of skin absorption; various factors (e.g., interaction of benzene with molecules within the skin) affect the transport of the solvent through the skin (Loden 1986).

In vivo experiments on 4 human volunteers, to whom 0.0026 mg/cm^2 of ^{14}C-benzene was applied to forearm skin, indicated that approximately 0.05% of the applied dose was absorbed (Franz 1984). Absorption was rapid, with more than 80% of the total excretion of the absorbed dose occurring in the first 8 hours after application. Calculations were based on urinary excretion data and no correction was made for the amount of benzene that evaporated from the applied site before absorption occurred. In addition, the percentage of absorbed dose excreted in urine that was used in the calculation was based only on data from rhesus monkeys and may not be accurate for humans. In another study, 35-43 cm^2 of the forearm was exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25-2 hours (Hanke et al. 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately 0.4 mg/cm^2/hour. The absorption due to

vapors in the same experiment was negligible. The results indicate that dermal absorption of liquid benzene is of concern, while dermal absorption from vapor exposure may not be of concern because of the low concentration of benzene in vapor form at the point of contact with the skin. No signs of acute intoxication due to liquid benzene dermally absorbed were noted. These results confirm that benzene can be absorbed through skin. However, non-benzene-derived phenol in the urine was not accounted for.

Studies of occupational exposure to benzene suggest that absorption occurs both by inhalation and dermally in many workplace settings. In a study conducted in 1992 in Finland, car mechanics' exposure to benzene was evaluated (Laitinen et al. 1994). Different workphases were measured at 5 Finnish garages. Blood samples from car mechanics (8 nonsmokers) were taken 3-9 hours after exposure to benzene. The results were approximated to the time point of 16 hours after exposure. Fourteen air samples were taken from the breathing zone and five stationary samples were collected from the middle of the garage for background concentration levels. The mechanics worked without protective gloves, and the risk of contamination and penetration through the skin was significant. The approximated benzene concentrations in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than could be expected according to the corresponding air measurements (8-hour TWA). The comparison of expected benzene concentrations in blood, if no dermal exposure was present, to the levels at the time point of 16 hours after the exposure showed that the dermal route must be the source of about 68% of exposure (range 1.l-88.2%). Two of eight workers had minimal exposure through the skin (0-1.1%). The other 6 workers showed high dermal exposure (79.4%).

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Section 2.3.1.l for a more detailed discussion). This exposure was 2-3.5 times higher than the mean 6-hour inhalation dose received by the sampling team members in other parts of the house.

In vitro experiments using human skin support the fact that benzene can be absorbed dermally. An experiment on the permeability of excised human skin with regard to benzene (specific activity 99.8 mCi/mmol; total volume of applied benzene not reported) resulted in the absorption of 0.17 mg/cm^2 after 0.5 hours and 1.92 mg/cm^2 after 13.5 hours (Loden 1986). Following application of 5, 120, 270, and 520 μL/cm^2 of benzene to human skin, total absorption was found to be 0.01, 0.24, 0.56, and 0.9 μL/cm^2, respectively. Thus, the total amount absorbed appears to increase linearly with dose. When exposure time (i.e., the time to complete evaporation) at each dose was measured and plotted as the ordinate of absorption, total absorption was found to increase linearly with exposure time. The percentage of the applied dose absorbed at each concentration was constant at about 0.2% (Franz 1984).

Using results from an *in vitro* study, it was estimated that an adult working in ambient air containing 10 ppm benzene would absorb 7.5 μL/hour from inhalation and 1.5 μL/hour from whole-body (2 m^2) dermal exposure (Blank and McAuliffe 1985). It was also estimated that 100 cm^2 of smooth and bare skin in contact with gasoline containing 5% benzene would absorb 7.0 μL/hour. Diffusion through the stratum corneum was considered the most likely rate-limiting step for dermal absorption because of benzene's low water solubility (Blank and McAuliffe 1985).

Based on an observational study of workers in a tire factory, it was estimated that a worker exposed to benzene as a result of direct skin contact with petroleum naphtha containing 0.5% benzene could absorb 4-8 mg of benzene per day through intact skin (Susten et al. 1985). This amount absorbed was compared with an estimated 14 mg of benzene absorbed as a result of inhalation of 1 ppm for an 8-hour day. The estimate for dermal absorption is exaggerated since in many facilities the concentration of benzene in rubber solvents such as petroleum naphtha is less than 0.5% and may be as low as 0.09%.

Benzene is also absorbed dermally by animals. In rhesus monkeys, minipigs, and hairless mice, dermal absorption was <1% following a single direct application of liquid benzene (Franz 1984; Maibach and Anjo 1981; Susten et al. 1985). As with humans, absorption appeared to be rapid, with the highest urinary excretion of the absorbed dose observed in the first 8 hours following exposure

(Franz 1984). Multiple applications, as well as application to stripped skin, resulted in greater skin penetration (Maibach and Anjo 1981). The percentage of absorption of the applied dose of benzene in each of these animals was approximately 2-3-fold higher than that of humans.

Data indicate that soil adsorption decreases the dermal bioavailability of benzene. A study in which male rats were treated dermally with 0.004 mg/cm^2 ^{14}C-benzene, with or without 1 g of clay or sandy soil, reported benzene absorption half-lives of 3.1, 3.6, and 4.4 hours for pure benzene, sandy soil, and clay soil, respectively (Skowronski et al. 1988).

Benzene in air was rapidly absorbed through the skin of hairless mice that were attached to respirators to avoid pulmonary uptake of the benzene vapors (Tsuruta 1989). The rate of absorption of benzene through the skin increased linearly with dose. The skin absorption rate for 200 ppm was 4.11 nmol/cm^2/hour (0.31 μg/cm^2/hour); at 1,000 ppm the rate was 24.2 nmol/cm^2/hour (1.89 μg/cm^2/hour); and at 3,000 ppm the rate was 75.5 nmol/cm^2/hour (5.90 μg/cm^2/hour). The skin absorption coefficient was 0.619 cm/hour.

In an *in vitro* experiment using Fischer 344 rat skin, the partition coefficient for skin:air was determined for benzene at 203 ppm (Mattie et al. 1994). The partition coefficient of a chemical in skin is an indicator of the capacity of the skin for the chemical, and may reflect the rate at which a chemical is absorbed through the skin and enters the circulation. Results indicated a partition coefficient of 35, with an equilibration time of 4 hours. This value more closely correlates with the permeability constant of 1.5 derived by McDougal et al. (1990), than does the commonly used octanol/water partition coefficient of 134.9, as derived by Leo et al. (1971). The skin:air partition coefficient is necessary for developing the dermal compartment of a PBPK model.

Based on data for skin absorption of benzene vapors in mice and occupational exposure data, Tsuruta (1989) estimated the ratio of skin absorption rate to pulmonary uptake to be 0.037 for humans. Dermal absorption could account for a relatively higher percentage of total benzene uptake in occupational settings where personnel, using respirators but not protective clothing, are exposed to high concentrations of benzene vapor.

2.3.2   Distribution

2.3.2.1   Inhalation Exposure

Information on the distribution of benzene in humans comes primarily from case studies. The data suggest that benzene is distributed throughout the body following absorption into blood. Since benzene is lipophilic, a high distribution to fatty tissue might be expected. Following inhalation exposure to benzene, the chemical has been detected in the biological fluids and tissues of the subjects (Pekari et al. 1992; Tauber 1970; Winek and Collom 1971; Winek et al. 1967). Fluid and tissue levels of benzene have been reported in cases of both accidental and intentional lethal exposures. Levels of 0.38 mg% in blood (mg% = mg per 100 mL of blood or mg per 100 g of tissue), 1.38 mg% in the brain, and 0.26 mg% in the liver were reported in a worker who died from exposure to very high air concentrations of the chemical (Tauber 1970). An autopsy (time after death not indicated) performed on a youth who died while sniffing reagent-grade benzene revealed benzene concentrations of 2.0 mg% in blood, 3.9 mg% in brain, 1.6 mg% in liver, 1.9 mg% in kidney, 1 mg% in stomach, 1.1 mg% in bile, 2.23 mg% in abdominal fat, and 0.06 mg% in urine (Winek and Collom 1971). Benzene crosses the human placenta and is present in the cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976).

Respiratory uptake (the amount of benzene absorbed from the lungs following inhalation of the vapors) in 6 male and female volunteers exposed to 52-62 ppm benzene for 4 hours was determined to be approximately 47% (Nomiyama and Nomiyama 1974a). Respiratory retention (the amount of benzene that is not excreted via the lungs following absorption) was determined to be about 30%. Although no sex differences in the final retention of benzene were observed in this study, another study in which male and female subjects were exposed to 25 ppm for 2 hours showed that benzene appeared to be retained longer in the females (Sato et al. 1975). The authors attributed these results to the higher fat content of females, but the difference in the total exposure period (4 hours as opposed to 2 hours) could have been responsible for the observed discrepancy in the results. -

Results from animal studies indicate that absorbed benzene is distributed throughout several compartments. The parent compound is preferentially stored in the fat, although the relative uptake in tissues also appears to be dependent on the perfusion rate of tissues by blood.

Following a 10-minute inhalation exposure to 2,000 ppm of benzene by pregnant mice, benzene and its metabolites were found to be present in lipid-rich tissues, such as brain and fat, and in wellperfused tissues, such as liver and kidney. Benzene was also found in the placenta and fetuses immediately following inhalation of benzene (Ghantous and Danielsson 1986). During inhalation exposure of rats to 500 ppm, benzene levels reached a steady-state concentration within 4 hours in blood (11.5 µg/mL), 6 hours in fat (164.4 µg/g), and less than 2 hours in bone marrow (37.0 µg/g) (Rickert et al. 1979). Benzene was also distributed to the kidney, lung, liver, brain, and spleen. The benzene metabolites phenol, catechol, and hydroquinone were detected in blood and bone marrow following 6 hours of exposure to benzene, with levels in bone marrow exceeding the respective levels in blood. The levels of phenol in blood and bone marrow decreased much more rapidly after exposure ceased than did those of catechol or hydroquinone, suggesting the possibility of accumulation of the latter two compounds.

Benzene was rapidly distributed throughout the bodies of dogs exposed via inhalation to concentrations of 800 ppm for up to 8 hours per day for 8-22 days (Schrenk et al. 1941). Fat, bone marrow, and urine contained about 20 times the concentration of benzene in blood; benzene levels in muscles and organs were l-3 times that in blood; and erythrocytes contained about twice the amount of benzene found in plasma. During inhalation exposure of rats to 1,000 ppm (2 hours per/day, for 12 weeks), benzene was stored longer (and eliminated more slowly) in female and male rats with higher body fat content than in leaner animals (Sato et al. 1975).

2.3.2.2   Oral Exposure

No studies were located regarding distribution in humans after oral exposure to benzene.

One animal study examined the distribution of benzene and its metabolites following oral exposure. In Sprague-Dawley rats administered a single dose of 0.15, 1.5, 15, 150, or 500 mg/kg of ^{14}C-benzene by gavage, benzene was rapidly absorbed and distributed to various organs and tissues within 1 hour of administration (Low et al. 1989). One hour after rats were dosed with 0.15 or 1.5 mg/kg of benzene, tissue distribution of benzene was highest in liver and kidney, intermediate in blood, and lowest in the Zymbal gland, nasal cavity tissue, and mammary gland. At higher doses, beginning with 15 mg/kg, benzene disproportionately increased in the mammary glands and bone marrow. Bone marrow and adipose tissue proved to be depots of benzene at the higher dose levels. The highest tissue

concentrations of benzene's metabolite hydroquinone 1 hour after administration of 15 mg/kg of benzene were in the liver, kidney, and blood, while the highest concentrations of the metabolite phenol were in the oral cavity, nasal cavity, and kidney. The major tissue sites of benzene's conjugated metabolites were blood, bone marrow, oral cavity, kidney, and liver for phenyl sulfate and hydroquinone glucuronide; muconic acid was also found in these sites. Additionally, the Zymbal gland and nasal cavity were depots for phenyl glucuronide, another conjugated metabolite of benzene. The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Therefore, it is reasonable to expect that lipophilic chemicals like benzene would partition readily into this gland. However, benzene did not accumulate in the Zymbal gland; within 24 hours after administration, radiolabel derived from ^{14}C-benzene in the Zymbal gland constituted less than 0.0001% of the administered dose.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of ^{14}C-benzene alone, or adsorbed to clay or sandy soil. Two hours after exposure, stomach tissue contained the highest amount of radioactivity, followed by fat in all treatment groups. No differences in tissue distribution patterns were detected for the three treatments.

2.3.2.3   Dermal Exposure

No studies were located regarding distribution in humans after dermal exposure to benzene.

A study of male rats treated dermally with 0.004 mg/cm^2 of ^{14}C-benzene, with and without 1 g of clay or sandy soil, revealed soil-related differences in tissue distribution following treatment. The ^{14}C activity (expressed as a percentage of initial dose per g of tissue) 48 hours after treatment with soiladsorbed benzene was greatest in the treated skin (0.059-0.119%), followed by the kidney (0.024%) and liver (0.013-0.015%), in both soil groups. In the pure benzene group, the kidney contained the largest amount of radioactivity (0.026%), followed by the liver (0.013%) and treated skin (0.11%) (Skowronski et al. 1988). In all 3 groups, less than 0.01% of the radioactivity was found in the following tissues: duodenum, fat, bone marrow, esophagus, pancreas, lung, heart, spleen, blood, brain, thymus, thyroid, adrenal, testes, untreated skin, and carcass.

2.3.3   Metabolism

There is no available evidence to suggest that the route of administration has any substantial effect on the subsequent metabolism of benzene in humans. There is, however, information from animal studies to indicate that the exposure route affects the disposition and metabolism (Sabourin et al. 1987, 1988, 1989a, 1989b).

Data regarding metabolism of benzene in humans have come primarily from studies using inhalation exposures. Benzene is excreted both unchanged via the lungs and as metabolites in the urine. The rate and percentage of excretion via the lungs are dependent on exposure dose and route. Qualitatively, the metabolism and elimination of benzene appear to be similar in humans and laboratory animals, but no directly comparable studies are available (Henderson et al. 1989; Sabourin et al. 1988).

Benzene metabolism in humans and animals follows many similar pathways. Benzene is metabolized by cytochrome P-450-dependent mixed-function oxidase enzymes. Cytochrome P-450s are ubiquitous in all tissues. However, since the predominant repository of cytochrome P-450 is the liver, benzene is primarily metabolized in the liver. The metabolism of benzene by hepatic cytochrome P-450 may play an important role in the bioactivation of benzene. Sammett et al. (1979) provided corroborative evidence for this role by showing that partial hepatectomy of rats diminished both the rate of metabolism of benzene and its toxicity, suggesting that a metabolite and/or metabolites formed in the liver are necessary for toxicity.

There are several toxification pathways and detoxification pathways (via conjugation) of benzene metabolism (Henderson et al. 1989). The first step in benzene metabolism is the formation of the epoxide benzene oxide via cytochrome P-450-dependent mixed-function oxidase (Jerina et al. 1968). After this occurs, two metabolic toxification pathways, one involving ring hydroxylation and the second involving ring opening, result in the formation of putative toxic benzene metabolites (Henderson et al. 1989). In the first pathway involving ring hydroxylation, acid-catalyzed opening of the epoxide ring is followed by aromatization resulting in the formation of phenol. Phenol is further converted into hydroquinone which is oxidized to benzoquinone. The conjugates formed from hydroquinone (hydroquinone glucuronide and hydroquinone sulfate) are markers for this toxification pathway leading to benzoquinone (Henderson et al. 1989). Phenol can also be metabolized to catechol

and trihydroxy benzene. Metabolism of benzene oxide by epoxide hydrolase leads to the formation of benzene dihydrodiol. Catechol can also be formed from benzene dihydrodiol via metabolism by cytosolic dehydrogenases (Henderson et al. 1989). The second pathway involving ring-opening leads to the formation of muconic acid via muconaldehyde. Mouse liver microsomes and cytosol have been shown to catalyze ring opening in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) *in vitro,* producing *trans,trans*-muconaldehyde, a six-carbon diene dialdehyde also referred to as muconic dialdehyde (Goon et al. 1993; Latriano et al. 1986), a known hematotoxin (Witz et al. 1985) and toxic metabolite of benzene (Henderson et al. 1989). Metabolism of benzene and *trans,trans*-muconaldehyde in the isolated perfused rat liver indicated that benzene was metabolized to muconic acid, a ring-opened metabolite of benzene (Grotz et al. 1994). *Trans,trans*-muconaldehyde was metabolized to muconic acid and three other metabolites. These studies indicate that ring-opening of benzene occurs in the liver. Other recent literature identifies the following metabolites after incubation of benzene with mouse liver microsomes: phenol, hydroquinone, *trans, trans*-muconaldehyde, *6*-oxo-*tram,trans*-2,4-hexadienoic acid, 6-hydroxy-*trans,trans*-2,4-hexadienal, and 6-hydroxy-*trans,trans*-2,4-hexadienoic acid (Zhang et al. 1995a). β-hydroxymuconaldehyde, a new metabolite, was also identified. Additional work by Zhang et al. (1995b) suggests that *cis,* cis-muconaldehyde is formed first, followed by *cis,trans*-muconaldhyde, and finally converted to *trans,trans*-muconaldehyde. Muconic dialdehyde has been shown to be metabolized *in vivo* in mice to muconic acid (Witz et al. 1990). These data suggest that muconic dialdehyde is the precursor of muconic acid in animals exposed to benzene. Small amounts of muconic acid were found in the urine of rabbits and mice that received oral doses of ^{14}C-benzene (Gad-El Karim et al. 1985; Parke and Williams 1953a). The percentage of this metabolite formed varied with the administered benzene dose and was quite high at low doses (17.6% of 0.5 mg/kg benzene administered to C57BL/6 mice) (Witz et al. 1990). Other studies in animals support these results (Brondeau et al. 1992; Ducos et al. 1990; McMahon and Birnbaum 1991; Schad et al. 1992). This pathway also appears to be active in humans (Bechtold and Henderson 1993; Ducos et al. 1990, 1992; Lee et al. 1993; Melikian et al. 1993, 1994). For instance, urine samples from male and female smokers and nonsmokers were obtained from subjects who applied for life insurance (Melikian et al. 1994). Samples from pregnant women were obtained during 7-35 weeks of pregnancy. Questionnaires were filled out on smoking history and occupation. The levels of muconic acid and cotinine (a biomarker for cigarette smoking) in the urine for the groups of pregnant and nonpregnant smokers and nonsmokers were compared with previously reported data in male smokers. Results showed the mean levels of muconic acid in the groups of male, female-nonpregnant, and female-pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher than the

mean concentration of this acid in the nonsmoking groups. The differences in the mean muconic acid concentrations between smoking and nonsmoking groups were significant in male, and nonpregnant and pregnant female smokers. Mean concentrations of muconic acid levels in nonpregnant female smokers are similar to that of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female smokers were 0.22± 0.03 and 0.24± 0.02 mg/g creatinine, or 0.13±0.06 and 0.13±1.07 mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant and 53 nonpregnant female smokers were 0.27±0.04 and 0.24±0.02 mg/g creatinine, or 0.24±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Because of its relative importance in benzene toxicity, additional modeling studies, including molecular orbital studies, have been conducted to further describe how *trans,trans*-muconaldehyde is transformed to muconic acid (Bock et al. 1994).

There are two detoxification pathways. One pathway leads to the formation of mercapturic acid via glutathione conjugates of benzene oxide, which are subsequently metabolized to prephenyl mercapturic acid and phenyl mercapturic acid and eliminated via biliary excretion (Henderson et al. 1989; Sabourin et al. 1988; Schafer et al. 1993; Schlosser et al. 1993; Schrenk et al. 1992; van Sittert et al. 1993). The major portion of benzene oxide is nonenzymatically rearranged into phenol (Parke and Williams 1953a). The second detoxification pathway involves the formation of water-soluble urinary metabolites, which are glucuronide or sulfate conjugates of phenol (Henderson et al. 1989; Wells and Nerland 1991). Further metabolites of phenol and benzene dihydrodiol (e.g., catechol, hydroquinone, and trihydroxy benzene) are excreted as sulfate or glucuronide conjugates, which are considered detoxification products of benzene metabolism (Henderson et al. 1989; Schrenk and Bock 1990). Electroanalytical methods have been used to further elucidate the metabolism of benzene and phenol by both microsomal and peroxidase enzymes (Lunte and Lunte 1990). The pathways for biotransformation of benzene are shown in Figure 2-3.

The ability of bone marrow to metabolize benzene independently of the liver has been tested *in situ* in male Fischer 344 rats. Benzene metabolism by bone marrow was complete and independent of metabolism by the liver, with concentrations of phenol greater than catechol and hydroquinone. Although the total metabolism by bone marrow was limited (total metabolites present were 25% of those in blood), the concentration of metabolites in the bone marrow exceeded that in the blood. The concentration of total metabolites in blood was 0.215 nmol/g as compared to 81.3 nmol/g for bone marrow (Irons et al. 1980). Similar studies have been conducted in mice (Ganousis et al. 1992).

Figure 2-3. Metabolic Pathways for Benzene

Adapted from Henderson et al. 1989

Fibroblasts had elevated levels of glutathione-S-transferase activity relative to macrophages, whereas macrophages had higher levels of UDP-glucuronyltransferase and peroxidase activity. These data suggest that cell-specific metabolism of benzene in the marrow may contribute to the toxicity of benzene in this tissue compartment. In addition, comparison of the detoxifying activities of rat and mouse bone marrow stromal cells indicates that rats have higher levels of glutathione and quinone reductase, which is known to play critical roles in modulating hydroquinone-induced toxicity; this suggests a metabolic basis for the observed increased susceptibility of mouse to benzene-induced hematotoxicity (Zhu et al. 1995).

Benzene has been found to stimulate its own metabolism, thereby increasing the rate of toxic metabolite formation. Pretreatment of mice, rats, and rabbits subcutaneously with benzene increased benzene metabolism *in vitro* without increasing cytochrome P-450 (CYP2El) concentrations (Arinc et al. 1991; Gonasun et al. 1973; Saito et al. 1973). In contrast, there was no significant effect on the metabolism of benzene when Fischer 344 rats and $B6C3F_1$ mice, pretreated with repeated inhalation exposure to 600 ppm of benzene, were again exposed to 600 ppm benzene (Sabourin et al. 1990). The rate of benzene metabolism can be altered by pretreatment with various compounds. Benzene is a preferential substrate of one particular cytochrome P-450 family, namely cytochrome $P-450_{ALC}$ (CYP2El), which also metabolizes alcohol and aniline. CYP2El can be induced by these substrates and is associated with the generation of hydroxyl radicals, probably via futile cycling of the cytochrome (Chepiga et al. 1990; Parke 1989; Snyder et al. 1993a). It is possible that hydroxy radical formation by cytochrome CYP2El may play a role in the benzene ring-opening pathway, leading to the formation of *trans,trans*-muconaldehyde. Phenol, hydroquinone, benzoquinone, and catechol have also been shown to induce P-450 in human hematopoietic stem cells (Henschler and Glatt 1995). Therefore, exposure to chemicals that stimulate the activity of this enzyme system prior to exposure to benzene could increase the rate of benzene metabolism.

Several studies examined the role of phenobarbital in benzene metabolism (Nakajima et al. 1985; Pawar and Mungikar 1975). Carworth Farms rats treated intraperitoneally with phenobarbital had higher activity levels of aminopyrine *N*-demethylase and acetanilide hydroxylase (determined in the liver extracts) than benzene-treated or control animals, indicating increased hepatic activity (Pawar and Mungikar 1975). However, when rats were orally pretreated with 1,400 mg/kg benzene and then treated intraperitoneally with phenobarbital, both the aminopyrine *N*-demethylase and acetanilide hydroxylase activity were similar to control levels. Other results indicate that phenobarbital

pretreatment of rats prior to treatment with benzene alters the drug metabolizing enzyme system producing more total and unconjugated phenol while decreasing benzene hematotoxicity (Gill et al. 1979). No increase in benzene metabolism was found in rats pretreated with phenobarbital and then exposed to a single inhalation dose of 500 ppm benzene (Nakajima et al. 1985). *In vitro* studies have also shown that phenobarbital may not increase metabolism of benzene and may have a protective effect against benzene-induced toxicity by enhancing the activity of conjugation reaction enzymes (Sato and Nakajima 1985).

Both NADPH-linked and ascorbate-induced lipid peroxidation activities induced *in vitro* were lowered 5.5% and 26%, respectively, in Carworth Farms rats following oral administration of 1,400 mg/kg/day of benzene for 3 days, followed by intraperitoneal injection of phenobarbital. It is concluded that benzene alters hepatic drug metabolism and lipid peroxidation. The decrease in lipid peroxidation could be due to the antioxidant property of the metabolites (Pawar and Mungikar 1975).

β-Diethyl amino ethyl diphenyl propyl acetate hydrogen chloride (SKF-525A) and toluene have inhibited benzene metabolism in rats (Gill et al. 1979; Ikeda et al. 1972). Carbon monoxide, aniline, aminopyrine, cytochrome C, and metapyrene inhibit benzene metabolism in mouse liver microsomes (Gonasun et al. 1973). Ethanol ingestion as well as dietary factors such as food deprivation and carbohydrate restriction, enhance the *in vitro* metabolism of benzene in rat liver microsomes (Nakajima et al. 1987).

In rats exposed to ethanol and benzene, it was found that benzene interfered with the disappearance of ethanol from the body (Nakajima et al. 1985). The results of further studies showed that ethanol treatment increased the production of hydroxylated benzene metabolites, phenol and hydroquinone, suggesting induction of benzene metabolism (Nakajima et al. 1987). Ethanol is known to be an inducer of cytochrome P-450 (CYP2El), the enzyme involved in the initial metabolism of benzene. The toxicity of alcohol, particularly necrosis of the gastrointestinal tract, is known to be associated with oxygen radical production. The possibility therefore exists of a synergism between-alcohol and benzene, and of the role of reactive oxygen and the formation of circulating lipid peroxides in the hematopoietic toxicity and carcinogenicity of benzene (Parke 1989).

Oral kinetic studies of pure, as opposed to soil-adsorbed, benzene were conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of radiolabeled benzene

alone, or radiolabeled benzene adsorbed to clay or sandy soil. Radioactivity was determined in plasma, stomach tissue, expired air, and urine. No difference in metabolism was detected with the treatments. However, the data suggested that loose binding of the benzene to the soils allowed more benzene to be available for metabolism, as opposed to being exhaled unchanged. This was especially true for the clay soil.

The dose of benzene affects both the total metabolism and the concentrations of individual metabolites formed. In mice, the percentage of hydroquinone glucuronide decreased as the dose increased. In both rats and mice, the percentage of muconic acid decreased as the dose increased. The shift in metabolism may affect the dose-response relationship for toxicity, and has been observed in all animal species studies thus far (Sabourin et al. 1989a, 1992; Witz et al. 1990).

Additional studies by Sabourin et al. (1987, 1988, 1989a, 1989b, 1992) showed that differences in species, routes of exposure, and dosing regimens will affect the disposition and metabolic fate of benzene. The metabolic fate of benzene follows two pathways that are currently thought to lead to toxic intermediates of benzene. Since they generate toxic products, they are also called toxification pathways. Each of the two pathways has its respective markers. One pathway of benzene metabolism involves the ring opening and is marked by the presence of *trans,trans*-muconaldehyde and muconic acid. Muconic acid has been postulated to be the marker of this pathway involving ring-opening. The second pathway involving ring hydroxylation includes the formation of ring-hydroxylated products such as phenol, hydroquinone, and hydroquinone conjugates. Consequently, hydroquinone and its conjugates have been postulated to be markers of this ring hydroxylation pathway. In addition, there are detoxification pathways involving mercapturic acid formation and phenyl conjugate formation. Therefore, markers of the detoxification pathways include phenyl conjugates such as prephenyl and phenyl mercapturic acid, phenyl sulfate, and phenyl glucuronide. The effect of species differences was evidenced by the fact that mice have a higher minute volume per kg body weight than rats (1.5 times higher). This caused the blood concentration of benzene to reach equilibrium more quickly in mice than in rats, but the steady-state level in blood was not influenced (Sabourin et al. 1987) Rats and mice were administered benzene by gavage at doses of 0.5-150 mg/kg/day (Sabourin et al. 1987). At doses below 15 mg/kg, >90% of the benzene was metabolized, while at doses above 15 mg/kg, an increasing percentage of orally administered benzene was exhaled unmetabolized. Total metabolites per unit body weight were equal in rats and mice at doses up to 50 mg/kg/day. However, total

metabolites in mice did not increase at higher doses, suggesting saturation of metabolic pathways (Sabourin et al. 1987).

The integrated dose to a tissue over a 14-hour period (6-hour exposure, 8 hours following exposure) was calculated for benzene metabolites in Fischer 344 rats and B6C3F$_1$ mice that were exposed to 50 ppm of radiolabeled (^{3}H) benzene (Sabourin et al. 1988). The major metabolic products in rats were detoxification products that were marked by phenyl conjugates. In contrast, mice had substantial quantities of the markers for toxification pathways (muconic acid, hydroquinone glucuronide, and hydroquinone sulfate) in their tissues. Muconic acid and hydroquinone glucuronide were also detected in mouse bone marrow. These results may explain why mice are more susceptible to benzene-induced toxicity than rats.

In a study by Orzechowski et al. (1995), hepatocytes from adult male Wistar rats and NMRI mice were incubated for 1 hour with 0.5 mM ^{14}C-benzene, and the supematant analyzed for metabolites. Formation of sulfate conjugates of benzene, hydroquinone, and 1,2,4-benzenetriol was also studied in a separate experiment. Mouse hepatocytes produced two metabolites (1,2,4-trihydroxybenzene sulfate and hydroquinone sulfate) that were not found in rat hepatocyte incubations. These sulfate metabolites were found in incubations including benzene, or the metabolites themselves, hydroquinone and 1,2,4-benzenetriol. Mouse hepatocytes were almost three times more effective in metabolizing benzene, compared to rat hepatocytes. This difference was accounted for in the formation of hydroquinone, hydroquinone sulfate, and 1,2,4-trihydroxybenzene sulfate. These *in vitro* experiments indicate there are both quantitative and qualitative differences in rodent metabolism of benzene.

Data produced *in vitro* by mouse and rat liver microsomes also indicate species differences in benzene metabolism (Schlosser et al. 1993). Quantitation of metabolites from the microsomal metabolism of benzene indicated that after 45 minutes, mouse liver microsomes from male B6C3F$_1$ mice had converted 20% of the benzene to phenol, 31% to hydroquinone, and 2% to catechol. In contrast, rat liver microsomes from male Fischer 344 rats converted 23% to phenol, 8% to hydroquinone, and 0.5% to catechol. Mouse liver microsomes continued to produce hydroquinone and catechol for 90 minutes, whereas rat liver microsomes had ceased production of these metabolites by 90 minutes. Muconic acid production by mouse liver microsomes was <0.04 and <0.2% from phenol and benzene, respectively, after 90 minutes.

There are quantitative differences in the benzene metabolites produced by different species (Sabourin et al. 1988). Fischer 344 rats exposed to 50 ppm benzene had undetectable amounts of phenol, catechol, and hydroquinone in the liver, lungs, and blood. The major water-soluble metabolites were muconic acid, phenyl sulfate, prephenyl mercapturic acid, and an unknown. The unknown was present in amounts equal to the amounts of phenyl sulfate in the liver; phenyl sulfate and the unknown were the major metabolites in the liver. $B6C3F_1$ mice exposed to 50 ppm benzene had detectable levels of phenol and hydroquinone in the liver, lungs, and blood; catechol was detectable only in the liver and not in the lungs or blood. As in the rat, the unknown was present in amounts equal to the amounts of phenyl sulfate in the liver. Mice had more muconic acid in the liver, which indicates a greater risk for them from *trans,trans*-muconaldehyde (Sabourin et al. 1988).

A physiologically based pharmacokinetics (PBPK) model based on the ventilation rate, cardiac output, tissue blood flow rates, and volumes as well as measured tissue/air and blood/air partition coefficients has been developed (Medinsky et al. 1989a; Travis et al. 1990). Experimentally determined data and model simulations indicated that during and after 6 hours of inhalation exposure to benzene, mice metabolized benzene more efficiently than rats (Medinsky et al. 1989a). After oral exposure, mice and rats appeared to metabolize benzene similarly up to oral doses of 50 mg/kg, above which rats metabolized more benzene than did mice on a per kg body weight basis (Medinsky et al. 1989b). This model may be able to predict the human response based on animal data. Benzene metabolism followed Michaelis-Menton kinetics *in vivo* primarily in the liver, and to a lesser extent in the bone marrow. Additional information on PBPK modeling is presented in Section 2.3.5.

The effect of dosing regimen on benzene metabolism was studied in Fischer 344 rats and $B6C3F_1$ mice that had either long inhalation exposures to low concentrations or short exposures to high concentrations of benzene (Sabourin et al. 1989a, 1989b). Inhalation occurred at 1 of 3 exposure regimens, all having the same integral amount of benzene: 600 ppm benzene for 0.5 hour, 150 ppm for 2 hours, or 50 ppm for 6 hours. Results indicated no dose-rate effect in rats. In mice, however, the fast exposure rate (0.5 hour times 600 ppm) produced less muconic acid in the blood, liver, and lungs. In the blood and lungs, less hydroquinone glucuronide and more prephenyl mercapturic acid were produced at the higher exposure rates. At the highest benzene exposure concentrations or fastest benzene exposure rate in mice, there was a reduction in the ratios of muconic acid and hydroquinone glucuronide to the metabolite phenylsulfate. Furthermore, with increased dose rate or increased exposure concentration, mice tended to shift a greater portion of their benzene metabolism toward

detoxification pathways. Likewise, the detoxification pathways for benzene appear to be low-affinity, high-capacity pathways, whereas pathways leading to the putative toxic metabolites appear to be high-affinity, low-capacity systems (Henderson et al. 1989). Accordingly, if the exposure dose regimen, via inhalation, extends beyond the range of linear metabolism rates of benzene ($\approx$200 ppm by inhalation) (Sabourin et al. 1989b), then the fraction of toxic metabolites formed relative to the amount administered will be reduced.

Kenyon et al. (1995) compared their urinary profile of metabolites in B6C3F$_1$ mice after oral dosing with phenol with the results of Sabourin et al. (1989a) who administered a comparable oral dose of benzene to B6C3F$_1$ mice. Their analysis indicated that phenol administration resulted in lower urinary levels of hydroquinone glucuronide, and higher levels of phenol sulfate and phenol glucuronide compared to benzene administration. They hypothesized that the differences in the urinary metabolite profiles between phenol and benzene after oral dosing were due to zonal differences in the distribution of metabolizing enzymes within the liver. Conjugating enzymes are more concentrated in the periportal area of the liver, the first region to absorb the compound, whereas oxidizing enzymes are more concentrated in the pericentral region of the liver. Based on this hypothesis, during an initial pass through the liver after oral administration, phenol would have a greater opportunity to be conjugated as it was absorbed from the gastrointestinal tract into the periportal region of the liver, thus resulting in less free phenol being delivered into the pericentral region of the liver to be oxidized. With less free phenol available for oxidation, less hydroquinone would be produced, relative to conjugated phenol metabolites. In contrast, benzene must be oxidized before it can be conjugated. Therefore, metabolism of benzene would be minimal in the periportal region of the liver, with most of the benzene reaching the pericentral region to be oxidized to hydroquinone. Based on this scheme, the authors suggest that benzene administration would result in more free phenol being delivered to oxidizing enzymes in the pericentral region of the liver than administration of phenol itself (Kenyon et al. 1995).

A number of investigators have suggested that covalent binding of benzene metabolites to cellular macromolecules is related to benzene's mechanism of toxicity, although the relationship between adduct formation and toxicity is not clear. Benzene metabolites have been found to form covalent adducts with proteins from blood in humans (Bechtold et al. 1992b). Benzene metabolites have been found to form covalent adducts with nucleic acids and proteins in rats (Norpoth et al. 1988); to covalently bind to proteins in mouse or rat liver, bone marrow, kidney, spleen, blood, and muscle

in vivo (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Longacre et al. 1981a, 1981b; Sun et al. 1990); to bind to proteins in perfused bone marrow preparations (Irons et al. 1980) and in rat liver DNA *in vivo* (Lutz and Schlatter 1977); and to bind to DNA in rabbit and rat bone marrow mitochondria *in vitro* (Rushmore et al. 1984). The inhibition of ribonucleic acid (RNA) synthesis in liver and bone marrow mitochondria has been correlated with covalent binding of benzene metabolites to DNA *in vitro* (Kalf et al. 1982). *In vitro* studies showed that benzene metabolites inhibited RNA synthesis and formed adducts similar to benzene; *p*-benzoquinone was the most effective inhibitor of RNA synthesis. When ^{14}C-hydroquinone was added to ^{3}H-deoxyguanosine, two adducts were formed. Two adducts were also formed when ^{14}C-hydroquinone was reacted with DNA from calf thymus and *M. lysodeikticus* (Snyder et al. 1987). Fischer 344 rats and B6C3F$_1$ mice were administered a single dose of 50, 100, 200, and 400 mg/kg ^{13}C/^{14}C-radiolabeled benzene in corn oil via gastric intubation (McDonald et al. 1994). Animals were sacrificed 24 hours after administration of labeled benzene. Radiolabeled (^{14}C/^{13}C) adducts in hemoglobin and bone marrow were monitored. Cysteine adducts of benzene oxide and 1,2- and 1,4-benzoquinone in the rats were assayed, and the proportions of cysteine-bound adducts to total protein binding were estimated. Increasing production of hemoglobin adducts was observed over all dose levels tested. Benzene oxide adducts with rat hemoglobin represented 27% of the total Hgb binding and 73% of the cysteinyl binding, whereas quinone adducts represented relatively small proportions. In the bone marrow, benzoquinone adducts were more abundant than those of benzene oxide. 1,2-benzoquinone adducts predominated in rat marrow with 9% of binding. The relationship between marrow adducts and dosage were linear. Protein adduct levels in the bone marrow were about 20% of the levels in rat hemoglobin. Comparisons of adduct production in the bone marrow indicated that 1,2-benzoquinone adducts were approximately 2.3-fold greater than those of 1,4-benzoquinone in the rat. In the mouse, increasing production of hemoglobin .adducts was observed over all dose levels tested. With mouse hemoglobin, the 1,4-benzoquinone adducts accounted for 5.5% of the total hemoglobin binding and 12.2% of the cysteinyl binding, while 1,2-benzoquinone and benzene oxide each accounted for less than 3% of the total. In the bone marrow, benzoquinone adducts were more abundant than those of benzene oxide. 1,4-Benzoquinone adducts were more abundant in the mouse with 21% of binding. Protein adduct levels in the bone marrow were about 60% of the levels in mouse hemoglobin. Comparisons of adduct production in the bone marrow indicated that 1,4-benzoquinone adducts were approximately 17-fold greater than those of 1,2-benzoquinone in the mouse.

The use of $^{13}C/^{14}C$-radiolabeled benzene enabled McDonald et al. (1994) to show that some phenol and hydroquinone adducts (those containing non-radiolabeled ^{12}C) were present prior to experimental exposure of the animals to benzene, most likely from non-benzene sources, i.e., background levels. Background levels of adducts (non-radiolabeled) of 1,2-benzoquinone and 1,4-benzoquinone with hemoglobin and bone marrow proteins of both rats and mice were much greater than those of the radiolabeled benzene-specific adducts. In rat hemoglobin, background levels of 1,2-benzoquinone and 1,4-benzoquinone were 68-fold and 473-fold higher, respectively, than the adduct formed from the administered radiolabeled benzene of 50 mg/kg, and 1l- and 25-fold higher at the 400 mg/kg dose. Similarly, mouse hemoglobin showed background levels of adducts that were 6-18-fold higher than the radiolabeled adducts at the low dose, and 2.7-5.2-fold higher at the high dose. When levels of benzoquinone adducts were expressed as ratios of the background levels, the levels of adducts from benzene exposure approached background levels at the two highest doses (200 and 400 mg/kg), but not at ≤100 mg/kg/day. The authors suggest that the background levels of quinone adducts accumulated over the life-span of the protein, and that a chronic steady-state exposure to benzene itself would result in levels of quinone adducts that would equal or exceed background levels. The existence of background levels of quinone adducts are important in assessing risk of exposure to benzene, especially at low doses.

In another study, Low et al. (1995) compared the benzene metabolizing activity of solid-tumor target and nontarget tissue homogenates from rats. Zymbal gland, nasal cavity, bone marrow, oral cavity, and mammary gland were the target organs; the liver, blood, brain and kidney were the nontarget organs. Target organs exhibited a greater ability to deconjugate but not to conjugate polar metabolites, to oxidize benzene to reactive intermediates, and to possess greater peroxidase activity than the nontarget organs. These results suggest that target organs may show increased susceptibility to the toxic effects of benzene through their ability to preferentially produce reactive intermediates.

Multiple mechanisms are involved in benzene toxicity and carcinogenicity. Such mechanisms may include synergism between metabolites (Eastmond et al. 1987; Snyder et al. 1989) or synergism between glutathione-depleting metabolites of benzene and oxygen radicals (generated by futile cycling of cytochrome P-450 or cycling of quinone metabolites) (Parke 1989).

The benzene metabolites hydroquinone and muconic dialdehyde can produce hematotoxic effects (Eastmond et al. 1987; Gad-El Karim et al. 1985; Latriano et al. 1986). The co-administration of

phenol (75 mg/kg/day) and hydroquinone (25-75 mg/kg/day) twice daily for 12 days to $B6C3F_1$ mice produced myelotoxicity similar to that induced by benzene (Eastmond et al. 1987). The proposed mechanism suggested that selective accumulation of hydroquinone occurred in the bone marrow after the initial hepatic conversion of benzene to phenol and hydroquinone. Additionally, phenol is thought to stimulate the enzymatic activity of myeloperoxidase, which uses phenol as an electron donor, thus producing phenoxy radicals. These radicals further react with hydroquinone to form 1,4-benzoquinone, a toxic intermediate that inhibits critical cellular processes (Eastmond et al. 1987).

Legathe et al. (1994) investigated the pharmacokinetics of hydroquinone and phenol in blood and recovery in urine after intraperitoneal administration of 75 mg/kg alone or in combination to $B6C3F_1$ mice. Combined administration resulted in a 2.6-fold increase in the area under the curve (AUC) for blood concentration of hydroquinone, and increased the half-life of hydroquinone from 9 to 15 minutes. The AUC of phenol was increased by a factor of 1.4, and clearance of phenol was decreased from 89 mL/min/kg when injected alone, to 62 mL/min/kg after co-administration. Recovery of conjugated metabolites in the urine indicated that both conjugation pathways were diminished. The authors suggest that enhanced myelotoxicity observed after co-administration is the result of reduced elimination of hydroquinone and phenol, leaving more phenol available for conversion to hydroquinone.

In studies on the erythroid cell line, Longacre et al. (1981b) demonstrated 60-80% decreases in the incorporation of iron (as ^{59}Fe) into bone marrow precursors of mice injected subcutaneously with 12-20 doses of 440 or 880 mg/kg of benzene. When given 48 hours prior to measuring ^{59}Fe uptake, benzene produced a dose-dependent decrease in erythroid cells (Snyder et al. 1989). Following intraperitoneal administration of benzene, hydroquinone, p-benzoquinone, and *trans,trans*smuconaldehyde, ^{59}Fe uptake was reduced. The combination of metabolites most effective in reducing ^{59}Fe uptake was hydroquinone plus *trans,trans*-muconaldehyde (Snyder et al. 1989).

2.3.4   Elimination and Excretion

2.3.4.1 Inhalation Exposure

Evidence indicates that in humans following inhalation exposure to benzene, the major route for elimination of unmetabolized benzene is via exhalation. Absorbed benzene is also excreted in humans

via metabolism to phenol and muconic acid followed by urinary excretion of conjugated derivatives (sulfates and glucuronides). Respiratory uptake (the amount of benzene absorbed from the lungs following inhalation of the vapors) in 6 male and female volunteers exposed to 52-62 ppm benzene for 4 hours was determined to be approximately 47% (Nomiyama and Nomiyama 1974a). Respiratory excretion (the amount of absorbed benzene excreted via the lungs) in a total of six male and female volunteers exposed to 52-62 ppm benzene for 4 hours was determined to be approximately 17%. No differences in respiratory excretion were observed between men and women (Nomiyama and Nomiyama 1974a, 1974b). Results from a study of 23 subjects that inhaled 47-110 ppm benzene for 2-3 hours showed that 16.4-41.6% of the retained benzene was excreted by the lungs within 5-7 hours (Srbova et al. 1950). The rate of excretion of benzene was the greatest during the first hour. The study also showed that only 0.07-0.2% of the retained benzene was excreted in the urine. Other studies suggest that benzene in the urine may be a useful biomarker of occupational exposure (Ghittori et al. 1993). Results of a study involving a single human experimental subject exposed to concentrations of benzene of 6.4 and 99 ppm for 8 hours and 1 hour, respectively, suggested that excretion of benzene in breath has three phases and could possibly have four phases. The initial phase is rapid and is followed by two (or three) slower phases (Sherwood 1988). The initial phase with a high exposure concentration (99 ppm) and a short-term exposure duration (1 hour) had a more rapid excretion rate (half-life = 42 minutes) and a greater percentage of the total dose excreted (17%) than did the initial phase with a low exposure concentration (6.4 ppm) and longer exposure duration (8 hours) (half-life = 1.2 hours, percentage of total dose excreted=9.3%). Subsequent phases showed an increase in the half-lives. These results also showed that urinary excretion of phenol conjugate was biphasic, with an initial rapid excretion phase, followed by a slower excretion phase. A greater proportion of the total dose was excreted in urine than in breath (Sherwood 1988). The urinary excretion of phenol in workers was measured following a 7-hour workshift exposure to l-200 ppm benzene. A correlation of 0.881 between exposure level and urinary phenol excretion was found (Inoue et al. 1986). Urine samples were collected from randomly chosen subjects not exposed to known sources of benzene, from subjects exposed to sidestream cigarette smoke, or from supermarket workers presumed exposed to benzene from polyvinyl chloride (PVC) meat packing wrap (Bartczak et al. 1994). Samples were analyzed for identification of muconic acid. Muconic acid concentrations of 8-550 ng/mL were found in all urines. Kok and Ong (1994) report blood and urine levels of benzene in nonsmokers as 110.9 and 116.4 ng/L, respectively, and in smokers, 328.8 and 405.4 ng/L, respectively. A significant correlation was found between benzene levels in blood and benzene levels in urine. Similar results were found for filling station attendants in Italy (Lagorio et al. 1994b).

Popp et al. (1994) reported a mean blood benzene level in car mechanics of 3.3 μg/L. Urinary muconic acid and S-phenyl-N-acetyl cysteine (PhAC) levels increased during the work shift, and were well correlated with the blood levels and the benzene air levels, which reached a maximum of 13 mg/m^3.

As discussed in Section 2.3.3, the mean urinary levels of muconic in groups of male, female-nonpregnant, and female-pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher than the mean concentration of this acid in the nonsmoking groups (Melikian et al. 1994). The differences in the mean muconic acid concentrations between smoking and nonsmoking groups were significant in male (p=0.00l), and nonpregnant (p=0.00l) and pregnant female smokers (p=0.002). Mean concentrations of muconic acid levels in nonpregnant female smokers are similar to that of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female smokers were 0.22± 0.03 and 0.24± 0.02 mg/g creatinine, or 0.13±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant and 53 nonpregnant female smokers were 0.27±0.04 and 0.24±l.02 mg/g creatinine, or 0.24±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of urinary cotinine in pregnant smokers were significantly lower than in the group of nonpregnant female smokers (1.13±0.12 mg/g creatinine compared to 1.82±0.14 mg/g creatinine). Data on excretion of benzene or its metabolites in human breast milk after inhalation exposure were not found.

Animal data show that exhalation is the main route for excretion of unmetabolized benzene and that metabolized benzene is excreted primarily in urine. Only a small amount of absorbed dose is eliminated in feces. A biphasic pattern of excretion of unmetabolized benzene in expired air was observed in rats exposed to 500 ppm for 6 hours, with half-times for expiration of 0.7 hour for the rapid phase and 13.1 hours for the slow phase (Rickert et al. 1979). The half-life for the slow phase of benzene elimination suggests the accumulation of benzene. The major route of excretion following a 6-hour nose-only inhalation exposure of rats and mice to various concentrations of ^{14}C-benzene appeared to be dependent on the inhaled concentration (Sabourin et al. 1987). At similar exposures to vapor concentrations of l0-1,000 ppm, the mice received 150-200% of the equivalent dose in rats on a per kg body weight basis. At all concentrations, fecal excretion accounted for <3.5% of the radioactivity for rats and <9% for mice. At lower exposure concentrations, (i.e., 13-130 ppm in rats and 1l-130 ppm in mice), less than 6% of the radioactivity was excreted in expired air. At the highest exposure concentrations (rats, 870 ppm; mice, 990 ppm), both rats and mice exhaled a

significant amount of unmetabolized benzene (48 and 14%, respectively) following termination of the exposure. The majority of the benzene-associated radioactivity that was not exhaled was found in the urine and in the carcass 56 hours after the end of exposure to these high concentrations. The radioactivity in the carcass was associated with the pelt of the animals. The authors assumed that this was due to contamination of the pelt with urine, since the inhalation exposure had been nose-only. Further investigation confirmed that the radioactivity was associated with the fur of the animals. Accordingly, the percentage of the total radioactivity excreted by these animals (urine and urine-contaminated pelt) that was not exhaled or associated with feces was 47-92% for rats and 80-94% for mice. At exposures of ≤260 ppm in rats, 85-92% of the radioactivity was excreted as urinary metabolites, while at exposures of ≤130 ppm in mice, 88-94% of the radioactivity was excreted as urinary metabolites. The total urinary metabolite formation was 5-37% higher in mice than in rats at all doses. This may be explained by the greater amount of benzene inhaled by mice per kg of body weight (Sabourin et al. 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm benzene vapors 6 hours per day, 5 days per week for 3 weeks (Dow 1992). The average concentration of phenol in the urine increased linearly with dose.

2.3.4.2 Oral Exposure

No studies were located regarding excretion in humans after oral exposure to benzene. Data on excretion of benzene or its metabolites in human breast milk after oral exposure were not found.

Radiolabeled benzene (≈340 mg/kg) was administered by oral intubation to rabbits; 43% of the label was recovered as exhaled unmetabolized benzene and 1.5% was recovered as carbon dioxide (Parke and Williams 1953). Urinary excretion accounted for about 33% of the dose. The isolated urinary metabolites were mainly in the form of conjugated phenols. Phenol was the major metabolite accounting for about 23% of the dose or about 70% of the benzene metabolized and excreted in the urine. The other phenols excreted (percentage of dose) were hydroquinone (4.8%), catechol (2.2%), and trihydroxybenzene (0.3%). L-phenyl-N-acetyl cysteine accounted for 0.5% of the dose. Muconic acid accounted for 1.3%; the rest of the radioactivity (5-10%) remained in the tissues or was excreted in the feces (Parke and Williams 1953).

Mice received a single oral dose of either 10 or 200 mg/kg radiolabeled benzene (McMahon and Birnbaum 1991). Radioactivity was monitored in urine, feces, and breath. At the low dose, urinary

excretion was the major route of elimination. Hydroquinone glucuronide, phenylsulfate, and muconic acid were the major metabolites at this dose, accounting for 40%, 28%, and 15% of the dose, respectively. At 200 mg/kg, urinary excretion decreased to account for 42-47% of the administered dose, while respiratory excretion of volatile components increased to 46-56% of the administered dose. Fecal elimination was minor and relatively constant over both doses, accounting for 0.5-3% of the dose.

The effect of dose on the excretion of radioactivity, including benzene and metabolites, following oral administration of ^{14}C-benzene (0.5-300 mg/kg) has been studied in rats and mice (Sabourin et al. 1987). At doses of <15 mg/kg for 1 day, ≥90% of the administered dose was excreted in the urine of both species. There was a linear relationship for the excretion of urinary metabolites up to 15 mg/kg; above that level, there was an increased amount of ^{14}C eliminated in the expired air. Mice and rats excreted equal amounts up to 50 mg/kg; above this level, metabolism apparently became saturated in mice. In rats, 50% of the 150 mg/kg dose of ^{14}C was eliminated in the expired air; in mice, 69% of the 150 mg/kg dose of ^{14}C was eliminated in expired air (Sabourin et al. 1987). The label recovered during exhalation was largely in the form of unmetabolized benzene, suggesting that saturation of the metabolic pathways had occurred. Dose also affected the metabolite profile in the urine. At low doses, a greater fraction of the benzene was converted to putative toxic metabolites than at high doses, as reflected in urinary metabolites.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al. 1988). Animals were gavaged with an aqueous suspension of benzene alone, or adsorbed to clay or sandy soil. Plasma concentration, half-life, tissue distribution, respiratory excretion, and urinary excretion were monitored. Excretion patterns were altered by the presence of the soils. Expired air was the primary excretion route for benzene alone, with lesser amounts eliminated in the urine during 48 hours after exposure. Urine and expired air were equal routes of exposure with sandy soil, while clay soil produced the results opposite to those seen with pure benzene. The increase in urinary excretion in the presence of soil suggests that the soil allows greater absorption, and thus greater opportunity for metabolism of benzene, compared to benzene alone. For clay soil, particularly, the increased potential for metabolism is supported by the significantly lower amount of unmetabolized benzene in expired air. Less than 2% of the dose was eliminated in the feces for all treatments, although sandy soil treatment resulted in the highest amount of benzene associated with fecal excretion. These result further suggest that clay soil binds benzene.

2.3.4.3   Dermal Exposure

Limited data on excretion of benzene after dermal exposure in humans were found. Four human male subjects were given a dermal application of 0.0024 mg/cm^2 ^{14}C benzene (Franz 1984). A mean of 0.023% (range, 0.006-0.054%) of the applied radiolabel was recovered in the urine over a 36-hour period. Urinary excretion of the radiolabel was greatest in the first two hours following skin application. More than 80% of the total excretion occurred in the first 8 hours. In another study, 35-43 cm^2 of the forearm were exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25-2 hours (Hanke et al. 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately 0.4 mg/cm^2/hour. The absorption due to vapors in the same experiment was negligible. Although there was a large variability in the physiological values, the amount of excreted phenol was 8.0-14.7 mg during the 24-hour period after exposure. It is estimated that approximately 30% of dermally absorbed benzene is eliminated in the form of phenol in the urine.

Data on excretion of benzene or its metabolites in human breast milk after dermal exposure were not found.

Monkeys and minipigs were exposed dermally to 0.0026-0.0036 mg/cm^2 of ^{14}C-benzene (Franz 1984). After application, the urine samples were collected over the next 2-4 days at 5-hour intervals. The rate of excretion was highest in the first two collection periods. The total urinary excretion of radioactivity was found to be higher in monkeys than in minipigs with the same exposure. Mean excretion in monkeys was 0.065% (range, 0.033-0.135%) of the applied dose compared to 0.042% (range, 0.030-0.054%) in minipigs.

Results of a study in which male rats were dermally treated with 0.004 mg/cm^2 of ^{14}C-benzene, with or without one gram of clay or sandy soil, showed that for all treatment groups the major routes of excretion were the urine and, to a lesser extent, the expired air (Skowronski et al. 1988). The highest amount of radioactivity in urine appeared in the first 12-24 hours after treatment (58.8%, 31.3%, and 25.1% of the absorbed dose, respectively, for pure benzene, sandy soil-adsorbed benzene, and clay soil-adsorbed benzene). In the group treated with pure benzene, 86.2% of the absorbed dose was excreted in the urine. Sandy soil and clay soil significantly decreased urinary excretion to 64.0% and

45.4%, respectively, of the absorbed dose during the same time period. Rats receiving pure benzene excreted 12.8% of the absorbed dose in expired air within 48 hours. Only 5.9% of the radioactivity was collected in expired air 48 hours after treatment with sandy soil-adsorbed benzene, while experiments with clay soil-adsorbed benzene revealed that 10.1% of the radioactivity was located in expired air. Less than 1% of the absorbed dose was expired as $^{14}CO_2$ in all groups. The ^{14}C activity in the feces was small (<0.5% of the applied radioactivity) in all groups 48 hours after treatment. Phenol was the major urinary metabolite detected in the 0-12-hour urine samples of all treatment groups. The percentage of total urinary radioactivity associated with phenol was 37.7% for benzene alone, 44.2% for benzene adsorbed to sandy soil, and 45.5% for benzene adsorbed to clay soil. Smaller quantities of hydroquinone, catechol, and benzenetriol were also detected (Skowronski et al. 1988).

2.3.4.4   Other Routes of Exposure

The metabolic fate of benzene can be altered in fasted animals. In nonfasted rats that received an intraperitoneal injection of 88 mg of benzene, the major metabolites present in urine were total conjugated phenols (14-19% of dose), glucuronides (3-4% of dose), and free phenol (2-3% of dose). However, in rats fasted for 24 hours preceding the same exposure, glucuronide conjugation increased markedly (18-21% of dose) (Cornish and Ryan 1965). Free phenol excretion (8-10% of dose) was also increased in fasted, benzene-treated rats. There was no apparent increase in total conjugated phenol excretion in fasted rats given benzene.

When ^{14}C-benzene (0.5 and 150 mg/kg) was injected intraperitoneally into rats and mice, most of the ^{14}C-benzene and ^{14}C-metabolites were excreted in the urine and in the expired air. A smaller amount of ^{14}C-benzene was found in the feces due to biliary excretion (Sabourin et al. 1987). Monkeys were dosed intraperitoneally with 5-500 mg/kg radiolabeled benzene, and urinary metabolites were examined (Sabourin et al. 1992). The proportion of radioactivity excreted in the urine decreased with increasing dose, whereas as the dose increased, more benzene was exhaled unchanged. This indicated saturation of benzene metabolism at higher doses. Phenyl sulfate was the major urinary metabolite. Hydroquinone conjugates and muconic acid in the urine decreased as the dose increased. When C57BL/6 mice and DBA/2 mice were given benzene subcutaneously in single doses (440, 880, or 2,200 mg/kg) for 1 day, or multiple doses (880 mg/kg) 2 times daily for 3 days, no strain differences were observed in the total amount of urinary ring-hydroxylated metabolites (Longacre et al. 1981a).

Although each strain excreted phenol, catechol, and hydroquinone, differences in the relative amounts of these metabolites were noted. The more sensitive DBA/2 mice excreted more phenol but less hydroquinone than the more resistant C57BL/6 mice, while both strains excreted similar amounts of catechol. DBA/2 mice excreted more phenyl glucuronide but less sulfate conjugate. Both strains excreted similar amounts of phenyl mercapturic acid (Longacre et al. 1981a).

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

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

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

The PBPK model for a chemical substance is developed in four interconnected steps: model representation, model parametrization, model simulation, and model validation (Krishnan and Andersen 1994). In the early 1990s validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The

numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

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

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where concentrations were higher or were administered in different species. Figure 2-4 shows a conceptualized representation of a PBPK model.

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

2.3.5.1   Summary of PBPK Models.

Four benzene PBPK models that describe the disposition of benzene in animals have been identified from the open literature since the late 1980s. These models are identified as the Medinsky model (Medinsky et al. 1989a, 1989b, 1989c), the Travis model (Travis et al. 1990), the Bois and Paxman model (Bois and Paxman 1992), and the Sun model (Sun et al. 1990). Based on the information presented in these four models, there appears to be sufficient evidence to suggest that PBPK models for benzene are fairly refined models which have a strong potential for use in human risk assessments. The PBPK model developed by Medinsky et al. (1989a, 1989b, 1989c) has provided a basic model for

Figure 2-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance
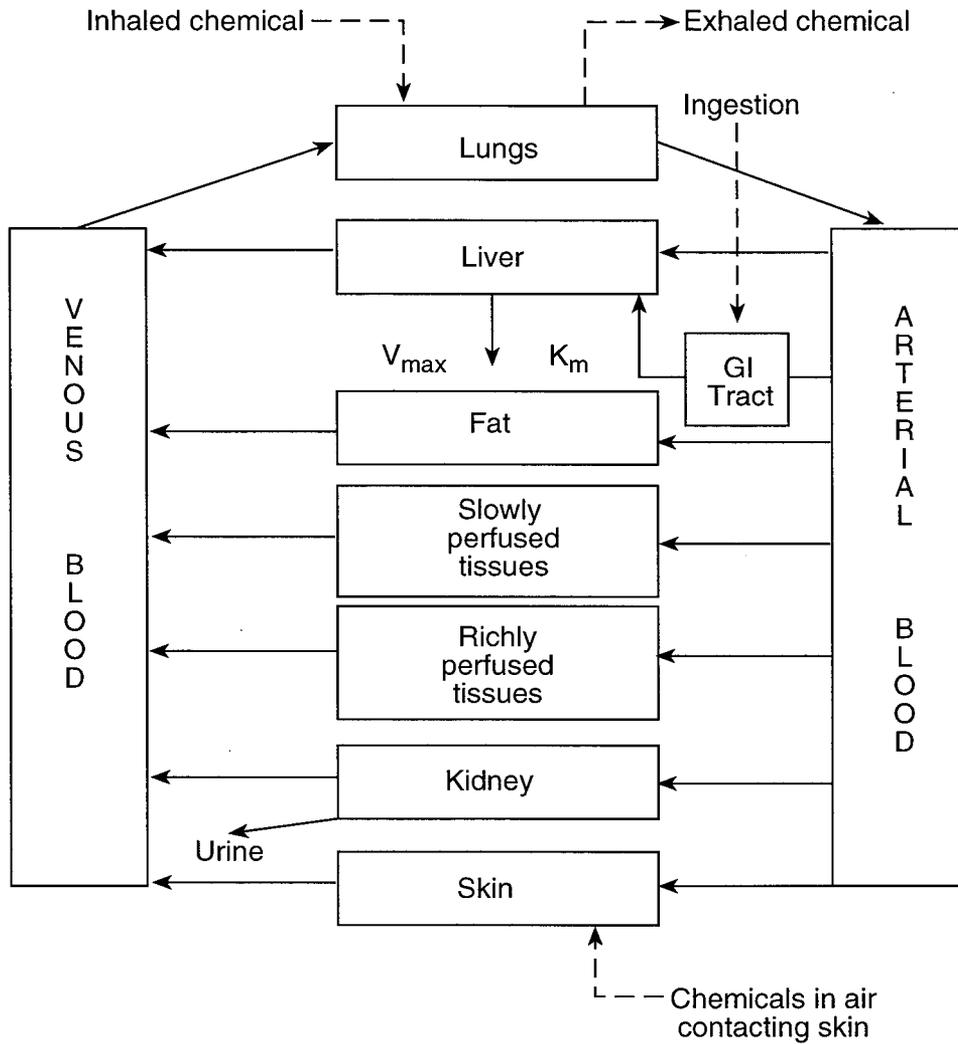
Source: adapted from Krishnan et al. 1992

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

the fate of benzene in laboratory animals. Using this model as a template, other more sophisticated and refined models have been developed that can be used in human risk assessment work.

2.3.5.2 Benzene PBPK Model Comparison.

The four PBPK models that are highlighted in this section of the profile have each contributed to the overall understanding of the pharmacokinetics of benzene. For instance, the Medinsky model addresses species differences in benzene kinetics using mice and rats. The Travis model specifically addresses human pharmacokinetics of benzene in comparison to experimental animal data, whereas the Bois and Paxman model addresses the effect of exposure rate on benzene metabolism. Finally, the Sun model addresses the formation of hemoglobin-benzene derived adducts in the blood, as a tool in monitoring benzene exposure. Each of these models in discussed in detail below.

2.3.5.3 Discussion of Models.

The Medinsky Model.

The Medinsky model (Medinsky et al. 1989a, 1989b, 1989c) is one of the original benzene PBPK models developed to describe and ultimately predict the fate of benzene in mice and rats and to determine if the observed differences in toxic effects could be explained by differences in pathways for metabolism of benzene or by differences in uptake of benzene.

Risk Assessment. This model successfully described the differences in disposition of benzene in mice and rats. Specifically, the model simulations indicated that for inhalation concentrations up to 1,000 ppm, mice metabolized at least 2-3 times as much benzene as did rats. Simulations of oral exposure indicated that rats metabolized more benzene on a kg body weight basis than did mice at doses greater than 50 mg/kg. Similarly, patterns of metabolites were different for the two species, with mice producing primarily hydroquinone glucuronide and muconic acid, metabolites-linked to toxic effects, and rats producing primarily phenyl sulfate, a detoxification product. These simulated results agree with experimental data, and provide a framework for understanding the greater sensitivity of the mouse to benzene toxicity.

Description of the Model. The Medinsky benzene PBPK model was based on an earlier PBPK model developed by Ramsey and Andersen (1984) to describe the disposition of styrene exposure in mice and rats. Disposition of benzene in humans was not originally represented in the Medinsky model (Medinsky et al. 1989a, 1989b), but human rate constants were later derived from the model (Medinsky et al. 1989c). Others have developed models that address human disposition of benzene (Travis et al. 1990). A schematic representation of the Medinsky model is shown in Figure 2-5, with oral and inhalation routes represented. The dermal route of exposure is not represented in this model, or in any other models found in the literature. Other models address the intraperitoneal route of exposure (Travis et al. 1990).

The tissue compartments included in the Medinsky model are: (a) the liver, presumed for the sake of the model to be the only organ where metabolism of benzene takes place; (b) a group of poorly perfused tissues including muscle and skin; (c) a group of richly perfused tissues including bone marrow, kidney, and intestines; and (d) a fat compartment. The poorly perfused tissues were important to the development of the model because of the large body mass they represent, the rapidly perfused tissues because of the large blood flow to these tissues, and the fat compartment because of its large partition coefficient. Large tissue mass, high blood flow, and increased solubility were assumed to result in significant amounts of benzene being distributed to that compartment, although the kinetics of distribution may be different for each compartment.

Physiologic, biochemical constants and partition coefficients used in the model are shown in Table 2-4. Physiologic constants (organ volume, blood flows, etc.) and tissue and blood coefficients were taken from Ramsey and Andersen (1984) or were taken from other literature sources. All metabolism of benzene, occurring only in the liver, and consisting of initial metabolism to benzene oxide, which is then further metabolized by one of four pathways, was modeled by Michaelis-Menten kinetic parameters. Metabolic rate constants were determined by fitting the results of model simulations to experimental data obtained by exposing mice and rats to benzene orally and by inhalation (Medinsky et al. 1989b; Sabourin et al. 1987). Human metabolic rate constants were derived by using the metabolic parameters obtained for mice, the more sensitive rodent species, and simulating 8-hour inhalation exposure over a range of concentrations. Model simulation results were compared to experimentally derived values for total metabolism of benzene and profiles of the individual major metabolites, including metabolism through benzene oxide to hydroquinone conjugates,

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Table 2-4. Parameters Used in the Medinsky PBPK Model for Benzene

Parameters	Mouse	Rat	Human
	Weights (kg):		
Body	0.030	0.288	70.0
	Percentage of body weight		
Liver	3.0	2.53	
Fat	11.0	6.30	
Rapidly perfused tissues	7.6	4.39	
Slowly perfused tissues	66.0	77.07	
Other[a]	12.4	12.4	
Gastrointestinal transfer (fraction/hr)	3.0	0.25	
	Flows (L/hr/kg)		
Alveolar ventilation	55.3	32.4	
Cardiac output	34.8	19.4	
	Percentage of cardiac output		
Liver	25.0	25.0	
Kidney	9.0	9.0	
Fat	2.0	5.0	
Rapidly perfused tissues	51.0	51.0	
Slowly perfused tissues	15.0	15.0	
	Partition coefficients		
Blood/air	18.0	18.0	
Liver/blood	1.0	1.0	
Fat/blood	28.0	28.0	
Rapidly perfused/blood	1.0	1.0	
Slowly perfused/blood	0.6	0.6	
	Metabolic and macromolecular binding constants		
V_{max} C (mg/hr/kg)	10.55	5.79	1.02
K_m (mg/L)	0.09	0.62	0.09
$V_{max, phc}$ C (mg/hr/kg)	12.12	4.77	0.17
$K_{m, phc}$ (mg/L)	0.08	0.05	0.08
$V_{max, pmc}$ C (mg/hr/kg)	7.04	1.25	0.67
$K_{m, pmc}$ (mg/L)	0.31	0.07	~ 0.31
$V_{max, hqc}$ C (mg/hr/kg)	1.48	0.10	0.15
$K_{m, hqc}$ (mg/L)	0.01	0.23	0.01
$V_{max, muc}$ C (mg/hr/kg)	0.23	0.19	0.07
$K_{m, muc}$ (mg/L)	0.01	0.01	0.01

[a]Includes tissues that are not perfused with blood such as bone (not including marrow); body fluids such as gastrointestinal tract contents, urine, bile, blood in major vessels not included in any tissue group; and hair.

Figure 2-5. Physiologic Model Used to Describe the Pharmacokinetics of Benzene in Mice and Rats During Inhalation and Oral Exposure
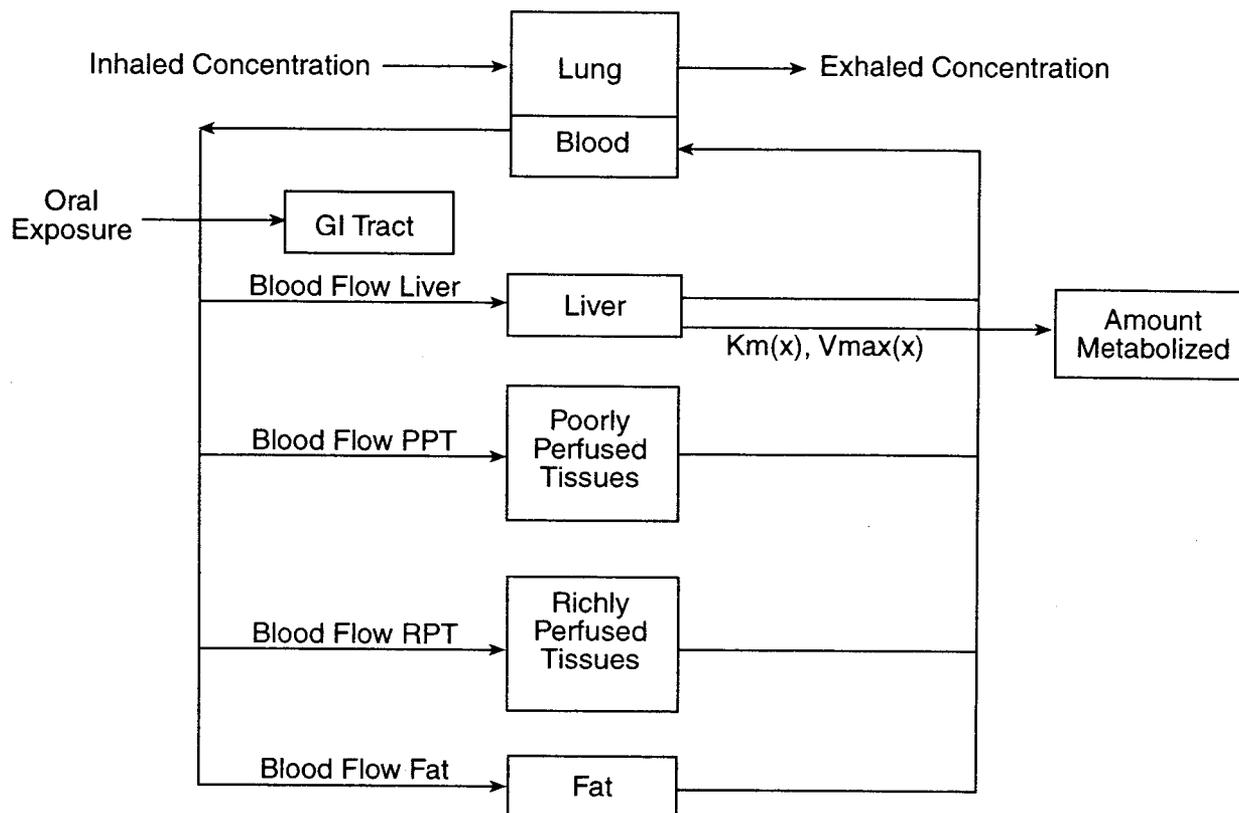
Adapted from Ramsey and Anderson 1984

phenol conjugates, muconic acid, or metabolites of the mercapturic pathway. Various parameters of the model were adjusted until the simulated data agreed with the experimental data.

Validation of the Model. Since the model was developed by Medinsky et al. (1989a) using the data of Sabourin et al. (1987), the model accurately reflects these data. Bois et al. (1991a) applied the data of Rickert et al. (1979) to the Medinsky model and compared to results obtained using data from Sabourin et al. (1987). In general, the Medinsky model tended to overestimate data to which it was not specifically fitted. It did correctly predict the percentage of a gavage dose administered to rats that was exhaled unchanged, or excreted as metabolites within 48 hours.

Target Tissues. The model correctly described metabolic differences observed between mice and rats. For instance, mice metabolize benzene more efficiently than rats during and after a 6-hour inhalation exposure. After oral exposure, mice and rats metabolized doses of benzene up to 50 mg/kg in a similar manner. At oral doses above 50 mg/kg, rats metabolized more benzene than did mice on a per kg body weight basis. Similar results were seen with the excretion of individual metabolites. Excretion of major metabolites, the phenyl conjugates, was similar for both species for oral doses up to 20 mg/kg, after which rats produced more phenyl conjugates than did mice. After inhalation, mice produced more phenyl conjugates at all exposure levels compared to rats when normalized to body weight. For hydroquinone metabolites, however, mice produce far more after both oral and inhalation exposure than rats. For muconic acid, the model predictions indicate that after inhalation exposure, mice produced more than rats; after oral exposure, the relative amount of muconic acid produced by the two species was similar.

Species Extrapolation. The Medinsky model used species-specific information to outline the model parameters; little extrapolation of information between mice and rats was required. Based on the parameters derived for mice, metabolism of benzene after inhalation by humans was simulated. The results indicate that at concentrations below 10 ppm, metabolism and formation of metabolites is linearly related to inhaled concentration, and that hydroquinone conjugates are the predominant metabolite. At concentrations above 10 ppm, the model predicts a change in the metabolic profile, with increased proportion of phenyl conjugates, associated with detoxification processes.

High-low Dose Extrapolation. High-low dose extrapolation was not specifically addressed in the Medinsky model. It is important to note however that the experiments used to determine some of the

model parameters spanned a wide concentration range and involved benzene concentrations spanning *at least* 1 order of magnitude for most experiments.

Interroute Extrapolation. The Medinsky model used two routes of administration, oral and inhalation, in rats and mice to describe the disposition of benzene. This model was used to predict human formation of benzene metabolites after inhalation exposure.

Strengths and Limitations of the Medinsky Model. The Medinsky model has many aspects which are desirable in a PBPK model. It uses more than one route of administration, it uses a range of concentrations and doses, it uses more than one species, and it specifically addresses the production of toxic metabolites, the putative agents of damage in benzene toxicity. However, the model is limited by the fact that it does not predict data other than that on which it was modeled very accurately (Bois et al. 1991a). In addition, because the model focuses on the prediction of metabolite kinetics, the kinetics of benzene itself suffer. This could be a problem in risk assessment if unmetabolized benzene plays a part in carcinogenesis as suggested by some researchers (Bois et al. 1991a). A major limitation of the model is its lack of inclusion of bone marrow activation of phenolic metabolites by myeloperoxidase-catalyzed reactions. In addition, the model bases the formation of muconaldehyde on the formation of muconic acid. Since muconaldehyde is a very reactive compound, it is a direct-acting alkylating agent which rapidly reacts with GSH and other cellular nucleophiles. Using muconic acid formation grossly underestimates muconaldehyde levels in tissues.

The Travis Model.

Risk Assessment. Risk assessment was not specifically addressed for this model.

Description of the Model. Travis et al. (1990) developed a model to describe the pharmacokinetics of benzene in mice, rats, and humans. The model contained five compartments, consisting of liver, fat, bone marrow, and muscle, and organs such as brain, heart, kidney, and viscera, connected by the arterial and venous blood pathways. Michaelis-Menten kinetics was assumed in all species, and occurred primarily in the liver, and to a lesser extent in the bone marrow. The speciesspecific physiological and chemical parameters were taken from the literature. The metabolic parameters were obtained by fitting the empirical data to the model.

Validation of the Model. The Travis model was validated using mouse and rat inhalation and gavage data from Sabourin et al. (1987), inhalation data for mice and rats from Snyder et al. (1981b), subcutaneous injection data from mice found in Andrews et al. (1977), intraperitoneal injection data from rats in Sato and Nakajima (1979a), and inhalation data in rats from Rickert et al. (1979) and Sato et al. (1975). Human inhalation exposure data from Berlin et al. (1980) and Sherwood (1972) were used for low concentration (5 ppm) modelling. For intermediate concentrations (25-57 ppm), the human data from Sato et al. (1975), Sherwood (1972), and Nomiyama and Nomiyama (1974a, 1974b) were used. For high concentrations (99-100 ppm) the data of Sherwood (1972) and Teisinger and Fiserova-Bergerova (1955) were used. The model successfully replicated the data, as discussed below.

Target Tissues. The Travis model successfully predicted the total amount of benzene in expired air and total metabolite produced in 48 hours following gavage administration in mice and rats, and inhalation administration in mice, but slightly overestimated these parameters in rats after inhalation exposure. Blood concentrations of benzene following inhalation exposure in mice and rats were adequately predicted. Bone marrow concentrations of benzene following subcutaneous injection in mice and inhalation exposure in rats were accurately predicted, as were blood concentrations of benzene in rats following intraperitoneal injection. For humans, the model slightly overestimated the data for benzene in expired air at the low concentration of 5 ppm. For the mid concentration of 25 ppm, excellent fit was obtained from the model for both benzene in expired air and blood benzene levels. Model agreement for expired benzene was also good at concentrations of 57 and 99 ppm. The model was also accurate in predicting the amount of phenol in the urine after a 5-hour exposure to 31.3 or 47 ppm.

Species Extrapolation. The Travis model used many of the same physiologic parameters that the Medinsky model used (average body weights, organ percentage of body weight, blood flow, etc.). Species-specific parameters were used, so extrapolation across species was not addressed.

High-low Dose Extrapolation. For human exposure, the Travis model successfully-predicted benzene in expired air at low (5 ppm), intermediate (25-57 ppm) and high concentrations of inhaled benzene. The animal studies used to validate the model employed a wide range of concentrations, encompassing at least one order of magnitude in concentration.

Interroute Extrapolation. The Travis model successfully predicted results from inhalation, oral, subcutaneous, and intraperitoneal administration of benzene.

Strengths and Limitations of the Travis Model. The Travis model has many aspects which are desirable in a PBPK model. It uses more than one route of administration, it uses a range of concentrations, it uses more than one species, and it successfully predicts blood and bone marrow concentrations of benzene after different routes of administration. However, the model is limited by the fact that it does not predict data other than that on which it was modeled very accurately (Bois et al. 1991a). For instance, the concentration of benzene in fat is poorly predicted by the Travis model. This could be a problem in risk assessment since benzene is fat soluble and is thought to accumulate in the fat (Bois et al. 1991a).

The Bois and Paxman Model.

Risk Assessment. The Bois and Paxman model (Bois and Paxman 1992) provided strong evidence that the exposure rate had a significant effect on the rate of formation of several important metabolites of benzene. The authors used the model to simulate metabolite production in human blood and bone marrow after an 8-hour inhalation exposure at the PEL of 1 ppm, and a 15-minute exposure at 32 ppm. The results of the simulation indicated that the levels of metabolites (hydroquinone, catechol, and muconaldehyde) were 20% higher (i.e., area under the curve) after the short-term exposure at the higher level, than after the long-term exposure at the lower level. These metabolites have been identified as being important in the genesis of bone marrow toxicity after benzene exposure (Eastmond et al. 1987). Since the STEL (Short Term Exposure Limit) is currently set at 5 ppm, or roughly l/6 of the simulated concentration, the authors concluded that the regulated level afforded an appropriate cushion of safety.

Description of the Model. Bois and Paxman (1992) produced a model that they used to explore the effect of exposure rate on the production of benzene metabolites. The model had three components, which described the pharmacokinetics of benzene and the formation of metabolites, using the rat as a model. Distribution and elimination of benzene from a five-compartment model, comprised of liver, bone marrow, fat, poorly perfused tissues, and well perfused tissues, made up the first component of the model. The five-compartment model included two sites for metabolism of benzene, liver and bone marrow. The bone marrow component was included for its relevance to

human leukemia. Parameter values for this component were derived from the literature and from the previously published work of Rickert et al. (1979) and Sabourin et al. (1987). The second component described the metabolic transformations of benzene and its by-products in the liver and bone marrow. The reactions were assumed to follow Michaelis-Menten kinetics, with the exception of the transformation of benzene oxide into phenol, which occurs spontaneously, and may be described by a first order reaction. The third component was the distribution of phenol. In addition to the compartments described for benzene, phenol was also assumed to distribute to the lung and gastrointestinal tract.

Validation of the Model. The model was validated against the data of Cassidy and Houston (1984), Sabourin et al. (1987, 1988, 1989a), and Sawahata and Neal (1983). An acceptable fit was observed for the data.

Target Tissues. The model was used to predict metabolite production for male rats exposed to benzene by nose-only procedures to three different vapor concentrations and at differing lengths of exposure, and compared to the experimental data of Sabourin et al. (1989a). Actual exposure regimens were 51 ppm for 6 hours, 153 ppm for 2 hours, and 588 ppm for 0.5 hours, which were established such that the product of the exposure level and duration of exposure was constant. Simulation results indicated that the model might over- or underestimate the level of urinary metabolites, although considering the variability inherent in the data, the authors were satisfied with the results.

Species Extrapolation. The Bois and Paxman model was used to model human metabolite formation after an 8-hour exposure at the PEL of 1 ppm, and at 32 ppm for 15 minutes, mimicking a high short-term exposure, the limit of which is currently 5 ppm. The model suggests that a higher level (i.e., area under the curve) of metabolites is produced after a high, short-term exposure compared to a low, longer-term exposure. This was true for both blood and bone marrow levels of metabolites.

High-low Dose Extrapolation. No high-low dose extrapolation was specifically addressed by the Bois and Paxman model. However, modeling of human occupational exposure (see above) did address both high and low concentrations.

Interroute Extrapolation. No interroute extrapolation was specifically addressed by the Bois and Paxman model.

Strengths and Limitations of the Bois and Paxman Model. The Bois and Paxman model has many aspects which are desirable in a PBPK model. For instance, it specifically addressed metabolism of benzene in the bone marrow, a primary site of toxicity. Specific limitations of the model were not apparent.

The Sun Model.

Risk Assessment. The Sun model (Sun et al. 1990) can be used to predict the levels of hemoglobin-benzene-derived adducts in the blood, and may be useful in predicting exposure levels that can be monitored through the hemoglobin adduct biomarker.

Description of the Model. The Medinsky model (1989a, 1989b, 1989c) was modified to account for the formation of hemoglobin adducts. It was assumed that hemoglobin adduct formation could proceed by both first-order and saturable pathways, and that benzene oxide would be the substrate for both pathways.

Validation of the Model. The model was validated using hemoglobin adduct formation data from rats after oral exposure of rats and mice (Sun et al. 1990). Formation of hemoglobin adduct after oral exposure in rats and mice was effectively simulated over a 6-fold range in exposure concentration. Inhalation exposure was validated against the data of Sabourin et al. (1989a), and was successfully predicted by the model.

Target Tissues. The model successfully predicted hemoglobin adduct formation after oral and inhalation exposure.

Species Extrapolation. No species extrapolation was specifically addressed by this model.

High-low Dose Extrapolation. No high-low dose extrapolation was specifically addressed by this model.

Interroute Extrapolation. The Sun model examined two routes of exposure, oral and inhalation. The model was found to be useful in predicting the concentrations of hemoglobin adducts in blood in rodents after oral and inhalation exposure.

Strengths and Limitations of the Sun Model. The Sun model has many aspects which are desirable in a PBPK model. It uses more than one route of administration, it uses more than one species, and it specifically addresses the production of hemoglobin adduct formation, a biomarker of effect for benzene toxicity. The limitations of the model were not apparent.

Other Applications of PBPK Modeling for Benzene. Other investigators have addressed the subject of PBPK modeling in relation to benzene toxicity. The approaches include comparing and refining the models to exclude some design parameters (Woodruff et al. 1992); modeling compound interactions after co-exposure (Purcell et al. 1990; Travis et al. 1992); kinetics in the presence of soil particles after oral exposure (Travis and Bowers 1990); modeling of metabolism (Schlosser et al. 1993); mathematical optimization of the model (Woodruff and Bois 1993); predictions of toxicity based on the parent and metabolite (Bois et al. 1991a); metabolite production in target tissues (Watanabe et al. 1994); modeling mechanism of action (Medinsky 1995); describing age-related changes in animal metabolism (McMahon et al. 1994) and mathematical analysis of the model itself (Spear et al. 1991). These applications of PBPK modeling are beyond the scope of this profile and will not be addressed.

2.4   MECHANISMS OF ACTION

Benzene toxicity has been studied extensively, and the current understanding of benzene toxicity includes its metabolic fate, mechanism of toxicity, pharmacokinetic models for disposition, and impact of exposure on human health. These aspects of benzene toxicity will be addressed in the following sections.

2.4.1   Pharmacokinetic Mechanisms

There have been numerous attempts to develop pharmacokinetic models of benzene disposition, in an effort to describe and predict benzene toxicity (see Section 2.3.5.1). Benzene disposition has been described as including three to five areas of distribution in the body: the liver; tissues that have a large

blood supply (brain, kidneys, heart, spleen); tissues that have a poor supply of blood (muscle and skin); and fat (Medinsky et al. 1989a; Sato and Nakajima 1979b). Benzene is absorbed into the blood from the lung, gastrointestinal tract, and skin. Metabolism is assumed to take place primarily in the liver, with some secondary metabolism in the bone marrow, the site of characteristic benzene toxicity. Benzene metabolism has been shown to be dose-dependent in all species studied thus fur, including humans. At low doses, more of the benzene is converted to putative toxic metabolites than at high doses. At high doses, benzene inhibits phenol metabolism to hydroquinone; phenol also inhibits benzene metabolism (Schlosser et al. 1993). This appears to occur through competition for a common site on the CYP2El enzyme to which catechol and hydroquinone also bond. Benzene is absorbed in the fat, and has been shown to bind to blood and tissue proteins. Excretion takes place from the lung and in the urine and feces. Biliary excretion also occurs.

Benzene metabolism to reactive intermediates follows two routes: ring-hydroxylation and ring opening (Sabourin et al. 1988; Snyder 1987; Snyder and Kocsis 1975; Snyder et al. 1993b). Hydroxylated metabolites form glucuronide or sulfate conjugates, and are excreted in the urine as detoxification products because the conjugate formation leads to elimination and the prevention of the formation of toxic metabolites. Ring opening leads to the formation of toxic intermediates (*trans,trans*-smuconaldehyde) that are also detoxified and excreted in the urine (Latriano et al. 1986).

2.4.2   Mechanisms of Toxicity

The most characteristic toxic effect of benzene in both human and animal models is the depression of the bone marrow, leading ultimately to aplastic anemia (Rozen and Snyder 1985; Snyder and Kocsis 1975; Snyder et al. 1993b). Rozen and Snyder (1985) have noted that abnormalities of humoral and cell-mediated immune responses following benzene exposure of C57BL mice by inhalation are presumably caused by a defect in the lymphoid stem cell precursors of both T- and B-lymphocytes. They also observed that bone marrow cellularity and the number of thymic T-cells increased, presumably as a compensatory response in these cell lines in response to benzene exposure. This compensatory proliferation may play a role in the carcinogenic response of C57BL mice to inhaled benzene.

The work of Longacre et al. (1981b) and Snyder et al. (1989) suggests that benzene and benzene metabolites may interfere with the incorporation of iron into bone marrow precursors. In studies on

the erythroid cell line, Longacre et al. (1981b) demonstrated 60-80% decreases in the incorporation of iron (as ^{59}Fe) into bone marrow precursors of mice injected subcutaneously with benzene. When given 48 hours prior to measuring ^{59}Fe uptake, benzene produced a dose-dependent decrease in erythroid cells (Snyder et al. 1989). Following intraperitoneal administration of benzene, hydroquinone, *p*-benzoquinone, and truns,trans-muconaldehyde, ^{59}Fe uptake was reduced. The combination of metabolites most effective in reducing ^{59}Fe uptake was hydroquinone plus *trans,trans*-muconaldehyde (Snyder et al. 1989).

Rao (1991) showed that hematin catalyzed the autoxidation of hydroquinone or 1,2,4-benzenetriol *in vitro,* producing reduced oxygen species that may be responsible for protein or DNA binding after benzene exposure. Further work along this line of research has provided evidence that *in vitro,* chelates of iron and hydroquinone or 1,2,4-benzenetriol are potent DNA cleaving agents (Rao 1996; Singh et al. 1994), and that 1,2,4-benzenetriol, but not hydroquinone, causes the release of iron from ferritin (Ahmad et al. 1995). 1,2,4-Benzenetriol or hydroquinone have also been shown to release reactive products from glutamate or DNA in the presence of copper ions (Rao and Pandya 1989).

Benzene also causes chromosomal abnormalities indicative of genetic damage, both in blood cells and the cells of other tissues (Forni et al. 1971a, 1971b; Hite et al. 1980; Tice et al. 1980, 1982). Benzene is carcinogenic in animals and leukemogenic in humans (Aksoy and Erdem 1978; Aksoy et al. 1987; Cronkite 1986; Cronkite et al. 1984, 1985, 1989; Farris et al. 1993; Huff et al. 1989; IARC 1982a; Maltoni et al. 1982a, 1982b, 1983, 1985, 1989; NTP 1986, 1994). Benzene has been shown to decrease the number of stem cells and committed stem cells (Baarson et al. 1984; Gill et al. 1980; Green et al. 1981a, 1981b; Seidel et al. 1989a). However, toxicity appears to occur only under conditions that allow metabolism to reactive intermediates.

The major portion of benzene metabolism takes place in the liver, mediated by cytochrome P-450 monooxygenase to form benzene oxide, a reactive intermediate. Muconaldehyde may also be formed in the liver, in addition to hydroquinone (Latriano et al. 1986). These metabolites are genotoxic, and Snyder et al. (1993b) suggest that, although it is unlikely that these metabolites formed in the liver, could reach the bone marrow to exert toxicity, they may be transported to target tissues by being bound to transport molecules. Evidence exists that benzene metabolites bind to blood proteins (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Irons et al. 1980; McDonald et al. 1994). Hydroquinone and catechol have been shown to accumulate in bone marrow, where they could

be substrates for myeloperoxidase, forming metabolites that could covalently bind to protein (Eastmond et al. 1987; Kalf et al. 1990; Snyder et al. 1993b; Subrahmanyam et al. 1990). This pathway has been shown to be possible using human myeloperoxidase (Eastmond et al. 1986; Smith et al. 1989; Subrahmanyam et al. 1991). Myeloperoxidase activity has been confirmed in both murine and human hematopoietic progenitor cells, further strengthening this mechanism of toxicity (Shattenberg et al. 1994).

In addition, hydroquinone and p-benzoquinone have been shown to bind to sulfhydryl groups of tubulin in the mitotic spindle, thereby preventing proper formation of microtubules (Irons 1985). Without adequate mitotic spindle support and function, some chromosomes may not complete segregation, giving rise to aneuploidy (Robertson et al. 1991a).

Carbonnelle et al. (1995) looked at the effect of hydroquinone on IL-l (IL-l) release from human monocytes in $vitro.$ Exposure of human monocytes to micromolar amounts of hydroquinone for 2 hours resulted in significantly decreased secretion of IL-l α and IL-l β at doses of 5 μM and above. RNA and protein synthesis were also inhibited, with a 50% inhibitory concentration at 21 μM for IL-l α and 10 μM for IL-l β. These results suggest that hydroquinone may contribute to benzene toxicity by inhibiting the production of cytokines by monocytes, which in turn may adversely affect regulation of hematopoiesis. Miller et al. (1994) observed that hydroquinone decreased the content of calpain, the enzyme responsible for the conversion of the precursor form of IL-l α to IL-l β, in murine bone marrow macrophages in culture. In addition, the work of Irons et al. (1992; Irons and Stillman 1993), who looked at the effect of pretreatment of murine bone marrow cells with hydroquinone on colony-forming response in the presence of growth stimulating factor, found evidence that hydroquinone alters differentiation in the myeloid progenitor cell population, which may be of relevance in the pathogenesis of leukemia secondary to benzene exposure.

Treatment of mice with 800 mg/kg benzene, intraperitoneally, 2 times daily for 2 days resulted in a significant increase in the production of nitric oxide by bone marrow cells stimulated by- lipopoly-saccharide and interferon γ, alone or in combination with macrophage colony-stimulating factor or granulocyte-macrophage colony-stimulating factor (Punjabi et al. 1994). Cells from benzene-treated mice showed increased sensitivity to the effects of the stimulating factors. Benzene, hydroquinone, 1,2,4-benzenetriol, and p-benzoquinone were evaluated for their effect on nitric oxide production by murine bone marrow leukocytes in $vitro$ (Laskin et al. 1995). Treatment of mice with benzene,

hydroquinone, 1,2,4-benzenetriol, or *p*-benzoquinone increased nitric oxide production in bone marrow leukocytes, in response to inflammatory mediators and growth factors (Laskin et al. 1995), with hydroquinone and 1,2,4-benzenetriol more effective than benzene or *p*-benzoquinone. Nitric oxide has been shown to suppress cell growth and, in the presence of molecular oxygen, can form a variety of nitrogen oxide intermediates that can react with primary and secondary amines to form nitrosamines, which are carcinogenic. Thus, nitric oxide may be important to the mechanism of toxicity of benzene and its metabolites.

In vitro studies of receptor-mediated phagocytosis and cytoskeletal integrity in mouse peritoneal macrophages indicate that benzene metabolites inhibit phagocytosis through some action on the filamentous action of the cell, thus adversely affecting host defense activities of the macrophages (Manning et al. 1994).

Schoeters et al. (1995) evaluated the hematopoietic and osteogenic toxicity of benzene, phenol, hydroquinone, and catechol *in vitro* using murine bone marrow cultures. Evaluation of toxicity to 3T3-fibroblasts was included to determine specific toxicity to marrow cells. Benzene and phenol showed little effect. However, hydroquinone inhibited proliferation of 3T3 cells and marrow precursor cells, and calcification of bone cells, although it was more specific for marrow cells. Catechol inhibited all cells, and showed no specificity.

Benzene, administered intraperitoneally at 600 mg/kg in corn oil, 2 times per day for 2 days to male C57BL/6J mice, caused a significant depression in the total number of nucleated bone marrow cells per femur, when measured on day 3 (Niculescu and Kalf 1995). Additional experiments with 7-day exposure revealed that there was an initial depression in erythroid cells on day 3 which remained constant; lymphocytes exhibited a progressively depressive effect, with the numbers of intermediate and terminally differentiated granulocytes exhibiting a progressive increase over the 7 days of exposure. Upon cessation of the benzene treatment, the bone marrow appeared to begin recovery, with the number of nucleated cells equal to control animals by day 7 after treatment ended. Concomitant administration of IL-l α prevented the decrease in nucleated bone marrow cells, whereas IL-l α administered after 2 days of benzene exposure significantly increased the rate of recovery of bone marrow cellularity.

The co-administration of phenol (75 mg/kg/day) and hydroquinone (25-75 mg/kg/day) twice a day for 12 days to B6C3F$_1$ mice produced myelotoxicity similar to that induced by benzene (Eastmond et al. 1987). The proposed mechanism suggested that selective accumulation of hydroquinone occurred in the bone marrow after the initial hepatic conversion of benzene to phenol and hydroquinone. Additionally, phenol is thought to stimulate the enzymatic activity of myeloperoxidase, which uses phenol as an electron donor, thus producing phenoxy radicals. These radicals further react with hydroquinone to form 1,4-benzoquinone, a toxic intermediate which inhibits critical cellular processes (Eastmond et al. 1987).

Benzene metabolites have been shown to bind to DNA and RNA, and have been shown to induce hyperphosphorylation of gene material, suggesting a mechanism for chromosomal damage and carcino-genesis (Dees and Travis 1994; Lutz and Schlatter 1977; Snyder et al. 1978b). Copper-mediated oxidation of hydroquinone to semiquinone anion radicals has been implicated in direct DNA damage (Li et al. 1995). In addition, benzene metabolites appear to be able to inhibit enzymes involved with DNA replication and repair, specifically the topoisomerase enzymes (Chen and Eastmond 1995). Singh et al. (1994) have studied the prooxidant and antioxidant properties of iron-hydroquinone and iron-1,2,4-benzenetriol complexes with respect to DNA degradation in rat bone marrow cells. Bleomycin-dependent DNA degradation was increased 1.5-fold in the presence of the iron hydroquinone chelate and 2.5-fold in the presence of the iron-1,2,4-benzenetriol chelate. However, the polyphenol chelates were less effective free radical generators than iron alone. Carcinogenesis may then be the result of aberrations of repair or replicating processes (Snyder et al. 1993).

In an attempt to elucidate the *in vivo* adduct formation by benzene metabolites, Reddy et al. (1989b) developed a highly sensitive nuclease P$_1$-enhanced ^{32}P-postlabeling assay for adduct detection at a frequency of one modification per 10^{9-10} DNA nucleotides. When DNA was prepared from Zymbal glands of rats given 200 mg/kg of benzene, orally, 5 days per week for 10 weeks, 3 potential adducts were discovered. However, no adducts were detected in the DNA from the liver, kidney, or mammary gland of rats at any dose (Reddy et al. 1989a). The adduct levels in the Zymbal gland corresponded to 4 lesions per 10^9 DNA nucleotides. The presence of adducts in the Zymbal DNA from benzenetreated rats may be related to the tumor formation in this organ following benzene treatment although this relationship is not clearly defined.

Benzene metabolites have also been shown to damage murine hematopoietic cells *in vitro* (Seidel et al. 1991). In addition, benzene has been shown to decrease mitochondrial respiration and increase superoxide radical production in isolated rat heart mitochondria (Stolze and Nohl 1994). The effects of exposure of HL-60 cells (human promyelocytic leukemic cells) to hydroquinone, *p*-benzoquinone, or 1,2,4-benzenetriol were studied by Rao and Snyder (1995). The cytotoxic effect of the metabolites on HL-60 cells, measured as cell viability, could be ranked as *p*-benzoquinone>hydroquinone> 1,2,4-benzenetriol, with viability from 50% to 70% after incubation with concentrations up to 100 pM for 4 hours. Basal levels of superoxide anion or nitric oxide production were not affected by incubation of the cells with the metabolites, but in the presence of TPA, each metabolite increased superoxide anion production; however, nitric oxide production was increased with hydroquinone and *p*-benzoquinone, but not 1,2,4-benzenetriol. HL-60 cells showed increased production of hydrogen peroxide after exposure to the three benzene metabolites. This study suggests that benzene metabolites may predispose the cells to oxidative damage by inhibiting or reducing antioxidant mechanisms within the cell.

Benzene leukemogenesis has been recently reviewed and summarized by Snyder and Kalf (1994). Detailed discussion of the proposed mechanisms of benzene toxicity is beyond the scope of this profile. However, the work of Snyder and Kalf (1994) in reviewing benzene toxicity can be summarized, in part, as follows. Chronic exposure to benzene results in the presence of benzene, hydroquinone, and other metabolites of benzene in the bone marrow. As a result of the toxic reaction of these compounds, lymphocytes are reduced, and granulocytes increased in the bone marrow. Chronic exposure to benzene might also result in leukemogenesis through activation of protein kinase C, involved in proliferation of human myeloid cells. In addition, activation of protein kinase C by chronic benzene exposure may result in overphosphorylation of cellular proteins, leading to an alteration in the activation and expression of some cellular protooncogenes. Benzene and its phenolic metabolites have been shown to induce oxidative DNA damage, which may play a role in leukemogenesis. As noted in Section 2.10.3, Ongoing studies, other possible mechanisms regarding leukemogenesis are currently under investigation.

2.4.3   Animal-to-Human Extrapolations

Recent PBPK models have tried to address benzene metabolism in an effort to derive animal-to-human extrapolations (Bois et al. 1991a; Medinsky 1995; Medinsky et al. 1989a; Spear et al. 1991; Travis et

al. 1990). Each model described a multicompartmental model that attempted to relate the generation of metabolites to end points of benzene toxicity. The generation of hydroquinone and muconaldehyde in the liver, with further metabolism in the bone marrow, has been addressed as well as the available data allow. However, the model is not sufficiently refined to allow it to accurately predict human metabolism. Thus, although PBPK modelling has provided a means to improve animal to human extrapolations, the models need to be improved.

2.5   RELEVANCE TO PUBLIC HEALTH

Overview.

Benzene is widely distributed in the environment. The exposure scenario of most concern to the general public is low-level inhalation over long periods. This is because the general population is exposed to benzene mainly through inhalation of contaminated air, particularly in areas of heavy traffic and around gas stations, and through inhalation of tobacco smoke from both active and passive smoking (Adlkofer et al. 1990; Appel et al. 1990; Chan et al. 1993, 1994; Dor et al. 1995; Fleming 1990; Fuselli et al. 1995; Gilli et al. 1994; Guerra et al. 1995; Lumley et al. 1990; Van Wijnen et al. 1995). Smoking has been identified as the single most important source of benzene exposure for the estimated 40 million U.S. smokers (Wallace 1989a, 1989b). Smoking accounts for approximately half of the total benzene exposure of the general population (Wallace 1989a). Individuals employed in industries that make or use benzene, or products containing benzene, are probably exposed to the highest concentrations of atmospheric benzene (Kawai et al. 1990, 1991; Moen et al. 1995a, 1995b; NOES 1990). In addition, benzene is a common combustion product, providing high inhalation exposure potential for firefighters (Burgess and Crutchfield 1995). Of the general population, those residing around certain chemical manufacturing sites or living near waste sites containing benzene may be exposed to concentrations of benzene that are higher than background air concentrations. In private homes, benzene levels in the air have been shown to be higher in homes with attached garages, or where the inhabitants smoke inside the house (Thomas et al. 1993).

Although low levels of benzene have been detected in certain foods, beverages, and tap water, these do not constitute major sources of exposure for most people. However, leakage from underground gasoline storage tanks and seepage from landfills and hazardous waste sites have resulted in significant benzene contamination of well water (ATSDR 1991; CEH 1983; Cline and Viste 1985; Plumb 1987;

Tucker et al. 1986). People with contaminated tap water can be exposed from drinking the water or eating foods prepared with it. In addition, exposure can also occur via inhalation during showering, bathing, or cooking with contaminated tap water (Lansari et al. 1992; Sidhu and Chadzynski 1994). Showering and bathing can also contribute significantly to dermal exposure.

The Benzene Subregistry Baseline Technical Report of the National Exposure Registry contains information on 1,143 persons who had documented exposure to benzene in their drinking water and were exposed for at least 30 days (ATSDR 1995). Although no causal relationship has been proposed for health conditions identified in the subregistry, continued follow-up of the population is planned to provide information on health effects of environmental exposure to benzene.

Several *in vivo* and *in vitro* studies conclusively demonstrate that benzene can be absorbed through human skin (Blank and McAuliffe 1985; Franz 1984; Susten et al. 1985; Tsuruta 1989). In general, skin absorption is considered a minor source of concern in the occupational environment as it occurs at a much lower rate and extent compared with benzene absorption through the respiratory system (OSHA 1989). However, benzene absorption through the skin as a result of benzene contamination in rubber solvents is a major route of exposure in tire building operations (Susten et al. 1985). Benzene is an irritant to the skin and, by defatting the keratin layer, may cause erythema, vesication, and dry and scaly dermatitis (Sandmeyer 1981). Therefore, it is important to avoid skin contact; training and education should be provided for occupationally exposed workers and protective clothing should be used.

Acute inhalation and oral exposures of humans to high concentrations of benzene have caused death (Cronin 1924; Greenburg 1926; Tauber 1970; Thienes and Haley 1972). These exposures are also associated with central nervous system depression (Flury 1928; Greenburg 1926). Chronic low-level exposures have been associated with peripheral nervous system effects (Baslo and Aksoy 1982). Abnormalities in motor conduction velocity were noted in 4 of 6 pancytopenic individuals occupationally exposed to adhesives containing benzene.

The most noted systemic effect resulting from intermediate and chronic benzene exposure is hematotoxicity. A common clinical finding in benzene hematotoxicity is cytopenia, which is a decrease in various cellular elements of the circulating blood manifested as anemia, leukopenia, or thrombocytopenia in humans (Aksoy 1991; Aksoy et al. 1971, 1972, 1987; Cody et al. 1993; Fishbeck

et al. 1978; Kipen et al. 1989; Midzenski et al. 1992; Townsend et al. 1978; Tsai et al. 1983; Xia et al. 1995; Yin et al. 1982, 1987b, 1987c) and in animals (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite et al. 1982, 1985, 1989; Dempster and Snyder 1991; Dow 1992; Farris 1993; Gill et al. 1980; Green et al. 1981b; Hsieh et al. 1988b, 1991; Huff et al. 1989; Li et al. 1986; Luke et al. 1988b; Maltoni et al. 1983, 1985; NTP 1986; Plappert et al. 1994a, 1994b; Rozen et al. 1984; Seidel et al. 1989b; Shell 1992; Snyder et al. 1978a, 1980, 1984; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956). Benzene-associated cytopenias vary and may involve a reduction in one (unicellular cytopenias) to all three (pancytopenia) cellular elements of the blood. Furthermore, a causal relation exists between benzene exposure and aplastic anemia in humans (Aksoy et al. 1972, 1974; Erf and Rhoads 1939; Yin et al. 1987c) and in animals (Cronkite et al. 1982, 1985, 1989; Luke et al. 1988b; Seidel et al. 1989b; Snyder et al. 1980, 1984; Toft et al. 1982; Ward et al. 1985). This disorder is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow. Aplastic anemia that results from benzene exposure is also associated with an increased risk of developing acute non-lymphocytic leukemia (Aksoy 1980; Aksoy et al. 1974).

Limited information is available on other systemic effects reported in humans and associated with benzene exposure. Respiratory effects have been noted after acute exposure of humans to benzene vapors (Avis and Hutton 1993; Midzenski et al. 1992; Winek and Collum 1971; Winek et al. 1967; Yin et al. 1987b). Cardiovascular effects, particularly ventricular fibrillation, have been suggested as the cause of death in fatal exposures to benzene vapor (Avis and Hutton 1993; Winek and Collum 1971). Gastrointestinal effects have been noted in humans after fatal inhalation exposure (congestive gastritis, Winek and Collum 1978) and ingestion (toxic gastritis and pyloric stenosis, Greenburg 1926) of benzene. Myelofibrosis was reported by a gasoline station attendant who had been exposed to benzene by inhalation, and probably also through dermal contact (Tondel et al. 1995). Myalgia was also reported in steel plant workers exposed to benzene vapors (Ruiz et al. 1994). Reports of renal effects in humans after benzene exposure consist of kidney congestion after fatal inhalation exposure (Winek and Collom 1971). Dermal and ocular effects including skin irritation and bums, and eye irritation have been reported after exposure to benzene vapors (Avis and Hutton 1993; Midzenski et al. 1992; Yin et al. 1987b). Swelling and edema have been reported to occur in a human who swallowed benzene (Greenburg 1926). Studies in animals show systemic effects after inhalation exposure, including cardiovascular (Magos et al. 1990; Nahum and Hoff 1934). Oral administration of benzene to animals has yielded information concerning hepatic effects (Pawar and Mungikar 1975). A study conducted in rabbits lends support to the finding that benzene is irritating and damaging to the skin

and also shows that it is irritating and damaging to the eyes following dermal or ocular application (Wolf et al. 1956).

Neurological effects have been commonly reported in humans after acute inhalation exposure (Cronin 1924; Davies 1929; Flury 1928; Greenburg 1926; Kraut et al. 1988; Midzenski et al. 1992; Tauber 1970; Tondel et al. 1995; Yin et al. 1987b). Fatal inhalation exposure has been associated with vascular congestion in the brain (Avis and Hutton 1993; Flury 1928). Chronic inhalation exposure has been associated with distal neuropathy in humans (Baslo and Aksoy 1982), difficulty in sleeping, and memory loss (Kahn and Muzyka 1973). Oral exposure of humans results in symptoms similar to inhalation exposure (Thienes and Haley 1972). Studies in animals suggest that inhalation exposure to benzene results in depressed electrical activity in the brain (Frantik et al. 1994), loss of involuntary reflexes and narcosis (Carpenter et al. 1944), decrease in hind-limb grip strength and tremors (Dempster et al. 1984), and narcosis (Evans et al. 1981), among other symptoms. Oral exposure to benzene has not been shown to cause significant changes in behavior (Hsieh et al. 1990, 1991). No neurological effects have been reported after dermal exposure to liquid benzene in either humans or animals.

Evidence of an effect of benzene exposure on human reproduction is not sufficient to demonstrate a causal association (Mukhametova and Vozovaya 1972; Stucker et al. 1994; Vara and Kinnunen 1946). Some animal studies provide limited evidence that benzene affects reproductive organs following inhalation exposure (Ungvary and Tatrai 1985; Ward et al. 1985; Wolf et al. 1956). Results from studies of benzene administered orally to rats and mice indicate no adverse effect on male or female reproductive organs at 17 weeks, but at 2 years, endometrial polyps were observed in female rats, preputial gland lesions were observed in male mice, and ovarian lesions were observed in female mice (NTP 1986). Results are conflicting or inconclusive as to whether inhalation of benzene vapors reduces the number of live fetuses (Ungvary and Tatrai 1985) and/or the incidences of pregnancy (Gofmekler 1968). Other studies are negative for effects on reproductive competence (Coate et al. 1984; Exxon 1986; Green et al. 1978; Kuna et al. 1992; Murray et al. 1979).

Epidemiological studies implicating benzene as a developmental toxicant have many limitations, and thus it is not possible to assess the effect of benzene on the human fetus (Budnick et al. 1984; Forni et al. 1971a; Funes-Cravioto et al. 1977; Goldman et al. 1985; Heath 1983; Olsen 1983). Results of inhalation studies conducted in animals are fairly consistent across species and demonstrate that, at

levels of ≥47 ppm, benzene is fetotoxic as evidenced by decreased fetal weight and/or minor skeletal variants (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). Benzene has also been shown to reduce pup body weight in mice (Seidenberg et al. 1986). A persistent decrease in number of erythroid precursors was found in mice exposed *in utero* (Keller and Snyder 1986, 1988). Benzene has not been shown to be teratogenic, but has been shown to be fetotoxic in animals at high concentrations that are maternally toxic.

Damage to both the humoral and cellular components of the immune system has been known to occur in humans following inhalation exposure. This is manifested by decreased levels of antibodies and decreased levels of leukocytes in workers (Aksoy et al. 1971, 1972, 1987; Cody et al. 1993; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Lange et al. 1973a, 1973b; Ruiz et al. 1994; Xia et al. 1995; Yin et al. 1987c). Animal data support these findings (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite 1986; Cronkite et al. 1982, 1985, 1989; Dow 1992; Fan 1992; Gill et al. 1980; Green et al. 1981a, 1981b; Hsieh et al. 1988b, 1990, 1991; Huff et al. 1989; Li et al. 1986, 1992; Maltoni et al. 1983, 1985; Neun et al. 1992; NTP 1986; Plappert et al. 1994a, 1994b; Rosenthal and Snyder 1985, 1987; Rozen et al. 1984; Shell 1992; Snyder et al. 1984, 1988; Stoner et al. 1981; Toft et al. 1982; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956; Yin et al. 1982).

In vivo and *in vitro* data from both humans and animals indicate that benzene and/or its metabolites are genotoxic. Chromosomal aberrations in peripheral lymphocytes and bone marrow cells are the predominant effects seen in humans (Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hartwich et al. 1969; Hedli et al. 1990; Karacic et al. 1995; Major et al. 1994; Picciano 1979; Popp et al. 1992; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Tompa et al. 1994; Tough and Court-Brown 1965; Tough et al. 1970; Turkel and Egeh 1994; Van den Berghe et al. 1979).

Many epidemiological and case studies correlate benzene exposure with leukemia, and some provide risk assessment of benzene exposure (Aksoy 1980, 1987; Aksoy and Erdem 1978; Bond et al. 1986b; Cody et al. 1983; Crump 1994; Hunting et al. 1995; Hurley et al. 1991; Infante 1978; Infante et al. 1977; Li et al. 1994; Ott et al. 1978; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987; Travis et al. 1994; Vigliani and Forni 1976; Wong 1995; Wong and Raabe 1995; Wong et al. 1983; Yin et al. 1987a, 1987c, 1989, 1994). These studies indicate that benzene is carcinogenic (leukemogenic).

Studies in humans following benzene exposure did not provide sufficient information on exposure levels and their correlation with observed effects. Data from animal studies have, therefore, been used to derive MRLs.

Minimal Risk Levels for Benzene.

Inhalation MRLs.

- An MRL of 0.05 ppm has been derived for acute-duration inhalation exposure (14 days or less) to benzene.

The acute-duration inhalation MRL of 0.05 ppm was derived from a LOAEL value of 10 ppm for the reduced lymphocyte proliferation following mitogen stimulation in mice (Rozen et al. 1984). The ratio of the blood/gas partition coefficients was assumed to be 1. The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10 ppm) by 6/24 to correct for less than a full day of exposure. The resulting adjusted LOAEL, 2.5 ppm, was then converted to a human equivalent concentration (HEC) by multiplying by the ratio of the mouse ventilation rate (2.21 L/hour) to its body weight (0.0316 kg) divided by the ratio of the human ventilation rate (833 L/hour) to human body weight (70 kg) (formula 4-l1, EPA 1990h; ventilation rate and body weight values taken from EPA 1988e). The resulting LOAEL$_{(HEC)}$ of 14.7 ppm was then divided by an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolation from animals to humans after adjusting for the human equivalent concentration, and 10 for human variability) to yield the MRL value of 0.05 ppm (see Appendix A). Increased number of MN-PCEs, decreased numbers of granulopoietic stem cells (Toft et al. 1982), lymphopenia (Cronkite et al. 1985), lymphocyte depression, and increased susceptibility to bacterial infection (Rosenthal and Snyder 1985) are among the adverse hematological and immunological effects observed in several other acute-duration inhalation studies. The study by Rozen et al. (1984) shows benzene immunotoxicity (reduced mitogen-induced lymphocyte proliferation) at a slightly lower exposure level than these other studies. C57BL/6J mice were exposed to 0, 10.2, 31, 100 and 301 ppm benzene for 6 days at 6 hours per day. Control mice were exposed to filtered, conditioned air only. Lymphocyte counts were depressed at all exposure levels; erythrocyte counts were elevated at 10.2 ppm, equal to controls at 31 ppm and depressed at 100 and 301 ppm. Femoral B-lymphocyte and splenic B-lymphocyte numbers were reduced at 100 ppm. Levels of circulating lymphocytes and mitogen-induced blastogenesis of femoral B-lymphocytes were depressed after

exposure to 10.2 ppm benzene for 6 days. Mitogen-induced blastogeneses of splenic T-lymphocytes were depressed after exposure to 31 ppm of benzene for 6 days. In another study, mice exhibited a 50% decrease in the population of CFU-E after exposure to 10 ppm benzene for 5 days, 6 hours per day (Dempster and Snyder 1991). In a study by Wells and Nerland (1991), groups of 4-5 male Swiss-Webster mice were exposed to 3, 25, 55, 105, 199, 303, 527, 1150, or 2290 ppm benzene for 6 hours/day for 5 days. The number of leukocytes in peripheral blood and spleen weights were significantly decreased compared with untreated controls at all concentrations ≥25 ppm. Therefore, 3 ppm was the NOAEL and 25 ppm was the LOAEL for these effects. These data support the choice of Rozen et al. (1984) as the study from which to derive the MRL.

- An MRL of 0.004 ppm has been derived for intermediate-duration inhalation exposure (15-365 days) to benzene.

The intermediate-duration inhalation MRL of 0.004 ppm was derived from a LOAEL value of 0.78 ppm for neurological effects of intermediate-duration inhalation exposure of mice to benzene (Li et al. 1992). The ratio of the blood/gas partition coefficients was assumed to be 1. The dose was adjusted for intermittent exposure by multiplying the LOAEL (0.78 ppm) by 2 hours/24 hours and by 6 days/7 days to adjust to continuous exposure. The resulting adjusted LOAEL 0.056 ppm was then converted to a human equivalent concentration (HEC) by multiplying by the ratio of the mouse ventilation rate (2.21 L/hour) to its body weight (0.0316 kg) divided by the ratio of the human ventilation rate (833 L/hour) to human body weight (70 kg) (formula 4-l1, EPA 1990h; ventilation rate and body weight values taken from EPA 1988e). The resulting LOAEL $_{(HEC)}$ of 0.33 ppm was then divided by an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans after adjusting for the human equivalent concentration, and 10 for human variability), yielding the MRL of 0.004 ppm (see Appendix A). Forty adult Kunming male mice were divided into 4 groups (5 per group) and exposed to 0, 0.78, 3.13, and 12.52 ppm benzene for 2 hours per day, 6 days per week for 30 days. Control mice were exposed to air. Benzene level was monitored by gas chromatography every 30 minutes for 3 days at the beginning of the experiment. Locomotor activity was measured in 15-minute sessions. A Y-maze, designed with a starting point and safety area, was used to measure rapid response. Motivation of the mice was accomplished by electrical shock. Fore-limb grip strength of mice was determined by persistent time (in seconds) while grasping a vertical iron stick. Body weight, food consumption, relative organ weight, blood and brain acetylcholinesterase activity, and bone marrow panel were determined. Body weight and food

consumption were not affected. Significant differences in organ-to-body weight ratios occurred only for the liver and spleen, but not for the kidney. A 12% increase in liver weight and a 26% decrease in spleen weight were observed at 12.52 ppm. The increase in liver weight was not considered to be adverse. There was decreased grip strength at 12.52 ppm, but increased grip strength occurred at the lower doses. Low level exposure also affected frequency of rapid response; at 0.78 ppm, the response was increased, whereas at the higher doses, the response was depressed. All of the changes were significantly different from the control values. Locomotor activity increased at the low dose, was similar to control values at the mid dose, and decreased at the high dose. However, these changes were not significantly different from the control value. A significant decrease of AChE activity in the brain was observed at 12.52 ppm, but the decrease was not large enough to be considered an adverse effect. Bone marrow profile showed decreased percentages of precursors of leukocytes and erythrocytes with increased concentrations of inhaled benzene; this effect was most consistent at the high dose. Myelocytes, premyelocytes, myeloblasts, and metamyelocytes were among the cell types most affected. Li et al. (1992) observed neurological changes at doses that were lower than those that caused the expected changes in bone marrow cells. Based on the observation of neurological changes in a study that also identified classic indicators of benzene toxicity, it seems reasonable to use the neurological effects observed by Li et al. (1992) as a basis for an intermediate-duration MRL. Benzene is known to cause adverse neurological effects in both humans and animals. Following acute inhalation of benzene, humans exhibited symptoms indicative of central nervous system effects (Cronin 1924; Flury 1928; Greenburg 1926; Midzenski et al. 1992). These symptoms, reported to occur at levels ranging from 300 to 3,000 ppm, included drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. Acute exposure (5-10 minutes) to higher concentrations of benzene (approximately 20,000 ppm) can result in death, which has been associated with vascular congestion in the brain (Avis and Hutton 1993; Flury et al. 1928). Lethal exposures are also associated with neurological symptoms similar to those reported for nonlethal exposures. These symptoms are similar to the consequences of exposure to multiple organic solvents and are reversible when symptomatic workers are transferred from the problem area (Kraut et al. 1988; Yin et al. 1987b). In reports of cases of benzene poisoning, subjects exhibited headaches, nausea, tremor, convulsions, and unconsciousness, among other neurological effects (Cronin 1924; Davies 1929; Greenburg 1926; Midzenski et al. 1992; Tauber 1970).

Chronic exposure to benzene has been reported to produce neurological abnormalities in humans. Of 8 patients (6 with aplastic anemia and 2 with preleukemia) with previous occupational exposure to

adhesives and solutions containing 9-88% benzene, 4 of the 6 patients with aplastic anemia showed neurological abnormalities (global atrophy of lower extremities and distal neuropathy of upper extremities) (Baslo and Aksoy 1982). Air concentrations in the workplace were reported to have reached levels of 210 ppm or higher. These findings suggest that benzene may induce toxic effects on the nervous system involving peripheral nerves and/or spinal cord. However, the limitations of this study are that benzene exposure levels were not monitored and that there was a possibility of an additional exposure to toluene (6.37-9.25%).

Chronic exposure to benzene and toluene was studied in 121 workers exposed to benzene for 2-9 years (Kahn and Muzyka 1973). The air concentration of benzene between 1962 and 1965 was 6-15.6 ppm (20-50 mg/m^3), while the toluene vapors did not exceed the 5 mg/m^3 level. Subsequently (the authors do not specify when), the air levels of both benzene and toluene have not exceeded the 5 mg/m^3 level. Of the examined workers, 74 complained of frequent headaches (usually at the end of the work day), became tired easily, had difficulties sleeping, and complained of memory loss. The limitations of this study are that workers were exposed to both benzene and toluene and that the precise dose and duration of exposure are not known.

The neurotoxicity of benzene has not been studied extensively in animals. Male albino SPF rats from a Wistar-derived strain exposed to benzene for 4 hours in glass chambers (dose not specified) exhibited depression of evoked electrical activity in the brain; the authors calculated the 30% effect level (depressed activity) as 929 ppm (Frantik et al. 1994). When female H strain mice were exposed to benzene for 2 hours, the 30% effect level for depression of evoked electrical activity in the brain was 856 ppm (Frantik et al. 1994). In rabbits, symptoms that occurred 3.7 minutes following acute exposure to benzene at 45,000 ppm were relaxation and light narcosis (Carpenter et al. 1944). As the time after exposure progressed so did the symptoms to include excitation and tremors (after 5 minutes), loss of pupillary reflex to strong light (after 6.5 minutes), pupillary contraction (after 12 minutes), and involuntary blinking (after 15.6 minutes). Behavioral tests of C57BL/6 mice showed significant increase in licking of sweetened milk after 1 week of exposure to 300 ppm; a 90% decrease in hind-limb grip strength after one exposure to 1,000 or 3,000 ppm (data for 100 ppm were not reported); and tremors after one exposure to 3,000 ppm that subsided 30 minutes after the exposure (Dempster et al. 1984). In another study, designed to reflect occupational exposure, male CD-l and C57BL/6 mice were exposed to 300 or 900 ppm of benzene 6 hours per day for 5 days followed by 2 weeks of no exposure after which the exposure regimen was repeated for an unspecified amount of

time (Evans et al. 1981). The following seven categories of behavioral activities were monitored in exposed and control animals: stereotypic behavior, sleeping, resting, grooming, eating, locomotion, and fighting. Only minimal and insignificant differences were observed between the two strains of mice. Increased behavioral activity was observed after exposure to benzene in both strains of mice. Mice exposed to 300 ppm of benzene had a greater increase than those exposed to 900 ppm, probably because of narcosis-like effects induced at the higher exposure level (Evans et al. 1981).

No chronic-duration MRLs were derived for benzene because of a lack of appropriate data on effects on chronic-duration inhalation exposure. The study by Tsai et al. (1983) was considered. In this study, a benzene cohort of 454 male workers employed at a large Texas refinery between September 15, 1952 and January 1978 was studied. Participants were included regardless of length of employment. Data of exposure to benzene for the years 1973-82 were examined since data prior to 1973 were inadequate for assessing worker exposure. Benzene exposures were <1 ppm, with a median exposure of 0.14 ppm for refinery workers, and 0.53 ppm for those in the benzene-related units. The relative risk for all causes of death decreased. There was no increased risk compared with either the U.S. general population or the general refinery workers. Evaluation of data for a 21-year period showed no significant changes in blood indices of selected employees. The study was not accepted for derivation of a chronic-duration inhalation MRL because there was no attempt to adjust for TWA exposure levels; most of the study was concerned with retrospective mortality results compared with the U.S. national rates; and in the medical surveillance study for hematological effects, benzene workers were defined by the supervisor of each unit, with no indication of how the subjects were selected, no controls, and no information regarding follow-up. Additional limitations included that the study was not originally designed to detect chronic health effects in the selected populations and focused more on mortality and cancer end points. It is unclear how the study subjects described in the medical testing portion were selected and followed. The study was judged inappropriate for deriving an MRL. In other chronic studies, the lowest LOAEL of 3 ppm was considered a serious LOAEL because pancytopenia was observed in humans exposed occupationally (Doskin 1971). The LOAEL is also higher than the $LOAEL_{(HEC)}$ of 0.33 ppm used as the basis for the intermediate-duration MRL.

Oral MRLs.

No acute-duration oral MRL was derived due to a lack of appropriate data on the effects of acute oral exposure to benzene.

In a study by Thienes and Haley (1972), central nervous system symptoms of giddiness, vertigo, muscular incoordination, unconsciousness, and death of humans have been reported when doses of 125 mg kg/body weight benzene or higher were ingested. The only lower doses in the acute oral data base are 50 mg/kg/day, at which pregnant rats experienced alopecia of hindlimbs and trunk (Exxon 1986), an endpoint not suitable for MRL derivation for benzene because the toxicological significance is not clear; and 88 mg/kg/day, at which slight non-dose-related central nervous system depression occurred in rats (Cornish and Ryan 1965).

No intermediate-duration oral MRL was derived. Examination of the literature indicated that studies by Hsieh et al. (1988b, 1991) and Wolf et al. (1956) might yield an intermediate-duration oral MRL. Adult male CD-l mice were exposed to 0, 8, 40, or 180 mg/kg/day benzene in the drinking water for 4 weeks (Hsieh et al. 1988b, 1991). Serious hematological and immunological effects occurred at the lowest dose, precluding its use for MRL derivation. In the study by Wolf et al. (1956), a NOAEL of 1 mg/kg/day and a LOAEL of 50 mg/kg/day for leukopenia were identified in rats given benzene in oil by gavage for 6 months. However, closer examination of the study by Wolf et al. (1956) revealed that the results were not adequately supported by the data and analysis presented in the paper. Therefore, Wolf et al. (1956) was not selected for derivation of the MRL.

No chronic-duration oral MRLs were derived for benzene because of lack of appropriate data on effects of oral exposure to benzene. The NTP (1986) carcinogenesis bioassay was considered. In the NTP (1986) bioassay, male rats were given 50, 100, or 200 mg/kg/day and female rats and mice of both sexes were given 0, 25, 50, and 100 mg/kg/day benzene in corn oil for 2 years. The dose of 25 mg/kg/day was a LOAEL for hematotoxicity and immunotoxicity in rats and mice, and is higher than the serious LOAEL of 8 mg/kg/day in the intermediate-duration data base. Therefore, the threshold for hematological and immunological effects of benzene has not been identified for oral exposure, precluding the derivation of a chronic MRL.

Death. Acute inhalation exposure to high concentrations of benzene has caused death Lethality in humans following inhalation exposure has been attributed to asphyxiation, respiratory arrest, central nervous system depression, or cardiac collapse (Avis and Hutton 1993; Hamilton 1922; Winek and Collom 1971). Many of the fatalities resulted from asphyxiation due to inhalation of high concentrations of benzene in enclosed places (Avis and Hutton 1993; Cronin 1924; Greenburg 1926; Tauber 1970). Based on case studies, it has been estimated that exposure to a benzene concentration

of 20,000 ppm for 5-10 minutes is likely to be rapidly fatal (Flury 1928). Animal studies lend support to human data that indicate that benzene has a relatively low toxicity following acute inhalation exposures. An LC_{50} of 13,700 ppm has been reported for rats after a 4-hour exposure (Drew and Fouts 1974). Similarly, exposure to 16,000 ppm for 4 hours was lethal for 4 of 6 rats (Smyth et al. 1962).

Concentrations of benzene in the workplace above 10 ppm are associated with hematological abnormalities, including aplastic anemia and leukemia, which have resulted in death (Aksoy and Erdem 1978; Ott et al. 1978; Rinsky et al. 1981, 1987). Cancer studies conducted in animals support the findings in humans that benzene is capable of shortening lifespan (Farris et al. 1993; Maltoni et al. 1982a, 1983; Paxton et al. 1994a; Snyder at al. 1978a).

Lethality in humans following oral exposure to benzene has been attributed to respiratory arrest, central nervous system depression, or cardiac collapse (Greenburg 1926). Oral lethal doses for humans have been estimated to be about 125 mg/kg (Thienes and Haley 1972). Accidental ingestion and/or attempted suicide with lethal oral doses have produced the following signs and symptoms: staggering gait, vomiting, shallow and rapid pulse, somnolence, and loss of consciousness, followed by delirium, pneumonitis, collapse, and then central nervous system depression, coma, and death (Thienes and Haley 1972). Animal studies lend support to human data indicating that there is a rapid progression of toxicity following high acute oral exposures to benzene. An LD_{50} of 930 mg/kg/day has been reported for rats after a l-day exposure (Cornish and Ryan 1965). No data are available on the effects of benzene on lifespan following intermediate and chronic oral exposures in humans. Increased mortality was observed in rats and mice after chronic-duration oral exposure to benzene at 250 mg/kg/day and 100 mg/kg/day, respectively (NTP 1986).

No data are available on death in animals following dermal exposure to benzene. Increased risk of death was associated with dermal exposure to gasoline by vehicle mechanics (Hunting et al. 1995).

Inhalation exposure to high concentrations of benzene in the occupational setting may lead to death from pancytopenia and leukemia. However, environmental exposure to benzene in the air, drinking water, soil, or food is unlikely to be fatal. People living near hazardous waste sites who are chronically exposed to contaminated air, water, or soil may be at a higher risk of death due to adverse health conditions, including leukemia and other types of cancer.

Systemic Effects.

Respiratory Effects. Respiratory effects have been reported after acute exposure to benzene vapors (Avis and Hutton 1993; Winek and Collum 1971). After a fatal occupational exposure to benzene vapors on a chemical cargo ship for only minutes, autopsy reports on three victims revealed hemorrhagic, edematous lungs (Avis and Hutton 1993). Similar results were noted after intentional inhalation resulting in death (Winek and Collum 1971). Mucous membrane irritation and dyspnea have been noted at occupational exposures of greater than 60 ppm for up to 3 weeks (Midzenski et al. 1992). Thus, benzene has been shown to have irritative properties; contact with benzene vapor is capable of damaging respiratory tissues. A dose of 500 mg/kg of benzene administered subcutaneously once daily, 5 days a week for 26 weeks to Wistar rats caused atrophy of the peribronchial lymphoidal tissue with blurring of the structure of the lymphoidal nodules in the lungs after 12 weeks of exposure (Bloch et al. 1990). After 26 weeks, a progression of these changes was noticed. Thus, exposure to benzene by routes other than inhalation could also cause respiratory effects.

Inhalation exposure benzene is likely to cause respiratory irritation. However, environmental exposure to benzene in the air, drinking water, soil, or food is unlikely to cause significant adverse respiratory effects. People living near hazardous waste sites who are chronically exposed to contaminated air, water, or soil may be at a higher risk for respiratory effects from exposure to the vapor, or through accumulation of benzene in the body after ingestion of contaminated food or water.

Cardiovascular Effects. In humans, ventricular fibrillation has been proposed as a contributing factor to death after acute inhalation exposure to benzene (Avis and Hutton 1993; Winek and Collom 1971). Electrocardiograms indicated that exposure of cats and monkeys to high but unspecified concentrations of benzene vapor caused extra systoles and ventricular tachycardia of the prefibrillation type in cats and monkeys (Nahum and Hoff 1934). Similar results have been observed for rats exposed to very high concentrations (3,526-8,224 ppm) for 15 minutes (Magos et al. 1990). A dose of 500 mg/kg of benzene administered subcutaneously once daily, 5 days a week for 26 weeks to Wistar rats resulted in muscle fiber necrosis in the left ventricle (Bloch et al. 1990).

The effect of benzene on the respiration of isolated heart mitochondria was studied by Stolze and Nohl (1994). Respiratory control and ATP/oxygen values decreased in the presence of benzene at 100 μM.

A concomitant increase in superoxide radical formation was observed. This suggests a mechanism for benzene-mediated damage to cardiovascular tissue and other tissues as well.

Based on this limited information, it is possible that inhalation of high concentrations of benzene vapors, such as those encountered in an occupational accident, could induce similar changes in humans in the industrial setting. Environmental exposure to low levels of benzene found in contaminated air, water, food, and soil is unlikely to cause these cardiovascular changes.

Gastrointestinal Effects. Few studies are available on the gastrointestinal effects of exposure to benzene. After intentional inhalation of benzene resulting in death, congestive gastritis was noted at the autopsy of an 18-year-old boy (Winek and Collum 1971). In the case report of a human who swallowed benzene, intense toxic gastritis and later pyloric stenosis were reported (Greenburg 1926). In long-term studies (2 years) in rats and mice, hyperkeratosis, acanthosis, and hyperplasia were observed in the forestomach after oral exposure by gavage to relatively high doses (200 mg/kg/day) of benzene (NTP 1986). Therefore, it is possible that accidental or intentional inhalation or ingestion of very high doses of benzene will cause these effects in humans. Environmental exposure to levels found in drinking water, soil, food, or air in or around hazardous waste sites is unlikely to cause adverse gastrointestinal effects in exposed populations.

Hematological Effects. Both human and animal studies have shown that benzene exerts toxic effects on various parts of the hematological system. All the major types of blood cells are susceptible (erythrocytes, leukocytes, and platelets). In the less severe cases of toxicity, specific deficiencies occur in individual types of blood elements. A more severe effect occurs when there is hypoplasia of the bone marrow, or hypercellular marrow exhibiting ineffective hematopoiesis so that all types of blood cells are found in reduced numbers. This is known as pancytopenia. A biphasic response (i.e., a hyperplastic effect in addition to destruction of the bone marrow cells) has been observed (Aksoy et al. 1972, 1974; Doskin 1971; Rozen et al. 1984). Severe damage to the bone marrow involving cellular aplasia is known as aplastic anemia and can occur with prolonged exposure to benzene. -This condition can lead to leukemia.

Studies on occupationally exposed populations demonstrate that these effects do indeed occur, but these studies do not provide sufficient data on exposure to determine a threshold level for effects. For these epidemiology studies, it was assumed that the major route of benzene exposure was through the

lungs. However, it is also likely that the people involved had skin contact with the substance, and it is possible that this also contributed to the observed toxicity. The human studies that did provide some estimate of levels of exposure show that adverse hematological effects occurred at levels greater than 10 ppm but generally not at levels less than 1 ppm (Aksoy 1991; Aksoy et al. 1971, 1972, 1987; Cody et al. 1993; Fishbeck et al. 1978; Kipen et al. 1989; Midzenski et al. 1992; Tsai et al. 1983; Xia et al. 1995; Yin et al. 1982, 1987b, 1987c). The animal studies support the findings in humans and can be used to provide more accurate data on the relationship between specific effects and exposure levels in air. Adverse hematological effects begin to appear in animals at concentrations of lo-100 ppm and above (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite et al. 1982, 1985, 1989; Dempster and Snyder 1991; Dow 1992; Farris 1993; Gill et al. 1980; Green et al. 1981b; Hsieh et al. 1988b, 1991; Huff et al. 1989; Li et al. 1986; Luke et al. 1988b; Maltoni et al. 1983, 1985; NTP 1986; Plappert et al. 1994a, 1994b; Rozen et al. 1984; Seidel et al. 1989b; Shell 1992; Snyder et al. 1980, 1984; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956).

Only one study was found that described hematological effects in humans after oral exposure to benzene (Selling 1916). No reports describing hematological effects in humans following direct dermal exposure to benzene were found. However, intermediate- and chronic-duration animal studies show that loss of blood elements occurs in animals exposed to benzene in drinking water or by gavage at doses as low as 8-25 mg/kg/day (Hsieh et al. 1988b, 1990; Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986; Shell 1992; Wolf et al. 1956).

Based on information found in the literature, it is reasonable to expect that adverse hematological effects might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to blood elements. Studies show that the hematological system is susceptible to chronic exposure at low levels, so people living in and around hazardous waste sites that may be exposed to contaminated air, drinking water, soil, or food may be at an increased risk for adverse hematological effects. Deficiencies in various types of blood cells lead to other disorders, such as hemorrhagic conditions from a lack of platelets, susceptibility to infection from the lack of leukocytes, and increased cardiac output from the lack of erythrocytes.

Several mechanisms to explain how benzene affects the bone marrow have been proposed. These include suppression of RNA and DNA synthesis (Moeschlin and Speck 1967; Post et al. 1985),

alkylation of cellular sulfhydryl groups (Irons 1985), disruption of the cell cycle (Irons et al. 1979; Snyder et al. 1981c), oxygen activation or free radical formation (Irons 1985), covalent binding of benzene metabolites to cellular macromolecules (Gill and Ahmed 1981; Longacre et al. 1981a; Snyder et al. 1978b), damage to stromal cells (Frash et al. 1976; Kalf et al. 1987), and loss of specific protein growth factors (Clark and Kamen 1987).

The effect of administration of benzene on iron metabolism was investigated by giving 0.5 mL/kg intraperitoneally or 1 ml/kg subcutaneously to female rats for 10 consecutive days (Ahmad et al. 1994). Serum from benzene-treated female rats offered less protection against iron-catalyzed lipid peroxidation of brain tissue than the serum of control female rats. Serum iron concentration and total iron binding capacity did not exhibit any significant change after benzene administration. Iron saturation of transfer&in was not affected by benzene.

Benzene, administered intraperitoneally at 600 mg/kg in corn oil, 2 times per day for 2 days to male C57BL/6J mice, caused a significant depression in the total number of nucleated bone marrow cells per femur, when measured on day 3 (Niculescu and Kalf 1995). Additional experiments with 7-day exposure revealed that there was an initial depression in erythroid cells on day 3 which remained constant; lymphocytes exhibited a progressively depressive effect, and the numbers of intermediate ant terminally differentiated granulocytes exhibited a progressive increase over the 7 days of exposure. Upon cessation of the benzene treatment, the bone marrow appeared to begin recovery, with the number of nucleated cells equal to control animals by day 7 after treatment ended. Concomitant administration of IL-l α prevented the decrease in nucleated bone marrow cells, whereas IL-l α administered after 2 days of benzene exposure significantly increased the rate of recovery of bone marrow cellularity.

There is general agreement among various investigators in the field of benzene toxicity that benzene metabolites, rather than benzene, are the primary toxic agents in the induction of hematotoxicity. This agreement has evolved as a result of studies in which agents known to alter benzene metabolism (toluene, phenobarbital, and ethanol) have also altered benzene toxicity (Andrews et al. 1977; Sammett et al. 1979). Toluene, Aroclor 1254, and phenobarbital appear to alleviate benzene toxicity, while ethanol generally increases benzene toxicity. Therefore, biotransformation is believed to be essential for benzene-induced bone marrow damage. However, there is disagreement as to whether benzene is activated in the marrow or activated elsewhere and transported to the marrow. When several of the

metabolites of benzene were compared with the parent compound in hematotoxicity studies, the metabolites were less toxic than benzene. Tunek et al. (1981) suggested that perhaps the metabolites tested conjugate strongly in the liver and other organs and do not reach the bone marrow in amounts sufficient to produce the expected effect. On the other hand, one metabolite of benzene, *trans,trans*-muconaldehyde (LD_{50}, 6.7-7.1 mg/kg), was highly toxic to the bone marrow of mice administered 2 mg/kg, intraperitoneally, for 10-16 days (Witz et al. 1985). Similarly, 6-hydroxy-truns,trans-2,4-hexadienal, a reactive intermediate of *trans,trans*--muconaldehyde, was administered to male CD-l mice intraperitoneally; leukocyte count increased in mice dosed at 5 mg/kg/day for 16 consecutive days, but bone marrow cellularity and red blood cell parameters were not affected (Zhang et al. 1995c). At 10 mg/kg/day for 16 consecutive days, a decrease in nonprotein sulfhydryl groups of the bone marrow, but no change in peripheral blood parameters, was noted. At 25 mg/kg/day for 3 consecutive days followed by 20 mg/kg for one day and sacrifice 24 hours later, a decrease in nucleated bone marrow cells and leukocyte count was noted. Iron incorporation in erythrocytes was inhibited by 3 consecutive doses of 20 or 30 mg/kg 6-hydroxy-*trans,trans*-2,4-hexadienal; 15 mg/kg had no effect on iron incorporation. The results of this study further support the concept that metabolites of benzene are hemotoxic, and can reach the bone marrow. Hedli et al. (1990) administered benzene metabolites phenol and hydroquinone intraperitoneally to rats and examined bone marrow cellularity and DNA adducts in the bone marrow. Data indicated that the metabolites were able to cause a decrease in bone marrow cellularity, and form DNA adducts in the bone marrow when administered *in vivo*. DNA adducts were observed at doses that did not affect bone marrow cellularity.

Carbonnelle et al. (1995) looked at the effect of hydroquinone on IL-l release from human monocytes *in vitro*. Exposure of human monocytes to micromolar amounts of hydroquinone for 2 hours resulted in significantly decreased secretion of IL- l α and IL-l β at doses of 5 μM and above. RNA and protein synthesis were also inhibited, with a 50% inhibitory concentration at 21 μM for IL-l α and 10 μM for IL-l β. Bone marrow mononuclear cells or purified hematopoietic progenitor cells were incubated with 30 μM hydroquinone alone or in the presence of IL-l β or tumor necrosis factor, and the-colonyforming ability of the cells (CFU-C) was evaluated (Colinas et al. 1995). Hydroquinone alone reduced CFU-C frequencies by approximately 60% for both bone marrow mononuclear cells and purified hematopoietic progenitor cells. Neither IL- 1 β or tumor necrosis factor protected the bone marrow mononuclear cells from the inhibitory activity of hydroquinone. For purified hematopoietic progenitor cells, however, treatment with tumor necrosis factor, but not IL-l β, provided protection against

inhibition of formation of progenitor cell colonies. Irons et al. (1992) looked at the effect of pretreatment of murine bone marrow cells with hydroquinone, phenol, catechol, and *trans,trans*-muconaldehyde on colony-forming response. Cells were stimulated with recombinant granulocyte/macrophage colony stimulating factor after exposure to the benzene metabolites. Pretreatment with hydroquinone for 30 minutes in the picomolar to micromolar range caused a 1.5-4.6-fold enhancement in colonies formed, with optimal enhancement at 1 μM hydroquinone. Synergism between hydroquinone and the growth factor was observed in cells not normally responsive to the growth factor, suggesting an effect of hydroquinone on cells not normally responsive to growth factors. Pretreatment with the other metabolites did not enhance growth factor response.

Equal numbers of male Swiss Webster and C57BL/6J mice were exposed to air or to 300 ppm benzene 6 hours per day, 4 days per week for 2 weeks (Neun et al. 1994). Bone marrow was isolated from the mice on the morning following final inhalation. Bone marrow cells from benzene-exposed and control mice were cultured with one of five benzene metabolites: 1,4-benzoquinone (BQ), catechol (C), hydroquinone (HQ), muconic acid (MA) or phenol (P); the abilities of these cultured bone marrow cells to produce erythroid (CFU-E) or granulocyte/macrophage colonies (GM-CFU-C) were assessed. Bone marrow cells isolated from both strains of mice exposed to benzene showed higher percentages of plated erythroid colony forming cells surviving culture with BQ, HQ, or muconic acid than marrow cells isolated from control mice. In contrast, marrow cells isolated from both strains of exposed mice showed decreased percentages of plated CFU-E surviving culture with catechol than cells isolated from control mice. *In vitro* treatment with phenol induced no differential effects on CFU-e isolated from exposed or control mice of either strain. Cells isolated from exposed Swiss Webster mice showed increased percentages of plated GM-CFU-c surviving culture with HQ than cells isolated from controls. In contrast, marrow cells isolated from exposed C57BL/6J mice showed decreased percentages of GM-CFU-C surviving culture with HQ than cells isolated from controls. Benzene exposure did not affect the total hepatic sulfhydryl (SH) concentrations in either mouse strain but the SH content of the bone marrow was more than doubled after benzene exposure in both strains.

Treatment of mice with 800 mg/kg benzene, intraperitoneally, 2 times daily for 2 days resulted in a significant increase in the production of nitric oxide by bone marrow cells stimulated by lipopolysaccharide and interferon γ, alone or in combination with macrophage colony-stimulating factor or granulocyte-macrophage colony-stimulating factor (Punjabi et al. 1994). Cells from benzene-treated mice showed increased sensitivity to the effects of the stimulating factors. Benzene,

hydroquinone, 1,2,4-benzenetriol, and *p*-benzoquinone were evaluated for their effect on nitric oxide production by murine bone marrow leukocytes *in vitro* (Laskin et al. 1995). Treatment of mice with benzene (800 mg/kg, intraperitoneally), hydroquinone (100 mg/kg), 1,2,4-benzenetriol (25 mg/kg), or *p*-benzoquinone (2 mg/kg) sensitized bone marrow leukocytes to produce more nitric oxide in response to stimulation from lipopolysaccharide, interferon-γ, and increased the sensitivity of the cells to

granulocyte/macrophage colony-stimulating factor and macrophage colony-stimulating factor. Cells from hydroquinone and 1,2,4-benzenetriol-treated animals were significantly more responsive than those from benzene or *p*-benzoquinone-treated animals. Nitric oxide has been shown to suppress cell growth and, in the presence of molecular oxygen, can form a variety of nitrogen oxide intermediates that can react with primary and secondary amines to form nitrosamines, which are carcinogenic. Thus, nitric oxide may be important to the mechanism of toxicity of benzene and its metabolites. Rao (1991) showed that hematin catalyzed the autoxidation of hydroquinone or 1,2,4-benzenetriol *in vitro,* producing reduced oxygen species that may be responsible for protein or DNA binding after benzene exposure. Further work along this line of research has provided evidence that *in vitro,* chelates of iron and hydroquinone or 1,2,4-benzenetriol are potent DNA cleaving agents (Rao 1996; Singh et al. 1994), and that 1,2,4-benzenetriol, but not hydroquinone, causes the release of iron from ferritin (Ahmad et al. 1995). 1,2,4-Benzenetriol or hydroquinone have also been shown to release reactive products from glutamate or DNA in the presence of copper ions (Rao and Pandya 1989). For a further discussion on biotransformation of benzene, see Section 2.3.3.

Scheding et al. (1992) have analyzed hematotoxic effects observed in mice in *in vivo* studies, using a mathematical model. Their results indicate that erythropoietic cells were the most sensitive, granulopoietic cells were about half as sensitive as erythropoietic cells, and hematopoietic stem cells exhibited a sensitivity somewhere between the two. A dose-response relationship between benzene exposure and hematotoxic effects was derived for doses up to 300 ppm; effects plateaued at higher doses. This study indicates that mathematical modeling represents a useful approach to investigate the action of hematotoxic agents.

Musculoskeletal Effects. There is little data describing musculoskeletal effects of benzene exposure. Two studies provide information describing musculoskeletal effects in humans after inhalation and possibly dermal exposure. Myelofibrosis was diagnosed in a 46-year-old man who had worked as a gasoline station attendant for 17 years (Tondel et al. 1995). The TWA concentration for gasoline station attendants was estimated to be ~0.2 ppm. Exposure was probably also dermal. Ruiz et al.

(1994) reported myalgia in employees from a steel plant of Cubatao, S. Paulo, Brazil, who presented with neutropenia due to benzene exposure. No histopathological lesions were found in bone tissues of rats or mice exposed to benzene orally for up to 2 years (NTP 1986). Although musculoskeletal effects may occur after chronic exposure to benzene, these effects are not likely to be of primary concern to people exposed occupationally or to the low levels that are likely to be found in ambient air, water, and soil, or in or around hazardous waste sites.

Hepatic Effects. No reliable studies were located regarding the hepatic effects of benzene in humans following inhalation, oral, or dermal exposures. Aksoy et al. (1972) reported enlarged livers in workers chronically exposed to benzene, but no additional data were given.

In animals, increased liver weight is the most common hepatic effect observed after inhalation or oral exposure to benzene (Pawar and Mungikar 1975; Tatrai et al. 1980a, 1980b), and histopathological examination of the liver of rats and mice exposed by gavage to doses up to 600 or 200 mg/kg/day, respectively, for up to two years revealed no treatment-related effects (Huff et al. 1989; NTP 1986).

Benzene is metabolized primarily in the liver by the cytochrome P-450 system (Parke 1989). It appears that the metabolism of benzene by the hepatic cytochrome P-450 system plays an important role in its bioactivation and toxicity. Sammett et al. (1979) provided corroborative evidence of this by showing that partial hepatectomy of rats diminished both the rate of metabolism of benzene and its toxicity. An increase in altered hepatic foci has been shown in male rats after benzene exposure in conjunction with initiator and promotor administration (Dragan et al. 1993). A dose of 500 mg/kg of benzene administered subcutaneously once daily, 5 days a week for 26 weeks to Wistar rats resulted in focal fine droplet fatty metamorphosis with accompanying lymphoidal infiltration in the liver after 12 weeks (Bloch et al. 1990). In some cases a proliferated histocyte-like cells formed clusters in the vicinity of the periportal fields. After 26 weeks, more diffuse steatosis, feathery degeneration of hepatocytes, single necrotic cells were seen.

The effect of administration of benzene, 0.5 mL/kg intraperitoneally or 1 mL/kg subcutaneously, to male and female rats for 10 consecutive days on free sulfhydryl content and lipid peroxidation in the liver was investigated (Ahmad et al. 1994). The free sulfhydryl content of the liver showed a significant decrease in groups dosed intraperitoneally, but not subcutaneously. The rate of lipid

peroxidation was increased in all benzene-treated animals. α-Tocopherol levels in the liver were not affected.

Based on the data found in the literature, hepatic effects do not appear to be of primary concern with regard to human health after exposure to benzene occupationally, in the environment, or at hazardous waste sites.

Renal Effects. Renal congestion has been noted at autopsy after a fatal human exposure by inhalation (Winek and Cullom 1971). No adverse renal effects were observed in animals administered benzene orally for acute, intermediate, or chronic durations (Exxon 1986; Hsieh et al. 1990; Huff et al. 1989; NTP 1986). Administration of benzene, 438 mg/kg intraperitoneally or 877 mg/kg subcutaneously, to male and female rats for 10 consecutive days resulted in a decrease in serum uric acid (Ahmad et al. 1994). Based on these limited data, adverse renal effects do not appear to be of primary concern with regard to human health after exposure to benzene occupationally, in the environment, or at hazardous waste sites.

Dermal Effects. Limited information is available on the dermal effects of benzene. Benzene was slightly irritating to the skin of rabbits following application one time per day for 4 weeks (Wolf et al. 1956). The skin showed moderate erythema, edema, and moderate necrosis. No dose information was provided in this study. Exposure of humans to benzene vapor in air has resulted in dermal effects that include erythema, edema, frank bums, and necrosis (Avis and Hutton 1993; Midzenski et al. 1992). Limited information also suggests that oral ingestion of high doses (near lethal) may cause swelling and edema (Greenburg 1926).

These data indicate that exposure to high levels of benzene in air, direct contact of the skin with benzene, and ingestion of high doses may result in dermal effects, but it is unlikely that levels of benzene in ambient air, in contaminated drinking water, or at hazardous waste sites are high enough to be of concern.

Ocular Effects. Eye irritation has been reported in workers exposed occupationally to benzene (Yin et al. (1987b), and lacrimation occurred in rats exposed to benzene in air (Shell 1980). Moderate conjunctival irritation and transient cornea1 damage were observed in rabbits subsequent to the placement of 2 drops of benzene into the eyeball (Wolf et al. 1956). Thus, eye irritation is a concern

for people exposed to high concentrations of benzene in air or if benzene is splashed in the eyes, but levels of benzene in the ambient air, in contaminated drinking water, or at hazardous waste sites are probably not high enough to be of concern.

Body Weight Effects. Relatively few studies report body weight effects after benzene exposure. Tondel et al. (1995) reported unspecified weight loss in a gasoline station attendant who had been exposed to benzene by inhalation and probably through dermal contact for 17 years. Decreases in body weight (15%) were seen in DBA/2 mice after exposure to 300 ppm benzene for 6 hours per day, 5 days per week for 2 weeks (Chertkov et al. 1992). Decreased body weight (16%) has also been noted in mice exposed to 50 or 200 ppm of benzene for 6 hours per day for 7 or 14 days (Aoyama 1986). C57BL mice exhibited a 50% decrease in body weight gain after exposure to 300 ppm benzene, 6 hours per day, 5 days per week for 70 weeks (Snyder et al. 1980). Other studies have shown decreased body weight in animals exposed by inhalation or orally (Exxon 1986; Green et al. 1978; Kuna and Kapp 1981; Maltoni et al. 1983, 1985; NTP 1986; Snyder et al. 1978a; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). However, in other animal studies, no change in body weight was seen after inhalation or oral exposure Green et al. 1981b; Hsieh et al. 1990; Kuna et al. 1992; Li et al. 1992; Shell 1992; Snyder et al. 1984; Taningher et al. 1995; Ward et al. 1985). Thus, body weight changes may not be primary indicators of benzene toxicity in humans.

Immunological and Lymphoreticular Effects. Benzene has been shown to have adverse immunological effects in humans following inhalation exposure for intermediate and chronic durations. Adverse immunological effects in animals occur following both inhalation and oral exposure for acute, intermediate, and chronic durations. The effects include damage to both humoral (antibody) and cellular (leukocyte) responses. Human studies of intermediate and chronic duration have shown that benzene causes decreases in the levels of circulating leukocytes in workers at low levels (30 ppm) of exposure (Aksoy et al. 1971, 1972, 1974) and decreases in levels of circulating antibodies in workers exposed to benzene at 3-7 ppm in a mixture with xylene and toluene (Lange et al. 1973b). Other studies have shown decreases in human lymphocytes and other blood elements after exposure (Aksoy et al. 1987; Cody et al. 1993; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Lange et al. 1973a; Ruiz et al. 1994; Xia et al. 1995; Yin et al. 1987c). Animal data support these findings (Aoyama 1986; Baarson et al. 1984; Chertkov et al. 1992; Cronkite 1986; Cronkite et al. 1982, 1985, 1989; Dow 1992; Fan 1992; Gill et al. 1980; Green et al. 1981a, 1981b; Hsieh et al. 1988b, 1990, 1991; Huff et al. 1989; Li et al. 1986, 1992; Maltoni et al. 1983, 1985; Neun et al. 1992; NTP 1986;

Plappert et al. 1994a, 1994b; Rosenthal and Snyder 1985, 1987; Rozen et al. 1984; Shell 1992; Stoner et al. 1981; Snyder et al. 1984, 1988; Toft et al. 1982; Ward et al. 1985; Wells and Nerland 1991; Wolf et al. 1956; Yin et al. 1982). Both humans (Yin et al. 1982) and rats (Li et al. 1986; Yin et al. 1982) have shown increases in leukocyte alkaline phosphatase activity. No studies regarding effects from oral or dermal exposure in humans were located. However, exposure to benzene through ingestion or dermal contact could cause immunological effects similar to those seen after inhalation exposure in humans and inhalation and oral exposure in animals.

Animal studies have also shown that benzene decreases circulating leukocytes and decreases the ability of lymphoid tissue to produce the mature lymphocytes necessary to form antibodies. This has been demonstrated in animals exposed for acute (Cronkite et al. 1982, Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1986; Rozen et al. 1984), intermediate (Aoyama 1986; Cronkite et al. 1982, 1985, 1989; Green et al. 1981a, 1981b; Ward et al. 1985; Wolf et al. 1956), or chronic periods (Snyder et al. 1980, 1984) via the inhalation route. This decrease in lymphocyte numbers is reflected in impaired cell-mediated immune functions in mice following intermediate inhalation exposure to 100 ppm of benzene (Rosenthal and Snyder 1987). The impaired cellular immunity after benzene treatment was observed both *in vivo* and *in vitro.* Mice exposed to 100 ppm for a total of 100 days were challenged with 104 polyoma virus-induced tumor cells (PYB6). Nine of 10 mice had reduced tumor resistance resulting in the development of lethal tumors. In the same study lymphocytes were obtained from spleens of benzene-treated mice and tested for their immune capacity *in vitro.* The results showed that two other immune functions, alloantigen response (capacity to respond to foreign antigens) and cytotoxicity, were also impaired (Rosenthal and Snyder 1987). Similar effects were noted in mice exposed to benzene via the oral route for intermediate time periods (Fan 1992; Hsieh et al. 1988b) and in rats and mice exposed for chronic time periods (Huff et al. 1989; NTP 1986). A decrease in spleen weight was observed in mice after acute-duration exposure to benzene at 25 ppm, the same dose levels at which a decrease in circulating leukocytes was observed (Wells and Nerland 1991). Similar effects on spleen weight and circulating leukocytes were observed in mice exposed to 12 ppm benzene 2 hours per day for 30 days (Li et al. 1992). A dose of 500 mg/kg of benzene administered subcutaneously once daily, 5 days a week for 26 weeks to Wistar rats resulted changes in the immunological and lymphoreticular system after 12 weeks (Bloch et al. 1990). The microscopic structure of most follicles became blurred, with damage to the germinal centers and an increase in volume of red pulp. After 26 weeks, a progression of the above changes were seen with atrophy of the white pulp, further expansion of the red pulp, and the appearance of an increased number of

fibroblasts. In the spleen, the lymphatic system showed signs of damage which became more pronounced in joint exposure to benzene and ethanol. The acute inhalation MRL was based on the study showing decreased mitogen-induced blastogenesis of B-lymphocytes following exposure to 10 ppm in mice (Rozen et al. 1984).

Selected measures of cellular immunity were evaluated in wild juvenile cotton rats after dietary protein restriction and intraperitoneal exposure benzene (McMurry et al. 1994a). Forty-seven-day-old juvenile cotton rats were maintained on one of two isocaloric diets containing either 4 or 16% crude protein for a 26-day experimental period. Animals were treated intraperitoneally with either 0 (corn oil), 100, 500, or 1,000 mg/kg benzene in corn oil for 3 consecutive days. The first dose was administered on day 15-17 of the experimental period. Animals were terminated on day 27. Relative weight of the spleen, but not the thymus, was greater in rats on the low protein diet and exposed to benzene, compared to the low protein control animals; no difference in spleen or thymus weight was noted between benzene-treated 16% protein diet rats and the 16% protein controls. With regard to peripheral leukocyte counts, control animals receiving 16% protein in their diet had more neutrophils and fewer lymphocytes than their 4% protein counterparts. Total neutrophils were higher in rats receiving benzene compared to controls. Splenocyte yield, although higher in control rats receiving 16% protein compared to the low protein diet group, were not affected by benzene treatment. Cellular immunity, measured *in vivo*, and splenocyte proliferation, measured *in vitro*, were not affected by benzene treatment. In a companion study, treatment of 21-day-old rats on a regular diet with 0, 100, or 1,000 mg/kg benzene intraperitoneally in corn oil for 3 consecutive days caused a depression in relative thymus weight, but no effect on relative spleen weight was observed (McMurry et al. 1994b). Benzene treatment had no effect on leukocyte counts, or viable splenocyte yields, cellular immunity, or splenocyte proliferation.

Benzene, administered intraperitoneally at 600 mg/kg in corn oil, 2 times per day for 2 days to male C57BL/6J mice, caused a significant depression in the total number of nucleated bone marrow cells per femur, when measured on day 3 (Niculescu and Kalf 1995). Additional experiments with 7-day exposure revealed that there was an initial depression in erythroid cells on day 3 which remained constant; lymphocytes exhibited a progressively depressive effect, with the numbers of intermediate and terminally differentiated granulocytes exhibited a progressive increase over the 7 days of exposure. Upon cessation of the benzene treatment, the bone marrow appeared to begin recovery, with the number of nucleated cells equal to control animals by day 7 after treatment ended. Concomitant

administration of IL-l α prevented the decrease in nucleated bone marrow cells, whereas IL-l α administered after 2 days of benzene exposure significantly increased the rate of recovery of bone marrow cellularity.

In vitro studies of the effect of benzene metabolites on receptor-mediated phagocytosis and cytoskeletal integrity in mouse peritoneal macrophages provides information on the immunotoxicity of benzene (Manning et al. 1994). In mouse peritoneal macrophage cultures, benzoquinone, 1,2,4-benzenetriol, and hydroquinone inhibited receptor mediated phagocytosis; catechol had no effect. Cells treated with benzoquinone or benzenetriol did not recover, even after overnight incubation in the absence of treatment. Additional observations suggest that for benzoquinone, at least, the inhibition of receptor-mediated phagocytosis is caused by a disruption of the filamentous action in the cell, although the specific effect is not known.

Based on information found in the literature, it is reasonable to expect that adverse immunological effects might occur in humans after inhalation, oral, or dermal exposure, since absorption of benzene through any route of exposure would increase the risk of damage to the immunological system. Studies show that the immunological system is susceptible to chronic exposure at low levels, so people living in and around hazardous waste sites that may be exposed to contaminated air, drinking water, soil, or food may be at an increased risk for adverse immunological effects

Neurological Effects. In humans, results of occupational studies indicate that there is a causeand-effect relationship between acute inhalation of benzene and symptoms indicative of central nervous system toxicity (Cronin 1924; Davies 1929; Flury 1928; Greenburg 1926; Midzenski et al. 1992; Tauber 1970). These symptoms, observed following both acute nonlethal and lethal exposures, include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. These symptoms are reversible when symptomatic workers are transferred from the problem area (Kraut et al. 1988; Yin et al. 1987b). Comparable toxicity in humans has been reported following ingestion of benzene at doses of 125 mg/kg and above (Thienes and Haley 1972). No studies were located regarding neurological effects in humans after dermal exposure to benzene. Occupational exposure to benzene has also been reported to produce neurological abnormalities in humans. Electromyographical and motor conduction velocity examinations were conducted on six patients with aplastic anemia, all of whom worked in environments where adhesives containing benzene were used (in one case, air concentrations bracketed around 210 ppm) (Baslo and Aksoy 1982). Abnormalities in motor

conduction velocity were noted in four of the six pancytopenic individuals and were thought to result from a direct effect of benzene on the peripheral nerves and/or spinal cord.

In its acute stages, benzene toxicity appears to be due primarily to the direct effects of benzene on the central nervous system, whereas the peripheral nervous system appears to be the target following chronic low-level exposures. In addition, because benzene may induce an increase in brain catecholamines, it may also have a secondary effect on the immune system via the hypothalamuspituitary-adrenal axis (Hsieh et al. 1988b). Increased metabolism of catecholamines can result in increased adrenal corticosteroid levels, which are immunosuppressive (Hsieh et al. 1988b).

Animal studies provide additional support that benzene affects the nervous system following acute inhalation and oral exposures. Effects reported include narcosis, nervous system depression, tremors, and convulsions (Carpenter et al. 1944; Cornish and Ryan 1965). Acute and intermediate inhalation exposures have also been reported to produce adverse neurological effects in animals including a reduction in hind-limb grip strength and evoked electrical activity in the brain, and behavioral disturbances (Dempster et al. 1984; Frantik et al. 1994). Effects of benzene on learning were investigated in male hooded rats of the Sprague-Dawley strain given 550 mg/kg of benzene in corn oil or corn oil without benzene, intraperitoneally, on days 9, 11, and 13 postpartum (Geist et al. 1983). The rats exhibited a significantly impaired learning ability when tested on problems of the closed-field, maze-learning task. This sign of neurotoxicity following benzene exposure was not observed in control animals. In another study, 47-day-old juvenile cotton rats were maintained on one of two isocaloric diets containing either 4% or 16% crude protein for a 26-day experimental period (McMurry et al. 1994a). Animals were treated intraperitoneally with either 0 (corn oil), 100, 500, or 1,000 mg/kg benzene in corn oil for 3 consecutive days. The first dose was administered on day 15-17 of the experimental period. Animals were terminated on day 27. During the experimental period, severe loss of coordination was observed in some rats on the low protein diet immediately after exposure to benzene, but this subsided.

Intermediate oral exposures resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes (Hsieh et al. 1988a). Mice exposed to 3 ppm for 2 hours per day for 30 days exhibited increased levels of acetylcholinesterase in the brain (Li et al. 1992). In the same experiment, grip strength was decreased at 12 ppm and rapid response was increased at

0.78 ppm. The intermediate inhalation MRL was based on this study, showing increased rapid response following exposure to 0.78 ppm in mice (Li et al. 1992).

In vitro studies suggest that benzene may have a direct effect on brain cells (Vaalavirta and Tahti 1995). Primary astrocyte cultures prepared from neonatal rat cerebella were treated with 3, 6, or 9 mmol/L benzene for 1 hour. ATPase and Mg^{2+}-ATPase activity were inhibited in a dose-related manner, and were detected at 78-92% of control values for ATPase, and 60-74% of control values for Mg^{2+}-ATPase.

These data suggest that humans exposed to benzene in the occupational setting for acute, intermediate, or chronic durations via the inhalation and oral routes are at risk of developing neurological effects. However, benzene levels in ambient air, drinking water, and at hazardous waste sites are not likely to be of concern.

Reproductive Effects. Menstrual disorders characterized by profuse or scanty blood flow and dysmenorrhea occurred in 12 of 30 women who worked in an environment containing benzene (Vara and Kinnunen 1946). Although air measurements of benzene were not reported, it is assumed that benzene levels at the work site may have been much higher than those in today's work areas. Ten of the 12 women were married. Of these 10 women, 2 had spontaneous abortions and no births occurred. However, the study failed to provide verification that the absence of birth was due to infertility. Gynecological exams revealed that scanty menstruations of 5 of the patients were due to ovarian atrophy. In a study of 360 female gluing operators exposed to petroleum (a major source of benzene) and chlorinated hydrocarbons, both dermally and by inhalation, no significant difference in fertility between exposed workers and unexposed controls was found (Mukhametova and Vozovaya 1972). However, incidences of spontaneous abortion and premature birth increased. Because of study limitations, no conclusions can be drawn concerning adverse reproductive effects of benzene in humans following inhalation exposure. The study limitations included limited follow-up, lack of appropriate control populations, and/or simultaneous exposure to other substances. No adverse effects on reproduction were detected in a study of paternal exposure to benzene in the chemical industry in France (Stucker et al. 1994).

Some effects on testes (atrophy/degeneration, decrease in spermatozoa, moderate increases in abnormal sperm forms) have been noted in experimental animals exposed by inhalation (Ward et al. 1985; Wolf

et al. 1956). Thus, results in humans are inconclusive, and results in animals indicate that inhalation of benzene vapors reduces the number of live fetuses (Murray et al. 1979; Ungvary and Tatrai 1985) and/or the incidences of pregnancy (Gofmekler 1968) only at high doses or not at all.

Evidence of an effect of benzene exposure on human reproduction is not sufficient to demonstrate a causal association. In addition, animal studies only provide suggestive evidence (i.e., effects on testes) that benzene affects reproduction following inhalation exposure, with most studies indicating no effect on reproductive indices even after high doses.

Developmental Effects. Epidemiological studies implicating benzene as a developmental toxicant have many limitations, so it is not possible to assess the effect of benzene on the human fetus. The few studies that do exist are limited by a lack of control incidences for end points, problems in identifying exposed populations, a lack of data on exposure levels, and/or concurrent exposure to multiple substances (Budnick et al. 1984; Forni et al. 1971a; Funes-Cravioto et al 1977; Goldman et al. 1985; Heath 1983; Olsen 1983). In a study conducted in the Love Canal area by Heath (1983), the outcome of pregnancy was evaluated in populations living in the proximity of waste sites where benzene and other hazardous substances had been identified. No clear increase in occurrence of spontaneous abortion, birth defects, or low infant birth weight was observed in women living next to the canal. The study limitations of inadequate sample size and lack of exposure history preclude an assessment of the significance of these findings. In another study conducted by Goldman et al. (1985) on the Love Canal area, birth weight was assessed in 239 infants exposed during gestation life. There was an association between low birth weight and hazardous waste exposure. However, there were inherent problems in the study design and methodology. One of the study groups was comprised of low-income renters and predominantly black individuals. Blacks have a high incidence of low birth weights. Information was retrieved by telephone survey, and some of the low-income renters may have lacked telephones. The methods also failed to include 235 families that were evacuated from the Love Canal area. Finally, no statistically significant clusters of birth defects were found in populations living around the Drake Superfund site in Pennsylvania, an area contaminated with benzene and other carcinogens (Budnick et al. 1984). However, the significance of this finding cannot be determined because of design methodology inadequacies including inadequate sample size and lack of quantification of exposure levels. One toxicokinetic study showed that benzene crosses the human placenta and is present in cord blood in amounts equal to or greater than those in maternal blood (Dowty et al. 1976).

Most of the animal data are from inhalation experiments because this has been the principal route of concern (Coate et al. 1984; Keller and Snyder 1986, 1988; Ungvary and Tatrai 1985). Results of inhalation exposure have been fairly consistent across species; they demonstrate that at high levels benzene is fetotoxic as evidenced by decreased fetal weight and/or minor skeletal variants. It has been suggested that benzene fetotoxicity is a function of maternal toxicity because the occurrence of a decrease in fetal weight and an increase in skeletal variants usually occurs when there is a decrease in maternal weight. However, the mechanisms of this toxicity have not been fully elucidated, and there are few data on the effects of benzene on maternal food consumption and on blood levels of benzene and its metabolites in the dams and their fetuses. None of the animal studies demonstrate that benzene is teratogenic at levels that induce maternal and fetal toxicity. Fetotoxic effects in rodents occurred at benzene levels ≥47 ppm (Coate et al. 1984; Green et al. 1978; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). However, there was evidence of transient hematopoietic anomalies in mice exposed *in utero* to benzene at 5 ppm and 10 ppm (Keller and Snyder 1986, 1988). *In utero* exposure of mice to 20 ppm benzene produced similar hematopoietic anomalies that persisted in 6-week-old animals (Keller and Snyder 1988). Thus, changes in the murine hematopoietic system can result from maternal exposure to low concentrations of benzene. This may have relevance for the human fetus after maternal exposure to benzene in that the human fetus is capable of metabolizing xenobiotics to a greater extent than the mouse fetus, thus possibly forming greater amounts of toxic metabolites responsible for the hematopoietic toxicity of benzene.

There are no data available on the developmental effects of benzene in humans or animals following dermal exposures. Oral data are limited to 2 animal studies. Benzene was shown to reduce pup body weight when mice were administered a high oral dose of 1,300 mg/kg/day during gestation (Seidenberg et al. 1986), indicating that benzene may induce fetotoxic effects in humans at high doses. However, no fetotoxicity was reported when rats were dosed orally with 1,000 mg/kg/day during gestation (Exxon 1986).

Benzene has been shown to be embryotoxic to rat embryos *in vitro* (Brown-Woodman et al. 1994). Day 10 Sprague-Dawley rat embryos were exposed to benzene in culture for 2 days, then evaluated. Benzene at 3.14 μmole/mL caused a significant decrease in yolk sac diameter and protein content of the embryo; crown-rump length and somite number were decreased at both 1.56 and 3.14 μmole/mL benzene. A no-effect level of 0.82 μmole/mL was determined. In another study of cultured rat embryos, Chapman et al. (1994) evaluated the embryotoxic effects of benzene and several of its

metabolites. Benzene at 1.6 mM produced little embryotoxicity, with or without hepatic activating enzymes, but phenol showed significant embryotoxicity in the presence of hepatic activation at concentrations as low as 0.01 nM. *Trans,trans*s-muconaldehyde was embryotoxic at 0.01 mM and embryolethal at 0.05 nM; hydroquinone, catechol, and benzoquinone were all 100% embryolethal at 0.1 mM.

Based on the available data, other than the possibility of hematological effects in the offspring, it is unlikely that persons living near hazardous waste sites are exposed to levels of benzene in the air, water, or soil high enough to cause fetotoxic effects.

Genotoxic Effects. The genotoxic effects of benzene have been studied extensively. The results are summarized in Tables 2-5 and 2-6. Benzene and/or its metabolites seem to be genotoxic to humans, causing primarily chromosomal aberrations (Ding et al. 1983; Forni and Moreo 1967, 1969; Forni et al. 1971a; Hartwich et al. 1969; Hedli et al. 1990; Karacic et al. 1995; Major et al. 1994; Picciano 1979; Popp et al. 1992; Rothman et al. 1995; Sardas et al. 1994; Sasiadek et al. 1989; Sellyei and Kelemen 1971; Tompa et al. 1994; Tough and Court-Brown 1965; Tough et al. 1970; Turkel and Egeh 1994; Van den Berghe et al. 1979). Peripheral lymphocytes and bone marrow cells appear to be the major targets. Inhalation, oral, and dermal routes are all potential pathways of exposure relevant to humans. However, available case-control studies reflect only inhalation and/or dermal exposure. This is because these studies are usually drawn from occupational settings in which inhalation and dermal exposure routes are most prevalent. In most of these studies, chromosome abnormalities were detected in workers exposed to high concentrations of benzene, sufficient to produce blood dyscrasias. The limitations of the occupational studies include lack of accurate exposure data, possible coexposure to other chemicals, and lack of appropriate control groups. The majority of these studies suggest that benzene is genotoxic, specifically clastogenic (i.e., capable of breaking the DNA molecule in a manner observable at the chromosome level).

Case studies involving individuals who were previously chronically exposed to benzene report significant chromosomal aberrations in bone marrow and lymphocytes (Forni and Moreo 1967, 1969; Hartwich et al. 1969; Major et al. 1992; Picciano 1979; Sellyei and Kelemen 1971; Tough and Court-Brown 1965; Tough et al. 1970; Van den Berghe et al. 1979; Yardley-Jones et al. 1990). Other mutagens in addition to benzene may have been involved since most of the workers were occupationally exposed to more than one chemical.

Table 2-5. Genotoxicity of Benzene *In Vivo*

Species (test system)	End point	Results	Reference
Prokaryotic cells:			
Escherichia coli (host mediated DNA repair)	DNA synthesis	–	Hellmer and Bolcsfoldi 1992a
Invertebrate animal cells:			
Drosophila melanogaster	Sex-linked recessive lethal	–	Kale and Baum 1983
D. melanogaster (spermatocytes)	Recombination	–	Kale and Baum 1983
D. melanogaster (spermatogonia)	Recombination	+	Kale and Baum 1983
D. melanogaster (spermatocytes)	Heritable translocation	–	Kale and Baum 1983
Mammalian cells:			
Mouse (spleen lymphocytes)	Chromosomal aberrations	+	Rithidech et al. 1987
Mouse (spleen lymphocytes)	Chromosomal aberrations	+	Au et al. 1991a
Mouse (spleen lymphocytes)	Mutations	+	Ward et al. 1992
Mouse (peripheral erythrocytes)	Micronucleus increase	+	Hayashi et al. 1992
Mouse (bone marrow)	Chromosomal aberrations	(+)	Tice et al. 1982
Mouse (bone marrow)	Chromosomal aberrations	(+)	Tice et al. 1980
Mouse (bone marrow)	Chromosomal aberrations	+	Siou et al. 1981
Mouse (bone marrow)	Chromosomal aberrations	+[a]	Meyne and Legator 1980
Mouse (bone marrow)	Chromosomal aberrations	+	Shelby and Witt 1995
Mouse (bone marrow)	Micronucleus increase	+	Shelby et al. 1993
Mouse (bone marrow)	Micronucleus increase	+	Shelby and Witt 1995
Mouse (bone marrow)	DNA adducts	–	Reddy et al. 1994
Mouse (bone marrow)	DNA adducts	+	Pathak et al. 1995
Mouse (liver)	DNA adducts	–	Reddy et al. 1994
Mouse (mammary gland)	DNA adducts	–	Reddy et al. 1994
Rat (bone marrow)	DNA oxidative damage	+	Kolachana et al. 1993
Rat (bone marrow)	Chromosomal aberrations	+	Styles and Richardson 1984

Table 2-5. Genotoxicity of Benzene *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Rat (bone marrow)	Chromosomal aberrations	+[b]	Anderson and Richardson 1981
Rat (bone marrow)	Chromosomal aberrations	+	Philip and Jensen 1970
Rat (bone marrow)	Chromosomal aberrations	+	Fujie et al. 1990
Rat (bone marrow)	Chromosomal aberrations	+	Hoechst 1977
Rat (bone marrow)	Chromosomal aberrations	−	Hoechst 1977
Chinese hamster (bone marrow)	Chromosomal aberrations	+	Siou et al. 1981
Rabbit (bone marrow)	Chromosomal aberrations	+	Kissling and Speck 1972
Rabbit (bone marrow)	Chromosomal aberrations	+	Kissling and Speck 1973
Human (lymphocytes)	Micronucleus increase	+	Robertson et al. 1991a
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	(+)	Yardley-Jones et al. 1990
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Sasiadek et al. 1989
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	−	Jablonická et al. 1987
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Forni et al. 1971a
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Ding et al. 1983
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Tough and Court Brown 1965
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Picciano 1979
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	+	Popp et al. 1992
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Tompa et al. 1994
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Sasiadek 1992
Human (occupational exposure/lymphocytes)	Chromosomal aberrations	+	Sasiadek and Jagielski 1990
Mouse (spleen lymphocytes)	Increased polyploidy	+	Rithidech et al. 1987
Mouse (peripheral blood PCEs)	Micronuclei increase	+[c,d]	Luke et al. 1988a
Mouse (peripheral blood NCEs)	Micronuclei increase	+[d,e]	Luke et al. 1988a
Mouse (peripheral blood NCEs)	Micronuclei increase	+	Rithidech et al. 1988
Mouse[f] (bone marrow PCEs)	Micronuclei increase	+[g]	Suzuki et al. 1989[h]

Table 2-5. Genotoxicity of Benzene *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Mouse (bone marrow PCEs)	Micronuclei increase	+[d]	Ciranni et al. 1988
Mouse (pregnant/bone marrow PCEs)	Micronuclei increase	(+)	Ciranni et al. 1988
Mouse (fetus/liver cells)	Micronuclei increase	+	Ciranni et al. 1988
Mouse (bone marrow PCEs)	Micronuclei increase	+	Erexson et al. 1986
Mouse (bone marrow PCEs)	Micronuclei increase	+	Toft et al. 1982
Mouse (bone marrow PCEs)	Micronuclei increase	+	Harper et al. 1984
Mouse (bone marrow PCEs)	Micronuclei increase	+	Siou et al. 1981
Mouse (bone marrow PCEs)	Micronuclei increase	+[a]	Meyne and Legator 1980
Mouse (bone marrow PCEs)	Micronuclei increase	+	Hite et al. 1980
Mouse (bone marrow PCEs)	Micronuclei increase	+	Barale et al. 1985
Mouse (bone marrow PCEs)	Micronuclei increase	+	Au et al. 1990
Mouse (peripheral blood NCEs)	Micronuclei increase	+	Barale et al. 1985
Mouse (peripheral blood NCEs)	Micronuclei increase	+	Choy et al. 1985
Mouse (bone marrow PCEs)	Micronuclei increase	+	Diaz et al. 1980
Chinese hamster (bone marrow)	Micronuclei increase	+	Siou et al. 1981
Rat (lymphocytes)	Micronuclei increase	+	Erexson et al. 1986
Mouse (bone marrow)	Sister chromatid exchange	+	Tice et al. 1982
Mouse (bone marrow)	Sister chromatid exchange	+	Tice et al. 1980
Mouse (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Mouse (pregnant/bone marrow)	Sister chromatid exchange	+	Sharma et al. 1985
Mouse (fetus/liver cells)	Sister chromatid exchange	+	Sharma et al. 1985
Rat (lymphocytes)	Sister chromatid exchange	+	Erexson et al. 1986
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	−	Yardley-Jones et al. 1988
Human (occupational exposure/lymphocytes)	Sister chromatid exchange	−	Seiji et al. 1990
Mouse (bone marrow)	DNA synthesis inhibition	+	Lee et al. 1988
Rabbit (bone marrow)	DNA synthesis inhibition	+	Kissling and Speck 1972
Mouse (bone marrow)	RNA synthesis inhibition	+	Kissling and Speck 1972

Table 2-5. Genotoxicity of Benzene *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Rat (liver mitochondria)	RNA synthesis inhibition	+	Kalf et al. 1982
Rat (liver cells)	DNA adducts	+	Lutz and Schlatter 1977
Mouse (spermatogonia)	Sperm head abnormality	+	Topham 1980

[a]This result was observed following both oral and intraperitoneal exposure.
[b]This result was observed following both inhalation and intraperitoneal exposure.
[c]Increase in micronuclei was exposure duration-independent.
[d]Males affected to a significantly greater degree than females.
[e]Increase in micronuclei was exposure duration-dependent.
[f]Two strains of mouse were tested; Ms/Ae and CD-1. The result applies to both strains.
[g]This result was observed following both oral and intraperitoneal exposure; however, oral exposure produced the greater effect.
[h]The authors of this study did not report statistical comparisons of their results.

+ = Positive result; − = negative result; (+) = weakly positive result; DNA = deoxyribonucleic acid; NCEs = normochromatic erythrocytes; PCEs = polychromatic erythrocytes; RNA = ribonucleic acid

Table 2-6. Genotoxicity of Benzene *In Vitro*

Species (test system)	End point	Result With activation	Result Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium (Ames test)	Gene mutation	–	–	De Flora et al. 1984
S. typhimurium (histidine reversion)	Gene mutation	+	–	Glatt et al. 1989
S. typhimurium (azaquanine reversion)	Gene mutation	+	No data	Seixas et al. 1982
S. typhimurium (azaquanine reversion)	Gene mutation	+	No data	Kaden et al. 1979
Bacillus subtilis (histidine reversion)	Gene mutation	–	–	Tannoka 1977
Escherichia coli (DNA polymerase 1/cell-free DNA synthetic system)	DNA synthesis inhibition	No data	–	Lee et al. 1988
E. coli (host meditated DNA repair)	DNA synthesis	No data	No data	Hellmer and Bolcsfoldi 1992b
Plasmid DNA *ϕ*X-174 RF I	DNA degradation	No data	+	Li et al. 1995
Eukaryotic organisms:				
Fungi:				
Aspergillus nidulans (methionine supressors)	Gene mutation	No data	–	Crebelli et al. 1986
Mammalian cells:				
Mouse (L5178Y cells/TK test)	Gene mutation	–	–	Oberly et al. 1984
Chinese hamster (ovary cell culture)	Chromosomal aberrations	–	–	Gulati et al. 1989
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	+	Morimoto 1976
Human (lymphoblastoid culture)	Intrachromosomal recombination	No data	+	Aubrecht et al. 1995
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	+	Eastmond et al. 1994
Human (lymphocyte cell culture)	Chromosomal aberrations	No data	–	Gerner-Smidt and Friedrich 1978
Human (leukemia cells)	DNA oxidative damage	No data	+	Kolachana et al. 1993
Chinese hamster (ovary cell culture)	Sister chromatid exchange	–	–	Gulati et al. 1989
Chinese hamster (ovary cell culture)	Sister chromatid exchange	–	–	Douglas et al. 1985
Human (lymphocyte cell culture)	Sister chromatid exchange	+	No data	Morimoto 1983
Human (lymphocyte cell culture)	Sister chromatid exchange	No data	–	Gerner-Smidt and Friedrich 1978
Chinese hamster (ovary cell culture)	Micronuclei increase	–	–	Douglas et al. 1985
Human (bone marrow)	DNA adducts	No data	+	Bodell et al. 1993
Human (leukemia cells)	DNA adducts	No data	+	Bodell et al. 1993
Human (bone marrow)	DNA adducts	No data	+	Levay and Bodell 1992
Human (leukemia cells)	DNA adducts	No data	+	Levay and Bodell 1992

Table 2-6. Genotoxicity of Benzene *In Vitro* (continued)

Species (test system)	End point	Result With activation	Result Without activation	Reference
Calf thymus DNA	DNA adducts	No data	+	Chenna et al. 1995
Rabbit (bone marrow mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Rat (liver mitoplasts)	DNA adducts	No data	+	Rushmore et al. 1984
Rat liver epithelial cells	DNA hyperphosphorylatioin	No data	+	Dees and Travis 1994
Rat (hepatocytes)	DNA breaks	No data	−	Bradley 1985
Chinese hamster (V79 cell culture)	DNA breaks	−	−	Swenburg et al. 1976
Chinese hamster (ovary cell culture)	DNA breaks	+	+	Douglas et al. 1985
Chinese hamster (ovary cell culture)	DNA breaks	+	+[a]	Lakhanisky and Hendricks 1985
Mouse (L5178Y cell culture)	DNA breaks	No data	−	Pellack-Walker and Blumer 1986
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	−	Probst and Hill 1985
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	−	Williams et al. 1985
Rat (hepatocyte culture)	Unscheduled DNA synthesis	No data	(+)	Glauert et al. 1985
Human (HeLa S3 cells)	Unscheduled DNA synthesis	−	−	Barrett 1985
Mouse (bone marrow cell culture)	DNA synthesis inhibition	No data	+	Lee et al. 1988
Mouse (bone marrow cell culture)	DNA synthesis inhibition	+	(+)	Lee et al. 1989
Calf (thymus DNA polymerase α/cell-free DNA synthetic system)	DNA synthesis inhibition	No data	+	Lee et al. 1988
Human (HeLa cells)	DNA synthesis inhibition	−	−	Painter and Howard 1982
Mouse (spleen lymphocytes)	RNA synthesis inhibition	No data	+	Post et al. 1985
Rat (liver mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Rabbit (bone marrow mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982
Cat (bone marrow mitoplasts)	RNA synthesis inhibition	No data	+	Kalf et al. 1982

[a]Benzene's effect on DNA breaks was reduced when metabolic activators were used.

− = negative results; + = positive results; (+) = weakly positive result; DNA = deoxyribonucleic acid; RNA = ribonucleic acid

In one epidemiological study testing benzene's effects on chromosomal aberrations, benzene appeared to exert a nonrandom effect on chromosomes one, two, four, and nine (Sasiadek et al. 1989). However, this study is limited because relatively few controls were used and all participants (control and exposed) were smokers. The effects of smoking may have confounded the results. In a later paper, extra break points were confirmed on chromosomes 2 and 4, only (Sasiadek and Jagielski 1990). Sister chromatid exchange was not found to be a significant effect of benzene exposure in humans (Seiji et al. 1990; Yardley-Jones et al. 1988); however, poor control selection was used in both studies. Refer to Table 2-5 for a further summary of these results.

In vivo animal studies provide convincing evidence of benzene's genotoxicity (Table 2-5). Furthermore, the finding that male mice are more sensitive than females to benzene-induced chromosomal damage is consistent among reports (Choy et al. 1985). Consistently positive findings for chromosomal aberrations in bone marrow and lymphocytes support the human case reports and epidemiological studies in which chromosomal damage was linked to benzene exposure. Micronuclei tests are popular methods for crudely analyzing DNA damage in animals. Positive results were observed in all studies testing for increased micronuclei frequencies. One of these micronuclei studies investigated the effects of different inhalation exposure durations on PCEs and NCEs in peripheral blood. The researchers found that PCEs are good indicators of recent and acute exposure, while NCEs are good indicators of accumulated long-term exposure (Luke et al. 1988a). Although no human studies were located that reported increased sister chromatid exchange in exposed individuals, increases in sister chromatid exchange were reported in mice and rats (Erexson et al. 1986; Sharma et al. 1985; Tice et al. 1980, 1982). *In vivo*, *trans,trans*-muconaldehyde (a metabolite of benzene) has also been shown to induce highly significant increases in sister chromatid exchanges in mice (Witz et al. 1990). In addition to oral and inhalation routes, many researchers tested subcutaneous and intraperitoneal routes as well; the results for these alternate routes of exposure were largely positive for chromosomal aberrations in bone marrow (Anderson and Richardson 1981; Kissling and Speck 1972, 1973; Kolachana et al. 1993; Meyne and Legator 1980; Philip and Jensen 1970), micronuclei in bone marrow (Diaz et al. 1980), and sister chromatid exchange in mouse fetus liver cells (Sharma et al. 1985). However, one study of intraperitoneal treatment of B6C3F$_1$ mice for 4 days with 500 mg/kg benzene in olive oil showed no DNA adduct formation in the liver, mammary gland, and bone marrow (Reddy et al. 1994). One study using intraperitoneal injections reported a dose-dependent increase in sperm head abnormalities in mice exposed to 0.5 or 0.6 mL benzene/kg/day (Topham 1980). The author views sperm head abnormality as a possible indication of heritable mutations. However, from

this study alone, it cannot be determined if benzene causes such transmissible genetic mutations. For these and other results from animal *in vivo* studies. see Table 2-5.

In vitro studies strongly imply that benzene's genotoxicity is derived primarily from its metabolites. Positive results were obtained for gene mutation in *Salmonella typhimurium* (Glatt et al. 1989; Kaden et al. 1979; Seixas et al. 1982) and sister chromatid exchange in human lymphocyte cell culture (Morimoto 1983) only when exogenous metabolic activators of benzene were used. Similarly, endogenous metabolic activation was required for effects to be seen on DNA synthesis in rat hepatocyte culture (Glauert et al. 1985), DNA adduct formation in rat liver mitoplasts (Rushmore et al. 1984), and RNA synthesis in rat liver mitoplasts and in rabbit and cat bone marrow mitoplasts (Kalf et al. 1982). Endogenous activation occurs naturally by enzymes already within the cells. Exogenous activation requires the addition of enzymes to cellular preparations. In one study using benzene and 13 possible metabolites, Glatt et al. (1989) found that *trans*-1,2-dihydrodiol (with metabolic activators) and the diol epoxides (with or without metabolic activators) produced histidine reversion in *S. typhimurium.* The same researchers also investigated the genotoxicity of these 13 proposed metabolites in V79 Chinese hamster cells; anti-diol epoxide, syn-diol epoxide, 1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, quinone, hydroquinone, catechol, phenol, and 1,2-dihydrodiol were found to produce genotoxic effects ranging from sister chromatid exchange and micronuclei increases to gene mutations (Glatt et al. 1989). Similar studies with truns,trans-muconaldehyde showed that this metabolite is strongly mutagenic in V79 cells and weakly mutagenic in bacteria (Glatt and Witz 1990). Chang et al. (1994) showed that muconaldehyde and its aldehyde metabolites 6-hydroxy-*trans,trans*-2,4-hexadienal and 6-oxo-*trans,trans*-hexadienoic acid were mutagenic in V79 cells. Another study discovered a possible connection between certain benzene metabolites and DNA damage via the formation of oxygen radicals (Lewis et al. 1988a). Researchers found that both 1,2,4-benzenetriol (BT) and hydroquinone (HQ) produce DNA strand breaks, BT to a greater degree than HQ. Consistent with these findings was the observation that BT generates a greater concentration of oxygen radicals than HQ. The study concluded that BT damages DNA by producing oxygen radicals, while HQ probably exerts its genotoxic effects by some other mechanism (Lewis et al. 1988a): Li et al. (1995) has proposed that hydroquinone exerts its genotoxic effect through the copper-mediated generation of semiquinone radicals that are responsible for DNA damage.

Chromosomal breaks and hyperdiploidy were also observed in human lymphocytes after exposure to hydroquinone *in vitro* (Eastmond et al. 1994). Benzene metabolites have been shown to form DNA

adducts in human bone marrow and HL-60 cells (Bode11 et al. 1993; Levay and Bode11 1992). Zhang et al. (1993) showed that 1,2,4-benzenetriol increased the frequency of micronuclei formation in human lymphocytes in culture, and in HL60 cells. The increase was dose-related. An increase in the level of oxidative damage to DNA was also noted in HL60 cells in culture. Extracts from human cells have been shown to have repair activity toward benzoquinone-DNA adducts *in vitro* (Chenna et al. 1995).

Chen and Eastmond (1995) showed that benzene metabolites can adversely affect human topoisomerases, enzymes involved in DNA replication and repair. No effect of any metabolite was seen on human topoisomerase I or for topoisomerase II for hydroquinone, phenol, 2,2'-biphenol, 4,4'-biphenol and catechol at concentrations as high as 500 μM. 1,4-Benzoquinone and 1,2,4-benzenetriol inhibited human topoisomerase II *in vitro,* at 500 and 250 μM without bioactivation. However, following bioactivation, phenol and 2,2'-biphenol showed inhibitory effects at doses as low as 50 μM, whereas 4,4'-biphenol inhibited topoisomerase II at concentrations of 10 μM.

Data also exist that suggest that benzene itself is genotoxic. Two studies reported that benzene produced DNA breaks in Chinese hamster ovary cells independent of metabolic activators (Douglas et al. 1985; Lakhanisky and Hendrickx 1985). In a study by Aubrecht et al. (1995), benzene was shown to induce intrachromosomal recombination in human lymphoblastoid cell culture. Therefore, benzene appears to have some genotoxic capabilities of its own, but its metabolites seem to be the primary genotoxins in systems in which normal metabolism is occurring. Refer to Table 2-6 for the results of these and other *in vitro* studies.

In summary, both structural and numerical chromosome aberrations have been found consistently in bone marrow cells of persons occupationally exposed to benzene. The conclusion, based on human epidemiological studies, that benzene is a human clastogen is well supported by *in vivo* animal studies and *in vitro* cell cultures and subcellular studies. Virtually all studies that looked for effects at the chromosomal level were positive when the ability to metabolize benzene was present. These experimental results are consistent with the chromosomal damage seen in exposed humans (see Sections 2.2.1.7, 2.2.2.7, and 2.2.3.7). The leukemia observed in some benzene-exposed persons may result from the appearance of a clone of chromosomally abnormal cells in the bone marrow. With respect to genetic effects, no safe human exposure level can be determined from available epidemiological data. Significant increases in sister chromatid exchanges were produced in bone

marrow cells and lymphocytes of animals. The significance of sister chromatid exchanges is unknown, but their production by a chemical is generally considered to indicate a genotoxic potential. Exposures generally occur via inhalation, and based on animal studies, effects following oral exposure may be greater than effects following inhalation exposure to comparable levels of benzene. Data presented in this section and elsewhere in this profile (Section 2.3) show that benzene metabolites are the genotoxic entities. It is possible that each metabolite causes a different genotoxic effect. Differences in metabolic capability are probably responsible for some of the variations in response to benzene seen in different test systems.

Cancer. It has been established that exposure to commercial benzene or benzene-containing mixtures can cause damage to the hematopoietic system including pancytopenia with subsequent manifestation of acute myelogenous leukemia (AML) (Aksoy et al. 1971, 1972, 1974). Numerous review articles can be found in the open literature that discuss benzene carcinogenesis (e.g., Aksoy 1991; Cox 1991; Gold et al. 1993; Snyder and Kalf 1994; Snyder et al. 1993b). In AML, there is diminished production of normal erythrocytes, granulocytes, and platelets, which leads to death by anemia, infection, or hemorrhage. The hallmark of AML is the appearance in the peripheral blood of cells morphologically indistinguishable from myeloblasts. These events can be rapid. Case reports and epidemiological studies of workers have established a causal relationship between benzene exposure and AML (Aksoy and Erdem 1978; Crump 1994; Infante 1978; Infante et al. 1977; Ott et al. 1978; Paxton et al. 1994a, 1994b; Rinsky et al. 1987; Travis et al. 1994; Wong 1987a, 198713, 1995; Wong and Raabe 1995). A series of studies (Infante 1978; Infante et al. 1977; Rinsky et al. 1981, 1987) analyzing the mortality of workers exposed to benzene at two rubber hydrochloride manufacturing locations demonstrated excess risk of leukemia. The most recent study demonstrated SMRs of 337 for leukemia and 409 for multiple myeloma. (An SMR of 100 is the normal value if an excess is not observed. An SMR of 200 represents 100% excess risk over normal.) The Rinsky et al. (1987) study included excellent follow-up; 96.6% of the employees in this cohort were traced to determine whether they were alive or dead and, if dead, the cause of death. The study also carefully analyzed extensive past-exposure data and was able to assign qualitative exposure levels-to individuals exposed l0-40 years in the past. The Rinsky et al. (1987) study also demonstrated a qualitative (i.e., ppm-years) dose-response relationship. The SMRs for exposed workers ranged from 105 to 6,637. The dose-response relationship increases the confidence in the results, provides a stronger basis for risk assessment, and provides measured (as opposed to extrapolated) evidence that lowering

exposure substantially reduces risk. Recent updates of this study support the original conclusions (Paxton et al. 1994a, 1994b).

Patients with MDS (disorders of stem cells characterized by maturation defects) may die of infection or hemorrhage (due to platelet/clotting abnormalities), or their condition may progress to acute leukemia. In a review by Rinsky et al. (1989), it was pointed out that the majority of benzene workers who died from hematologic disorders, including aplastic anemia, acute myelocytic anemia, and blood dyscrasia with secondary anemia and leukemoid reaction, may actually have been MDS patients, (i.e., patients whose stem cells had undergone malignant transformation, but who died of anemia plus complications before frank acute leukemia became manifest). MDS patients should be included in the population at risk of developing leukemia if a significant time interval (years) has elapsed between the last benzene exposure and death.

While some studies have implicated other types of leukemia or even lymphomas, only AML and its variants have consistently been in excess in groups of workers with high benzene exposure (Goldstein 1988).

Benzene has also been shown to cause leukemia and lymphoma in laboratory animals. Snyder and coworkers (Snyder et al. 1980, 1988) administered a continuous lifetime inhalation concentration of 300 ppm benzene to C57BL/6 male mice and observed a significant increase in hematopoietic neoplasms that included 6 cases (15%) of thymic lymphomas. Myelogenous leukemia was found in Sprague-Dawley rats exposed to 100 ppm for life (Snyder et al. 1984). A highly significant increase in thymic and nonthymic lymphomas was found in C57BL/6 mice exposed to 300 ppm (Cronkite et al. 1984, 1985), and leukemia was found in CBA/Ca mice exposed to ≥100 ppm (Cronkite et al. 1986, 1989) of benzene by inhalation for 16 weeks. Lymphoma was also observed in CBA/Ca mice exposed to 300 ppm benzene for 16 weeks (Farris et al. 1993) and in $B6C3F_1$ mice treated by gavage with benzene at ≥25 mg/kg/day for 2 years (NTP 1986).

Experimental data in animals and studies of human cases of benzene intoxication indicate a link between the decrease in bone marrow cellularity and the development of leukemia. Many cases of benzene-induced leukemia appear to have been preceded by aplastic anemia (Toft et al. 1982). The compensatory response (regenerative hyperplasia) observed in the bone marrow, thymus, and spleen of

exposed animals may play a role in the carcinogenic response (Rozen and Snyder 1985; Snyder 1987; Snyder and Koscis 1975; Snyder et al. 1984).

One of the favored mechanisms for benzene toxicity or carcinogenicity is related to the covalent binding of benzene metabolites to cellular macromolecules. In mice administered radiolabeled benzene for relatively short durations, metabolites have been found covalently bound to liver, bone marrow, kidney, spleen, blood, and fat (Bechtold and Henderson 1993; Bechtold et al. 1992a, 1992b; Gill and Ahmed 1981; Irons et al. 1980; Longacre et al. 1981a; McDonald et al. 1994; Snyder et al. 1978b). The label was bound to the nucleic acids and other macromolecules of the mitochondria (Gill and Ahmed 1981), and as the levels of covalently bound and water-soluble metabolites increased, so did benzene toxicity (Longacre et al. 1981a). Hydroquinone and catechol may accumulate in bone marrow to act as substrates for myeloperoxidase, forming metabolites that could bind to protein (Eastmond et al. 1987; Kalf et al. 1990; Snyder et al. 1993b; Subrahmanyam et al. 1991). Since myeloperoxidase activity has been confirmed in both murine and human hematopoietic progenitor cells (Shattenberg et al. 1994), this mechanism of toxicity is of more interest.

Phenol, hydroquinone, catechol, benzoquinone, and 1,2,4-trihydroxybenzene form adducts in bone marrow mitochondria, resulting in the inhibition of the synthesis of mitochondrial proteins that are necessary for mitochondrial function (Kalf et al. 1982).

Recent research has focused on the molecular mechanism by which benzene metabolites adversely affect cells. Production of reactive oxygen species that may bind to DNA (Rao 1991), formation of chelates with iron that may act as DNA cleaving agents (Rao 1996; Singh et al. 1994), causing the release of iron from ferritin (Ahmad et al. 1995), and producing reactive products from glutamate or DNA in the presence of copper (Rao and Pandya 1989) are only a few of the areas of investigation into the mechanism of toxicity of benzene metabolites.

Hydroquinone has been extensively studied. Carbonnelle et al. (1995) have investigated the effect of hydroquinone on IL-l release from human monocytes, in an effort to show that reduced interleukin release my inhibit cytokine release by monocytes, thereby adversely affecting the regulation of hematopoiesis. Support for this mechanism comes from the work of Miller et al. (1994) who showed that hydroquinone decreased the enzyme responsible for the conversion of interleukin precursors in murine bone marrow macrophages. In addition, hydroquinone has been shown to alter differentiation

of myeloid progenitor cells which may be important to benzene leukemogenesis (Irons and Stillman 1993; Irons et al. 1992).

Nitric oxide, which has been shown to suppress cell growth and form reactive intermediates that lead to carcinogenic products, has been detected in bone marrow cells treated with benzene (Laskin et al. 1995; Punjabi et al. 1994) and may be important to the mechanism of benzene toxicity.

The ultimate mechanism for benzene-induced suppression of cellular growth and the development of leukemia is not known at this time; it could be one of the mechanisms discussed above (see also Section 2.4.2) but most likely the mechanism involves a combination of factors. Identification of the mechanism would be facilitated by clear identification of the specific target cell and intracellular target of benzene and its metabolites (Andrews and Snyder 1986).

Although only acute myelogenous leukemia and its variants have consistently been in excess in groups of workers exposed to benzene, other studies in humans also suggest increased risks of kidney, brain, and bladder cancer and soft-tissue sarcoma (Greenland et al. 1994; Hunting et al. 1995; Lagoreost et al. 1994b; Serraino et al. 1992; Steineck et al. 1990). Furthermore, benzene is a multipotential carcinogen in animals, as evidenced by the array of treatment-related increased incidences of neoplasms in rats and mice exposed by inhalation or orally (Cronkite et al. 1989; Huff et al. 1989; Maltoni et al. 1982a, 1982b, 1983, 1985; NTP 1986; Snyder et al. 1984). These include cancers of the liver, Zymbal gland, harderian gland, skin, mammary gland, lungs, oral cavity, nasal cavity, forestomach, ovary, and preputial gland.

Benzene is considered to be a human carcinogen by the Department of Health and Human Services (DHHS), International Agency for Research on Cancer (IARC), EPA, American Conference of Governmental and Industrial Hygienists (ACGIH) and OSHA. The DHHS has determined that benzene is a known human carcinogen (NTP 1994). EPA (IRIS 1996) has verified the weight-of-evidence classification for carcinogenicity of benzene as EPA Group A, based on a sufficient level of human evidence supported by a sufficient level of animal evidence. The American Conference of Governmental Industrial Hygienists (ACGIH) classified benzene as an Al-substance-a confirmed human carcinogen (ACGIH 1996a). There are still questions regarding both the mechanisms of benzene carcinogenesis and the most appropriate models for developing human risk estimates. EPA (IRIS 1996) has reviewed the human and animal carcinogenicity data on benzene. The following

paragraphs are a summary of the EPA calculations of unit risk values for leukemia based on human epidemiological studies. It should be noted that these values are estimates of human risk since the true human risk at low doses cannot be accurately identified.

The cancer potency of benzene has been estimated based on three separate epidemiological studies (Ott et al. 1978; Rinsky et al. 1981; Wong et al. 1983). Equal weight was given to cumulative dose and weighted cumulative dose, as well as relative and absolute model forms. EPA (IRIS 1996) estimated a unit risk value of 2.7×10^{-4} for leukemia from a lifetime inhalation exposure to 1 ppm benzene. Based on this value, the exposure levels associated with individual lifetime upper-bound risks of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} have been calculated to be 4×10^{-3}, 4×10^{-4}, 4×10^{-5}, and 4×10^{-6} ppm, respectively. The unit risk should not be used if the air concentration exceeds $100\ \mu g/m^3$ (31 ppb) since above this the unit risk may not be appropriate. One ppm of benzene ($3,190\ \mu g/m^3$) exceeds the upper limit of 31 ppb. A peer review workshop conducted in 1989 to review the EPA's risk assessment of benzene, recommended that the epidemiological studies, including their updates, be reviewed, with consideration given to appropriate disease end points, dose-response model, the use of animal as opposed to human data, relative as opposed to absolute risk, and other factors (Bond et al. 1986b; Rinsky et al. 1987; Voytek and Thorslund 1991; Wong 1987a). Additional independent reviews of the epidemiological data and risk assessment can also be found in the open literature (Brett et al. 1989; Cox and Ricci 1992; Crump 1994; Crump et al. 1993; Hallenbeck and Flowers 1992; Hawkins 1991; Johnson and Lucier 1992; Paxman and Rappaport 1990).

EPA has used cancer risk data from human epidemiological studies to derive risk factors associated with oral exposure to benzene. The oral dose levels associated with specific carcinogenic risks have been extrapolated by converting the risk value of 2.7×10^{-2} for a lifetime inhalation exposure to 1 ppm to a slope factor of 2.9×10^{-2} for oral exposure of 1 mg/kg/day and assuming identical levels of absorption of benzene into the body. The slope factor corresponds to a drinking water unit risk of 8.3×10^{-3} per mg/L (ppm). Using the method described in the EPA report (IRIS 1996), the dose levels associated with individual upper-bound estimates of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} have been-calculated to be 1×10^{-1}, 1×10^{-2}, 1×10^{-3}, and 1×10^{-4} mg/L (ppm), respectively, which are equivalent to dose levels of 3×10^{-3}, 3×10^{-4}, 3×10^{-5} and 3×10^{-6} mg/kg/day, respectively. The unit risk should not be used if the water concentration exceeds $10,000\ \mu g/L$ (ppb) since above this concentration the slope factor may no longer be accurate.

Based on the above information, it is reasonable to assume that benzene could cause cancer in humans if inhaled or ingested in sufficient quantities. The increased risk that individuals may have by living near waste sites through contaminated air, drinking water, or soil cannot be ascertained until exposure estimates of benzene at these locations become better defined.

2.6   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).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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 (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 benzene are discussed in Section 2.6.1.

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

be directly adverse but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by benzene are discussed in Section 2.6.2.

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 2.8, Populations That Are Unusually Susceptible.

2.6.1   Biomarkers Used to Identify or Quantify Exposure to Benzene

Biomarkers of exposure are available for benzene, and have been reviewed (Ong and Lee 1994). Adequate monitoring of benzene exposure may depend on the use of a series of biomarkers with correlation of the results. Endpoints specific for benzene and endpoints not specific for benzene have been used. One non-specific test is the urinary sulfate ratio test, which is based on the premise that with increasing exposure there will be an increase in benzene metabolites conjugated with sulfate moieties (Hammond and Herman 1960). Estimates of benzene exposure can be made by comparing the ratio of inorganic to organic sulfates in the urine. Inorganic sulfate levels amounting to 80-95% of total urinary sulfates are considered normal background, 70-80% levels indicate some exposure to benzene, 60-70% levels indicate a dangerous level of exposure, and 0-60% levels indicate an extremely hazardous exposure. Urinary sulfate levels are, however, quite variable, and they have not been used to identify exposure levels of benzene associated with minimal toxic effects. Additionally, the test for urinary sulfates is not specific for benzene.

Urinary phenol measurements have routinely been used for monitoring occupational exposure to benzene (OSHA 1987b), and there is some evidence that urinary phenol levels can be correlated with exposure level (Astier 1992; Inoue et al. 1986, 1988b; Karacic et al. 1987; Pagnotto et al. 1961; Pekari et al. 1992). However, correlating urinary phenol with benzene exposure is complicated-by potentially high and variable background levels of phenol that result from ingestion of vegetables, exposure to other aromatic compounds, ingestion of ethanol, and inhalation of cigarette smoke (Nakajima et al. 1987). Relatively high urinary phenol levels (5-42 mg/L) have also been found in persons with no known exposure to benzene (NIOSH 1974). In addition, coexposure to toluene, a common solvent, has been shown to inhibit transformation of benzene to phenol (Inoue et al. 1988b).

Changes in urinary phenol levels (pre- and post-shift sample results) were observed in examinations carried out on 33 female workers (23-54 years old) employed at a shoe factory for 5-30 years (Karacic et al. 1987). Their exposure was to solvents, glues, and paints known to contain benzene. Workers were known to be coexposed to toluene, which was listed as an ingredient of several of the glues. Urine samples were collected on Monday mornings before work (M_1) and on Wednesdays before work (W_1) and after work (W_{11}). Additionally, venous blood was taken on W_{11} only. All samples were coupled with controls from 29 females (19-58 years old) who were not occupationally exposed to solvents. Results indicated there was no difference in the urinary phenol concentrations measured on M_1 and W_1, but W_{11} was about 2 times that of W_1. Both benzene and toluene were found in the blood samples of exposed workers but were not detected in control blood samples. The difference in pre- and post-shift phenol concentrations in the urine was found to be correlated (regression coefficient, $r=0.465$; $p<0.01$) with benzene concentrations in the blood. The study did not assess the impact that concomitant exposure to toluene had on the data. In another study, urinary phenol levels were found to correlate well with benzene exposure, especially when these levels were expressed in terms of creatinine excretion (Inoue et al. 1986, 1988b). The calculated mean correlation coefficient was approximately 0.858-0.891.

In a small group of chemical workers whose TWA exposure to benzene was 0.4 ppm, no correlation was found between the TWA exposure and urinary phenol excretion (Perbellini et al. 1988). Additionally, analysis of data collected from 49 chemical workers in the coke oven industry led the examiners to conclude that urinary phenol could not be used as an indicator of benzene uptake for exposures of about 1 ppm or less (Drummond et al. 1988). Concomitant exposure to other chemicals (e.g., toluene) may have affected the metabolism of benzene.

The data suggest that variations in urinary phenol due to other factors interfere with determination of phenol formed from low levels of benzene. Therefore, benzene exposures of 10 ppm or less would be difficult to monitor by measuring urinary phenol levels, and consequently, correlations between phenol levels and LOAELs would not be likely for effects occurring in this dose range. Additionally, for exposures of ≤1 ppm, workplace standard, urinary phenol is an inadequate assay to determine extent of benzene exposure. Nevertheless, the measurement of urinary phenol is still useful in determining whether or not an individual has been exposed to substantial levels of benzene. In such situations, biological exposure indices (BEI) are directly correlated with threshold limit values. The ACGIH has established 50 mg phenol/g creatinine in the urine as the BE1 for benzene exposure in the workplace

(ACGIH 1996b). The BE1 is primarily an index of exposure and not a level at which health effects might occur from exposure to benzene. ACGIH has indicated that the BE1 for benzene will be changed to 25 µg PhAC/g creatinine in the urine (ACGIH 1996b).

In addition to phenol, other phenolic metabolites have been investigated as biomarkers of benzene exposure (Inoue et al. 1988a). Results from data collected on 152 chemical workers showed a linear relationship between the concentration of benzene in breathing zone air (when greater than 10 ppm) and urinary concentrations of catechol and hydroquinone. Workers who had an average work-site exposure of 10 ppm benzene showed no significant differences in the concentration of urinary catechol or hydroquinone when compared to a group of unexposed subjects. Coexposure to toluene suppressed hydroquinone formation but had little effect on formation of catechol (Inoue et al. 1988b). However, there was more overlap in measured values of urinary catechol between subjects in the exposed and unexposed groups than occurred in the hydroquinone measurements (Inoue et al. 1988a).

Urinary muconic acid has also been investigated as an indicator of acute exposure to benzene (Ducos et al. 1990, 1992; Inoue et al. 1989; Lee et al. 1993; Melikian et al. 1993, 1994). A study of 152 shoe and paint manufacturers exposed to benzene reported that urinary muconic acid correlated linearly with the TWAs of benzene (Inoue et al. 1989). Measurements of muconic acid were able to distinguish between workers exposed to benzene at concentrations of 6-7 ppm and nonexposed workers. The high sensitivity of muconic acid measurement is due to the very low urinary background levels of muconic acid in the general population, in contrast to the high background levels found for phenol, catechol, and hydroquinone. However, the authors concluded that, while muconic acid measurements were useful for determining group exposures to benzene, they could not be used to monitor individual exposure because of the large variations in individual urinary muconic acid values. In addition, urinary muconic acid excretion was suppressed by coexposure to toluene. Urine samples from male and female smokers and nonsmokers were obtained from subjects who applied for life insurance (Melikian et al. 1994). Samples from pregnant women were obtained during 7-35 weeks of pregnancy. Questionnaires were filled out on smoking history and occupation. The levels of muconic acid and cotinine (a biomarker for smoking) in the urine for the groups of pregnant and nonpregnant smokers and nonsmokers were compared with previously reported data in male smokers. Results showed the mean levels of muconic acid in the groups of male, and female nonpregnant and pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher, respectively, than the mean concentration of this acid in the nonsmoking groups. The differences in the mean muconic acid concentrations between smoking

and nonsmoking groups were significant in male (p=0.00l), nonpregnant (p=0.00l) and pregnant smokers (p=0.002). Mean concentrations of muconic acid levels in female nonpregnant smokers are similar to those of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female nonpregnant smokers were 0.22±0.03 and 0.24±0.02 mg/g creatinine, or 0.13±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of muconic acid in groups of 63 pregnant smokers and 53 nonpregnant female smokers were 0.27±0.04 and 0.24±0.02 mg/g creatinine, or 0.24±0.06 and 0.13±0.07 mg/mg cotinine, respectively. Mean concentrations of urinary cotinine in pregnant smokers were significantly lower than in the group of nonpregnant smokers (1.13±0.12 mg/g creatinine compared to 1.82±0.14 mg/g creatinine).

Recently, a sensitive gas chromatography/mass spectrometry (GC/MS) assay was developed to detect and quantitate muconic acid in the urine after benzene exposure (Bechtold and Henderson 1993; Bechtold et al. 1991). The detection limit of the assay is also greater, and muconic acid at levels greater than 100 ng/mL can be detected. A high performance liquid chromatography method also exists, and has been used to detect muconic acid in urine (Schad et al. 1992). Although muconic acid is used as a marker for benzene exposures, muconic acid in the urine can also result from ingestion of sorbic acid, a common food preservative (Ducos et al. 1990).

Another urinary metabolite of benzene is *S*-phenyl-*N*-acetyl cysteine (PhAC) (Jongeneelen et al. 1987). No background excretion of PhAC was found in rats or in humans. However, the authors concluded that biological monitoring of industrial exposure to benzene by the determination of PhAC in the urine is not better than the determination of phenol in urine. The work by van Sittert et al. (1993) in refinery workers indicates a strong correlation between 8-hour exposure to airborne benzene at concentrations of 0.3 ppm and higher) and urinary PhAC concentrations in end of shift samples. A strong correlation was also found between urinary phenol and PhAC concentrations. Similar results with regard to benzene concentrations in air and blood, and urinary concentrations of muconic acid and PhAC were observed by Popp et al. (1994) in car mechanics. PhAC has also been suggested as a useful biomarker in both humans and animals by Stommel et al. (1989). As noted above, ACGIH intends to change the BE1 to 25 μg PHAC/g creatinine in urine (ACGIH 1996b). In a recent study, a very sensitive gas chromatography/mass spectroscopy assay was developed to quantify the binding of the benzene metabolite benzene-oxide to cysteine groups in hemoglobin (Bechtold and Henderson 1993; Bechtold et al. 1992a). Using this assay, the hemoglobin adduct *S*-phenylcysteine was detected in the blood of mice and rats exposed by inhalation or by gavage to benzene. Further work necessary

for the application of this method to humans is in progress. *S*-phenylcysteine was not detected in the hemoglobin of women exposed to up to 28 ppm benzene in a rubber factory (Bechtold et al. 1992a). However, albumin adducts of *S*-phenylcysteine were detected in these women, and levels were linearly correlated with exposure, although the data showed great variability (Bechtold et al. 1992b). An immunoassay has also been developed to detect hemoglobin adducts formed after benzene exposure (Grassman and Haas 1993). Hemoglobin adducts formed with hydroquinone, catechol, and trihydroxybenzene were detectable in mice treated with radiolabeled compound. Further development of this assay is needed to prove its usefulness as a tool to detect exposure to benzene. Hemoglobin adducts may be useful in evaluating cumulative exposure to benzene.

Ong et al. (1995) evaluated the various biomarkers of benzene exposure for their relationship with environmental benzene levels. Muconic acid in the urine correlated best with environmental benzene concentrations. Urinary hydroquinone levels were the most accurate biomarker of exposure for the phenolic metabolites of benzene, followed by phenol and catechol. No correlation was found between environmental benzene levels and unmetabolized benzene in the urine, although other studies suggest that benzene in the urine may be a useful biomarker of occupational exposure (Ghittori et al. 1993).

Benzene is partially excreted in expired air, and breath levels have been used as a measure of exposure. An early study on humans found that benzene could be detected in expired air 24 hours after a 100 ppm exposure (Hunter 1468). Additionally, this study was among the first to suggest that it may be possible to extrapolate back to the concentration in the inspired air. However, the amount of benzene in expired air varies, and benzene is not exhaled in large quantities until the capacity of the body to metabolize benzene is exceeded. ACGIH has established BEIs for benzene in exhaled air as 0.12 ppm for end-exhaled air and 0.08 ppm for mixed-exhaled air prior to next workshift (ACGIH 1996b). Monitoring of expired benzene as a marker of exposure is still being explored (Pekari et al. 1992).

Comparative studies of residents in urban and rural areas revealed higher levels of benzene in the expired air of urban dwellers (Wester et al. 1986). For nonsmokers, benzene breath levels were 2.5±0.8 ppb in the urban area and 1.8±0.2 ppb in the rural area. However, in both cases breath levels were higher than ambient air levels (1.4±l.l and 1.0±0.l ppb, respectively), suggesting that there were other sources of exposure to benzene.

In another study, benzene levels measured in the breath of occupationally exposed workers 16 hours after exposure ended were elevated as compared to benzene levels in the breath of people not occupationally exposed to benzene (Brugnone et al. 1989). A group of coal tar distillation workers with TWA exposure to benzene between 0.2 and 4.1 ppm had measurable benzene vapor in their breath 16 hours after exposure and showed a progressive build-up of benzene in their expired air during the working week (Money and Gray 1989).

Benzene levels in blood and expired air were determined in a population of 168 men divided into 4 groups: blood donors, hospital staff, and chemical workers with and without benzene exposure (Brugnone et al. 1989). Workers with a TWA benzene exposure of 0.4 ppm had measurable blood and alveolar concentrations of benzene that were significantly different from the other 3 groups. However, blood levels would be expected to provide a more accurate assessment of internal dose and, thus, a more accurate prediction of target organ effects than other monitoring end points. In addition, baseline levels of benzene in the blood of environmentally sensitive individuals not known to have exposure to benzene have been estimated. These could provide a baseline for monitoring exposure to benzene (DeLeon and Antoine 1985). Similar determinations have been made with smokers and nonsmokers (Angerer et al. 1991). Determination of benzene in postmortem blood has been used to determine glue-sniffing as the cause of death (Chao et al. 1993).

As discussed in Chapter 6, the primary method of analyzing benzene in body fluids and tissues is gas chromatography (GC) in conjunction with either mass spectrometry (MS), photoionization detection (PID), or flame ionization detection (FID). For detection of benzene metabolites, both GC/FID and high-performance liquid chromatography (HPLC) with ultraviolet detection (UV) have been used. A recent article describes the development of simple and sensitive methods for determination of blood and urinary benzene levels using gas chromatography headspace method (Kok and Ong 1994).

The presence of benzene metabolite-DNA adducts may be useful as a biomarker of exposure. DNA adducts with benzene metabolites have been found after benzene exposure (Hedli et al. 1990; Lutz and Schlatter 1977; Reddy et al. 1989a). However, the feasibility of using DNA adducts themselves as biomarkers of exposure is not clear at this time. Norpoth et al. (1988) found that degradation products of DNA adducts could be detected in the urine of rats after high-dose exposure. Assay of these degradation products may prove to be a more useful method of biomonitoring benzene exposure.

2.6.2   Biomarkers Used to Characterize Effects Caused by Benzene

In addition to using levels of benzene and benzene metabolites for monitoring purposes, various biological indices might also be helpful in characterizing the effects of exposure to benzene. As with monitoring for benzene exposure, monitoring for effects may best be accomplished through the use of a series of biomarkers with correlation of the results. Decreases in erythrocyte and leukocyte counts have been used as an indicator of high occupational exposures. Monitoring of benzene workers has included monthly blood counts, with workers being removed from areas of high benzene exposure when leukocyte counts fell below $4,000/mm^3$ or erythrocyte counts fell below $4,000,000/mm^3$ (ITII 1975; OSHA 1987b). Hayes (1992) indicates that benzene-related leukopenia, commonly thought of as an intermediate end point in the process of developing benzene-related leukemia, is considered a biomarker of benzene poisoning in China, not necessarily related to leukemia. Additional studies planned by Hayes seek to define the role of benzene-related leukopenia in the disease process initiated by benzene exposure (Hayes 1992). Leukocyte alkaline phosphatase (LAP) activity was increased in benzene workers exposed to about 31 ppm for a chronic time period (Yin et al. 1982). The change in LAP activity could be used in the diagnosis of benzene poisoning since it was more sensitive than the change in the leukocyte count, although it is not a biomarker that is specific for benzene. Additionally, it seems reasonable that chromosomal aberrations in bone marrow and peripheral blood lymphocytes and sister chromatid exchanges could be used to monitor for benzene effects (Eastmond et al. 1994; Van Sittert and de Jong 1985). Benzene metabolites have also been found to form adducts with DNA (Chenna et al. 1995; Lutz and Schlatter 1977; Norpoth et al. 1988; Rushmore et al. 1984; Snyder et al. 1987).

Exposure to benzene causes toxic effects in the bone marrow via its metabolites and possibly by benzene via solvent effects (Eastmond et al. 1987; Gad-El Karim et al. 1985; Hedli et al. 1990; Irons et al. 1980). Therefore, it is possible that hematological tests could be used as markers of hematotoxicity. To date, surveillance and early diagnosis of benzene hematotoxicity rely primarily on the complete blood count, including hemoglobin, hematocrit, erythrocyte count, leukocyte count, and differential and platelet counts. In effect, complete blood counts and marrow exams should be good for early detection of preleukemic lesions. Additionally, cytogenetic tests of marrow cell are being used more often but are not yet diagnostic. Workers exposed to benzene in the air have shown elevated levels of delta-aminolevulinic acid in erythrocytes and elevated coproporphyrin in the urine (Kahn and Muzyka 1973). These may be biomarkers for disruption of porphyrin synthesis and may be

early indicators of adverse hematological effects. These effects are not specific for benzene. Hedli et al. (1990) observed that in rats, benzene metabolite-DNA adducts were observed in the bone marrow at doses that did not affect bone marrow cellularity, and suggested that monitoring of the bone marrow DNA adducts might be a sensitive bioassay of genotoxic effects effect of benzene exposure. Work by other researchers also suggests that monitoring DNA adducts or products of DNA damage might be useful (Bode11 et al. 1993; Chen et al. 1994; Lagorio et al. 1994a; Reddy et al. 1989a).

2.7   INTERACTIONS WITH OTHER CHEMICALS

Studies have been conducted on the interaction of benzene with other chemicals, both *in vivo* and in the environment. Benzene metabolism is complex, and various xenobiotics can induce or inhibit specific routes of detoxification and/or activation in addition to altering the rate of benzene metabolism and clearance from the body. Toluene, Aroclor 1254, phenobarbital, acetone, and ethanol are known to alter the metabolism and toxicity of benzene.

Agents that alter benzene metabolism may also modify benzene toxicity. Ethanol and benzene induce the formation of the hepatic cytochrome P-450 isoenzyme, CYP2E1, in rabbits and rats (Gut et al. 1993; Johansson and Ingleman-Sundberg 1988), although benzene derivatives, such as toluene and xylene, can inhibit the enzymatic activity of the isozyme (Koop and Laethem 1992). Ethanol enhances both the metabolism *(in vitro)* and the toxicity *(in vivo)* of benzene in animals (Baarson et al. 1982; Nakajima et al. 1985). When ethanol was administered to benzene-treated mice, it increased the severity of benzene-induced anemia, lymphocytopenia, and bone marrow aplasia and produced transient increases in normoblasts and peripheral blood atypia (Baarson et al. 1982). The modulating effects of benzene were dose dependent. The enhancement of the hematotoxic effects of benzene by ethanol is of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985). Benzene can interfere with the disappearance of ethanol from the body. Accordingly, increased central nervous system disturbances (e.g., depression) may occur following concurrent exposure to benzene and ethanol.

Other chemicals that induce specific isoenzymes of cytochrome P-450 can increase the rate of benzene metabolism and may alter metabolism pathways favoring one over another. Ikeda and Ohtsuji (1971) presented evidence that benzene hydroxylation was stimulated when rats were pretreated with phenobarbital and then exposed to 1,000 ppm of benzene vapor for 8 hours per day for 2 weeks.

Additionally, phenobarbital pretreatment increased the rate of metabolism by 40% in rats and 70% in mice (Pawar and Mungikar 1975). In contrast, rats exposed to phenobarbital showed no effects on the metabolism of micromolar amounts (35-l12.8 μmol) of benzene *in vitro* (Nakajima et al. 1985).

Co-administration of toluene inhibited the biotransformation of benzene to phenol in rats (Ikeda et al. 1972; Inoue et al. 1988b). This was due to competitive inhibition of the oxidation mechanisms involved in the metabolism of benzene. Phenobarbital pretreatment of the rats alleviated the suppressive effect of toluene on benzene hydroxylation by the induction of oxidative activities in the liver. This effect has been observed in other studies in rats (Purcell et al. 1990).

Mathematical models of benzene and phenol metabolism suggest that the inhibition by benzene of phenol metabolism, and by phenol on benzene metabolism, occurs through competition for a common reaction site, which can also bind catechol and hydroquinone (Schlosser et al. 1993). Flavonoids have been shown to inhibit phenol hydroxylase or increase phenol hydroxylase activity in a dose-dependent manner, dependent on the oxidation potential of the flavonoid (Hendrickson et al. 1994).

SKF-525A and carbon monoxide are classic inhibitors of cytochrome P-450s. The binding between P-450 and carbon monoxide or SKF-525A is coordinate covalent. Carbon monoxide inhibits all cytochrome P-450 isoenzymes since it binds to the heme component of cytochrome P-450, whereas SKF-525A inhibits specific types. SKF-525A inhibited benzene metabolism in the rat (Ikeda et al. 1972). Injection of 80 mg/kg of SKF-525A in rats resulted in a depression of phenol excretion. It also prolonged phenol excretion and interfered in the conversion of benzene to glucuronides and free phenols. Carbon monoxide, aniline, aminopyrine, cytochrome C, and metyrapone inhibited benzene metabolism *in vitro* by mouse liver microsomes (Gonasun et al. 1973).

2.8   POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to benzene than will most persons exposed to the same level of benzene in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxification or excretion of benzene, or compromised function of target organs affected by benzene. Populations who are at greater risk due to

their unusually high exposure to benzene are discussed in Section 5.6, Populations With Potentially High Exposure.

Ethanol can increase the severity of benzene-induced anemia, lymphocytopenia, and reduction in bone marrow cellularity, and produce transient increases in normoblasts in the peripheral blood and atypical cellular morphology (Baarson et al. 1982). The enhancement of the hematotoxic effects of benzene by ethanol is of particular concern for benzene-exposed workers who consume alcohol (Nakajima et al. 1985). Accordingly, increased central nervous system disturbances (e.g., depression) may be expected following concurrent exposure to benzene and ethanol.

Individual susceptibility is one of the most important variables in the development of chronic benzene toxicity. Aksoy et al. (1976) suggested familial susceptibility as one of the main factors in the frequency of chronic benzene toxicity. Two patients with pancytopenia associated with chronic exposure to benzene were cousins. One of these two patients died of aplastic anemia; the son of this patient presented with leukopenia a short time after exposure to this chemical. Genetic polymorphism with regard to the P-450 enzymes that metabolize benzene to its reactive metabolites may render some individuals at higher risk to the toxic effects of benzene (Kato et al. 1995). Although there are possible indications of genetic susceptibility to benzene, it is believed that all humans are susceptible to the pancytopenic effects of this agent.

There is a possible relationship between thalassemia (abnormal hemoglobins) and exposure to benzene. Aksoy (1989) presented a series of 44 pancytopenic patients, 4 of which had β-thalassemia heterozygotes. Two of these four patients had high levels of fetal hemoglobin, while a third patient had pseudo-Pelger-Huet anomaly. Thus, some forms of β-thalassemia may increase the deleterious effects of benzene on the hematopoietic system.

The occurrence of aplastic anemia in chronic benzene toxicity may be accelerated in individuals with viral hepatitis (Aksoy 1989). Furthermore, children and fetuses may be at increased risk-because their hematopoietic cell populations are expanding and dividing cells are at a greater risk than quiescent cells.

In vitro studies of benzene-caused chromosomal aberrations in human lymphocytes taken from people with different health practices suggests that "unhealthy lifestyles, " including smoking, excessive

alcohol consumption, inadequate physical exercise and sleep, excessive stress, and inadequate
nutritional balance, could make cells more sensitive to the production of chromosomal aberrations after
exposure (Morimoto et al. 1983).

2.9   METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects
of exposure to benzene. 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 benzene. When
specific exposures have occurred, poison control centers and medical toxicologists should be consulted
for medical advice. The following references provide specific information about treatment following
exposures to benzene: "Benzene" in *Occupational Medicine,* 3rd edition, 1994, Mosby, St. Louis, Carl
Zenz et al., eds.; "Benzene" in *Goldfrank's Toxicologic Emergencies,* 5th edition, 1994, Norwalk, C.K.
Aaron and M.A. Howland; "Benzene and the Aromatic Hydrocarbons" in *Clinical Management of
Poisoning and Drug Overdose,* 2nd edition, 1990, W.B. Saunders, Philadelphia, L.M. Haddad and J.F.
Winchester, eds.; *Benzene Contamination in Well Water,* Perdido, Alabama, CEH, 1983.

2.9.1   Reducing Peak Absorption Following Exposure

Human exposure to benzene can occur by inhalation, oral, or dermal contact. General
recommendations for reducing absorption of benzene following acute high-level inhalation exposure
have included moving the patient to fresh air and monitoring for respiratory distress (Bronstein and
Currance 1988; Haddad and Winchester 1990; HSDB 1996; Kunisaki and Augenstein 1994; Stutz and
Janusz 1988). The administration of 100% humidified supplemental oxygen with assisted ventilation,
when required, has also been suggested (Bronstein and Currance 1988; Haddad and Winchester 1990;
HSDB 1996; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). In the case of eye exposure,
irrigation with copious amounts of water or saline has been recommended (Bronstein and Currance
1988; Haddad and Winchester 1990; HSDB 1996; Stutz and Janusz 1988). The removal of
contaminated clothing and a thorough washing of exposed areas with soap and water have also been
recommended (Bronstein and Currance 1988; Ellenhom and Barceloux 1988; Haddad and Winchester
1990; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Some sources suggest the
administration of water or milk to the victim after ingestion of benzene (Stutz and Janusz 1988).
Emesis may be indicated following recent substantial ingestion of benzene unless the patient is or

could rapidly become obtunded, comatose, or convulsive (Ellenhorn and Barceloux 1988; HSDB 1996); however, other sources do not recommend the use of emetics because of the risk of aspiration pneumonitis (Bronstein and Currance 1988; Haddad and Winchester 1990). Some sources recommend gastric lavage if indicated (Haddad and Winchester 1990; Kunisaki and Augenstein 1994;). While lavage may be useful, induction of emesis should be regarded with great caution because of the rapid onset of central nervous system depression. Although of unproven value, administration of a charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, has also been suggested as a way to stimulate fecal excretion of the chemical before it is completely absorbed by the body (HSDB 1996; Kunisaki and Augenstein 1994; Stutz and Janusz 1988). Diazepam and phenytoin may be helpful in controlling seizures (Bronstein and Currance 1988; HSDB 1996; Stutz and Janusz 1988). Administration of epinephrine or other catecholamines has not been recommended because of the possibility of myocardial sensitization (Haddad and Winchester 1990; HSDB 1996; Nahum and Hoff 1934).

2.9.2   Reducing Body Burden

Following absorption into the blood, benzene is rapidly distributed throughout the body. Since benzene is lipophilic, it is preferentially distributed to lipid-rich tissues. The initial stage of benzene metabolism is the formation of benzene oxide via P-450 mixed-function oxidases. Detoxification pathways generally involve the formation of glutathione conjugates of benzene oxide and glucuronide or sulfate conjugates of phenol or its subsequent metabolites, catechol, hydroquinone, and trihydroxybenzene. Other metabolites of benzene also have known toxic effects. Exhalation is the main route for excretion of unmetabolized benzene, while metabolized benzene is excreted primarily in the urine. Studies in humans and animals indicate that both exhalation and urinary excretion occur in several phases, with half-lives of minutes to hours. Hence, benzene and its metabolites have relatively short half-lives in the body, and while some of these metabolites are clearly toxic, substantial body burdens are not expected.

No methods are currently used for reducing the body burden of benzene. It is possible that methods could be developed to enhance the detoxification and elimination pathways, such as ensuring sufficient glutathione stores in the body by the administration of N-acetyl-L-cysteine.

2.9.3   Interfering with the Mechanism of Action for Toxic Effects

Since ethanol can increase the severity of benzene-induced hematotoxicity (Baarson et al. 1982; Nakajima et al. 1985) and ethanol and benzene are both considered strong nervous system depressants (Carpenter et al. 1944; Cornish and Ryan 1965) avoidance of ethanol following benzene exposure may help to reduce the toxic effects of benzene.

Administration of indomethacin, a nonsteroidal anti-inflammatory drug, has been shown to prevent benzene-induced myelotoxicity in mice and the accompanying increase in prostaglandin E in the bone marrow (Kalf et al. 1989; Renz and Kalf 1991). Co-administration of indomethacin also prevented an increase in the number of micronucleated polychromatic erythrocytes in peripheral blood. The authors suggest that these results suggest a role for prostaglandin synthetase in benzene-induced myelotoxic and genotoxicity, and a way to interfere with that process with substances such as indomethacin. Prostaglandins have been shown to inhibit hematopoiesis (Kalf et al. 1989). Additionally, prostaglandin synthetase could be involved in the oxidation of phenol and/or hydroquinone to toxic metabolites (Kalf et al. 1989).

The use of indomethacin to block benzene toxicity has led to data that indicate that myelotoxicity may involve the destruction of stromal macrophages that produce IL-l, a cytokine essential for hematopoiesis (Renz and Kalf 1991). External administration of recombinant IL-l to mice prior to benzene administration prevents the myelotoxicity, presumably by providing a source of the cytokine (Renz and Kalf 1991). Further indication that IL-1 is affected by benzene exposure comes from the work of Carbonnelle et al. (1995), who showed that exposure of human monocytes to micromolar amounts of hydroquinone for 2 hours resulted in significantly decreased secretion of IL-l α and IL-l β at concentrations of 5 μM and above. RNA and protein synthesis were also inhibited. Additional research in this area indicates that tumor necrosis factor may provide protection against the inhibitory effects of hydroquinone on human hematopoietic progenitor cells (Colinas et al. 1995). The research of Shankar et al. (1993) determined that pretreatment with Protein A, a glycoprotein that acts as a multipotent immunostimulant, modulated the toxicity of benzene. Groups of 6 female albino rats (Swiss Wistar) were injected intraperitoneally with 1.0 ml/kg/body weight (879 mg/kg) benzene once daily for 3 consecutive days. Another group (6 per group) was administered intravenously with 60 μg/kg Protein A twice weekly for 2 weeks and then injected with 879 mg/kg benzene intraperitoneally once daily for 3 consecutive days. Controls were injected with normal saline. All of

the animals were killed 24 hours after receiving the last benzene injection. Blood was collected from the jugular vein for enumeration of total leukocyte counts. Routine autopsy was performed on all animals and the liver, thymus, spleen, and kidney organs were collected for organ weights. In benzene-only treated animals, there was a significant decrease in the total leukocyte counts in the peripheral blood as well as a significant decrease in the number of lymphocytes, a decrease in the gross organ weights of thymus and spleen, a significant increase in the iron content and lipid peroxidation of the liver and bone marrow, and an increase in low molecular weight iron in the bone marrow. Pretreatment with Protein A prevented these parameters from changing.

The covalent binding of benzene metabolites to nucleic acids and proteins is thought to play a major role in the toxicity and carcinogenicity of benzene (Gill and Ahmed 1981; Irons et al. 1980; Longacre et al. 1981a, 1981b; Lutz and Schlatter 1977; Norpoth et al. 1988; Rushmore et al. 1984; Snyder et al. 1978b; Sun et al. 1990). In addition to the covalent binding to cellular macromolecules, several other mechanisms have been proposed to explain how benzene affects the bone marrow. These include suppression of RNA and DNA synthesis (Moeschlin and Speck 1967; Post et al. 1983, alkylation of cellular sulfhydryl groups (Irons 1985), disruption of the cell cycle (Irons et al. 1979; Snyder et al. 1981c), oxygen activation or free radical formation (Irons 198.5), damage to stromal cells (Frash et al. 1976; Kalf et al. 1987), and loss of specific protein growth factors (Clark and Kamen 1987). Further studies to determine the significance and details of these possible mechanisms could lead to additional approaches for reducing the toxic effects of benzene. These approaches could include ways to reduce binding as well as a more direct interference with the toxic effects caused by benzene metabolites/macromolecule complexes.

Other mechanisms may also be involved in benzene toxicity and carcinogenicity. Such mechanisms may include synergism between metabolites (Eastmond et al. 1987; Snyder et al. 1989) or synergism between glutathione-depleting metabolites of benzene and oxygen radicals (Parke 1989). It may be possible to develop methods to interfere with these mechanisms as well. Work by Zhu et al. (1995) indicated that in mice and rats, both glutathione and quinone reductase were important in modulating the hematotoxicity of hydroquinone. Mice, which exhibit a greater susceptibility to benzene-induced hematotoxicity, had less glutathione in bone marrow stoma1 cells, compared to rats. Induction of endogenous glutathione and quinone reductase, or pretreatment of mice with glutathione ethyl ester provided dramatic protection against hydroquinone toxicity.

Benzene also affects the central nervous system. Effects noted include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness (Flury 1928; Greenburg 1926; Kraut et al. 1988; Yin et al. 1987b). Since benzene may induce an increase in brain catecholamines, it may also have a secondary effect on the immune system via the hypothalamus-pituitary-adrenal axis (Hsieh et al. 1988b). Increased metabolism of catecholamines can result in increased adrenal corticosteroid levels, which are immunosuppressive (Hsieh et al. 1988b). Further studies to determine the molecular mechanism of these effects could lead to additional approaches for reducing the toxic effects of benzene.

2.10   ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA 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 benzene 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 benzene.

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.

2.10.1   Existing Information on Health Effects of Benzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to benzene are summarized in Figure 2-6. The purpose of this figure is to illustrate the existing information concerning the health effects of benzene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific

Figure 2-6. Existing Information on Health Effects of Benzene

Human

Animal

● Existing Studies

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.

Virtually all of the information regarding health effects in humans comes from studies of workers exposed to benzene-containing solvents and/or adhesives. Exposures to benzene occurred at rotogravure printing shops; at shoe, rubber, and raincoat manufacturing plants; and during chemical manufacturing processes. Case reports and cohort studies describe both acute and chronic health effects. The predominant route of exposure in these studies is inhalation. Dermal contact is also suspected as a possible route of exposure in these studies.

As seen in Figure 2-6, inhalation information for humans is available regarding death; acute-, intermediate- and chronic-duration systemic effects; immunologic, neurologic, reproductive, developmental, and genotoxic effects; and cancer. However, as mentioned above, human exposure to benzene in specific work environments probably occurs not only by inhalation but also by the dermal route. Limited information is available regarding direct skin contact with benzene by humans. Additionally, oral studies in humans are limited to isolated case reports of death and acute-duration systemic effects subsequent to accidental or intentional ingestion of benzene, although one study (Hunting et al. 1995) described effects in vehicle maintenance workers who siphoned gasoline by mouth. There is limited information on effects of dermal exposure of humans to benzene, including death, acute-duration effects, and cancer.

Inhalation and oral studies in animals provide data on death; systemic effects after acute-, intermediate-, and chronic-duration exposure; and immunologic, neurologic, reproductive, developmental, genotoxic, and cancer effects. Furthermore, data exist regarding acute- and intermediate-duration systemic effects and cancer in animals after dermal exposure to benzene.

2.10.2   Identification of Data Needs

Acute-Duration Exposure. There are reports on the health effects resulting from acute exposure of humans and animals to benzene via the inhalation, oral, and dermal routes. The primary target organs for acute exposure are the hematopoietic system, nervous system, and immune system. Acute effects on the nervous system and immune system are discussed below under Neurotoxicity and

Immunotoxicity. Information is also available for levels that cause death in humans (e.g., Cronin 1924; Flury 1928; Greenburg 1926; Tauber 1970; Thienes and Haley 1972) and in animals (e.g., Cornish and Ryan 1965; Drew and Fouts 1974; Smyth et al. 1962) following inhalation and oral exposures.

No acute human or animal data on hematological effects from oral or dermal exposure are available. However, there are acute inhalation data that characterize the effects of benzene on the hematological system in humans and animals. Data regarding effects on the human hematological system following acute inhalation exposure to benzene are scant, but indicate leukopenia, anemia, and thrombocytopenia after more than 2 days occupational exposure to more than 60 ppm benzene (Midzenski et al. 1992). Data for hematological effects in animals after acute-duration inhalation oral exposure are more extensive. Changes in peripheral erythrocytes (Chertkov et al. 1992; Cronkite et al. 1985; Rozen et al. 1984; Ward et al. 1985), in peripheral leukocytes (Aoyama et al. 1986; Chertkov et al. 1992; Gill et al. 1980; Green et al. 1981b; Li et al. 1986; Ward et al. 1985; Wells and Nerland 1991) and in bone marrow cells (Chertkov et al. 1992; Cronkite et al. 1989; Dempster and Snyder 1991; Gill et al. 1980; Green et al. 1981b; Neun et al. 1992; Toft et al. 1982) were seen in rats and mice. An acute inhalation MRL of 0.05 ppm was determined based on the LOAEL for immunologic effects in the mouse (Rozen et al. 1984) (discussed below under Immunotoxicity). No acute oral studies were suitable for deriving MRLs. Additional animal or human data on hematological effects following acute oral or dermal exposure would be useful to provide threshold levels for populations living near hazardous waste sites. However, acute dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

Intermediate-Duration Exposure. There is sufficient information in humans and animals to identify the hematopoietic, nervous, and immunological systems as targets for benzene toxicity. The intermediate effects on the nervous system and immune system are discussed in the sections below titled Neurotoxicity and Immunotoxicity. Data on adverse hematological effects in humans are available following intermediate- and chronic-duration exposures to benzene in the workplace (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1987; Townsend et al. 1978; Yin et al. 1987c). However, the exposure levels and durations were not well defined and, therefore, could not be used to calculate an MRL. Studies in rats and mice following inhalation exposure can be used to define NOAELs and LOAELs for hematological effects (e.g., Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Farris et al. 1993; Green et al. 1981b; Luke et al. 1988b; Plappert et al. 1994a, 1994b; Seidel et

al. 1989b; Snyder et al. 1978a, 1980; Toft et al. 1982; Vacha et al. 1990; Ward et al. 1985; Wolf et al. 1956), but none were considered appropriate for calculating an MRL. An intermediate-duration inhalation MRL of 0.004 ppm was derived based on the LOAEL for neurological effects in mice (Li et al. 1992), which are discussed below under Neurotoxicity. Data on hematological effects from oral exposures in animals are also available (Hsieh et al. 1988b, 1990, 1991; NTP 1986; Shell 1992; Wolf et al. 1956), but again, no intermediate-duration oral MRL could be derived because the threshold for hematological (and immunological) effects could not be identified. Additional animal or human data on hematological effects following intermediate oral or dermal exposure would be useful to provide threshold levels for populations living near hazardous waste sites. However, intermediate dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

Chronic-Duration Exposure and Cancer. The primary target for adverse systemic effects of benzene following chronic exposure is the hematological system. Hematological toxicity was reported in studies of humans chronically exposed to benzene in the air in the workplace (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974, 1987; Cody et al. 1993; Doskin 1971; Erf and Rhoads 1939; Goldwater 1941; Greenburg et al. 1939; Kipen et al. 1989; Li et al. 1994; Townsend et al. 1978; Yin et al. 1987c). No human data are available to evaluate hematological effects following oral exposure. Chronic animal studies are available for hematological effects following inhalation exposure (Snyder et al. 1978a, 1980, 1984) and oral exposure (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986). No chronic inhalation or oral MRLs were derived. Although the study of occupational inhalation exposure to benzene by Tsai et al. (1983) defines a NOAEL for hematological effects, no MRL could be derived because the study did not address TWA exposure levels, there were no controls, and no follow-up was done, in addition to other deficiencies in the study design. In other chronic studies, the lowest LOAEL value was serious (pancytopenia) (Doskin 1971). No chronic-duration oral MRL was derived since the most extensive study found (NTP 1986) did not conclusively define a NOAEL or less serious LOAEL for end points that can be used to derive and MRL. Since the hematological system is a target organ, further human and animal data regarding the effects of benzenein the air and drinking water are important for the purposes of defining threshold levels for populations living near hazardous waste sites, and to derive MRLs. Dermal data for humans and animals were not available. However, chronic dermal exposure at levels that are likely to be found in the environment and at hazardous waste sites is not likely to cause adverse health effects.

Benzene can induce leukemia. The leukemogenic potential of benzene for humans has been estimated from data from three separate occupational epidemiological studies and their analyses (Bond et al. 1986b; Infante 1977; Ott et al. 1978; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987; Wong et al. 1983). Additional studies of workers in China may be found in the literature (Li et al. 1994; Travis et al. 1994; Yin et al. 1987a, 1987b, 1989). The older data are limited because of the lack of complete records documenting benzene levels in workplace air, individual worker exposure histories, and other confounding factors. Newer data are more informative. Animal data show that benzene is leukemogenic following inhalation exposure (Cronkite et al. 1984; Snyder et al. 1984). AML is the most common leukemia associated with benzene exposure in humans (animals develop leukemia and lymphomas and other neoplasms in numerous tissues that have not been associated with benzene exposure in humans) (Crump 1994; Wong 1995; Wong and Raabe 1995). However, there is a need for more information on the mechanism of benzene induction of AML. For this, an appropriate animal model needs to be identified. Development of a suitable animal model to conduct mechanistic studies of benzene carcinogenesis has been suggested as a data need (Goldstein and Warren 1993). Additionally, there is a possibility that some of the metabolites of benzene may interact with each other to produce effects leading to toxicity or to cancer. More research is needed to define what these interactions are and how they enhance the toxicity and carcinogenicity of benzene. Data are sufficient to validate the carcinogenic potential of benzene in animals following inhalation exposure (Cronkite 1986; Cronkite et al. 1989; Maltoni et al. 1982a, 1982b, 1983; Snyder et al. 1980, 1984, 1988) and oral exposure (Huff et al. 1989; Maltoni et al. 1989; NTP 1986). Newer literature is addressing these issues (see Section 2.10.3, Ongoing Studies). Although the exact mechanism of benzene carcinogenicity is not known, it has been postulated that some benzene metabolites are capable of forming adducts with DNA and are responsible for reduced immune function which could potentially lead to cancer. DNA adduct formation could occur with both inhalation and oral exposures (Ding et al. 1983; Sasiadek et al. 1989). Questions that need to be answered with regard to the mechanism of benzene carcinogenesis include how do benzene metabolites produce greater-than-additive effects, what are the critical target genes, is aplastic anemia essential to the development of leukemia, and what is the role of cytokines and growth factor pathways in benzene toxicity. Bioassays using oral or dermal exposures in animals should provide quantitative data to make the establishment of the dose-response relationship more precise for each route. However, dermal exposure is not likely to be the most relevant route of exposure for humans at hazardous waste sites.

Genotoxicity. Evidence for the genotoxicity of benzene in humans comes from studies of occupationally exposed populations (Ding et al. 1983; Eastmond 1993; Forni et al. 1971a; Major et al. 1992; Popp et al. 1992; Sasiadek 1992; Sasiadek and Jagielski 1990; Sasiadek et al. 1989; Tompa et al. 1994; Tough and Court-Brown 1965; Tough et al. 1970; Yager et al. 1990; Yardley-Jones et al. 1990). These exposures have occurred primarily via inhalation, although some dermal exposure cannot be ruled out. In spite of the lack of accurate exposure data, exposure to multiple chemicals, and often inappropriate control groups, the association between benzene exposure and the appearance of structural and numerical chromosome aberrations in human lymphocytes suggests that benzene can be considered a human clastogen. Benzene-induced cytogenetic effects, including chromosome and chromatid aberrations, sister chromatid exchanges, and micronuclei, have been consistently found in *in vivo* animal studies (Anderson and Richardson 1981; Au et al. 1991a; Erexson et al. 1986; Fujie et al. 1992; Kolachana et al. 1993; Siou et al. 1981; Toft et al. 1982; Ward et al. 1992). Inhalation exposure of mice has yielded identifiable benzene-derived hemoglobin adducts (Sabourin et al. 1990). Ward et al. (1992) have shown that intermediate-duration exposure of mice to benzene by inhalation at levels below the current PEL may induce gene mutations in the lymphocytes.

Standard short-term *in vitro* assays indicate that benzene's genotoxicity is derived primarily from its metabolites, although benzene has been shown to produce DNA breaks in Chinese hamster ovary cells independent of metabolic activators (Douglas et al. 1985; Eastmond et al. 1994; Lakhanisky and Hendrickx 1985; Zhang et a1.1993). There is good evidence that benzene affects cell cycle progression, RNA and DNA synthesis, as well as DNA binding (Forni and Moreo 1967, 1969; Hartwich et al. 1969; Sellyei and Kelemen 1971; Van den Berghe et al. 1979). Chen and Eastmond (1995) showed that benzene metabolites can adversely affect human topoisomerases (enzymes involved in DNA replication and repair); however, evidence exists that some repair of DNA binding with benzene metabolites occurs in human cells (Chenna et al. 1995).

There are currently no reliable data on the dose-response relationship of benzene exposure and chromosomal effects. In light of the data of Ward et al. (1992), further investigation of mutational effects at low doses seems appropriate. Additional data on the quantitative relationship between measured exposures and clastogenic effects might provide an alternative biomarker of effect.

Reproductive Toxicity. Reproductive data are available on women occupationally exposed to benzene (Mukhametova and Vozovaya 1972; Vara and Kinnunen 1946). The data suggest

spontaneous abortions, menstrual disturbances, and ovarian atrophy. These studies are limited by the difficulty in identifying appropriate controls, problems in controlling for concomitant exposures to other chemicals, and inadequate follow-up. Only one study was found that described the reproductive effects on men exposed to benzene (Stucker et al. 1994). There are some data available from inhalation studies on reproductive effects of benzene in animals. Although there are data on adverse gonadal effects (e.g., atrophy/degeneration, decrease in spermatozoa, moderate increases in abnormal sperm forms) (Ward et al. 1985; Wolf et al. 1956), data on reproductive outcomes are either negative (Coate et al. 1984; Green et al. 1978; Kuna et al. 1992; Murray et al. 1979; Tatrai et al. 1980a) or inconclusive or conflicting (i.e., number of live fetuses, incidences of pregnancies) (Murray et al. 1979; Ungvary and Tatrai 1985). Gofmekler (1968) showed infertility in female rats after intermediate-duration inhalation exposure to 210 ppm, but the results are poorly reported. Thus, they provide only suggestive evidence that benzene may have an adverse effect on reproductive outcomes. No data were located on reproductive effects following oral exposure to benzene in humans. Negative effects on reproductive outcome have been reported in one oral study in animals (Exxon 1986). NTP (1986) reported neoplastic changes in the reproductive tissues of female rats and male and female mice after chronic oral exposure. Given the paucity of available data across all exposure routes, it would be useful to have additional 90-day studies conducted by the oral and inhalation routes that assess reproductive organs histologically or cytogenetically. Although dermal contact is not likely to be the most relevant route of exposure for humans at hazardous waste sites, data on dermal exposure would also be useful. If the results of the suggested inhalation or oral studies indicate reproductive toxicity, multigeneration or continuous breeding studies for oral and inhalation exposures would help clarify the potential for benzene to cause reproductive effects in humans.

Developmental Toxicity. Based on epidemiological studies at hazardous waste sites, an increased susceptibility to benzene of pregnant women or their offspring has not been demonstrated (Budnick et al. 1984; Goldman et al. 1985; Heath 1983; Olsen 1983). However, these studies have several limitations that make it impossible to assess the effect of benzene on the human fetus. For example, the few studies that do exist are limited by a lack of control incidences for end points, problems in identifying exposed populations, a lack of data on exposure levels, and/or exposure to multiple substances (Budnick et al. 1984; Forni et al. 1971a; Funes-Cravioto et al. 1977; Goldman et al. 1985; Heath 1983; Olsen 1983). In the occupational setting, however, there may be stronger evidence of increased susceptibility to benzene of pregnant women and/or their offspring (Forni et al. 1971a; Funes-Cravioto et al. 1977). For example, severe pancytopenia and increased chromosomal aberrations

occurred in a pregnant worker exposed to benzene (levels not reported) during the entire pregnancy. She gave birth to a healthy boy (Forni et al. 1971b). On the other hand, increased frequency of chromatid and isochromatid breaks and sister chromatid exchanges was found in lymphocytes from 14 children of female workers exposed to benzene (levels not reported) and other organic solvents during pregnancy (Funes-Cravioto et al. 1977). Irrespective of the hematological effects reported in the pregnant worker or the 14 children of female workers, the lack of complete and detailed medical records and the lack of follow-up limit the significance of effects with regard to post exposure morbidity.

A number of investigations have evaluated the developmental/maternal toxicity of benzene in animals following inhalation exposures. In these investigations, fetotoxicity was evidenced by reduced fetal weight and/or minor skeletal variations at concentrations ≥47 ppm (Coate et al. 1984; Green et al. 1978; Kimmel and Wilson 1973; Kuna and Kapp 1981; Murray et al. 1979; Tatrai et al. 1980a, 1980b; Ungvary and Tatrai 1985). However, persistently altered fetal hematopoiesis occurred in mice at 20 ppm (Keller and Snyder 1986, 1988). Additional information designed to assess the hematopoietic system of the developing fetus (human or animal) following low-level *in utero* exposures to benzene is needed. Oral data are limited to two animal studies in which benzene was shown to reduce pup body weight when mice were administered a high single oral dose (Seidenberg et al. 1986) or have no effect after gestational exposure (Exxon 1986). Additional oral studies designed to assess the developmental effects (human or animal) of low-level exposures to benzene would be useful. No data are available on the developmental toxicity of benzene following dermal exposure. Although these data would be useful, the most likely routes of exposure for humans at hazardous waste sites are the inhalation and oral routes.

Immunotoxicity. The immune system is known to be a target for benzene toxicity. The effects do not appear to be route- or species-specific. The evidence for immunotoxicity in humans comes from workers exposed by inhalation of intermediate and chronic durations. A series of studies demonstrated decreases in numbers of circulating leukocytes (Aksoy et al. 1971, 1972, 1974), but levels of exposure were not well documented. In other studies, alterations in human serum immunoglobulins were observed, but there was concomitant exposure to xylene and toluene (Lange et al. 1973a, 1973b). There is a need to test for subtle alterations in the immune system and immune competence in workers with intermediate- and chronic-duration exposure to benzene. Animal studies support the findings of immune dysfunction and indicate that additional parameters of the immune system are affected by

exposure to benzene in the air for acute (Aoyama 1986; Chertkov et al. 1992; Cronkite 1986; Cronkite et al. 1982, 1985, 1989; Gill et al. 1980; Green et al. 1981a; Li et al. 1986; Neun et al. 1982; Rozen et al. 1984; Rosenthal and Snyder 1985; Toft et al. 1982; Ward et al. 1985; Wells and Nerland 1991), intermediate (Baarson et al. 1984; Cronkite et al. 1982, 1985, 1989; Dow 1992; Gill et al. 1980; Green et al. 1981a, 1981b; Li et al. 1992; Plappert et al. 1984b; Rosenthal and Snyder 1987; Rozen and Snyder 1985; Seidel et al. 1990; Snyder et al. 1980; Stoner et al. 1981; Ward et al. 1985; Wolf et al. 1956; Yin et al. 1982), and chronic (Snyder et al. 1980, 1984, 1988) periods. An acute inhalation study found depressions of proliferative responses of bone-marrow-derived B-cells and splenic T-cells in mice at 10 ppm (Rozen et al. 1984) and an acute inhalation MRL has been derived based on this study. No human data on the oral route were available, but animal data showed immunological effects after intermediate (Fan 1992; Hsieh et al. 1988b, 1990, 1991; Shell 1992; Wolf et al. 1956) and chronic exposures (Huff et al. 1989; Maltoni et al. 1983, 1985; NTP 1986) via the oral route. Since the immune system is known to be a target organ, it is important to have information on subtle alterations in immune competence in people exposed to benzene in the air and in the drinking water. A decrease in cell-mediated immune functions, including alloantigen response and cytotoxicity, was reported in mice following intermediate inhalation exposure to 100 ppm of benzene (Rosenthal and Snyder 1987). This impaired cell-mediated immune function was also apparent in other *in vivo* studies (Stoner et al. 1981). Mice exposed to 100 ppm of benzene for a total of 100 days had reduced tumor resistance when challenged with syngeneic tumor cells and developed tumors that were lethal (Rosenthal and Snyder 1987). Further animal studies would also be useful in defining more NOAEL and LOAEL values. No data are available that document immunotoxicity in humans or animals exposed by dermal application. Dermal sensitization tests may also provide useful data on the likelihood of an allergic response in humans, since skin contact may occur in the workplace and at hazardous waste sites.

Neurotoxicity. In humans, the nervous system is a target of benzene toxicity following both inhalation and oral exposures. No data are available that demonstrate neurologic effects in humans or animals exposed dermally. There are sufficient data to suggest that it is the central nervous system which is affected following acute exposures. Neurological symptoms reported in humans following acute oral and inhalation exposures are similar and include drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness (Cronin 1924; Davies 1929; Flury 1928; Greenburg 1926; Midzenski et al. 1992; Tauber 1970; Thienes and Haley 1972). Acute- and intermediate-duration inhalation and oral animal studies provide supportive evidence that benzene effects the central nervous

system (Carpenter et al. 1944; Cornish and Ryan 1965; Evans et al. 1981; Frantik et al. 1994). Effects observed include narcosis, hyperactivity, tremors, tonic-clonic convulsions, decreased evoked electrical activity in the brain, and slight nervous system depression. Behavioral changes were found in mice after 1 week of exposure to 300 ppm, and decreased grip strength was found after three exposures to 1,000 ppm (Dempster et al. 1984). Acute oral exposures of 950 mg/kg benzene altered neurotransmitter levels in the brains of rats (Kanada et al. 1994). Intermediate oral exposures of ≥8 mg/kg/day resulted in changes in the levels of monoamine transmitters in the brain without treatment-related behavioral changes (Hsieh et al. 1988a). An intermediate-duration inhalation study found increased rapid response in mice at 0.78 ppm (Li et al. 1992), and an intermediate-duration MRL has been derived from this LOAEL.

One chronic-duration occupational study reported neurological abnormalities of the peripheral nervous system (global atrophy of lower extremities and distal neuropathy of upper extremities) in four of six patients with aplastic anemia (Baslo and Aksoy 1982). Additional studies are needed to verify the peripheral nervous system effects that might occur following chronic exposures to low doses of benzene.

Although there are sufficient data to indicate that the nervous system is a target of benzene toxicity, the neurotoxicity of benzene has not been extensively studied. Additional studies in animals are needed to identify the thresholds of neurotoxicity following acute, intermediate, and chronic inhalation and oral exposures.

Epidemiological and Human Dosimetry Studies. A large segment of the U.S. population is exposed to benzene. This exposure occurs primarily as a result of benzene emitted to the air from tobacco smoke, gasoline stations, and.automobile exhaust. Benzene has been found in about a third of the NPL hazardous waste sites. The magnitude of exposure is greater for those occupationally exposed.

The predominance of available case studies on humans are occupational studies and associated analysis (Aksoy and Erdem 1978; Aksoy et al. 1971, 1972, 1974; Baslo and Aksoy 1982; Bond et al. 1986a; Ciccone et al. 1993; Cody et al. 1993; Dosemeci et al. 1994; Doskin 1971; Crump 1994; Infante 1978; Infante et al. 1977; Kipen et al. 1989; Ott et al. 1978; Paustenbach et al. 1992; Paxton et al. 1994a, 1994b; Rinsky et al. 1981, 1987; Travis et al. 1994; Utterback and Rinsky 1995; Wong 1987b; Wong

et al. 1983; Yin et al. 1989, 1994). While these studies provide data on hematological and neurotoxic effects and evidence of carcinogenicity, they only offer information on the effects of inhalation exposure. Retrospective and prospective studies of populations that have been identified as being exposed to contaminated groundwater or drinking water could provide information on long-term health effects from oral exposures. The Benzene Subregistry Baseline Technical Report of the National Exposure Registry contains information on 1,143 persons who had documented exposure to benzene in their drinking water and were exposed for at least 30 days (ATSDR 1995). Although no causal relationship has been proposed for health conditions identified in the subregistry, continued follow-up of the population is planned to provide information on health effects of environmental exposure to benzene. Hematopoietic and immune system end points are fairly easy to measure and could provide information on the possible progression of the disease to leukemia and lymphoma in humans.

Few well conducted epidemiology studies of exposed populations exist. A recently published epidemiology study of a population with chronic exposure to two NPL hazardous waste sites illustrates the problems inherent in assessing adverse health effects from waste site exposure (Dayal et al. 1995). Benzene was only 1 of 20 compounds or groups of compounds identified as being chemicals of concern in the waste site. The authors note that assessment of long-term exposure to a dumpsite is problematic because of a lack of dumping history, the complexity of the waste components, the interaction of the components over time, and the emphasis on statistically significant versus biologically significant effects. The study had certain limitations, including lifestyle characteristics, the use of mailed-in responses, subjective symptom-reports without independent confirmation, and various problems with exposure estimation. A relationship was detected between exposed individuals and neurological symptoms, including learning difficulties, nervousness, numbness in fingers or toes, sleeping difficulties, unusual fatigue, and decreased sense of smell. Chest pains, irregular heartbeat, skin rashes, and detection of a peculiar odor or taste also occurred more often in the exposed population. However, no effort was made to relate the symptoms to particular chemicals in the waste site. Clearly, more studies of exposed populations surrounding waste sites would be warranted. Other recent studies include the series describing workers in China (Dosemeci et al. 1994; Yin-et al. 1987c, 1994), and reanalysis of data from the Pliofilm cohort (Crump 1994; Paxton et al. 1994a, 1994b; Utterback and Rinsky 1995). A recent publication by Lioy and Pellizzari (1995) outlines an appropriate framework for the National Human Exposure Assessment Survey, using benzene as a candidate compound. Other recent articles also address population-based exposure models and measurements (Macintosh et al. 1995; Pellizzari et al. 1995).

Analysis of benzene dosimetry in animal studies suggests that the metabolic pathways leading to the production of the putative toxic metabolites appear to be low-capacity, high-affinity pathways that are saturated at relatively low-exposure concentrations (Henderson et al. 1992). The authors suggest that this could also be true for humans. Additional dosimetry studies are necessary to further define the role of toxic metabolites in the development of benzene-related leukemia.

Most, but not all studies of chromosomal damage have been done on workers with preexisting hematological effects and cancer (Ding et al. 1983; Forni et al. 1971a; Picciano 1979; Yardley-Jones et al. 1988). Chromosomal aberration data could be useful in monitoring possible adverse effects in individuals living near hazardous waste sites if more quantitative data were available regarding the relationship of dose to clastogenic effect.

Studies to determine whether benzene causes solid tumors and hematological malignancies other than acute nonlymphocytic leukemia in humans have been suggested as a data need (Goldstein and Warren 1993). These data could be obtained by revisiting already established cohorts of exposed individuals, or by examining new cohorts.

Biomarkers of Exposure and Effect.

Exposure. There is no clinical disease state unique to benzene toxicity. However, the effects on the hematopoietic and immune systems are well recognized, and analytical methodologies exist for monitoring benzene levels in expired breath and blood (Brugnone et al. 1989, 1992; DeLeon and Antoine 1985; Pellizzari et al. 1988). Several biomarkers of exposure to benzene exist, but they are not unique to benzene exposure. Estimates of benzene exposure can be made by comparing the ratio of inorganic to organic sulfates in the urine (Hammond and Herman 1960). However, urinary sulfate levels are variable, and they have not been used to identify exposure levels of benzene associated with minimal toxic effect. Other more specific biomarkers, including adducts with hemoglobin and albumin, in addition to benzene levels, themselves, may be useful as biomarkers (Bechtdd et al. 1992a, 1992b; Bechtold and Henderson 1993; Pekari et al. 1992; Schad et al. 1992).

Phenol measurements have routinely been used for monitoring occupational exposures, and there is evidence that urinary phenol levels can be correlated with exposure levels (Astier 1992; Inoue et al. 1986; Pekari et al. 1992). Correlating urinary phenol with benzene exposure is complicated by high

and variable background levels in nonexposed persons. The data suggest that variations in urinary phenol will obscure phenol formed from low levels of benzene (Ong and Lee 1994; Perbellini et al. 1988). For exposures to 1 ppm or less, the current workplace standard, urinary phenol is not an adequate assay to determine the extent of benzene exposure. In addition, urinary excretion of phenol is lowered by coexposure to toluene (Inoue et al. 1988b). In many exposure situations (e.g., occupational settings and hazardous waste sites), toluene and other chemicals are present and could interfere with the metabolism and elimination of benzene. Studies that attempt to identify another biomarker that is specific to benzene, such as S-phenyl-N-acetyl cysteine (PhAC), the proposed urinary BE1 (ACGIH 1996b), may be helpful for medical surveillance.

The metabolism of benzene to ring-opened compounds (e.g., muconic acid) may be pertinent to the development of a biomarker of benzene exposure and effect (Ducos et al. 1990, 1992; Inoue et al. 1989; Lee et al. 1993; Melikian et al. 1993; Ong and Lee 1994; Witz et al. 1990). However, the dose-response curve for metabolism of benzene to urinary muconic acid in humans needs to be determined. If urinary muconic acid increases in humans at low benzene exposures, as it does in mice, then it may be a valid biomarker of benzene exposure. Excretion of urinary muconic acid in humans has also been shown to be lowered by coexposure to toluene (Inoue et al. 1989). The effect of coexposure to toluene and other chemicals on muconic acid formation needs to be assessed in order to determine the usefulness of muconic acid as a biomarker of benzene exposure.

Breath levels of benzene have been used as a measure of exposure (Brugnone et al. 1989; Money and Gray 1989; Nomiyama and Nomiyama 1974a, 1974b; Ong and Lee 1994; Pekari et al. 1992). However, the amount of benzene lost in expired air will vary not only with the dose, duration, and time from exposure, but also with the extent of metabolism in the body. Benzene levels in blood have been measured. However, blood levels rapidly decrease after exposure (Brugnone et al. 1989; DeLeon and Antoine 1985; Schrenk et al. 1941).

Benzene metabolites also form DNA adducts (Popp et al. 1992). The use of hemoglobin adducts formed from benzene metabolites as a biomarker of benzene exposure has been developed by Sun et al. (1990). Bechtold and colleagues have conducted further studies using this model (Bechtold et al. 1992a; Bechtold and Henderson 1993). Further refinement of this model would be useful to quantitate cumulative low-level exposures to benzene. Development of specific markers of exposure to use in epidemiological studies to clarify the shape of the dose-response curve in the low-dose region has been

suggested as a data need (Goldstein and Warren 1993). Ongoing epidemiological studies are currently focusing on this data need (see Section 2.10.3).

Effect. Monitoring of benzene workers has included monthly blood counts. Toxic effects occur in the bone marrow and are either from benzene via a solvent effect, or via its metabolites (Gad-El Karim et al. 1985; Irons et al. 1980). Hematological tests could be used as markers of hematotoxicity, but medical laboratories lack tests specific to benzene hematotoxicity. The tests cannot be relied upon to find preclinical disease but can identify the subtle changes that are early indicators of effects. Furthermore, these markers of effect may not be useful for long periods following cessation of exposure, nor do they distinguish between acute and chronic exposures. As stated above, additional studies are needed to define the role of benzene-related leukopenia in the disease process initiated by benzene exposure, to determine if it is a biomarker of effect, or an intermediate end point in the development of leukemia (Hayes 1992). Ongoing studies are currently focusing on this data need (see Section 2.10.3).

Absorption, Distribution, Metabolism, and Excretion. Data from both humans and animals consistently indicate that benzene is rapidly absorbed through the lungs (Eutermoser et al. 1986; Nomiyama and Nomiyama 1974a; Sabourin et al. 1987; Schrenk et al. 1941; Srbova et al. 1950). Although experimentally acquired data are not available on oral absorption of benzene in humans, case reports of accidental or intentional poisoning suggest that benzene is rapidly absorbed from the gastrointestinal tract (Thienes and Haley 1972). The efficient absorption of oral doses in animals is well documented (Cornish and Ryan 1965; Parke and Williams 1953; Sabourin et al. 1987). Benzene can be absorbed through the skin, but the rate of absorption is much lower than that for inhalation (Maibach and Anjo 1981; Susten et al. 1985; Tsuruta 1989). Following absorption into the body, benzene is widely distributed to tissues, with the relative uptake dependent on the perfusion of the tissue by blood, and the total potential uptake dependent on fat content and metabolism (Sato et al. 1975; Tauber 1970).

There is no evidence to suggest that the route of administration has any substantial effect on the subsequent metabolism of benzene, either in humans or animals. Benzene is metabolized primarily in the liver and to a lesser extent, in the bone marrow. Benzene is classified as a substrate of both cytochrome P-448 and cytochrome P-450 (Parke 1989). Within the cytochrome P-450 family, benzene is a preferential substrate of cytochrome P-450ALc (P450IIE). This same family of cytochromes also

metabolizes alcohol. The induction of cytochrome P450IIE by benzene with subsequent generation of oxygen radicals, circulating lipid peroxides, and hydroxyl radicals could be associated with hematopoietic toxicity and carcinogenicity of benzene (Parke 1989). Metabolites have been identified consequent to cytochrome P-450 induction by benzene (Henderson et al. 1989). However, it would be of value to know if reconstituted cytochromes P-4501, P-450IIB, and P-45011E metabolize benzene to different products. Cytochromes P-450 are not confined to the liver, but are ubiquitous to all tissue. Benzene metabolism in bone marrow is not well understood; additional data regarding the initial oxidation step and the comparatively low levels of cytochrome P-450 activity in bone marrow would be useful in identifying the mechanisms of benzene's hematotoxicity. This aspect of metabolism may have implications for long-term exposures, which could be explored in chronic exposure studies. The intermediary metabolites of benzene are responsible for many of the toxic effects observed (Eastmond et al. 1987; Gad-El Karim et al. 1985). Biotransformation is believed to be essential for benzeneinduced bone marrow damage. However, there is disagreement as to whether benzene is activated in the marrow, activated elsewhere and transported to the marrow, or metabolized in the liver and the metabolites activated in the marrow. Further studies on the metabolism of benzene would help define its mechanism of action. Additionally, more information is needed on the pathways of metabolism in humans, the chemical nature of the toxic metabolites, and the mechanism of toxicity. Recently published data comparing urinary metabolite profiles of orally administered benzene and phenol in mice suggest that zonal differences in metabolism in the liver may be responsible for relative differences in the production of hydroquinone, thus explaining the higher toxicity observed after benzene administration compared with phenol administration (Kenyon et al. 1995). Additional work in this area would aid in further understanding the kinetic determinants of benzene toxicity. Ethanol and dietary factors such as food deprivation and carbohydrate restriction enhance the hematotoxic effects of benzene. Therefore, more information regarding differences in metabolic pattern according to sex, age, nutritional status, and species, and correlation to differences in health effects would be useful. Ongoing studies are currently focusing on problems in toxicokinetics (see Section 2.10.3).

Humans and animals both excrete inhaled benzene via expiration. Additionally, benzene metabolites are excreted primarily in the urine in both humans and animals. No studies in humans exist for excretion of oral doses of benzene. Studies in several animal species indicate that the route of excretion of benzene and/or its metabolites is a function of exposure level and the saturation of metabolic systems (Henderson et al. 1989). Data regarding excretion following dermal exposure in humans are limited. However, the major route of excretion in both humans and animals following

dermal exposure is the urine. More information from animal model systems is needed on excretion following oral and dermal exposure to benzene.

Comparative Toxicokinetics. Qualitatively, absorption, distribution, metabolism, and excretion appear to be similar in humans and laboratory animals. However, quantitative variations in the absorption, distribution, metabolism, and excretion of benzene have been observed with respect to exposure routes, sex, nutritional status, and species. Further studies that focus on these differences and their implications for human health would be useful. Additionally, *in vitro* studies using human tissue and further research into PBPK modeling in animals would contribute significantly to the understanding of the kinetics of benzene and would aid in the development of pharmacokinetic models of exposure in humans. These topics are being addressed in ongoing studies (see Section 2.10.3).

Methods for Reducing Toxic Effects. Development of methods and practices that are specific for benzene is needed for reducing peak absorption, body burden and for interfering with the mechanism of action following benzene exposures. Since benzene metabolites are thought to play the major role in the toxicity and carcinogenicity, more information is needed about their covalent binding to nucleic acids and cellular macromolecules. This information would help the development of methods for possible prevention of benzene-induced toxicity. Current lines of investigation in other areas include the use of non-steroidal anti-inflammatory drugs to block prostaglandin and prostaglandin synthetase-mediated activity after benzene exposure, and the support of IL-1 cytokine activity to prevent depression of hematopoiesis.

2.10.3   Ongoing Studies

Ongoing studies were found in abstract form in the open literature. These studies include the investigation of hemopoietic effects in adult mice at low exposures (Fart-is et al. 1995), strain differences in metabolism (Grotz and Witz 1995), analysis of benzene metabolites and glutathione in the bone marrow of mice (Henderson et al. 1995), comparison of pulmonary and hepatic-microsomal metabolism of benzene in rats (Chaney and Carlson 1995), chromosomal aberrations or DNA repair in exposed humans (Anderson et al. 1995; Hallberg et al. 1995; Mills et al. 1994; Smith et al. 1995), DNA adduct formation (Hedli et al. 1995), effects of chronic, low dose benzene on liver enzymes in mice (Daiker et al. 1995), effects of hydroquinone on T-cell proliferation (Li et al. 1995), and influence of variations in metabolism on benzene toxicity (Seaton et al. 1995). Weisel and Yu (1995)

are studying exhaled breath of benzene and urinary muconic acid after environmental benzene exposure in humans. Lox (1995) is investigating effects in coagulant activity in male rats after inhalation exposure to benzene, as a marker of pathogenesis. Additional work is being conducted on the PBPK model for benzene and its metabolites (Kenyon and Medinsky 1995). Current research aimed at substituting glucose for benzene as a starting material in many industrial applications may eventually limit worker exposure to benzene (Black 1995). The most recent ongoing research spans many different approaches, and includes deriving potency factors for benzene and some of its metabolites (Davidson and Faust 1996); cell-specific metabolism of benzene in progenitor cells in human bone marrow (Ross et al. 1996); hematotoxicity of 1,2,4-benzenetriol in mice (Hedli et al. 1996); use of protein adduct in rat blood to study reactive intermediates of benzene (Yeowell-O'Connell et al. 1996); inhibition of human topoisomerase II by the benzene metabolite diphenoquinone (Frantz and Eastmond 1996); benzene metabolism and toxicity in transgenic mice (Valentine et al. 1996); effects of benzene and unleaded gasoline coexposures on benzene metabolism and toxicity (Seaton et al. 1996); mechanistic studies of benzene toxicity related to DNA-protein crosslinking (Schoenfeld et al. 1996); effects of benzoquinone, a benzene metabolite, on human T-cell function (Geiselhart and Freed 1996); the role of iron in hydroquinone and catechol-induced inhibition of lymphoblast proliferation (Li and Freed 1996); and molecular cytogenetics of humans exposed to benzene (Smith et al. 1996).

Recent symposia have addressed the subject of benzene toxicity. "Benzene '95," an international conference on the toxicity, carcinogenesis, and epidemiology of benzene, was held June 17-20, 1995, at Rutgers University, New Brunswick, New Jersey. Proceedings from the symposium, to be published in Environmental Health Perspectives, were in press during the development of this profile. Examination of the symposium program indicates that the field of benzene toxicity is moving rapidly. Specific areas of interest include toxicity and leukemogenesis, chromosomal and genetic damage, metabolism, reactive intermediates, exposure assessment, results of human exposure studies in China and Europe, pharmacokinetics and modelling, and risk assessment. Areas of investigation highlighted in the symposium may be summarized in part below.

Epidemiological studies included surveys of workers in China (Hayes 1996; Linet et al. 1996; Rothman et al. 1996; Yin et al. 1996), and in other areas (Raabe and Wong 1996; Rushton 1996). Leukemia mortality and biomarkers were the focus of these investigations. Snyder and Hedli (1996) presented an overview of benzene metabolism, and benzene metabolism in different species was

discussed by Henderson (1996). Additional work on benzene metabolism addressed cell-specific activation and detoxification in mouse and human bone marrow (Ross et al. 1996), phase II metabolism of benzene (phenylsulfate and phenylglucuronide) (Schrenk et al. 1996), reactive ringopened aldehyde metabolites in benzene toxicity (Witz et al. 1996), and the role of cytochromes P-450 (CYP2El and CYP2Bl) in benzene metabolism and toxicity (Gut et al. 1996). Kinetic modeling was a focus of several presentations, including incorporation of mechanistic information into a PBPK model (Medinsky et al. 1996), and model fitting to human data (Bois et al. 1996). DNA adduct formation after benzene exposure was studied in $B6C3F_1$ mice (Bode11 et al. 1996; Li et al. 1996), and *in vitro* (Bleasdale et al. 1996). Work defining the mechanism of toxicity of benzene includes the impact of metabolites on bone marrow progenitor cells (Irons et al. 1996), inhibition of human topoisomerase II by hydroquinone and other metabolites (Frantz et al. 1996; Hutt and Kalf 1996), and the effect of benzene and its metabolites on the hemopoietic system (Abraham 1996; Farris et al. 1996; Hazel et al. 1996; Laskin and Laskin 1996). Effects of benzene exposure on DNA and other cytogenetic effects were also an important area of discussion (Angelosanto et al. 1996; Forni 1996; Smith et al. 1996; Tunca and Egeli 1996; Zhang et al. 1996); Weisel et al. (1996) are looking at the practical application of using benzene in exhaled breath and urinary muconic acid as biomarkers of environmental exposure to benzene in tobacco smoke. Exhaled breath concentrations of benzene were used to validate a PBPK model. Similar research is ongoing at DOE's Pacific Northwest Laboratories, where a mass spectrometric procedure with nearly instantaneous results has been developed (Thrall 1997). Additional work on biomarkers includes monitoring *S*-phenylcysteine in albumin (Bechtold et al. 1996), a comparison of S-phenyl mercapturic acid muconic acid in the urine (Boogaard et al. 1996), and formation of phenyl guanine found in the urine (Norpoth et al. 1996). Risk estimation was addressed by Cox (1996), Ward et al. (1996), Paxton (1996), and Crump (1996).

Ongoing studies regarding the health effects of benzene were also reported in the FEDRIP (1996) and the CRISP Data Base of National Institutes of Health. These studies may further elucidate the mechanism of action of benzene and further elaborate on the toxicokinetics and intermediate metabolism of benzene. These research efforts may add to our ability to perform more accurate risk assessments for benzene. These research grant studies are listed in Table 2-7. In addition, the Benzene Subregistry Baseline Technical Report of the National Exposure Registry contains information on 1,143 persons who had documented exposure to benzene in their drinking water and were exposed for at least 30 days; continued follow-up of the population is planned to provide information on health effects of environmental exposure to benzene (ATSDR 1995).

Table 2-7. Ongoing Studies on the Health Effects of Benzene

Investigator	Affiliation	Research description	Sponsor
Smith, M.T.	Univ of California Warren Hall, Berkeley, CA	Biomarkers of benzene exposure and genotoxicity	NIEHS
Freed, B.M.	Albany Medical College Albany, NY	Immunotoxicology of benzene metabolites	NIEHS
Lawrence, D.A.	Wadsworth Center for Labs and Research Albany, NY	Hematoxic effects of benzene and doxorubicin	NIEHS
Ross, D.	University of Colorado Health Sciences Center Denver, CO	Metabolic basis of benzene-induced myelotoxicity	NIEHS
Witz, G.	University of Medicine and Dentistry of New Jersey Piscataway, NY	Muconaldehyde in relation to benzene hematotoxicity	NIEHS
Smith, M.T.	University of California Berkeley, CA	Biomarkers of genetic damage in human cells	NIEHS
Wiencke, J.K.	University of California Berkeley, CA	Molecular biomarkers of occupational benzene exposure	NIEHS
Bodell, W.J.	University of California Berkeley, CA	DNA adducts as molecular dosimeters of genotoxins	NIEHS
Melikian, A.A.	American Health Foundation Valhalla, NY	Biological markers for tobacco smoke exposure to benzene	NCI
Hoffmann, D.	American Health Foundation	Experimental tobacco carcinogenesis	NCI
Christiani, D.	Harvard University Boston, MA	Petrochemical exposure and reproductive outcomes	NIOSH
Naughton, G.	Marrow Tech Inc.	*In vitro* models for hematoxicity and hepatoxicity	DHHS
Fischer, L.	Michigan State Univeristy East Lansing, MI	Health hazards from groundwater contamination	NIEHS
Turtletaub, K.	University of California Berkeley, CA	Protein and DNA adducts following low dose exposure by accelerator mass spectrometry	NIEHS
Kalman, D.	University of Washington Seattle, WA	Dosimetry assessment of exposure to volatile compounds	NIEHS

2. HEALTH EFFECTS

Table 2-7. Ongoing Studies on the Health Effects of Benzene (continued)

Investigator	Affiliation	Research description	Sponsor
Anthony, D.C.	Brigham & Women's Hospital	Hemoglobin adducts—dosimeter of internal exposure	NIEHS
Burlingame, A.L.	University of California Berkeley	Detection and characterization of protein and DNA adducts by mass spectrometry	NIEHS
Omiecinski, C. J.	University of Washington	Fingerprinting of cytochrome P-450 profiles as biomarkers of chemical exposure	NIEHS
Kavanaugh, T. J.	University of Washington	Glutathione biosynthesis as a biomarker of toxic exposure	NIEHS
Carlson, G.P.	Purdue University	Ethanol and xenobiotic metabolism and toxicity in lung	NIEHS
Smith, A.	University of California Berkeley	Mutagenesis and carcinogenesis	NIEHS
Adams, D. O.	Duke University	Immunotoxicology—environmental agents and macrophages	NIEHS
King, M.	University of California Berkeley	Human genetics, environmental interactions	NIEHS
Sehnert, S.S.	Johns Hopkins University	Pollutant-particle-lung surfactant interactions	NIEHS
Ehrlich, M.	Tulane University of Louisiana	Sensitive assays to assess various types of genotoxicity	NIEHS
Rappaport, S.M.	University of North Carolina	Development and application of biomarkers of exposure	NIEHS
Hertz-Picciotto, I.	University of North Carolina	Core—epidemiology core	NIEHS
Benjamin, S.A.	Colorado State University	Chemical mixtures as promoters of hepatocarcinogenesis	NIEHS
Yang, R.S.H.	Colorado State University	Toxicological interaction studies in chemical mixtures—pharmaco-kinetics	NIEHS

Table 2-7. Ongoing Studies on the Health Effects of Benzene (continued)

Investigator	Affiliation	Research description	Sponsor
Billings, R.E.	Colorado State University	Mechanisms of toxic chemical interaction in the liver—hepatotoxicity	NIEHS
Lowndes, H. E.	Rutgers State University	Glutathione conjugation and its role in neurotoxicity	NIEHS
Barrett, J.	NIEHS	Role of mutagenesis in carcinogenesis	NIEHS
French, J. E.	NIEHS, NIH	Karyotype of malignant skin tumors of tg.ac (zeta-globin promoted V-ha-ras) mice	NIEHS
Singer, B.	University of California-Lawrence Berkeley Laboratory	Chemical basis of carcinogenic risk from benzene	NIEHS
Spalding, J. W.	NIEHS, NIH	A transgenic mouse model for dermal carcinogenesis	NIEHS
Blot, W.	NCI	Analytical cancer studies, including field studies in high-risk areas	Division of Cancer Etiology
Irons, R.	University of Colorado Denver, CO	Mechanism(s) of chemical leukemogenesis	NIEHS
Henderson, R.	Inhalation Toxicology Research Institute Albuquerque, NM	Evaluation of bone marrow metabolites for dosinetry and biomarkers	American Petroleum Institute
Irons, R.	University of Colorado Denver, CO	Biological mechanism of benzene leukemogenesis	American Petroleum Institute
Kalf, G.	Jefferson Medical College Philadelphia, PA	Influence of hydroquinone on benzene leukemogenesis	American Petroleum Institute
Bechtold, W.F.	Inhalation Toxicology Research Institute Albuquerque, NM	Use of benzene metabolite adducts with (S-phenylcysteine) as a marker of exposure	Health Effects Institute
Melikian, A.A.	American Health Foundation	Quantification of urinary metabolites; metabolic activation pathways	Health Effects Institute
Turteltaub, K.W.	Livermore National Laboratory and University of California at San Francisco San Francisco, CA	In vivo metabolic studies; characterization of DNA and protein adducts in bone marrow	Health Effects Institute

2. HEALTH EFFECTS

Table 2-7. Ongoing Studies on the Health Effects of Benzene (continued)

Investigator	Affiliation	Research description	Sponsor
Eastmond, D.A.	University of California Riverside, CA	Chromosomal alterations induced in mice and humans	Health Effects Institute
Eastmond, D.A.	University of California	Chromosomal mechanisms in environmental carcinogenesis	U.S. Department of Agriculture
Cross, T.A.	Florida State University	Protein stability—catalytic and noncatalytic solvents	National Institute of General Medical Sciences
Trager, W.F.	University of Washington	Isotope Effects— cytochrome P-450 catalyzed oxidations	National Institute of General Medical Sciences
Hanzlik, R.P.	University of Kansas	Mechanisms of P-450 catalyzed C oxidations	National Institute of General Medical Sciences
Grimes, P.	Charles R. Drew University	Phenolic chemicals and viral agents in the pathogenesis of immune abberations	National Institute of General Medical Sciences
Gonzalez, F .J.	NCI, NIH	Transgenic mice, gene knockout mice, and cytochrome P-450 function	Division of Cancer Etiology
Shields, P. G.	NCI, NIH	Molecular epidemiology of human lung cancer	Division of Cancer Etiology
Nims, R. W.	NCI, NIH	Mechanisms and interspecies differences in tumor promotion	Division of Cancer Etiology
Poirier, M. C.	NCI, NIH	Use of immunological techniques to study the interaction of carcinogens with DNA	Division of Cancer Etiology
Gail, M. H.	NCI, NIH	Consulting on epidemiologic methods	Division of Cancer Etiology

DHHS = Department of Health and Human Services; ORD = Office of Research and Development; NCI = National Cancer Institute; NIEHS = National Institute of Environmental Health; NIH = National Institutes of Health; NIOSH = National Institute for Occupational Safety and Health

3. CHEMICAL AND PHYSICAL INFORMATION

3.1   CHEMICAL IDENTITY

Information regarding the chemical identity of benzene is shown in Table 3-l. Although the term benzol is found in older literature for the commercial product, benzene is the name presently approved by the International Union of Pure and Applied Chemistry (IUPAC), the Chemical Manufacturers Association (CMA), and the American Society for Testing and Materials (ASTM) for the pure product (HSDB 1996).

3.2   PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of benzene is shown in Table 3-2. The major impurities found in commercial products are toluene, xylene, phenol, thiophene, carbon disulfide, acetylnitrile and pyridine (NIOSH 1974). Commercial refined benzene-535 is free of hydrogen sulfide and sulfur dioxide, but contains a maximum of 1 ppm thiophene and a maximum of 0.15% nonaromatics. Refined nitration-grade benzene is free of hydrogen sulfide and sulfur dioxide. Benzene is also commercially available as thiophene-free, 99 mole%, 99.94 mole% and nanograde quality (HSDB 1996).

Table 3-1. Chemical Identity of Benzene

Characteristic	Information	Reference
Chemical name	Benzene	HSDB 1996
Synonym(s)	Annulene, benzeen (Dutch), benzen (Polish), benzol, benzole; benzolo (Italian), coal naphtha, cyclohexatriene, fenzen (Czech), phene, phenyl hydride, pyrobenzol, pyrobenzole	HSDB 1996
Registered trade name(s)	Polystream	IARC 1982
Chemical formula	C_6H_6	Merck 1989
Chemical structure		Merck 1989
Identification numbers:		
CAS registry	71–43–2	HSDB 1996
NIOSH RTECS	CY–1400000	HSDB 1996
EPA hazardous waste	NA	
OHM/TADS	7216601	HSDB 1996
DOT/UN/NA/IMCO shipping	UN1114; IMO3.2	HSDB 1996
HSDB	35	HSDB 1996
NCI	C55276	HSDB 1996
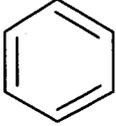
CAS = Chemical Abstracts Service; DOT/UN/NA/IMO = Dept. of Transportation/United Nations/ North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

3. CHEMICAL AND PHYSICAL INFORMATION

Table 3-2. Physical and Chemical Properties of Benzene

Property	Information	Reference
Molecular weight	78.11	Merck 1989
Color	Clear, colorless liquid	Merck 1989
Physical state	Rhombic prisms	HSDB 1996
Melting point	5.5 °C	Merck 1989
Boiling point	80.1 °C	Merck 1989
Density at 15 °C, g/cm^3	0.8787	Merck 1989
Odor	Aromatic	NFPA 1994
Odor threshold:		HSDB 1996
Water	2.0 mg/L	HSDB 1996
Air	4.9 mg/m^3	
Taste threshold:	0.5–4.5 mg/L	HSDB 1996
Solubility:		
Water at 25 °C	w/w: 0.188%	Merck 1989
Organic solvents	Alcohol, chloroform, ether, carbon disulfide, acetone, oils, carbon, tetrachloride, glacial acetic acid	Merck 1989
Partition coefficients:		
Log K_{ow}	2.13	HSDB 1996; Karickhoff
Log K_{oc}	1.8–1.9	1981; Kenaga 1980
Vapor pressure at 20 °C	75 mm Hg	NFPA 1994
Henry's law constant at 25 °C	5.5×10^{-3} atm-m^3/mol	Mackay and Leinonen 1975
Autoignition temperature	498 °C	NFPA 1994
Flashpoint	−11 °C (closed cup)	Merck 1989
NFPA Hazard Classification:		
Health	2.2	HSDB 1996
Flammability	3.3	
Reactivity	0.0	
Flammability limits in air	1.2% (lower limit; 7.8% (upper limit)	NFPA 1994
Conversion factors	1 ppm = 3.24 mg/m^3 at 20 °C and 1 atm pressure; 1 mg/m^3 = 0.31 ppm	HSDB 1996
Explosive limits	1.4% (lower limit); 8% (upper limit)	HSDB 1996

NFPA = National Fire Protection Association

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

4.1 PRODUCTION

In 1825, Faraday isolated benzene from a liquid condensed by compressing oil gas. Benzene was first synthesized by Mitscherlich in 1833 by distilling benzoic acid with lime. Benzene was first commercially recovered from light oil derived from coal tar in 1849 and from petroleum in 1941 (IARC 1982a). Several years after the end of World War II, the rapidly expanding chemical industry created an increased demand for benzene that the coal carbonization industry could not fulfill. To meet this demand, benzene was produced by the petroleum and petrochemical industries by recovery from reformat and liquid by-products of the ethylene manufacturing process (Purcell 1978).

Currently, benzene is commercially recovered from both coal and petroleum sources. More than 98% of the benzene produced in the United States is derived from the petrochemical and petroleum refining industries (OSHA 1987b). These sources include refinery streams (catalytic reformats), pyrolysis gasoline, and toluene hydrodealkylation. Catalytic reformat is the major source of benzene (Greek 1990). Between 1978 and 1981, catalytic reformats accounted for approximately 44-50% of the total U.S. benzene production (Fishbein 1988a). During catalytic reforming, cycloparaffins (also known by the obsolescent term "naphthenes") such as cyclohexane, methyl cyclohexane, and dimethylcyclohexane are converted to benzene by isomerization, dehydrogenation, and dealkylation, and paraffins in naphtha (such as hexane) are converted to benzene by cyclodehyrogenation. The process conditions and the catalyst determine which reaction will predominate. The benzene is recovered by solvent extraction (e.g., with sulpholane or tetraethylene glycol). Pyrolysis gasoline is a liquid by-product produced by the steam cracking of lower paraffins (gas oil) or heavier hydrocarbons (heavy naphtha). Pyrolysis gasoline contains unsaturated aliphatic hydrocarbons (such as ethylene and propylene) and aromatics. Several integrated pyrolysis gasoline treatment processes are available including partial hydrogenation and extractive distillation; hydrogenation, hydrodesulfurization, and solvent extraction; or partial hydrogenation, desulfurization, hydrocracking, hydrodealkylation, and distillation for the optimization of benzene yield and the recovery of benzene (IARC 1982a; Purcell 1978). In the toluene hydrodealkylation process, toluene or toluene/xylene mixtures are reacted with hydrogen at temperatures up to 730 °C and demethylated to produce benzene and methane. Another process whereby toluene is converted to benzene and xylenes by transalkylation or disproportionation is also used for the production of benzene (Purcell 1978). Small quantities of benzene are also

produced from destructive distillation of coal used for coke manufacture. Benzene is derived from the light oil fraction produced during the coking process (Greek 1990; Purcell 1978). New coking, gasification, and liquefaction processes for coal are all potential sources of benzene (IARC 1982a). Of the total U.S. production capacity of 17.2 billion pounds in 1993, catalytic reformats constituted 45%, toluene and xylene 29%, pyrolysis gasoline 23% and coke oven <3% (SRI 1993).

In 1994, benzene was the 17th highest volume chemical produced in the United States, compared to the 18th and 17th ranks in 1993 and 1992, respectively (C&EN 1994; Kirschner 1995; Reisch 1994). Based on actual and preliminary production data and excluding tar distillers and coke-oven operators, the U.S. production of benzene was 14.7 billion pounds in 1994, 12.3 billion pounds in 1993, 1.64 billion gallons (12.0 billion pounds) in 1992, and 1.57 billion gallons (11.5 billion pounds) in 1991 (C&EN 1995). Production data from 1984 to 1994 indicate that the production of benzene increased by about 4% annually (C&EN 1995). Preliminary production data reported for the first and second quarters of 1995 show potential towards continuing this trend: 3.8 billion L (7.4 billion pounds) were produced (USITC 1995).

SRI (1994) lists the following companies as producers of benzene in the United States in 1994: Amoco Oil Co., Texas City, Texas; Ashland Oil Co., Catlettsburg, Kentucky; BP Oil, Alliance, Louisiana, and Lima, Ohio; Chevron Chemical Co., Pascagoula, Mississippi, Philadelphia, Pennsylvania, and Port Arthur, Texas; CITGO Petroleum Corp., Corpus Christi, Texas, and Lake Charles, Louisiana; Coastal Eagle Point Oil Co., Westville, New Jersey; Coastal Refining and Marketing, Inc., Corpus Christi, Texas; Dow Chemical, USA, Freeport, Texas, and Plaquemine, Louisiana; Exxon Chemical Co., Baton Rouge, Louisiana, and Baytown, Texas; Fina Oil and Chemical Co., Port Arthur, Texas; Hess Oil Virgin Islands Corp., St. Croix, U.S. Virgin Islands; Huntsman Chemical Corp., Bayport, Texas; Koch Refining Co., Corpus Christi, Texas; Lyondell-CITGO Refining Co. Ltd., Houston, Texas; Lyondell Petrochemical Co., Channelview, Texas; Marathon Oil Co., Texas City, Texas; Mobil Oil Corp., Beaumont, Texas, and Chalmette, Louisiana; Occidental Petroleum Corp., Chocolate Bayou, Texas, and Corpus Christi, Texas; Phibro Energy USA, Inc., Houston, Texas; Phillips Petroleum Co., Sweeny, Texas; Phillips Puerto Rico Core Inc., Guayama, Puerto Rico; Quantum Chemical Co., La Porte, Texas; Shell Chemical Co., Deer Park, Texas, and Wood River, Illinois; Southwestern Refining Co., Inc., Corpus Christi, Texas; Sun Co., Inc., Marcus Hook, Pennsylvania and Toledo, Ohio; Texaco Chemical Co., El Dorado, Kansas; and the UNO-VEN Co., Lemont, Illinois. In addition, the United States International Trade Commission lists the following

companies as producers: Oxy Petrochemicals, Inc., Dallas, Texas, and Kalama Chemical, Inc., Seattle, Washington (USITC 1995). Facilities listed as being responsible for producing the top 50% of regional capacity in the United States include: Chevron Chemical (18%); Exxon Chemical Company (8%); Shell Chemical Company (7%); Dow Chemical Company (7%); BP Oil Company (7%); and Occidental Petroleum Corporation (7%) (SRI 1995).

According to the Toxics Release Inventory (TRI), 472 facilities in the United States produced or processed benzene in 1993 (TRI93 1995). Table 4-l lists the facilities in each state that manufacture or process benzene, the intended use, and the range of maximum amounts of benzene that are stored on site. The TRI data listed in Table 4-l should be used with caution since only certain types of facilities were required to report. Therefore, this is not an exhaustive list.

4.2   IMPORT/EXPORT

The import of benzene into the United States is dependent on domestic production and demand. Imports of benzene (both petroleum and coal tar derived) into the United States were approximately 200 million L (387 million pounds) in 1990, 305 million L (591 million pounds) in 1991, 272 million L (528 million pounds) in 1992, 376 million L (729 million pounds) in 1993, and 304 million L (589 million pounds) in 1993 (USDOC 1995). The largest exporters of benzene to the United States during 1991-93 were Canada, Brazil, the Netherlands, and Mexico (USDOC 1994). As in the case of import, the export of benzene from the United States to other countries is dependent on domestic and world production and demand. The 1990-94 data indicate a decline in export volumes for benzene during this period. Exports of benzene (both petroleum and coal-tar derived) to other countries were approximately 289 million L (560 million pounds) in 1990, 54 million L (105 million pounds) in 1991, 63 million L (123 million pounds) in 1992, 3.3 million L (6.4 million pounds) in 1993, and 59.6 million L (115 million pounds) in 1994 (USDOC 1995). The largest importers of benzene from the United States are Canada, the Netherlands, Taiwan, and South Korea (USDOC 1994).

Table 4-1. Facilities That Manufacture or Process Benzene

State[a]	Number of facilities	Range of maximum amounts on site in thousands of pounds [b]	Activities and uses [c]
AK	4	0-10000	1, 3, 4, 8, 10
AL	18	0-50000	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13
AR	4	1-10000	1, 3, 4, 7, 13
AZ	2	10-1000	1, 6, 10, 13
CA	35	0-50000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13
CO	2	1000-10000	1, 3, 4, 6, 7
DE	4	1-50000	1, 6, 7, 9, 12, 13
GA	6	0-10000	1, 2, 4, 8, 9, 10, 11
HI	2	1000-10000	1, 2, 6
IA	1	1000-10000	1, 4
IL	26	1-100000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
IN	18	1-100000	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 13
KS	10	0-50000	1, 3, 4, 5, 6, 7, 8, 9, 10, 13
KY	12	1-50000	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13
LA	40	0-500000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13
MD	3	1-10000	7, 8, 9
MI	28	0-10000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13
MN	3	1-100000	1, 3, 4, 6, 8, 9
MO	7	0-100	8, 9, 13
MS	6	10-50000	1, 3, 4, 6, 7, 8
MT	4	100-500000	1, 2, 3, 4, 6, 7, 8, 9
NC	3	0-1000	1, 5, 11
ND	1	10000-50000	1, 2, 3, 4, 7
NE	1	0-1	11
NJ	12	1-50000	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13
NM	4	1-10000	1, 3, 4, 6, 8, 13
NY	5	1-1000	1, 2, 4, 5, 9, 11, 13
OH	25	0-100000	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 13
OK	6	1-50000	1, 2, 3, 4, 5, 6, 7, 8, 9, 13
OR	1	1000-10000	10
PA	21	0-50000	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 13
PR	5	100-50000	1, 2, 3, 5, 6, 7, 8
SC	3	0-1000	1, 5, 7, 12
TN	9	0-10000	1, 3, 5, 6, 7, 8, 9, 12, 13
TX	94	0-500000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
UT	8	10-50000	1, 2, 3, 4, 6, 7, 8, 9, 10, 13
VA	5	1-1000	1, 2, 3, 5, 6, 7, 9, 11
VI	1	100000-500000	1, 2, 3, 4, 7

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

Table 4-1. Facilities That Manufacture or Process Benzene (continued)

State[a]	Number of facilities	Range of maximum amounts on site in thousands of pounds [b]	Activities and uses [c]
WA	6	100-100000	1, 2, 3, 4, 6, 7, 8
WI	4	1-10000	1, 6, 9, 12, 13
WV	8	1-10000	1, 4, 5, 6, 7, 8, 9, 13
WY	5	0-10000	1, 3, 4, 5, 6, 7, 8, 10

Source: TRI93 1995

[a] Post office state abbreviations used
[b] Data in TRI are maximum amounts on site at each facility
[c] Activities/Uses:

1. Produce	8. As a formulation component
2. Import	9. As a product component
3. For on-site use/processing	10. For repackaging only
4. For sale/distribution	11. As a chemical processing aid
5. As a by-product	12. As a manufacturing aid
6. As an impurity	13. Ancillary or other uses
7. As a reactant	

4.3 USE

Benzene has been used extensively as a solvent in the chemical and drug industries, as a starting
material and intermediate in the synthesis of numerous chemicals, and as a gasoline additive (NTP
1994).

Benzene recovered from petroleum and coal sources is used primarily as an intermediate in the
manufacture of other chemicals and end products. The major uses of benzene are in the production of
ethylbenzene, cumene, and cyclohexane. Ethylbenzene (55% of benzene production volume) is an
intermediate in the synthesis of styrene, which is used to make plastics and elastomers. Cumene
(24%) is used to produce phenol and acetone. Phenols are used in the manufacture of phenolic resins
and nylon intermediates; acetone is used as a solvent and in the manufacture of pharmaceuticals.
Cyclohexane (12%) is used to make nylon resins. Other industrial chemicals manufactured from
benzene include nitrobenzene (5%), which is used in the production of aniline, urethanes, linear
alkylbenzene sulfonates, chlorobenzene, and maleic anhydride (Eveleth 1990; Greek 1990; HSDB
1996). Benzene is also a component of gasoline since it occurs naturally in crude oil and since it is a
byproduct of oil refining processes (Brief et al. 1980; Holmberg and Lundberg 1985). Benzene is
especially important for unleaded gasoline because of its anti-knock characteristics. For this reason,
the concentration of aromatics, such as benzene, in unleaded fuels has increased (Brief et al. 1980).
The percentage by volume of benzene in unleaded gasoline is approximately l-2% (NESCAUM
1989).

The EPA has listed benzene as a hazardous air pollutant and a hazardous waste (EPA 1977, 1981~).
In addition, there is sufficient evidence to support classifying benzene as a human carcinogen
(Group A) (IRIS 1996). One result of EPA's action is that the widespread use of benzene as a solvent
has decreased in recent years. Many products that used benzene as solvents in the past have replaced
it with other organic solvents; however, benzene may still occur as a trace impurity in these products.
Less than 2% of the amount produced is used as a solvent in such products as trade and industrial
paints, rubber cements, adhesives, paint removers, artificial leather, and rubber goods. Benzene has
also been used in the shoe manufacturing, and rotogravure printing industries (EPA 1978b; Farm
Chemical Handbook 1993; OSHA 1977). In the past, certain consumer products (such as some paint
strippers, carburetor cleaners, denatured alcohol, and rubber cement used in tire patch kits and arts and
crafts supplies) contained small amounts of benzene (Young et al. 1978). Other consumer products

that contained benzene were certain types of carpet glue, textured carpet liquid detergent, and furniture wax (Wallace et al. 1987).

The Consumer Products Safety Commission (CPSC) withdrew an earlier proposal to ban consumer products, except gasoline and laboratory reagents, that contained benzene as an intentional ingredient or as a contaminant at 0.1% or greater by volume. The withdrawal of the rulemaking was based on CPSC findings that benzene was no longer used as an intentional ingredient and that the contaminant levels remaining in certain consumer products were unlikely to result in significant exposures (NTP 1994). Products containing more that 5% benzene, and paint solvents and thinners containing 10% or more of petroleum distillates such as benzene, are required to meet established labeling requirements. In a guidance document targeting school science laboratories, the CPSC recommended that benzene not be used or stored in schools. The document identified benzene as a carcinogen and ascertained that the hazards posed by its use in high school laboratories may be greater that its potential usefulness.

The use of benzene in certain pesticides has been canceled (HSDB 1996). Under the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA), the EPA established a voluntary cancellation of registered products containing benzene as an active ingredient (HSDB 1996; IRIS 1996). Benzene was once used alone or in formulations to control screwworms on animals, and as an early fumigant for grain (HSDB 1996). The EPA also required labeling on pesticides containing benzene as an inert ingredient. The U.S. Food and Drug Administration (FDA) regulates benzene as an indirect food additive under the Food, Drug, and Cosmetics Act (FDCA). Under the FDCA benzene is restricted to use only as a component of adhesives used on articles intended for packaging, transport, or holding foods (FDA 1977a).

4.4   DISPOSAL

Benzene-containing wastes, such as commercial chemical products, manufacturing chemical intermediates, and spent solvents, are subject to federal and/or state hazardous waste regulations (HSDB 1996). Regulations governing the treatment and disposal of benzene-containing wastes are presented in Chapter 7. Waste by-products from benzene production processes include acid and alkali sludges, liquid-solid slurries, and solids (EPA 1982f; Saxton and Narkus-Kramer 1975). In the past, landfilling and lagooning have been the major methods of disposal of benzene-containing industrial

wastes (EPA 1982f). In addition to biodegradation, a portion of the benzene is expected to be lost due to volatilization. Unfortunately benzene, along with other hazardous contaminants, also leaches into groundwater from the lagooned wastes. Currently, the recommended method of disposal is to incinerate solvent mixtures and sludges at a temperature that ensures complete combustion. The recommended methods for combustion are liquid injection incineration at a temperature range of 650-1,600 °C and a residence time of 0.1-2 seconds; rotary kiln incineration at a temperature range of 82&1,600 °C and residence times of seconds for liquids and gases, and hours for solids; fluidized bed incineration at a temperature range of 450-980 °C and residence times of seconds for liquids and gases and longer for solids (HSDB 1996). Since benzene bums with a very smoky flame, dilution with alcohol or acetone is suggested to minimize smoke. Small quantities of benzene waste can be destroyed by chemical reaction. For example, treating benzene with dichromate in strong sulfuric acid for l-2 days is sufficient for total destruction (HSDB 1996). Small quantities can also be ignited after pouring on dry sand or earth (OHM/TADS 1990).

Underground injection also appears to be an important disposal method in some states. Approximately 356,000 pounds of benzene (2.7% of the total environmental release) was disposed of by underground injection. This release via underground injection in 1992 was lower than the amount (654,000 pounds) released in 1992 (TRI90 1992). In addition, 340,000 pounds of benzene (2.6% of the total environmental release) was disposed of via land disposal (TRI92 1994). The amount discharged to soil in 1992 was less than half the amount (724,000 pounds) discharged in 1990 (TRI90 1992).

Several methods exist for the treatment of waste water that contains benzene: biological treatment (aeration or activated sludge process), solvent extraction, air and/or steam stripping, and activated carbon process (EPA 1994r; IRPTC 1985). Full-scale chemical treatability studies have demonstrated 95-100% reductions in benzene concentrations for industrial waste waters receiving biological treatments (HSDB 1996). A combination of steam stripping and air stripping, and a vapor extraction system that removes the separated benzene vapor may be suitable for the treatment of contaminated groundwater and soil (Naft 1992). An *in situ* bioremediation process has been used to decontaminate a site by delivering a controlled amount of nitrate (to accelerate biodegradation of benzene) to the site under hydraulic control (Kennedy and Hutchins 1992).

5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Benzene is released to the environment by both natural and industrial sources, although the anthropogenic emissions are undoubtedly the most important. Emissions of benzene to the atmosphere result from gasoline vapors, auto exhaust, and chemical production and user facilities. EPA's estimate of nationwide benzene atmospheric emissions from various sources was ≈34,000 metric tons/year (EPA 1989d). According to the Toxics Release Inventory, releases to the air from manufacturing and processing facilities were ≈10.2 million pounds (≈4,600 metric tons) in 1993 (TRI93 1995). Releases to air accounted for about 96.2% of the total industry-related releases to the environment (TRI93 1995). Benzene is released to water and soil from industrial discharges, landfill leachate, and gasoline leaks from underground storage tanks.

Chemical degradation reactions, primarily reaction with hydroxyl radicals, limit the atmospheric residence time of benzene to only a few days, and possibly to only a few hours. Benzene released to soil or waterways is subject to volatilization, photooxidation, and biodegradation. Biodegradation, principally under aerobic conditions, is the most important environmental fate process for water- and soil-associated benzene.

Benzene is ubiquitous in the atmosphere. It has been identified in air samples of both rural and urban environments and in indoor air. Although a large volume of benzene is released to the environment, environmental levels are low because of efficient removal and degradation processes. Benzene partitions mainly into air (99.9%) and inhalation is.the dominant pathway of human exposure; inhalation accounts for more than 99% of the total daily intake of benzene (Hattemer-Frey et al. 1990). The general population is exposed to benzene primarily by tobacco smoke (both active and passive smoking) and by inhaling contaminated air (particularly in areas with heavy motor vehicle traffic and around filling stations). Air around manufacturing plants that produce or use benzene arid air around landfills and hazardous waste sites that contain benzene are additional sources of exposure. Exposure to benzene can also result from ingestion of contaminated food or water. Use of contaminated tap water for cooking, showering, etc., can also be a source of inhalation exposure since benzene can volatilize from water. Compared to inhalation, dermal exposure probably accounts for a minor portion of the total exposure of the general population. The magnitude of exposure is greatest for those

individuals occupationally exposed to benzene; however, a far greater number of individuals are exposed as a result of benzene released from gasoline filling stations, from smoking tobacco products, and from auto exhaust. Smoking was found to be the largest anthropogenic source of background human exposure to benzene (Hattemer-Frey et al. 1990).

Benzene has been identified in at least 816 of the 1,428 current or former NPL hazardous waste sites (HazDat 1996). However, the number of sites evaluated for benzene is not known. The frequency of these sites within the United States can be seen in Figure 5-1. Of these sites, 813 are located in the United States, 1 is located in the Virgin Islands, and 2 are located in the Commonwealth of Puerto Rico (not shown).

5.2   RELEASES TO THE ENVIRONMENT

5.2.1   Air

Benzene is released into the atmosphere from both natural and industrial sources. Natural sources include crude oil seeps, forest fires, and plant volatiles (Brief et al. 1980; Graedel 1978). Major anthropogenic sources of benzene include environmental tobacco smoke, automobile exhaust, automobile refueling operations, and industrial emissions. Using source exposure modeling, it was estimated that benzene emissions were highest from coke oven blast furnaces (Edgerton and Shah 1992). Other sources that contributed to emissions of benzene are automobiles, petrochemical industries, waste water treatment plants, and petroleum industries (Edgerton and Shah 1992). Under California legislation, the City of Los Angeles prepared air toxics emissions inventories at its four waste water treatment plants. The two facilities with greater potential to emit toxics had benzene emission rates of 163 and 108 kg/year. The other facilities had benzene emission rates of 1 and 3 kg/year (Mayer et al. 1994). EPA (1989e) estimates of recent nationwide benzene atmospheric emissions, in metric tons/year (kkg/year), from various industrial sources include: (1) coke by-product recovery plants (17,000 kkg/year), (2) benzene waste operations (5,300 kkg/year), (3) gasoline marketing systems, including bulk gasoline terminals and plants, service stations, and delivery tank trucks (4,800 kkg/year), (4) transfer operations at chemical production facilities, bulk terminals, and coke by-product recovery plants (4,600 kkg/year), (5) benzene storage vessels (620-l,290 kkg/year), (6) industrial solvent use (450 kkg/year), (7) chemical manufacturing process vents (340 kkg/year), and (8) ethylbenzene/styrene process vents (135 kkg/year). The 1984 benzene emission inventory for

Figure 5-1. Frequency of NPL Sites with Benzene Contamination

SITES
- ▦ 1-4
- ▨ 5-10
- ▩ 11-20
- ▤ 21-83
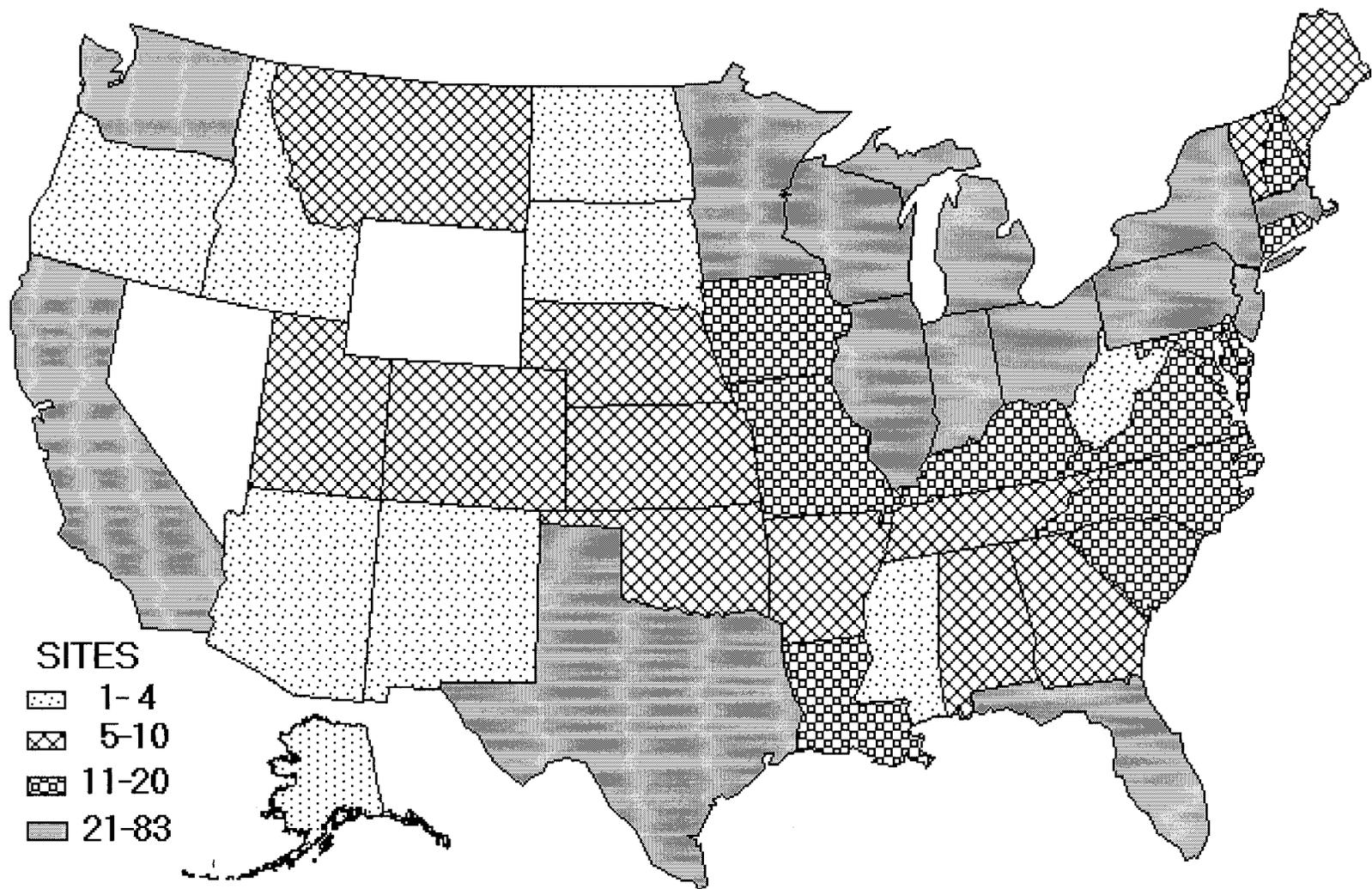
Derived from HazDat 1996

California totaled 19,350 metric tons/year (17,500 kkg/year), with motor vehicle exhaust accounting for 71% of the total emissions (Allen 1987). Benzene is present in passenger car tailpipe emissions at a composition ranging from 2.9% to 15% of the total tailpipe hydrocarbon composition (Black et al. 1980). The contribution of mobile source hazardous air pollutant emissions has been compared to that of stationary sources in the Seattle-Tacoma area. Mobile sources were estimated to contribute approximately 83% of the benzene from stationary area and mobile sources combined, with major stationary point sources excluded (Mangino and Jones 1994). Another source of atmospheric benzene is cigarette smoke and exhaled breath of smokers (Wallace 1989a, 1989b; Wallace and Pellizzari 1986; Wester et al. 1986). Other releases of benzene to the atmosphere include structural fires (Lowry et al. 1985) off-gassing from particle board (Glass et al. 1986), vaporization from oil spills, and emissions from landfills (Bennett 1987; HSDB 1996; Wood and Porter 1987). According to TRI, an estimated total of 10.2 million pounds of benzene, amounting to approximately 96.2% of the total environmental release, was discharged to air from manufacturing and processing facilities in the United States in 1993 (TRI93 1995). Table 5-l lists the amounts released from these facilities. The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

There is a potential for atmospheric release of benzene from hazardous waste sites. Benzene has been detected in air in 136 of the 816 current and former NPL sites where benzene has been detected in some medium (HazDat 1996).

5.2.2   Water

Benzene is released to water from the discharges of both treated and untreated industrial waste water, leachate from landfills and other contaminated soils, gasoline leaks from underground storage tanks, and from accidental spills during marine transportation of chemical products (CDC 1994; Crawford et al. 1995; EPA 1979a; NESCAUM 1989; Staples et al. 1985). A fire in a tire dump site in western Frederick county, Virginia, produced a free-flowing oily tar containing benzene among ether chemicals. The seepage from this site contaminated nearby surface water (EPA 1993j). Between 1986 and 1991, 3,000 gallons of benzene were accidentally released into Newark Bay and its major tributaries. Another 3,000 gallons were released in 1991 (Crawford et al. 1995). According to TRI, approximately 18,700 pounds of benzene, amounting to approximately 0.2% of the total environmental release, were discharged to water from manufacturing and processing facilities in the United States in

Table 5-1. Releases to the Environment from Facilities That Manufacture or Process Benzene

Range of reported amounts released in pounds per year [a]

State [b]	Number of facilities	Air	Water	Land	Underground injection	Total environment [c]	POTW transfer	Off-site waste transfer
AK	4	10-39505	0-9	0-250	0	10-39516	0-5	0-1588
AL	18	45-134800	0-250	0-2	0-13	45-134800	0-501	0-282935
AR	4	250-69879	0	0	0-5	255-69879	0	0-95600
AZ	2	94-260	0	0	0	94-260	0-2	0-229
CA	35	0-26000	0-630	0-250	0-197	0-26630	0-30000	0-2814
CO	2	4900-20075	0-1	0	0	4901-20075	0	0-175
DE	4	65-47996	0-550	0-100	0	65-48011	0	20-235
GA	6	0-32114	0-5	0-250	0	0-32115	0	0-12190
HI	2	17600-29100	0	0-5	0-5	17605-29105	0	0-10
IA	1	15700	0	0	0	15700	0	173
IL	26	44-246000	0-750	0-320	0	44-246000	0-2232	0-900000
IN	18	0-80411	0-250	0-1400	0-7000	0-80411	0-3700	0-25414
KS	10	89-47000	0-230	0-3084	0	89-47230	0-1833	0-4182
KY	12	15-185634	0-250	0-250	0	15-185702	0-70	0-801433
LA	40	1-178000	0-680	0-370	0-6700	1-178025	0	0-23157
MD	3	58-3400	0	0-675	0	58-4075	0-30	0-65560
MI	28	4-102693	0-30	0	0-9000	4-102693	0-11949	0-280363
MN	3	180-174500	0-13	0	0	180-174500	0	0-5
MO	7	3-770	0	0	0	3-770	0-250	0-840
MS	6	250-112000	0-250	0-250	0	500-112058	0-6	0-240
MT	4	3750-18900	0-5	0-76	0	3754-18910	0-5	5-48
NC	3	950-88427	0-1	0-1	0	950-88427	0	0-360
ND	1	44500	0	1	0	44501	0	49
NE	1	500	0	0	0	500	0	0
NJ	12	30-68300	0-2403	0-10	0	30-68300	0-75000	0-183000
NM	4	10-24758	0	0-500	0	10-25008	0	0-755
NY	5	5-4425	0-5	0-250	0	5-4430	0-250	0-6614
OH	25	5-188924	0-250	0-350	0-3	5-188924	0-124	0-11700

Table 5-1. Releases to the Environment from Facilities That Manufacture or Process Benzene (continued)

		Range of reported amounts released in pounds per year [a]						
State[b]	Number of facilities	Air	Water	Land	Underground injection	Total environment[c]	POTW transfer	Off-site waste transfer
OK	6	312-38100	0-46	0-250	0	312-38355	0	0-1334
OR	1	15003	0	0	0	15003	206	65
PA	21	100-170000	0-250	0-428	0	100-170130	0-65000	0-286081
PR	5	250-125696	0	0-1089	0	250-125696	0	0-29
SC	3	1600-262768	0-198	0	0	1600-262966	0	0-52038
TN	9	0-41900	0-5	0	0-1	0-41900	0-4631	0-32800
TX	94	1-372368	0-1520	0-2531	0-117517	5-372990	0-39664	0-633368
UT	8	1440-10222	0	0-100	0	1440-10222	0-11000	0-25
VA	5	44-302000	0-1360	0	0	44-302002	0-456	0-200
VI	1	86389	819	1454	0	88662	0	448
WA	6	8995-33400	0-10	0-250	0	9005-33655	0	0-255
WI	4	10-7441	0	0-334	0	10-7656	0	0-3700
WV	8	12-243000	0-310	0-250	0	12-243005	0	0-2534
WY	5	1290-8470	0-5	0-500	0	1290-8480	0	0-250

Source: TRI93 1995

[a] Data in TRI are maximum amounts released by each facility.
[b] Post office state abbreviations used
[c] The sum of all releases of the chemical to air, land, water, and underground injection wells by a given facility

POTW = publicly owned treatment works

1993 (TRI93 1995). Another 363,700 pounds were released to publicly owned waste water treatment plants (TRI93 1995). Although the major portion of benzene will biodegrade during the treatment processes, a small but undetermined portion will volatilize and a portion will remain unaltered. The ultimate discharge of the treated waste water will release benzene to natural bodies of water. Table 5-l lists the amounts released from these facilities. The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

There is a potential for release of benzene to water from hazardous waste sites. Benzene has been detected in groundwater samples collected at 686 of the 816 current and former NPL sites, in surface water samples collected at 172 of the 816 sites, and in leachate samples collected at 112 of the 816 sites where benzene has been detected in some medium (HazDat 1996).

5.2.3   Soil

Benzene is released to soils through industrial discharges, land disposal of benzene-containing wastes, and gasoline leaks from underground storage tanks. In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil (Mushrush et al. 1994). According to TRI, approximately 21,500 pounds of benzene were released to land from manufacturing and processing facilities in the United States in 1993 (TRI93 1995). An additional   364,000 pounds of benzene were released to soil by underground injection from manufacturing and processing facilities in the United States in 1993 (TRI93 1995). These combined releases amount to approximately 3.6% of the total environmental release. Table 5-l lists the amounts released from these facilities. The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list.

There is a potential for release of benzene to soil from hazardous waste sites. Benzene has been detected in soil samples collected at 312 of the 816 current and former NPL sites, in soil gas samples collected at 69 of the 816 sites, and in sediment samples collected at 125 of the 816 sites where benzene has been detected in some medium (HazDat 1996).

5.3   ENVIRONMENTAL FATE

5.3.1   Transport and Partitioning

The high volatility of benzene is the controlling physical property in the environmental transport and partitioning of this chemical. Benzene is considered to be highly volatile with a vapor pressure of 95.2 mm Hg at 25 °C. Benzene is slightly soluble in water, with a solubility of 1,780 mg/L at 25 °C, and the Henry's law constant for benzene (5.5×10^{-3} atm-m^3/mole at 20 °C) indicates that benzene partitions readily to the atmosphere from surface water (Mackay and Leinonen 1975). Mackay and Leinonen (1975) estimated a volatilization half-life for benzene of 4.81 hours for a l-meter-deep body of water at 25 °C. Even though benzene is only slightly soluble in water, some minor removal from the atmosphere via wet deposition may occur. A substantial portion of any benzene in rainwater that is deposited to soil or water will be returned to the atmosphere via volatilization.

Benzene released to soil surfaces partitions to the atmosphere through volatilization, to surface water through runoff, and to groundwater as a result of leaching. A useful parameter for investigating the leachability of a chemical is the soil organic carbon sorption coefficient (K_{oc}). According to Kenaga (1980), compounds with a K_{oc} of <100 are considered to be moderately to highly mobile. Benzene, with a K_{oc} value of 60-83 (Karickhoff 1981; Kenaga 1980), would be considered highly mobile. Other parameters that influence leaching potential include the soil type (e.g., sand versus clay), the amount of rainfall, the depth of the groundwater, and the extent of degradation. In a study of the sorptive characteristics of benzene to groundwater aquifer solids, benzene showed a tendency to adsorb to aquifer solids. Greater adsorption was observed with increasing organic matter content (Uchrin and Mangels 1987). An investigation of the mechanisms governing the rates of adsorption and desorption of benzene by dry soil grains revealed that periods of hours are required to achieve equilibrium and that adsorption is much faster than desorption (Lin et al. 1994). A model developed to predict the environmental fate of benzene following leakage of gasoline from an underground storage tank at Vero Beach in Florida indicated that most (67%) of the benzene in the gasoline would volatilize from this shallow sandy soil within 17 months. Of the remaining benzene, 29% would leach to groundwater, 3% would remain in the soil, and 1% would be degraded (Tucker et al. 1986). According to the model, the rate of volatilization and leaching would be the principal factors in determining the persistence of benzene in sandy soils.

The bioconcentration bioaccumulation potential of benzene in aquatic organisms of the open coastal ocean was investigated by sampling final effluent from the Los Angeles County waste water treatment plant quarterly from November 1980 to August 1981 (Gossett et al. 1983). The benzene concentration was 0.22 ppm. The results show that the bioconcentration is related to the *n*-octanol/water partition coefficient   (K_{ow}). Benzene has a relatively low partition coefficient (log K_{ow} = 2.13 or 2.15) (Gossett et al. 1983; HSDB 1996) and although its concentration in the effluent water was high, its bioaccumulation in fish liver was low (0.001-0.052 μg/g wet weight). In the alga *Chlorella,* a bioaccumulation factor of 30 was determined experimentally and a bioconcentration factor of 40 was estimated from regression equation using a value of K_{ow} (Geyer et al. 1984). These findings suggest that bioaccumulation/bioconcentration in marine organisms is not significant and can be estimated by using the *n*-octanol/water partition coefficient. Similar results were reported by Miller et al. (1985) who used a log octanol-water partition coefficient of 2.13 and an estimated bioconcentration factor (BCF) of 24 to conclude that benzene is not expected to bioconcentrate to any great extent in aquatic organisms. An experimental BCF of 4.27 was measured in goldfish reared in water containing 1 ppm of benzene (Ogata et al. 1984). Based on these estimated and measured values, bioconcentration/ bioaccumulation of benzene in the aquatic food chains does not appear to be important. There is no evidence in the literature of biomagnification of benzene in aquatic food chain (e.g., increased accumulation from algae to algae-eating fish).

Evidence exists for the uptake of benzene by cress and barley plants from soil (Scheunert et al. 1985; Topp et al. 1989). BCFs for barley plants after 12, 33, 71, and 125 days were 17, 2.3, 2.9, and 4.6, respectively. BCFs for cress plants after 12, 33, and 79 days were 10, 2.3, and 1.9, respectively. The relative decrease in the BCFs with time was attributed to growth dilution (Topp et al. 1989). However, since benzene exists primarily in the vapor phase, air-to-leaf transfer rather than root uptake is considered to be the major pathway of vegetative contamination (Hattemer-Frey et al. 1990). Based on an equation to estimate vegetative contamination, the total concentration of benzene on exposed food crops consumed by humans and used as forage by animals was estimated to be 587 ng/kg, 81% of which was from air-to-leaf transfer and 19% from root uptake (Hattemer-Frey et al. 1990).
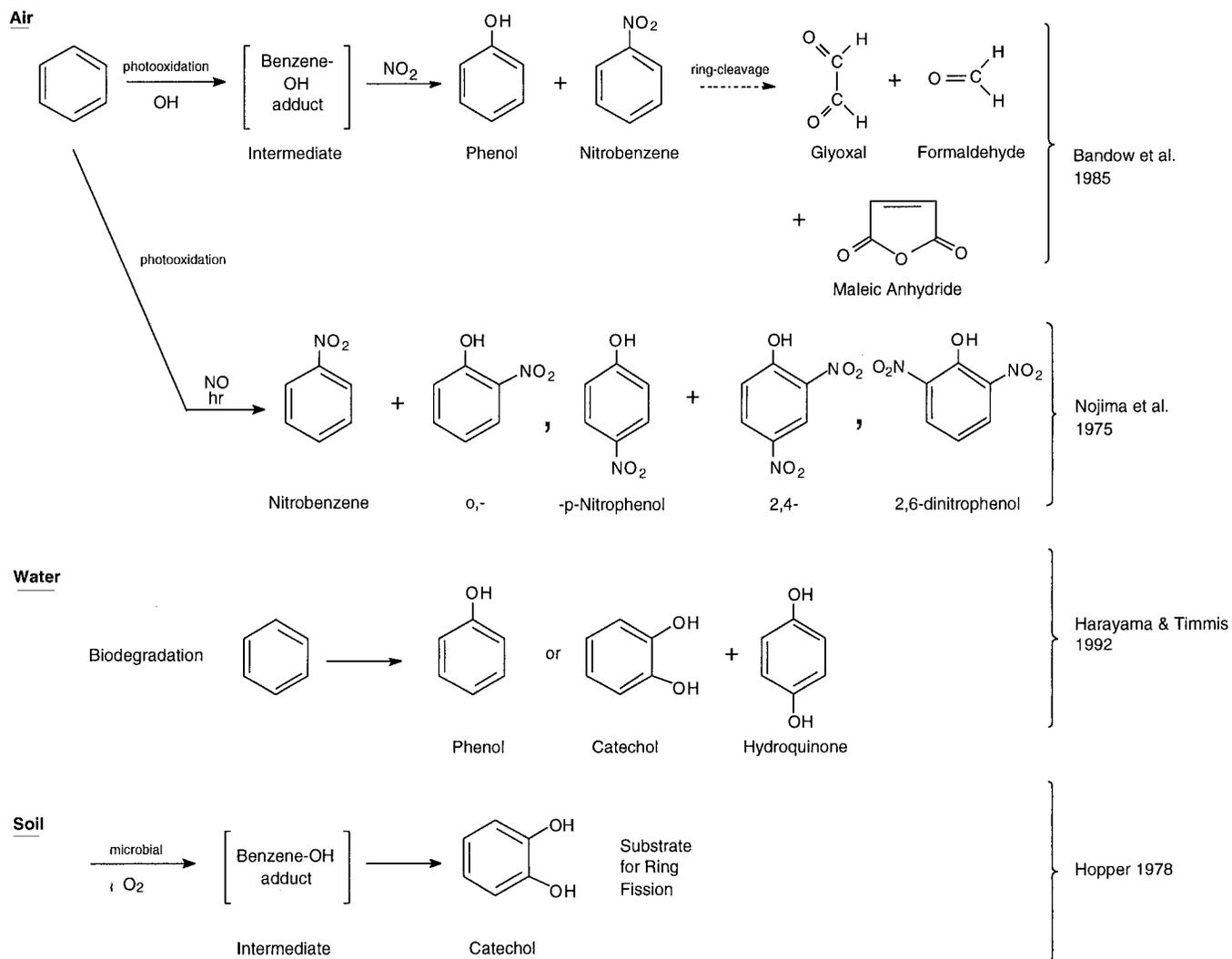
5.3.2   Transformation and Degradation

Benzene undergoes a number of different transformation and degradation reactions in the environment
as discussed in the following sections. The resulting environmental transformation products within
different media are shown in Figure 5-2.

5.3.2.1   Air

Benzene in the atmosphere exists predominantly in the vapor phase (Eisenreich et al. 1981). The most
significant degradation process for benzene is its reaction with atmospheric hydroxyl radicals. The
rate constant for the vapor phase reaction of benzene with photochemically produced hydroxyl radicals
has been determined to be 1.3×10^{-2} cm^3/molecule-second, which corresponds to a residence time of
8 days at an atmospheric hydroxyl radical concentration of 1.1×10^6 molecules/cm^3 (Gaffney and
Levine 1979; Lyman 1982). With a hydroxyl radical concentration of 1×10^8 molecules/cm^3,
corresponding to a polluted atmosphere, the estimated residence time is shortened to 2.1 hours (Lyman
1982). Residence times of 472 years for rural atmospheres and 152 years for urban atmospheres were
calculated for the reaction of benzene with ozone (O_3) using a rate constant for O_3, of
7×10^{-23} cm^3/molecule-second (Pate et al. 1976) and atmospheric concentrations for O_3 of
9.6×10^{11} molecules/cm^3 (rural) and 3×10^{12} molecules/cm^3 (urban) (Lyman 1982).

The reaction of benzene and nitric oxide in a smog chamber was investigated to determine the role of
benzene in photochemical smog formation (Levy 1973). The results showed that benzene exhibited
low photochemical smog reactivity in the four categories tested: rate of photooxidation of nitric oxide,
maximum oxidant produced, eye-irritation response time, and formaldehyde formation. The authors
concluded that benzene probably does not play a significant role in photochemical smog formation
(Levy 1973). However, data show that, in the presence of active species such as nitrogen oxides and
sulfur dioxide, the rate of photodegradation of benzene in the gas phase was greater than that in air
alone. Its half-life in the presence of such active species (100 ppm benzene in the presence of
10-110 ppm NO$_x$ or l0-100 ppm SO$_2$) was 4-6 hours with 50% mineralization to carbon dioxide in
approximately 2 days (Korte and Klein 1982). Some of the products of the reaction of benzene with
nitrogen monoxide gas (e.g., nitrobenzene, *o*- and *p*-nitrophenol, and 2,4- and 2,6-dinitrophenol) may
have potentially adverse effects on human health (Nojima et al. 1975). Photooxidation of benzene in a

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Figure 5-2. Environmental Transformation Products of Benzene in Various Media

Air

Intermediate · Phenol · Nitrobenzene · Glyoxal · Formaldehyde · Maleic Anhydride — Bandow et al. 1985

Nitrobenzene · o,- · -p-Nitrophenol · 2,4- · 2,6-dinitrophenol — Nojima et al. 1975

Water

Biodegradation — Phenol · Catechol · Hydroquinone — Harayama & Timmis 1992

Soil

microbial, O_2 — Benzene-OH adduct (Intermediate) → Catechol (Substrate for Ring Fission) — Hopper 1978

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nitrogen monoxide/nitrogen dioxide-air system formed formaldehyde, formic acid, maleic anhydride, phenol, nitrobenzene, and glyoxal (Bandow et al. 1985).

Direct photolysis of benzene in the atmosphere is not likely because the upper atmosphere effectively filters out wavelengths of light less than 290 nm, and benzene does not absorb wavelengths of light greater than 260 nm (Bryce-Smith and Gilbert 1976).

5.3.2.2 Water

McAllister and Chiang (1994) have summarized the natural attenuation of benzene, toluene, ethylbenzene, and o-, *m-,* and *p*-xylene (BTEX) in groundwater. They report that aerobic biodegradation is expected to be the primary mechanism for degradation of BTEX. Dispersion will reduce the concentration of benzene (or any contaminant) as the contaminated water migrates from the source. Volatilization is expected to account for approximately 5-10% of natural attenuation at most sites (McAllister and Chiang 1994). Sensitivity testing of groundwater models with subsurface characteristics and monitoring well data have shown that 80-100% of BTEX in plumes must have been removed by biotransformation processes within l-l.5 years. The main factor limiting biodegradation was the dissolved oxygen concentration in the groundwater (Salanitro 1993).

A half-life of 16.9 days was reported for photolysis of benzene dissolved in oxygen-saturated deionized water and exposed to sunlight (Hustert et al. 1981). For reaction with hydroxyl radicals, an estimated half-life of 0.71 years has been calculated, which is much slower than the same reaction in air (Anbar and Neta 1967).

Benzene is biodegradable in surface water and groundwater. Microbial degradation of benzene in aquatic environments is influenced by many factors such as microbial population, dissolved oxygen, nutrients, other sources of carbon, inhibitors, temperature, and pH. One study reported biodegradation half-lives for benzene in surface water (river water) and groundwater of 16 and 28 days,-respectively. Benzene was found to be resistant to biodegradation in surface water taken from a harbor and supplemented with either nutrients (nitrogen and phosphorus) or acclimated microbes. Biodegradation did occur, with a half-life of 8 days, in surface water enriched with both nutrients and microbes (Vaishnav and Babeu 1987). In another study, Davis et al. (1994) observed rapid aerobic biodegradation of benzene in aquifer groundwater samples and measured times for 50% disappearance

ranging from 4 days for an initial benzene concentration of =l mg/kg to 14 days for an initial benzene concentration of ≈10 mg/kg.

Under aerobic conditions (pH 5.3, 20 °C), benzene was completely microbially degraded in 16 days in groundwater taken from a shallow well (Delfino and Miles 1985). The aerobic biodegradation of benzene is also influenced by the presence of other aromatic hydrocarbons. A bacterial culture grown with aromatic hydrocarbons plus nitrogen-, sulfur-, and oxygen-containing aromatic compounds was much less efficient in degrading benzene than the culture grown with aromatic hydrocarbons alone. Pyrrole strongly inhibited benzene degradation. Benzene degradation was high when toluene and xylene were present (Arvin et al. 1989).

An analysis of benzene, toluene, and xylene (BTX) in groundwater from a field site indicated that the amount of BTX in groundwater was inversely related to the availability of dissolved oxygen (Chiang et al. 1989). Results of biodegradation experiments in laboratory microcosms using groundwater from the field site showed the following results at BTX levels of 120-16,000 ppb: BTX degradation was 80-100% (half-life of 5-20 days) when the dissolved oxygen level was ≥2 ppm; BTX degradation was slowed (half-life of 20-60 days) when the dissolved oxygen level was <2 ppm; and little or no degradation of BTX occurred when the dissolved oxygen level was 0, 0.1, or 0.5 ppm (Chiang et al. 1989).

Several pure cultures of microorganisms isolated from water including several *Pseudomonas sp.* (Chang et al. 1993; Harayama and Timmis 1992), *Mycobacterium vaccae* (Burback and Perry 1993), and a strain of *Pseudomonas fluorescens* (Mikesell et al. 1993), can catabolize benzene in water under aerobic conditions. A strain of *P. fhorescens* can catabolize benzene under oxygen-limiting conditions (initial dissolved oxygen concentration of about 2 mg/L) with nitrate as alternate electron acceptor (Mikesell et al. 1993; Olsen et al. 1994). If the bacteria contain ring-hydroxylating monooxygenases (e.g., *P. fluorescens*) they catabolize benzene to phenol; while ring dioxygenases (e.g., *P. urviella)* catabolize benzene to pyrocatechol and hydroquinone (Harayama and Timmis 1992). Ring cleavage by dioxygenases requires aromatic compounds carrying two hydroxyl groups, one in the ortho or para position (Harayama and Timmis 1992).

Laboratory studies on microbial degradation of benzene with mixed cultures of microorganisms in gasoline-contaminated groundwater revealed that both oxygen and nitrogen concentrations are major

controlling factors in the biodegradation of benzene. A natural mix of adapted microorganisms, obtained by selective enrichment, was used as the inoculum to accelerate the biochemical breakdown of benzene in groundwater. The inoculum plus nitrogen amendments enhanced the biodegradation rate of benzene 4.5-fold at 23 °C using a shaker flask system. More than 95% of the benzene in groundwater was removed through microbial action within 73.5 hours (Karlson and Frankenberger 1989). Similarly, a mixed culture consortia obtained from subsurface sediments degraded 1 mg/L benzene in water at an initial rate of 167 μg/L/day to below detectable limits (detection limit not given) by a continuously recycled bioreactor within 8-10 days (Korde et al. 1993). Results of a biochemical oxygen demand (BOD) test determined that benzene was completely biodegradable after the second week of static incubation at 25 °C at benzene concentrations of 5 and 10 mg/L using domestic waste water as the microbial inoculum (Tabak et al. 1981). A study of the degradation of benzene by the microbial population of industrial waste water at 23 °C using a shaker flask system showed that after 6 hours, only 4 mg/L of benzene remained of the 50 mg/L dose (Davies et al. 1981). Produced water from a petroleum production site has been successfully biotreated for complete removal of benzene using a flocculated culture of *T. denitrificans* strain F and mixed heterotrophs (Rajganesh et al. 1995).

When dissolved oxygen is depleted, an alternative electron acceptor such as nitrate, carbonate, or iron(II1) must be available, and microbes capable of using the alternative electron acceptor to degrade the benzene must be present (McAllister and Chiang 1994). Benzene biodegradation under anaerobic conditions is much slower than under aerobic conditions. No significant benzene biodegradation was reported during the first 20 weeks of incubation under anaerobic conditions at 17 °C; however, after 40 weeks of incubation, benzene concentrations were reduced by 72% (Wilson et al. 1986). After 120 weeks of incubation, over 99% degradation had taken place (Wilson et al. 1986). No degradation of benzene was observed in 96 days under anaerobic conditions (20 °C) using raw water intake from a water treatment plant (Delfino and Miles 1985). Use of water as an oxygen source in the anaerobic degradation of benzene has been demonstrated. Experiments indicated that incorporation of ^{18}O from ^{18}O-labeled water is the initial step in the anaerobic oxidation of benzene by acclimated-methanogenic cultures. Phenol was the first major product (Vogel and Grbić-Galić 1986). BTX was degraded under denitrifying conditions in anaerobic batch microcosms (10 °C) containing shallow aquifer material. BTX loss was greater with the addition of nitrate. Some degradation occurred without the addition of nitrogen or oxygen (Major et al. 1988).

5.3.2.3   Sediment and Soil

Benzene is biodegraded in soil under aerobic conditions. Microbial metabolism of benzene proceeds through the formation of *cis*-dihydrodiols and, with further metabolism, to catechols, which are the substrates for ring fission (Gibson 1980; Hopper 1978). *Pseudomonas putida* oxidized benzene through cis-1,2-dihydroxy-1,2-dihydrobenzene (Gibson 1977; Hopper 1978). *Norcardia* species and *Pseudomonas* species, after acclimation, effectively degraded benzene to carbon dioxide after 7 days (45-90%). A strain of *Rhodococcus* isolated from a contaminated river sediment mineralized 71% of benzene at an initial concentration of 0.7 mg/L in 14 days (Malachowsky et al. 1994). The soil bacterium *Nitrosomonas europaea* catabolized benzene to phenol and hydroquinone (Keener and Arp 1994a). A strain of *Pseudomonas putida* developed by encoding of genes completely mineralized benzene (Lee et al. 1994). Another new mixotrophic bacteria, a strain of *Pseudomonas sp.* isolated from contaminated soil, grew under both anaerobic and aerobic conditions and used benzene for its growth (Morikawa and Imanaka 1993).

Salanitro (1993) has summarized the aerobic degradation rates for BTEX in laboratory subsoilgroundwater slurries and aquifers. The data indicate that decay rates for benzene are highest (19-52% per day) for benzene concentrations less than 1 ppm when initial dissolved oxygen levels are about 8 ppm. Rates are significantly reduced (0-l .l% per day) when benzene levels are l-2 ppm, and no degradation was"observed when benzene levels were greater than 2 ppb.

In situ microcosms (ISM) and laboratory batch microcosms (LBM) have been used to determine the first-order degradation rate constants of benzene in an aerobic aquifer (Nielsen et al. 1996). Degradation rate constants for benzene for three ISMs ranged from 0.2 to 0.5 day^{-1} with lag times of l-6 days. In the 3 corresponding LBMs, the degradation rate constants ranged from 0.07 to 0.2 day^{-1} with lag times of 0-5 days. The first-order degradation rate constants measured in this study are much lower than the rate constant of 18 day^{-1} obtained from column experiments in another study (Angley et al. 1992).

Benzene has been shown to be anaerobically transformed by mixed methanogenic cultures derived from ferulic acid-degrading sewage sludge enrichments. In most of the experiments, toluene or benzene were the only semicontinuously supplied energy sources in the defined mineral medium (Grbic-Galic and Vogel 1987). At least 50% of the substrates were converted to CO_2 and methane.

An initial lag time of 11 days occurred before the onset of gas production with 15 mM benzene. The intermediates were consistent with benzene degradation via initial oxidation by ring hydroxylation.

It has been demonstrated that when mixtures of benzene, toluene, xylenes, and ethylbenzene are present in an anaerobic environment, there is a sequential utilization of the substrate hydrocarbons, with toluene usually being the first to be degraded, followed by the isomers of xylene in varying order. Benzene and ethylbenzene tend to be degraded last if they are degraded at all (Edwards and Grbic-Galic 1992). To test the hypothesis that benzene degradation was prevented by the presence of other more suitable substrates, a study was carried out using sediment microcosms amended with benzene only. Benzene at initial concentrations ranging from 40 to 200 μM was depleted in all microcosms, with degradation rates ranging from 0.36 to 3.7 μM/day depending upon substrate concentration and the presence of other carbon sources (Edwards and Grbic-Galic 1992).

The biodegradation of 2 mg of radiolabeled benzene in 100 g of soil with a mixed microbial population transformed only 47% of the added radioactivity to carbon dioxide after 10 weeks (Haider et al. 1981). The authors concluded that specific organisms that mineralize benzene were present in the soil in only small numbers. ULTRA, a fate and transport model used to predict the environmental fate of benzene following leakage of gasoline from an underground storage tank into shallow sandy soil, indicated that only about 1% of the benzene in the gasoline would be degraded over a 17-month period, and 3% would remain in the soil (Tucker et al. 1986). Of the benzene that leaked in soil, the highest percentages would either volatilize (67%) or get into groundwater (29%) according to this model.

5.4   LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to benzene depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. In reviewing data on benzene levels monitored in the environment, it should also be noted that the amount of-chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

5.4.1   Air

Benzene is ubiquitous in the atmosphere. It has been identified in outdoor air samples of both rural and urban environments and in indoor air. Table 5-2 lists benzene levels in outdoor air from various cities in the United States.

Volatile organic compounds, including benzene, were measured at 11 monitoring sites in Anchorage, Alaska, in a year-long study ending in April 1994 (Taylor and Morris 1995). The study was initiated in response to concerns by residents about petroleum odors from several large fuel storage facilities located just north and west of an Anchorage neighborhood. Average annual benzene concentrations ranged from a minimum of 1.15 parts per billion by volume (ppbv) in a low density residential area to 5.44 ppbv near a major midtown intersection. In the neighborhood where residents were complaining of petroleum odors, the highest benzene concentrations (annual average 3.74 ppbv) were measured on a cliff over the petroleum tank farm, within 50 meters of a petroleum storage tank. This site also showed peak benzene concentrations in summer, indicating warm weather evaporative emissions, while those in other areas showed peaks in winter, suggesting mobile sources as the main contributors.

Ambient air samples from 44 sites in 39 U.S. urban areas were collected from 6 a.m. to 9 a.m. during June through September of 1984, 1985, and 1986. Benzene was present in every sample. The median benzene site concentrations ranged from 4.8 to 35 ppb, with the overall median being 12.6 ppb (detection limit = 0.04 ppb). The data indicated that mobile sources were the major source of benzene in the vast majority of samples (EPA 1987e). In California, motor vehicle exhaust and motor vehicle evaporation accounted for 79.8% of the population exposure to ambient benzene (Allen 1987). The 1984 population-weighted average benzene concentration in California was estimated to be 3.3 ppb (Allen 1987).

The following daily median benzene air concentrations were reported by the Volatile Organic Compound National Ambient Database (1975-1985): remote (0.16 ppb), rural (0.47 ppb), suburban (1.8 ppb), urban (1.8 ppb), indoor air (1.8 ppb), and workplace air (2.1 ppb). The outdoor air data represent 300 cities in 42 states. The indoor air data represent 30 cities in 16 states (Shah and Singh 1988).

5. POTENTIAL FOR HUMAN EXPOSURE

Table 5-2. Benzene Levels in Air Samples

Location	Concentration (ppb)	Comments	References
Outdoor air			
San Francisco, CA	0.8–5.2 (range); 2.6 ± 1.3[a]	Data from six different urban locations in 1984; N = 25	Wester et al. 1986
Stinson Beach, CA	0.38 ± 0.39[a]	Data from remote coastal area in 1984; N = 21	Wester et al. 1986
Houston, TX	7.5–112.3 (range); 22.5[b]	EPA survey, urban area; N = 14; summer 1986	EPA 1987e
Houston, TX	0.55–6.3; 1.73[b]	Semirural area; N = 22; 1990–1991	Kelly et al. 1993
St. Louis, MI	3.8–72.7 (range); 11.1[b]	EPA survey, urban area; N = 18; summer 1985	EPA 1987g
Denver, CO	17.9–39.5 (range); 24.5[b]	EPA survey, urban area; N = 13; summer 1986	EPA 1987g
Philadelphia, PA	1.9–17.9 (range); 6.0[b]	EPA survey, urban area; N = 14; summer 1985	EPA 1987g
New York (Manhattan), NY	5.3–31.8 (range); 10.5[b]	EPA survey, urban area; N = 12; summer 1986	EPA 1987g
Chicago, IL	3.8–30.3 (range); 20.7[b]	EPA survey, urban area; N = 14; summer 1986	EPA 1987g
Boston, MA	0.69–3.1; 1.06[b]	Urban area; N = 22; 1990–1991	Kelly et al. 1993
New York (Staten Island), NY	0.1–34 (range); 4.4 ± 6.6[a]	Urban area; spring 1984	Singh et al. 1985
73 km northwest of Denver, CO	0.02–0.85 (range)	Rural area; May 1981–Dec. 1982	Roberts et al. 1985
Elizabeth and Bayonne, NJ	Night 2.7[c], 28.5 (max); day, 3.0[c], 13.8 (max)	Night, N = 81–86; day, N = 86–90; fall 1981	Wallace et al. 1985
Personal air			
Elizabeth and Bayonne, NJ	Night 9.7[c], 159.6 (max); day, 8.5[c], 84.5 (max)	Night, N = 346–348; day, N = 339–341; fall 1981	Wallace et al. 1985

[a]Average ± standard deviation
[b]Median
[c]Weighted average

EPA = Environmental Protection Agency; max = maximum; N = number of samples

Analysis of ambient air samples collected in industrial areas showed benzene levels ranging from 0.4 to 16 μg/m^3 (0.13-5 ppb) in Iberville Parish, Louisiana, an area that included many organic chemical and petroleum producer, user, and storage facilities located along the Mississippi River (Pellizzari 1982). Indoor and outdoor air measurements were made in August 1987 in the Kanawha Valley region of West Virginia, which is the center of a heavily industrialized area known for its chemical manufacturing. The mean, maximum, and median indoor concentrations of benzene were 2.1, 14.9, and 0.64 ppb, respectively. The median outdoor ambient air concentration of benzene was 0.78 ppb (Cohen et al. 1989).

Concentrations of volatile organic compounds were measured in 12 northern California office buildings with three different types of ventilation. Benzene concentrations ranged from ~0.1 to 2.7 ppb, with a geometric mean of 0.98 ppb (Daisey et al. 1994).

Population-weighted personal exposures to benzene exceeded the outdoor air concentrations in data from EPA's Total Exposure Assessrhent Methodology (TEAM) study. The overall mean personal exposure was about 15 μg/m^3 (4.7 ppb), compared to an overall mean outdoor concentration of only 6 μg/m^3 (1.9 ppb) (Wallace 1989a). The study also reported that the median level of benzene in 185 homes without smokers was 7 μg/m^3 (2.2 ppb), and the median level of benzene in 343 homes with one or more smokers was 10.5 μg/m^3 (3.3 ppb) (Wallace 1989a). This finding points to the possible significance of passive smoking as a source of benzene exposure. Indoor air samples taken from a smoke-filled bar contained 8.1-11.3 ppb of benzene (Brunnemann et al. 1989). A study conducted by R.J. Reynolds Tobacco Company in smoking and nonsmoking homes revealed that benzene levels were elevated in smoking homes. In 24 nonsmoking homes, the mean benzene concentration was 3.86 μg/m^3 with a maximum of 18.96 μg/m^3. In 25 smoking homes, the mean benzene concentration was 5.54 μg/m^3, with a maximum of 26.96 μg/m^3. However, benzene was not significantly correlated or associated with 3-ethenylpyridine, a proposed vapor phase environmental tobacco smoke marker (Heavner et al. 1995).

An analysis of landfill gas from 20 Class II (municipal) landfills revealed a maximum concentration of 32 ppm for benzene (Wood and Porter 1987). Benzene was measured in the vicinity of the BKK landfill, a hazardous waste landfill in California, at a maximum concentration of 3.8 μg/m^3 (1.2 ppb) (Bennett 1987). Maximum estimated levels of benzene in air near uncontrolled (Superfund) hazardous

waste sites were 190 μg/m³ (59.5 ppb) at the Kin-But Landfill (Edison, New Jersey) and 520 μg/m³ (162.8 ppb) in Love Canal basements (Niagara Falls, New York) (Bennett 1987; Pellizzari 1982).

5.4.2   Water

Measured benzene levels in open ocean samples in the Gulf of Mexico in 1977 in relatively unpolluted waters ranged from 5 to 15 ng/kg (5-15 parts per trillion [ppt]); levels in polluted waters of the open ocean ranged from 5 to 40 ng/kg (5-40 ppt) (Sauer 1981). Benzene levels measured in coastal surface waters of the Gulf of Mexico were 6 ng/kg (6 ppt) in relatively unpolluted waters and 50-175 ng/kg (50-175 ppt) in polluted coastal waters (Sauer 1981). Benzene has been detected in rainwater in the United Kingdom at a concentration of 87.2 ppb (Colenutt and Thorbum 1980).

Data from EPA's STOrage and RETrieval (STORET) database (1980-82) showed that benzene was positively detected in 15% of the surface water samples collected at 1,271 observation stations at a median concentration of 5 ppb. The sampling sites in the STORET database include both ambient and pipe sites. Ambient sites include streams, lakes, ponds, wells, reservoirs, canals, estuaries, and oceans and are intended to be indicative of general U.S. waterway conditions. Pipe sites refer to municipal or industrial influents or effluents (Staples et al. 1985).

Benzene levels in water in the vicinity of five industrial facilities using or producing benzene ranged from <l ppb to a high of 179 ppb. The highest level (179 ppb) was found in plant effluent. In general, benzene in plant effluents quickly dispersed in rivers or streams to levels of l-2 ppb or less (EPA 1979a). The maximum benzene levels observed in monitoring wells in plumes from fuel spills at gasoline service stations ranged from 1,200 to 19,000 ppb (Salanitro 1993). A monitoring well in the vicinity of a bulk storage facility had a maximum benzene level of 45,000 ppb (Salanitro 1993). In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil. A dichloromethane extract of groundwater from a monitoring well in the same area gave a benzene concentration of 52.1 ppm (Mushrush et al. 1994). Benzene has been detected at concentrations ranging from 16 to 110 ppb in landfill leachate from a landfill that accepted both municipal and industrial wastes (Cline and Viste 1985).

Composite data from the Comprehensive Emergency Response, Compensation, and Liability Act (CERCLA) monitoring program indicate that benzene was detected at a frequency of 11.2% in

groundwater in the vicinity of 178 inactive hazardous waste disposal sites (Plumb 1987). Data from a 1980 national survey by the Council on Environmental Quality on groundwater and surface water contamination showed benzene concentrations in contaminated drinking water wells in New York, New Jersey, and Connecticut ranged from 30 to 330 ppb, with the highest concentration in drinking water from surface water sources reported to be 4.4 ppb (Burmaster 1982). Benzene has also been identified but not quantified as one of the major organic constituents in commercially bottled artisan water in the United States (Dowty et al. 1975). A survey of 182 samples of bottled drinking water, which consisted of 86 packaged (transparent plastic or glass) spring waters, 61 packaged (transparent plastic or glass) mineral waters, and 35 miscellaneous bottled waters, was conducted in Canada in 1990 (Page et al. 1993). At detection limits ranging from 0.2 to 1.0 ppb, benzene from unknown source(s) was detected in only one sample of bottled spring water at a concentration of 2 ppb.

5.4.3   Sediment and Soil

Benzene levels ranging from <2 to 191 ppb were recorded in the vicinity of five industrial facilities using or producing benzene (EPA 1979a). Sediment levels ranging from 8 to 21 ppb were detected in Lake Pontchartrain in Louisiana (Ferrario et al. 1985). In northern Virginia, approximately 200,000 gallons of liquid hydrocarbons were released from a fuel-storage terminal into the underlying soil. Soil gases measured at a depth of 10 feet about 1,000 feet from the storage terminal had a benzene concentration of 1,500 ppm (Mushrush et al. 1994). Data from EPA's STORET database (1980-1982) showed that benzene had been positively detected in sediment samples taken at 9% of 355 observation stations with a median level of <5 ppb (Staples et al. 1985). The concentration of benzene in soil near factories where benzene was produced or used ranged from 2 to 191 ppb (IARC 1982a).

5.4.4   Other Environmental Media

Benzene was detected in oysters and clams at levels of 220 and 260 ppb (wet weight), respectively, from Lake Pontchartrain in Louisiana in 1980 (Ferrario et al. 1985). Data on the occurrence of benzene in food are limited. Benzene has been reported to occur in fruits, fish, vegetables, nuts, dairy products, beverages, and eggs (EPA 1982e). Although benzene has been detected in dairy products, there is no evidence of the presence of benzene in either cow's milk or human breast milk (Hattemer-Frey et al. 1990). In many foods, including dairy products where benzene has been detected, the

presence of benzene is likely to be due to uptake from the air (Grob et al. 1990). This conclusion was supported by the facts that the uptake decreased with decrease in exposed surface area of foods and contact time with air (Grob et al. 1990). Only a small number of these foods have been analyzed quantitatively. Eggs had the highest concentrations (2,100 ppb [uncooked] and 500-1,900 ppb [hard-boiled]), followed by haddock (100-200 ppb), Jamaican rum (120 ppb), irradiated beef (19 ppb), heat-treated canned beef (2 ppb), and butter (0.5 ppb). Lamb, mutton, veal, and chicken all had <10 ppb benzene (when the meats were cooked) (EPA 1980b, 1982e). A more recent survey of more than 50 foods collected and analyzed from 1991 to early 1992 (McNeal et al. 1993) revealed that foods (including eggs without added benzoates contained benzene at levels ≤2 ng/g. The level of benzene) in foods containing added benzoates in addition to ascorbates (for example, imitation strawberry preserves, taco sauce, and duck sauce) ranged from <l to 38 ng/g.

As part of a program to identify possible exposures that may be important in the high incidence of lung cancer among women in Shanghai, Pellizzari et al. (1995) qualitatively identified the volatile components emitted during heating of cooking oils to 265 °C. This study found that, on a relative basis, the intensity of the benzene peak in the total ion current chromatogram of vapors from Chinese rapeseed oil (commonly used in wok cooking) was 14-, 6.6-, and 1.7-fold greater than in vapors from peanut, soybean, and other canola (rapeseed) oils, respectively.

The amount of benzene measured in mainstream smoke ranged from 5.9 to 73 μg/cigarette (Brunnemann et al. 1990). Larger amounts of benzene were found in sidestream smoke, ranging from 345 to 653 μg/cigarette (Brunnemann et al. 1990).

5.5   GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The Total Exposure Assessment Monitoring (TEAM) studies, carried out by the EPA between 1980 and 1990, suggested that for many chemicals, including benzene, the most important sources of pollution are small and close to the person, and that exposures are not clearly correlated-with emissions. For example, the TEAM study findings indicated that, although nearly 85% of atmospheric benzene in outdoor air is produced by cars burning petroleum products and the remaining 15% is produced by industry, about half of the total national exposure to benzene comes from cigarette smoke (Wallace 1995). In fact, breath measurements of benzene provided by the TEAM study between 1979 and 1988 identified smoking as the single most important source of benzene exposure for about
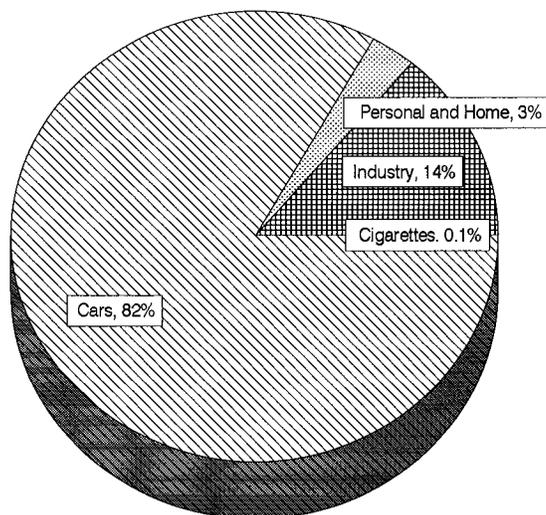
40 million U.S. smokers (Wallace 1989b). Even passive exposure to cigarette smoke is responsible for more benzene exposure (about 5% of the total) than the emissions from the entire industrial capacity of the United States (about 3% of the total) (Wallace 1995). A breakdown of the emissions and exposure sources for benzene that was derived from the Los Angeles TEAM study data (Wallace et al. 1991) is given in Figure 5-3. The reason that a relatively small source of emissions can have such a large effect on exposure is the efficiency of delivery. Wallace (1995) reports that one cigarette delivers an average of 55 μg of benzene with nearly 100% efficiency to the smoker. Benzene from industrial sources is dissipated into the atmosphere.

Smokers (n=200) in the TEAM study had a mean breath concentration of 15 μg/m^3 (4.7 ppb), almost 10 times the level of 1.5-2 μg/m^3 (0.47-0.63 ppb) observed in more than 300 non-smokers (Wallace 1989b). Smokers also had about 6-10 times as much benzene in their blood as non-smokers (Wallace 1995). In another study, benzene concentrations were compared in the breath of smokers and nonsmokers and in ambient air in both an urban area of San Francisco and in a more remote area of Stinson Beach, California (Wester et al. 1986). In the urban area, benzene in smokers' breath (6.8±3.0 ppb) was greater than in nonsmokers' breath (2.5±0.8 ppb) and smokers' ambient air (3.3±0.8 ppb). In the remote area, the same pattern was observed: benzene in smokers' breath (12.1±9.6 ppb) was higher than in nonsmokers' breath (1.8±0.2 ppb) and ambient air (1.0±0.1 ppb). Benzene in the nonsmokers' breath (2.5±0.8 ppb) in the urban area was greater than in the nonsmokers' breath (1.8±.02 ppb) in the remote area. The results also showed that, in the urban area, benzene in the breath of nonsmokers (2.5±0.8 ppb) was greater than in nonsmokers' ambient air (1.4±0.l ppb). The same was true in the remote area, where benzene in nonsmokers' breath (1.8±0.2 ppb) was greater than in ambient air (1.0±0.1 ppb). This suggests an additional source of benzene other than outdoor ambient air (Wester et al. 1986). In 10 of 11 homes inhabited by tobacco smokers, mean indoor and personal benzene concentrations were 2-5 times higher than outdoor levels (Thomas et al. 1993).

Assuming that the average sales-weighted tar and nicotine cigarette yields 57 μg benzene in mainstream smoke, Wallace (1989a) estimates that the average smoker (32 cigarettes per day) takes in about 1.8 mg benzene per day from smoking. This is nearly 10 times the average daily intake of nonsmokers (Wallace 1989a). On the assumption that intake of benzene from each cigarette is 30 μg, Fishbein (1992) has calculated that a smoker who consumes 2 packs of cigarettes per day will have an additional daily intake of 1,200 μg.

Figure 5-3. Benzene Emissions and Exposures

Benzene Emissions

Personal and Home, 3%

Industry, 14%

Cigarettes. 0.1%

Cars, 82%

Benzene Exposures

Cigarettes, 40%

Industry, 3%

Personal Activities, 18%

ETS, 5%

Car Exhaust, 18%
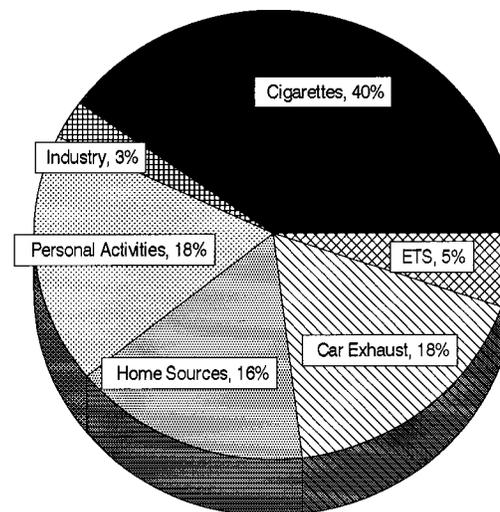
Home Sources, 16%

Note: A comparison of benzene emission sources and benzene exposure sources (home sources include paints and potroleum products; personal activities include driving and use of consumer products that contain benzene. Data taken from Wallace et al. 1991.

Virtually all (99.9%) of the benzene released into the environment finally distributes itself into the air. The general population may be exposed to benzene through inhalation of contaminated air, particularly in areas of heavy motor vehicle traffic and around gas stations. Compared to inhalation, dermal exposure probably constitutes a minor portion of benzene exposure for the general population. Personal sources account for 18% of the total exposure of the general population to benzene. The main personal sources (other than smoking cigarettes) are driving or riding in automobiles and using products that emit benzene (paints, adhesives, marking pens, rubber products, and tapes) (Wallace 1989a).

Benzene constitutes l-2% of most blends of gasoline and is released as a vapor from vehicular emissions. Since benzene is a constituent of auto exhaust and fuel evaporation, people who spend more time in cars or in areas of heavy traffic have increased personal exposure to benzene. Assuming an average benzene concentration of 40 $\mu g/m^3$ (12.5 ppb) for a moving automobile and an exposure duration of 1 hour/day, the calculated intake for driving or riding in an automobile is 40 $\mu g/day$ (Wallace 1989a). Pumping gasoline can also be a significant source of exposure. One study reported a benzene concentration of 1 ppm at the breathing-level of a person pumping gas (Bond et al. 1986a). Using this concentration and an estimated 70 minutes/year of time spent pumping gasoline, a benzene intake of 10 $\mu g/day$ has been calculated (Wallace 1989a). In an investigation of exposure to methyl *tertiary*-butyl ether (MTBE) in oxygenated gasoline in Stamford, Connecticut, venous blood samples were collected from 14 commuters and from 30 other persons who worked in the vicinity of traffic or automobiles. In addition to MTBE, the samples were analyzed for five chemicals, including benzene. Levels of benzene in the blood of 11 nonsmoking men and women commuters ranged from 0.10 to 0.20 $\mu g/L$ (ppb). In the blood of three smoking commuters, benzene levels ranged from 0.14 to 0.58 $\mu g/L$. In 13 nonsmoking car repair workers, blood benzene levels ranged from 0.11 to 0.98 $\mu g/L$; in 8 smoking car repair workers, levels ranged from 0.17 to 0.67 $\mu g/L$. Three nonsmoking male gasoline attendants had blood benzene levels ranging from 0.32 to 0.47 $\mu g/L$ (White et al. 1993). Gasoline vapors vented into the home from attached garages can also increase indoor air exposure to benzene (Wallace 1989a, 1989b). Depending on airflow from garage to living areas, mean indoor benzene concentrations in houses with a garage were 2-5 times higher than outdoor levels in most homes (Thomas et al. 1993). Benzene levels in four garages during different times in a day ranged from 3 to 196 $\mu g/m^3$. The higher concentrations of benzene in these garages were not only from vehicular activity, but also in varying proportions from stored gasoline, paints, and benzene-containing consumer products (Thomas et al. 1993). Inhalation exposure to off-gassing from benzene-containing

products and to evaporative emissions from automobiles in attached garages has been estimated to be 150 μg/day (Wallace 1989a).

All outdoor sources, including automobile exhaust and stationary source emissions, account for only about 20% of the total population exposure to benzene (Wallace 1989b). The main outdoor source is likely to be automobile exhaust (Wallace 1995). Average air intake of benzene for urban/suburban residents (assuming a typical concentration range of 2.8-20 ppb and an intake of 20 m^3 air/day) is 180-l,300 μg/day .

Another source of exposure to benzene for the general population is the use of domestic wood stoves. It has been estimated that approximately 10% of the space heating in urban areas of the northern United States is from wood burning, with up to 50% in smaller, rural towns (Larson and Koenig 1994). Benzene has been found to be a major component of the emissions from wood burning, especially from efficient flame combustion, and constituted roughly lo-20% by weight of total nonmethane hydrocarbons (Barrefors and Peterson 1995). It should be noted, however, that chimney emissions result in much lower human exposure than equally large emissions at the ground level.

Other sources of inhalation exposure to benzene include air around hazardous waste sites, industrial facilities, off-gassing from particle board, and off-gassing from contaminated water during showering and cooking. Based on the TEAM study findings, it appears that the following are not important sources of exposure to benzene on a nationwide basis: chemical manufacturing facilities, petroleum refining operations, oil storage tanks, drinking water, food, and beverages (Wallace 1989a).

Average water intake of benzene (assuming a typical drinking water concentration of 0.1 ppb and a consumption of 2 L/day) is 0.2 μg/day (HSDB 1996). According to another estimate, the daily intakes of benzene for a non-smoking individual (not exposed to secondary smoke) are l-550 μg (Fishbein 1992).

Individuals employed in industries that use or make benzene or products containing benzene may be exposed to the highest concentrations of benzene. The National Occupational Exposure Survey (NOES), conducted by NIOSH from 1981 to 1983, estimated that approximately 272,300 workers employed in various professions were potentially exposed to benzene in the United States. Approximately half of these workers were employed in general medical and surgical hospitals, and

their occupations included nurses and aides, physicians, technicians, technologists, therapists, dietitians, pharmacists, and janitors (NIOSH 1989). The NOES database does not contain information on the frequency, concentration, or duration of exposure; the survey provides only estimates of workers potentially exposed to chemicals in the workplace. The current OSHA permissible limit for an 8-hour TWA exposure to benzene is 1 ppm and a short-term exposure limit in any 15minute period is 5 ppm (OSHA 1987a). The NIOSH recommended exposure limit is 0.1 ppm for an &hour TWA and 1 ppm for short-term exposure (NIOSH 1992b). In 1987, OSHA estimated that approximately 238,000 workers were exposed to benzene in seven major industry sectors, including petrochemical plants, petroleum refineries, coke and coal chemicals, tire manufacturers, bulk terminals, bulk plants, and transportation via tank trucks (Table 5-3) (OSHA 1987b). Approximately 10,000 workers were estimated to be exposed to TWA concentrations in excess of the 1 ppm standard. This estimate did not include firms covered by the exclusions, firms under jurisdiction of other agencies, or firms involved in the use of products containing small quantities of benzene. The uptake of benzene by workers in a municipal waste incinerator in Germany was assessed by measuring benzene levels in blood (Angerer et al. 1991). No significant difference (p< 0.05) in blood benzene levels between workers and controls were detected (mean 0.22 µg/L for non-smoking workers versus 0.25 µg/L for non-smoking controls). OSHA requires the use of engineering controls and/or respiratory protection *in situ*ations where compliance with the TWA is not feasible (OSHA 1987b).

5.6   POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Other than individuals who are occupationally exposed, discussed in the preceding section, populations exposed to higher than background concentrations of benzene in the air include those living near certain chemical manufacturing sites, cigarette smokers, passive smokers, and individuals living near hazardous waste sites. Individuals who live near hazardous waste sites or near leaking underground fuel storage tanks might be exposed to potentially high concentrations of benzene in their drinking water if they obtain tap water from wells located near these sources. In a private residence using gasoline-contaminated groundwater (approximately 300 µg/L of benzene), a series of experiments was performed to assess potential benzene exposures that might occur in various parts of the home during a 20-minute shower. Maximum benzene concentrations were observed in the shower stall (758-1,670 µg/m^3) (237-523 ppb) and bathroom (366-498 µg/m^3) (115-156 ppb) during and immediately after the shower. The total benzene dose resulting from the shower was estimated to be

Table 5-3. Percentage of Employees Exposed to Benzene by Exposure Level and Industry Division[a]

Industry sector	Percentage of observations in each exposure category according to range of 8-hour TWA benzene concentrations (ppm)						Total number of employees
	0.0–0.1	0.11–0.5	0.51–1.0	1.1–5.0	5.1–10	10+	
Petrochemical plants[b]		74.6		23.0	2.4	0.0	4,300
Petroleum refineries[c,d]	64.6	26.1	4.6	3.8	0.5	0.4	47,547
Coke and coal chemicals[e]	0.0	39.3	27.6	27.5	4.4	1.3	947[f]
Tire manufacturers[c]	53.4	37.5	6.3	2.8	0.0	0.0	65,000
Bulk terminals[c]	57.8	32.8	5.3	3.7	0.3	0.1	27,095
Bulk plants[c]	57.8	32.8	5.3	3.7	0.3	0.1	45,323
Transportation via tank truck[c]	68.4	23.1	5.3	2.9	0.1	0.2	47,600
Total							237,812

[a] Derived from OSHA 1987b
[b] Percentages represent the portion of workers whose average exposures are in each category.
[c] Percentages represent the portion of sampling results in each category.
[d] Data do not reflect respirator use and sampling biases.
[e] Percentages represent the portion of workers whose average exposures are in each category.
[f] Excludes workers employed at the coke ovens.

TWA = time-weighted average

approximately 281 μg, with 40% via inhalation and 60% via the dermal pathway (Lindstrom et al. 1993).

The major source of exposure to benzene is cigarette smoke. A smoker of 32 cigarettes per day (the U.S. average per smoker) would have a benzene intake of approximately 1.8 mg/day (at least 10 times the average nonsmoker's intake) (Wallace 1989a). Median benzene concentrations in 343 homes with smokers averaged 3.3 ppb compared to 2.2 ppb in 185 homes without smokers. This represents a 50% increase in benzene exposure for nonsmokers exposed to passive smoke compared to nonsmokers not exposed to passive smoke (Wallace 1989a).

5.7   ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA 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 benzene is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of benzene.

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 or eliminate 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.

5.7.1   Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of benzene are well characterized and allow prediction of the transport and transformation of the compound in the environment.

Production, Import/Export, Use, and Release and Disposal. In 1994, benzene was the 17th
highest volume chemical produced in the United States, compared to the 18th and 17th ranks in 1993
and 1992, respectively (C&EN 1994; Kirschner 1995; Reisch 1994). In 1994, the U.S. production
volume of benzene was 14.7 billion pounds (C&EN 1995). The production volume during the
1984-94 period has increased by 4% annually (C&EN 1995). Preliminary production data reported for
the first and second quarters of 1995 indicate that this trend will continue; (3.8 billion L [7.4 billion
pounds] produced) (USITC 1995). Imports of benzene into the United States have generally ranged
from 387 to 729 million pounds during 1990-94 (USDOC 199). Exports decreased from 560 million
pounds in 1990 to 6.4 million pounds in 1993 but increased to 115 million pounds in 1994 (USDOC
199). The major use of benzene is in the production of other chemicals (primarily ethylbenzene,
cumene, and cyclohexane), accounting for approximately 91% of benzene production volume.
Benzene is also used in chemical laboratories as a solvent and a reactant (OSHA 1977, 1987b), as an
anti-knock agent in unleaded gasoline (Brief et al. 1980; EPA 19858). The widespread use of benzene
as a solvent has decreased in recent years. Many products that used benzene as a solvent in the past
have replaced it with other organic solvents; however, benzene may still occur as a trace impurity in
these products. Less than 2% of the amount of benzene produced is used as a solvent in such
products as trade and industrial paints, rubber cements, adhesives, paint removers, artificial leather, and
rubber goods. Benzene has also been used in the shoe manufacturing, and rotogravure printing
industries (EPA 1978b; Farm Chemical Handbook 1993; OSHA 1977). In the past, certain consumer
products (such as some paint strippers, carburetor cleaners, denatured alcohol, and rubber cement used
in tire patch kits and arts and crafts supplies) contained small amounts of benzene (Young et al. 1978).
Other consumer products that contained benzene were certain types of carpet glue, textured carpet
liquid detergent, and furniture wax (Wallace et al. 1987). The use of benzene in certain pesticides has
been canceled. Benzene-containing wastes, such as commercial chemical products, manufacturing
chemical intermediates, and spent solvents, are subject to federal and/or state hazardous waste
regulations (HSDB 1996). Currently, the recommended method of disposal is to incinerate solvent
mixtures and sludges at a temperature that ensures complete combustion. No additional information
on the production, import/export, use, release, or disposal of benzene is needed at this time.

Environmental Fate. Benzene released to the environment partitions mainly to the atmosphere
(Mackay and Leinonen 1975). However, the compound can also be found in surface water and
groundwater. Benzene is mobile in soil (Karickhoff 1981; Kenaga 1980); however, there is a need for
more information on the leachability potential of benzene to groundwater in different soil types.

Benzene is transformed in the atmosphere by photooxidation. Biodegradation, principally aerobic, is the most important fate process of benzene in water (Delfino and Miles 1985; McAllister and Chiang 1994; Salanitro 1993) and soil (Gibson 1980; Hopper 1978; Salanitro 1993). Benzene can persist in groundwater. No additional information on the environmental fate of benzene is needed at this time.

Bioavailability from Environmental Media. Benzene can be absorbed following oral exposure (Thienes and Haley 1972), dermal exposure (Blank and McAuliffe 1985; Franz 1984; Laitinen et al. 1994; Lindstrom et al. 1993; Loden 1986; Susten et al. 1985), and inhalation exposure (Ashley et al. 1994; Avis and Hutton 1993; Boogaard and van Sittert 1995; Brunnemann et al. 1989; Byrd et al. 1990; Etzel and Ashley 1994; Fustinoni et al. 1995; Ghittori et al. 1995; Gordian and Guay 1995; Hajimiragha et al. 1989; Hanzlick 1995; HazDat 1996; Karacic et al. 1995; Kok and Ong 1994; Lagorio et al. 1994a; Laitinen et al. 1994; Lauwerys et al. 1994; Lindstrom et al. 1993; Mannino et al. 1995; Nomiyama and Nomiyama 1974a; Ong et al. 1994, 1995; Pekari et al. 1992; Popp et al. 1994; Rauscher et al. 1994; Rothman et al. 1995; Ruppert et al. 1995; Scherer et al. 1995; Shamy et al. 1994; Srbova et al. 1950). These routes of exposure may be of concern to humans because of the potential for benzene to contaminate the air (Allen 1987; Bennett 1987; Black et al. 1980; Brief et al. 1980; Edgerton and Shah 1992; EPA 1989e; Glass et al. 1986; Graedel 1978; Mangino and Jones 1994; Mayer et al. 1994; TRI93 1995; Wallace 1989a, 1989b; Wallace and Pellizzari 1986; Wester et al. 1986; Wood and Porter 1987), drinking water (CDC 1994; EPA 1979a), and soil (HazDat 1996; Mushrush et al. 1994; TRI93 1995). Information on inhalation exposure and on the absorption of benzene following ingestion of plants grown in contaminated environments near hazardous waste sites would be helpful in determining bioavailability of the compound in these media.

Food Chain Bioaccumulation. Benzene has an estimated low-to-moderate bioconcentration potential in aquatic organisms (Miller et al. 1985; Ogata et al. 1984) and some plants (Geyer et al. 1984). Most of the benzene accumulation on vegetation results from air-to-leaf transfer. Root uptake is not believed to be important (Hattemer-Frey et al. 1990). Biomagnification in aquatic food chains does not appear to be important (Ogata et al. 1984). No further information is needed.

Exposure Levels in Environmental Media. Benzene is widely distributed in the environment and has been detected in air (EPA 1987e), water (Sauer 1981), soil (EPA 1979a; Ferrario et al. 1985; Staples et al. 1985), sediment, and some foods (EPA 1980b, 1982e). The levels of benzene in air and water are well documented, but there is a need for more current information. Benzene is not expected

to be a significant contaminant in aquatic foods (Geyer et al. 1984; Gossett et al. 1983; Miller et al. 1985; Ogata et al. 1985); however, some contamination of food crops consumed by humans may occur, primarily from air-to-leaf transfer (Hattemer-Frey et al. 1990). The total concentration of benzene on exposed food crops consumed by humans was estimated to be 587 ng/kg (Hattemer-Frey et al. 1990). Humans are at risk of exposure to benzene because of its widespread distribution in the environment, particularly in the atmosphere. Releases to the air from gasoline, smoking, and automobile exhaust constitute the major risk of potential exposure for the general population (Wallace 1995). Additional data characterizing the concentration of benzene in drinking water, air, and soil surrounding hazardous waste sites would be helpful in assessing human exposure for populations living near these waste sites. In addition, more current data on levels of benzene in foods would be helpful in estimating intake of benzene from food.

Exposure Levels in Humans. Benzene has been detected in human body fluids and tissues such as blood, urine, and fat (Brugnone et al. 1989; Chao et al. 1993; Karacic et al. 1987). Most of the monitoring data have come from occupational studies of specific worker populations exposed to benzene (Inoue et al. 1989; Karacic et al. 1987; OSHA 1987b). Biological monitoring studies exist for the general population (Melikian et al. 1994). There is information for background levels in breath of smokers and nonsmokers (Wallace 1989b), baseline blood levels (Karacic et al. 1987), and levels of urinary metabolites in unexposed people (Inoue et al. 1989). Information on exposure levels for populations living in the vicinity of hazardous waste sites would be helpful in estimating exposure in these groups. This information is necessary for assessing the need to conduct health studies on these populations.

Exposure Registries. In 1995, 1,142 people were included in the Benzene subset of the Volatile Organics Compounds subregistry of the National Exposure Registry. These people were exposed to benzene at a site in Texas. Demographic and health information was obtained on all the exposed persons; the information will be updated longitudinally. For those who were identified as exposed and were deceased, a death certificate will be obtained to ascertain cause of death. This activity is carried out by the Exposure and Disease Registry Branch (EDRB), Division of Health Studies (DHS), ATSDR. The data will become part of public-user data files maintained by ATSDR. The statistical analyses of the baseline data were scheduled to be completed and published, and the CD-ROM released in 1995 (Burg and Gist 1995). 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 the exposure to this substance.

5.7.2   Ongoing Studies

As part of the Third National Health and Nutrition Evaluation Survey (NHANES III), the Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control and Prevention, will be analyzing human blood samples for benzene and other volatile organic compounds. These data will give an indication of the frequency of occurrence and background levels of these compounds in the general population.

The Federal Research in Progress (FEDRIP 1996) database provides additional information obtainable from a few ongoing studies that may fill some of the data needs identified in Section 5.7.1. New methods for the remediation of groundwater and soil contaminated by benzene are being investigated by A.M. Anderson of University of California, Riverside, California; L.J. Fischer of Michigan State University, East Lansing, Michigan; J.W. Tester of M.I.T., Cambridge, Massachusetts; and M. Tomkiewicz of Brooklyn College, Brooklyn, New York. D.R. Lovely of the University of Massachusetts, Amherst, Massachusetts, is investigating hydrological, geochemical and microbiological controls on anaerobic degradation of aromatic hydrocarbons (including benzene) in petroleumcontaminated groundwater. A high-efficiency activated carbon for removing benzene from drinking water is being developed by R. Mieville of Mega-Carbon, Mt. Prospect, Illinois. The kinetics of vapor phase transport of benzene in soils under natural and forced air advection is being studied by L.M. Abriola of Michigan State University, East Lansing, Michigan. Properties of organic compounds and of soils that govern how aging soil affects bioavailability are being determined by M. Alexander of Cornell University, Ithaca, New York. Several studies are being conducted to investigate the kinetics of biodegradation of benzene under different conditions and to apply this information to the development of bioreactors capable of removing benzene from water and from subsurface aquifers (P.J. Alvarez of the University of Iowa, Iowa City, Iowa; B.R. Folson of Envirogen Inc., Lawrenceville, New Jersey; J.S. Herman of University of Virginia, Charlottesville, Virginia; J.R. Hunt of University of California, Berkeley, California; N.G. Love of Virginia Polytechnic Institute, Blacksburg, Virginia; R.H. Olsen, J.M. Tiedje, and W.J. Weber of Michigan State University, East Lansing, Michigan; K.F. Reardon of Colorado State University, Fort Collins, Colorado). H. Wijmans of Membrane Technology and Research, Inc., is developing a membrane module for a natural gas

dehydration process to eliminate benzene emissions. Methods are being developed to validate the feasibility of using new biomarkers as a measure of exposure to benzene (D.L. Eaton of University of Washington, Seattle, Washington; A.A. Melikian of American Health Foundation, Valhalla, New York; M.T. Smith and J.H. Weincke of University of California, Berkeley, California). The bioaccumulation potential of benzene in fish from natural bodies of water with contaminated sediments and the relative importance of dietary versus aqueous exposure to benzene are being investigated by W.H. Clements of Colorado State University, Fort Collins, Colorado.

No other ongoing studies for benzene were located.

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 benzene, its metabolites, and other biomarkers of exposure and effect to benzene. 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 SAMPLES

Analytical methods have been developed to measure benzene levels in exhaled breath, blood, and various body tissues. The primary method of analyzing for benzene in exhaled breath, body fluids and tissues is gas chromatography (GC) coupled with either flame ionization detection (FID), photoionization detection (PID), or mass spectrometry (MS). Rigorous sample collection and preparation methods must be followed when analyzing for benzene to prevent contamination of the sample. A summary of commonly used methods of measuring benzene in biological samples is presented in Table 6-l.

Breath samples are collected on a solid sorbent (Gruenke et al. 1986; Pellizzari et al. 1988; Wallace 1986; Wallace et al. 1985), in canisters (Thomas et al. 1991) or collected in a breath sampling tube and analyzed directly (Sherwood and Carter 1970). Samples collected on Tenax sorbent are subjected to a thermal desorption/cryofocussing step prior to analysis by capillary GC/MS (Pellizzari et al. 1988; Wallace 1986; Wallace et al. 1985). Techniques involving headspace analysis of benzene adsorbed on silica gel has also been used (Gruenke et al. 1986). MS detection generally provides the most sensitivity, from the low to sub-ppb. The selectivity of the methods is improved if capillary GC columns are used (Pellizzari et al. 1988). Extraction of benzene from blood is frequently accomplished by either purge-and-trap or headspace analysis. In purge-and-trap analysis, an inert gas such as helium or nitrogen is passed through the sample, and purged volatiles are trapped on an

Table 6-1. Analytical Methods for Determining Benzene in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath	Collection on Tenax GC; thermal desorption	HRGC/MS (IARC Method 5)	3 ppt	70–130 estimated	Pellizzari et al. 1988
Breath	Collection in sampling tube; direct injection of sample	GC/FID	100 ppb	100	Sherwood and Carter 1970
Breath	Collection on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/MS	1.6 ppb (5-L sample)	86–90	Wallace et al. 1986, 1985
Breath	Collection in bags, adsorption on silica gel; desorption to headspace vial; analysis of headspace gases	GC/MS-SIM	0.1 ppb	NR	Gruenke et al. 1986
Blood	Purge and trap	HRGC/MS	30 ppt	112–128	Ashley et al. 1992; 1994
Blood	Heparinization; transfer to isotonic saline in headspace vial; equilibration with heat	HRGC/PID	0.4 µg/L	NR	Pekari et al. 1989
Blood	Collection and transfer to headspace vial; analysis of headspace gases	GC/MS-SIM	2 µg/L	NR	Gruenke et al. 1986
Blood	Purging with nitrogen; collection on Tenax GC-silica gel	GC/MS	0.5 µg/L	NR	Antoine et al. 1986
Blood	Extraction with toluene + HCl; centrifugation; analysis of toluene layer	GC/FID	100 µg/L	98–100	Jirka and Bourne 1982
Blood	Extraction device coupled to MS	ITMS	90 ppt	NR	St-Germain et al. 1995
Urine	Incubation; analysis of headspace gases	GC/PID	0.51 nmol/L	>90	Kok and Ong 1994
Tissues (bone marrow, fat)	Homogenization with internal standard; centrifugation; analysis of supernatant	GC/MS-SIM	NR	NR	Rickert et al. 1979
Tissues (lung, liver)	Homogenization in buffer; centrifugation; analysis of supernatant	RID-preparative HPLC/UV	20 pg/g	NR	Bechtold et al. 1988

FID = flame ionization detection; GC = gas chromatography; HCl = hydrochlorid acid; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; ITMS = ion trap mass spectrometry; MS = mass spectrometry; NR = not reported; PID = photoionization detection; RID = reverse isotope dilution; SIM = selected ion monitoring; UV = ultraviolet detection

appropriate solid sorbent (Antoine et al. 1986; Ashley et al. 1992, 1994; Michael et al. 1980). Recent improvements in the method have resulted in excellent sensitivity (300 ppt) and acceptable precision and accuracy (Ashley et al. 1992, 1994). The purge-and-trap method has also been used to analyze breast milk for other volatile organic compounds and could be used for analyzing benzene in breast milk (Michael et al. 1980). For headspace analysis, the samples are placed in a special vial, and the gas generated above the liquid sample under equilibrium conditions is analyzed (Gruenke et al. 1986; Pekari et al. 1989). Sensitivity is in the sub- to low-ppb range. A third method of sample preparation involves extraction of the blood sample with an organic solvent (Jirka and Boume 1982) and analysis of the organic fraction. These methods are generally less sensitive, with reported detection limits usually in the low- to mid-ppb range. Selectivity is improved with use of high resolution gas chromatography (HRGC). Accuracy and precision could not be adequately compared given the limited data available.

Screening methods are available for analysis of benzene in feces and urine (Ghoos et al. 1994) and body fluids (Schuberth 1994). Both employ analysis by capillary GC with an ion trap detector (ITD). Benzene in urine has been determined by trapping benzene stripped from the urine on a Carbotrap tube, followed by thermal desorption GC/flame ionization detection (FID). The detection limit is 50 ng/L and the average recovery is approximately 82% (Ghittori et al. 1993). Benzene in urine has also been determined using headspace analysis with capillary GCYphotoionization detection (PID). The detection limit is 40 ng/L (Kok and Ong 1994).

Methods are also available for determining metabolites of benzene in urine. A summary of available methods is shown in Table 6-2. Both GC/FID or GC/MS and high-performance liquid chromatography (HPLC) with ultraviolet detection (UV) have been used to measure urinary metabolites.

The primary metabolite of benzene is phenol. Phenol is excreted as glucuronide and sulphate conjugates in urine. Total phenolic metabolites (phenol, phenyl sulfate, and phenyl glucuronide) have been determined by hydrolyzing urine samples either enzymatically or by acid, then extracting the phenol with solvent. Phenol is then measured by GC or HPLC techniques. Enzymatic hydrolysis coupled with GC/FID has been reported; the detection limit is 1 mg/L and recovery is excellent (92-98%) (Buchet 1988). Acid hydrolysis followed by HPLC provides quantitative recovery (~100%) and a detection limit of 0.01 nmol/g (Murray and Adams 1988). Sulfate and glucuronide conjugates

Table 6-2. Analytical Methods for Determining Metabolites of Benzene in Urine

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (phenol, phenyl sulfate, phenyl glucuronide)	Centrifugation	HPLC/UV	4 mg/L (PS); 5 mg/L (PG)	100.5 (PS); 101.8 (PG)	Ogata and Taguchi 1987
Urine (phenol, phenyl sulfate, phenyl glucuronide)	Digestion (enzymatic and with acid); extraction with diethyl ether	GC/FID (IARC Method 6)	1 mg/L	92–98	Buchet 1988
Urine (phenols and cresols)	Hydrolysis with perchloric acid; extraction with diisopropyl ether	GC/FID	NR	NR	NIOSH 1974
Urine (phenols and cresols)	Hydrolysis with perchloric acid; saturation with NaCl; extraction with diisopropyl ether	GC/FID	2 mg/L	NR	Roush and Ott 1977
Uriine (phenol)	Enzymatic reaction	HPLC/fluorometric detection	50 ppb	97.7	Jen and Tsai 1994
Urine (trans,trans-muconic acid)	Mixing with methanol; centrifugation	HPLC/UV	0.1 mg/L	NR	Inoue et al. 1989
Urine (muconic acid, phenol)	Mixing with formic acid; extraction (twice) with ethyl ether; evaporation of combined extracts	GC/MS	10 µg/L	NR	Bechtold et al. 1991
Urine (trans,trans-muconic acid)	Cleanup on anion exchange resin; derivitization	GC/MS	0.01 mg/L	93–106	Ruppert st al 1995
Urine (S-phenyl-mercapturic acid	Solid-phase extraction; hydrolysis; derivatization	HPLC	1 µg/L	NR	Einig and Dehnen 1995

FID = flame ionization detection; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; MS = mass spectrometry; NaCl = sodium chloride; NR = not reported; PG = phenyl glucuronide; PS = phenyl sulfate; UV = ultraviolet detection

have been determined directly by HPLC/UV (Ogata and Taguchi 1987). The normal baseline levels of urinary phenolic metabolites from humans are usually 2-18 mg/L (Ong and Lee 1994). The available methods are sensitive enough to measure these relatively high amounts accurately.

Analysis of urinary *trans,trans*-muconic acid (t,t-MA) seems to be a better indicator than phenol for assessing exposure to low levels of benzene (Ducos et al. 1990). However, muconic acid is a minor metabolic route and background levels of muconic acid in urine are much lower than levels of phenolic metabolites and are frequently below the limit of detection of the method used to determine them (Inoue et al. 1989). The detection of low levels of t,t-muconic acid in urine was difficult by earlier methods because of low recovery of t,t-muconic acid (37% with ether) by the commonly used solvent extraction method (Gad-El Karim et al 1985). An improved method for the determination of urinary t,t-muconic acid utilizes solid phase extraction with SAX sorbent in combination with the HPLC/UV for quantitation. The detection limit is 0.06-0.1 mg/L and recovery is very good (90%) (Boogaard and van Sittert 1995; Ducos et al. 1990). The relative standard deviation of the method was 5% in the concentration range 1-20 ng/L. t,t-muconic acid has been determine directly by HPLC/UV with similar sensistivity (detection limit = 0.1 mg/L (Inoue et al. 1989). The detection limit and specificity for the determination of urinary t,t-muconic acid may be improved by using HPLC with diode array detector, GC/FID of the methylated product, or GC/MS of trimethylsilyated product (Bartczak et al. 1994). Both GC/FID and HPLC/diode array detection are capable of detecting urinary t,t-muconic acid at concentrations above μg/L, but GC/MS is capable of detecting t,t-muconic acid at concentrations below 40 μg/L (Bartczak et al. 1994).

The metabolite S phenyl-N-acetyl cysteine may be an indicator of exposure to benzene. It can be detected at low levels (1 μg/L) in urine using solid phase extraction and determination by HPLC (Teinig and Dehnen 1995).

6.2   ENVIRONMENTAL SAMPLES

Methods exist for determining benzene in air (ambient, occupational, and industrial), water, sedminent, soil, foods, cigarette smoke, gasoline, and jet fuel. Most involve separation by GC with detection by FID, PID, or MS. HPLC/UV and spectrophotometry have also been used. Table 6-3 summarizes several of the methods that have been used to analyze for benzene in environmental samples

Table 6-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Sample trapped on silica gel; thermal desorption	GC/MS	0.1 ppb	88–105	Gruenke et al. 1986
Air	Cryogenically trap; thermal desorption	GC/FID	NR	85–115	Singh et al. 1985
Air	Direct on-line analysis	GC/FID	NR	NR	Bayer et al. 1988
Air (ambient)	Direct injection of ambient air	GC/PID	0.25 ppb	NR	Clark et al. 1984
Air (ambient)	Direct analysis of ambient air	Electrochemical	NR	NR	Stetter et al. 1986
Air (ambient)	Sample collection in Tedlar bag; Cryogenically trap; thermally desorb	GC/PID	0.5 ppb	NR	Kowalski et al. 1985
Air (ambient)	Sample collection in stainless steel canisters or sorbent tubes; trap cryogenically; thermal desorption	HRGC/PID or HRGC/FID	5 ppt (PID) 24 ppt (FID)	97–104 (PID) 96–104 (FID)	Reineke and Bächmann 1985
Air (ambient)	Collect on charcoal (vapor badge or tube); desorb with carbon disulfide	GC/FID	0.3 ppb (estimated)	NR	Fung and Wright 1986
Air (ambient)	Cryogenically trap; thermal desorption	GC/PID/FID	1 ppt	70–130	Nutmagul and Cronn 1985
Air (ambient)	Collection in canisters; preconcentration using 2-stage trap	HRGC/ITD	sub-ppb level	8–13% bias	Kelly et al. 1993
Air (ambient)	Direct analysis	ALMS	250 ppb	NR	Hadeishi et al. 1985
Air (ambient)	Sample collection onto on-column cryogenic sample loop or into stainless steel containers	GC/PID/FID	1 ppt	70–130	Nutmagul and Cronn 1985
Air (ambient)	Sample trapped on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC	<0.1 ppb	69–126	EPA 1979a
Air (ambient)	Sample collected on Tenax GC; thermal desorion to on-column cryogenic trap	GC/FID	0.03 ppb	56–144	EPA 1980a
Air (ambient)	Sample trapped on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/FID; conf. by HRGC/MS	3 ppt	NR	Roberts et al. 1984

Table 6-3. Analytical Methods for Determining Benzene in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (consumer products)	Sample trapped on solid sorbent; thermal desorption	GC/MS	NR	NR	Bayer et al. 1988
Air (at waste sites and landfills)	Sample trapped on Tenax GC; thermal desorption	HRGC/FID/ECD; conf. by HRGC/MS	0.05 ppb	NR	Harkov et al. 1985
Air (occupational)	Sample trapped on charcoal; desorption with carbon disulfide	GC/FID (NIOSH Methods 1500 and 1501)	10–100 ppb	NR	NIOSH 1984
Air (occupational)	Sample collection on silica gel; desorption with ethanol	GC/FID	100 ppb	90	Sherwood and Carter 1970
Air (occupational)	Sample collection in Tedlar bag; direct injection	GC/PID (NIOSH Method 3700)	50 ppb	NR	NIOSH 1987
Air (occupational)	Sample collection on charcoal disk in miniature passive dosimeter; thermal desorption	GC/PID	60 ppb	85–115	Gonzalez and Levine 1986
Air (occupational; jet fuel fumes)	Sample collection on charcoal; desorption with methyl chloride-ethyl acetate	HPLC/UV	0.08 ppm	94–112	Dibben et al. 1989
Soil air	Sample collection on activated charcoal; desorption with carbon disulfide	GC/FID	NR	97–100	Colenutt and Davies 1980
Drinking water	Purge and trap	GC/MS	0.2 µg/L	NR	Brass et al. 1977
Drinking water	Purge and trap	HRGC/MS (EPA Method 524.2)	0.03 - 0.04 µg/L	97–99	EPA 1992b
Water	Purge and trap onto Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/MS	0.1–10 µg/L	74–78	Michael et al. 1988
Water	Purge and trap on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC	<1 µg/L	69–126	EPA 1979a
Water	Purge and trap onto Tenax GC; thermal desorption	GC/MS	NR	85–125	Harland et al. 1985
Water	Solvent extraction with dichloromethane; concentration	GC/MS	15 µg/L	NR	Sporstøl et al. 1985

Table 6-3. Analytical Methods for Determining Benzene in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Purge and trap on adsorbent column; thermal desorption	GC/PID (EPA Method 602)	0.2 µg/L	81–119	EPA 1984d
Water	Purge and trap on activated carbon; desorption with carbon disulfide	GC/FID; conf. by GC/MS	NR	96–99	Colenutt and Thorburn 1980
Water	Purge and trap on Tenax; thermal desorption	GC/FID	0.001 µg/L	94–111	Hammers and Bosman 1986
Water	Permeation of benzene through a silicone polycarbonate membrane into an inert gas stream	GC/FID	7.2 µg/L	NR	Blanchard and Hardy 1986
Wastewater	Purge and trap onto adsorbent column; thermal desorption	GC/MS (EPA Method 624)	4.4 µg/L	90–110	EPA 1984d
Wastewater	Addition of isotopically labeled benzene analog; purge and trap onto adsorbent column; thermal desorption	GC/IDMS (EPA Method 1624)	10 µg/L	65–141	EPA 1984d
Water, industrial effluents	Purge and trap on Tenax; thermal desorption	GC/MS	<5 µg/L	95–106	Pereira and Hughes 1980
Landfill leachate	Purge sample and trap on Tenax-silica gel; thermally desorb	GC/FID/FID	1 µg/L	NR	Stuart et al. 1984
Landfill leachate	Extract sample with pentane	GC/MS	1,000–10,000 µg/L	NR	Schultz and Kjeldsen 1986
Solid wastes	Purge and trap	HRGC/PID (EPA Method 8021A)	0.009 µg/L	99	EPA 1994z
Soil	Sample mixed with NaOH solution; equilibration; analysis of headspace gases	HRGC/FID; conf. by HRGC/MS	≤0.02 ng/mL	75–98	Kiang and Grob 1986
Soil	Purge and trap on Tenax; thermal desorption to on-column cryogenic trap	HRGC	<0.1 ppb	69–126	EPA 1979a
Soil	Purge and trap on Tenax; thermal desorption	GC/FID	1 ppt	52	Hammers and Bosman 1986
Soil	Supercritical fluid extraction	HRGC/FID	low ppb	77–81	Burford et al. 1994

Table 6-3. Analytical Methods for Determining Benzene in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sediment and biota	Purge and trap on Tenax GC-silica gel; thermal desorption	HRGC/MS	NR	NR	Ferrario et al. 1985
Sediment	Purge and trap on Tenax; thermal desorption	GC/MS	NR	64	Harland et al. 1985
Fruits and vegetables	Mix with water and methanol; filter; distill azeotrope	GC/FID	NR	84–96	Kozioski 1985
Shellfish	Tissue homogenized; purge with inert gas and trap on Tenax GC-silica gel; thermally desorb	GC/MS	NR	NR	Ferrario et al. 1985
Mainstream cigarette smoke	Collection on filters and impingers; [^{2}H$_6$]-benzene added to impinger	HRGC/IDMS-SIM	0.05 µg/cigarette	75–85 (trapping efficiency)	Byrd et al. 1990
Cigarette smoke	Mainstream smoke filtered and analyzed directly; side-stream smoke and smoke-polluted air filtered and collected in cryogenic methanol-filled impingers	HRGC/MS-SIM	0.1 µg/cigarette	NR	Brunnemann et al. 1989; 1990
Gasoline	Dilute sample with hexane	GC/FID	NR	NR	Poole et al. 1988
Gasoline	Dilute sample with methanol; elute benzene to analytical column with 50% methanol; back-flush guard column with 100% methanol	HPLC/UV	NR	NR	Ludwig and Eksteen 1988

ECD = electron capture detection; EPA = Environmental Protection Agency; FID = flame ionization detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; IDMS = isotope dilution mass spectrometry; ITD = ion trap mass spectrometry; LRS = laser Raman spectroscopy; MS = mass spectrometry; NaOH = sodium hydroxide; NIOSH = National Institute of Occupational Safety and Health; NR = not reported; PID = photoionization detection; SIM = selected ion monitoring; UV = ultraviolet detection

Numerous methods exist for detecting and measuring benzene in ambient air. Air samples for benzene analysis may be preconcentrated by passing the sample through a trap containing a solid adsorbent (Bayer et al. 1988; EPA 1979a, 1980a; Fung and Wright 1986; Gruenke et al. 1986; Harkov et al. 1985; Reineke and Bachmann 1985; Roberts et al. 1984). Commonly used adsorbents are Tenax resins (e.g., Tenax TA, Tenax GC), silica gel, activated carbon, and carbonaceous polymeric compounds. Benzene in ambient air can be collected in stainless steel canisters (EPA 1988f; Kelly et al. 1993) or Tedlar bags (Kowalski et al. 1985) and can be analyzed with or without preconcentration. Preconcentration of benzene can be accomplished by direct on-column cryogenic trapping (Holdren et al. 1985; Kowalski et al. 1985; Nutmagul and Cronn 1985; Reineke and Bachmann 1985; Singh et al. 1985), or samples may be analyzed directly without preconcentration (Bayer et al. 1988; Clark et al. 1984).

The most common methods of analysis for benzene in air are GC/PID, GC/FID, and GC/MS. The limit of detection for GC/FID and GC/PID ranges from low ppb to low ppt. GC/MS is generally considered to be more reliable than GC/FID or GC/PID in identifying benzene in samples, particularly those containing multiple components having similar GC elution characteristics. Benzene has been quantified in ambient air samples at sub-ppb levels by GC/MS (Gruenke et al. 1986) and ion trap mass spectrometry (Kelly et al. 1993). The ion trap detector has the advantage of remaining largely unaffected by water vapor in the sample. A continuous monitoring instrument using Fourier transform infrared (FTIR) spectroscopy is being developed for measuring the levels of benzene or other toxic chemicals in exhaust emissions from hazardous waste incinerators (Demirgian 1992).

Several analytical methods are available for determining atmospheric levels of benzene in the workplace. The OSHA recommended procedure involves the collection of the sample vapors on charcoal adsorption tubes, then desorption followed by GC/MS analysis (OSHA 1985). Samples desorbed from charcoal are also analyzed by GC/FID (NIOSH 1984) or HPLC/UV (Dibben et al. 1989). Detection limits are in the ppb range (Dibben et al. 1989; NIOSH 1984). Passive dosimeters are also utilized, with GC/PID quantitation; detection limits are in the ppb range (Gonzales and Levine 1986). Other acceptable methods include portable direct reading instruments and real-time continuous monitoring systems; these methods generally have a sensitivity in the ppm range.

The most frequently used analytical methods for water samples containing benzene are GC/MS, GC/FID, and GC/PID (Blanchard and Hardy 1986; Colenutt and Thorbum 1980; EPA 1984f, 1992b;

Hammers and Bosman 1986; Harland et al. 1985; Lysyj et al. 1980; Michael et al. 1988; Pereira and Hughes 1980; Sporstøl et al. 1985; Stuart et al. 1984). Benzene is usually isolated from aqueous media by the purge-and-trap method (Brass et al. 1977; Colenutt and Thorbum 1980; EPA 1979a, 1984f, 1992b; Hammers and Bosman 1986; Harland et al. 1985; Michael et al. 1988; Stuart et al. 1984). An inert gas such as nitrogen is used to purge the sample. The purged benzene is trapped on an adsorbent substance, such as Tenax GC or activated charcoal, and thermally desorbed. Recovery, where reported, ranges from acceptable ($\approx70\%$) (EPA 1979a, 1984f; Michael et al. 1988) to very good ($\geq90\%$) (Colenutt and Thorbum 1980; EPA 1984f, 1992b; Hammers and Bosman 1986). Detection limits in the sub-ppb to ppt range may be attained with HRGC/MS techniques (EPA 1992); Michael et al. 1988). Liquid-liquid extraction procedures (Harrison et al. 1994; Schultz and Kjeldsen 1986; Sporstøl et al. 1985) are less commonly used, having been replaced by more sensitive purge-and-trap methods. Interference from contamination can occur with all methods if extreme care is not used in the handling of samples and cleaning of all equipment.

Solid samples, such as soil, sediment, and foods, are most frequently prepared for analysis using the purge-and-trap method (EPA 1979a, 1994w; Ferrario et al. 1985; Hammers and Bosman 1986; Harland et al. 1985), although supercritical fluid extraction has recently been utilized (Burford et al. 1994). Detection and quantitation of benzene may be GC/FID, GC/PID or GC/MS. Detection limits as low as 1 ppt have been reported, but recoveries and precision have frequently been low. Improvements in the method, including analysis by HRGC/PID, have resulted in low detection limits (9 ppt) and excellent recovery (99%) for benzene (EPA 1994w). Screening methods are available for benzene; some may be used at field sites. Immunoassay may be used as a screening and semiquantitative tool (Van Emon and Gerlach 1995).

Methods exist for detection of benzene in other environmental media such as cigarette smoke, gasoline, and jet fuel and its fumes (Brunnemann et al. 1989; Byrd et al. 1990; Ludwig and Eksteen 1988; Poole et al. 1988). HPLC/UV, GC/FID, and GC/MS separation and detection techniques have been used for these analyses. Sensitivity and reliability of these methods cannot be compared because of the lack of data. Few methods have been reported for measurement of benzene in foods; performance data are generally lacking.

6.3   ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA 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 benzene is available. Where adequate information is not
available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of
research designed to determine the health effects (and techniques for developing methods to determine
such health effects) of benzene.

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 or eliminate 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. Methods exist for measuring
benzene in breath (Gruenke et al. 1986; Pellizzari et al. 1988; Sherwood and Carter 1970; Wallace et
al. 1986), blood (Antoine et al. 1986; Ashley et al. 1992, 1994; Gruenke et al. 1986; Jirka and Boume
1982; Pekari et al. 1989), and tissues (Bechtold et al. 1988; Rickert et al. 1979). The methods for
breath are sensitive and accurate for determining exposure levels of benzene at which health effects
have been observed to occur, as well as for background levels in the general population. The methods
are relatively precise and selective. Methods for determining benzene in blood are sensitive; based on
the limited recovery data available, they appear to be accurate. More information on the performance
obtained with different methods would be helpful. The application of GC/MS techniques to the
analysis of blood specimens has resulted in a rapid, cost-effective, clinical screening tesefor common
volatile organic compounds, including benzene (DeLeon and Antoine 1985). This test, the VOST
(Volatile Organics Screening Test), has demonstrated the presence (down to 0.1 ppb) of a variety of
toxic volatile organics in the blood of environmentally sensitive patients and has provided preliminary
baseline concentration levels for the test population (DeLeon and Antoine 1985). The data on
determination of benzene in urine and tissue samples are very limited. In general, the available

methods have limits of detection that are too high to be useful in other than acute exposure situations. Methods that could be used to measure low levels in human tissues would be useful for determining the relationship between chronic low-level exposure and the effects observed in specific tissues.

Methods are available for measuring phenolic benzene metabolites in urine (Bechtold et al. 1991; Buchet 1988; Jen and Tsai 1994; NIOSH 1974; Ogata and Taguchi 1987). Available methods for determining most benzene metabolites in urine are sufficiently sensitive and reliable to allow measurement of background concentrations in nonoccupationally exposed individuals. However, the phenolic metabolites are not unique to benzene. Improved methods to detect phenolic metabolites are not needed. Sensitive assays have been developed for detection of urinary t,t-muconic acid (detection limit 10 μg/L) (Bechtold et al. 1991; Ruppert et al. 1995). Since urinary t,t-muconic acid concentration can be correlated with benzene exposure, this may provide a useful biomarker of exposure on an individual basis (Bechtold et al. 1991; Buckley et al. 1992). In addition, information is needed to assess the effect of co-exposure to other chemicals (e.g., toluene) on urinary muconic acid levels. Also needed are specific biomarkers of cumulative exposure to benzene, based on albumin or hemoglobin adducts, and lymphocyte DNA adducts of N-7-phenylguanine. It would also be useful to develop specific biomarkers of acute- and chronic-duration exposure to benzene based on adducts of muconaldehyde. The levels of such biomarkers formed *in vivo* would be useful later for correlation with toxic effects of acute- or chronic-duration exposure to benzene.

Methods for determining benzene in breath, blood, and tissues and for determining its metabolites in urine could also be used as biomarkers of effect. However, efforts to correlate these measures with observed toxic effects of benzene exposure have been unsuccessful. Other biomarkers of effect (e.g., complete blood cell counts, red and white blood cell counts, chromosomal aberrations, sister chromatid exchanges, and examination of bone marrow) have been suggested for benzene, but they are not specific for benzene exposure. Further development of methods for determining reliable unique biomarkers of effect for benzene are needed.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining benzene in air (Clark et al. 1984; Gruenke et al. 1986) and water (Brass et al. 1977; EPA 1979a, 1984f: Hammers and Bosman 1986; Pereira and Hughes 1980), the media of most concern for human exposure, are sensitive enough to measure background levels in the environment and levels at which health effects might occur. Their reliability

is limited primarily by the ubiquitous presence of benzene in the environment, which makes contamination a constant problem. The accuracy and precision of some methods for water analyses (e.g., GC/MS) need to be improved to produce more reliable results. Methods for soil and other solid media appear to have the same problems as those for air and water. In addition, there is a lack of information on methods for determining benzene in media such as shellfish, fish, foods, and plants. Although exposure to benzene via ingestion of food is believed to be minimal, standardized methods for these media are needed to better assess the extent of benzene contamination in the environment and the resulting risk of exposure.

6.3.2   Ongoing Studies

The Federal Research in Progress (FEDRIP 1996) database provided information on a few ongoing studies that may fill some of the data gaps discussed in Section 6.3.1. A personal vapor detection device (wear badge) is being developed by M. Druy of Foster-Miller Inc., New York, NY, to determine the accumulated exposure of benzene in the workplace. A fiber optic derivative ultraviolet absorption spectroscopic probe is being developed for *in situ* monitoring of groundwater contaminated with benzene (J.W. Haas of ORNL, Oak Ridge, TN). A fiber optic absorption spectrometer system is being developed by P.E. O'Rourke of Westinghouse Savannah River Co., Aiken, SC, to measure the concentration of benzene in radioactive waste. A method is being developed by A.A. Burlingame of University of California, Berkeley, CA, to separate and quantitate DNA adducts of benzene and its metabolites in blood/serum and to study the feasibility of using these adducts as biomarkers for the detection of exposure to benzene by humans.

The Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control and Prevention, is developing methods for the analysis of benzene and other volatile organic compounds in blood. These methods use purge and trap methodology, high resolution gas chromatography, and magnetic sector mass spectrometry which gives detection limits in the low parts per trillion (ppt) range.

Dr. Karla Thrall of DOE's Pacific Northwest Laboratories has been developing a sampling and analytical method that provides nearly instantaneous readouts of chemical concentrations in exhaled air (Thrall 1997). It uses a MS devise and has been tested and validated in animals and humans for chloroform and carbon tetrachloride. In the summer of 1997 there are tests planned in which samples

will be tested specifically for benzene. Dr. Thrall will have a section devoted to this method in the

NAS-sponsored document on biomarkers that is scheduled for publication in early 1998.

7. REGULATIONS AND ADVISORIES

The international, national, and state regulations and guidelines regarding benzene in air, water, and other media are summarized in Table 7-l.

ATSDR has derived an acute inhalation MRL of 0.05 ppm for benzene based on a LOAEL for immunological effects in mice exposed to 10.2 ppm benzene for 6 hours a day for 6 consecutive days (Rozen et al. 1984).

ATSDR has derived an intermediate inhalation MRL of 0.004 ppm for benzene based on a LOAEL for neurological effects in mice exposed to 0.78 ppm benzene for 2 hours a day, 6 days a week for 30 days (Li et al. 1992).

The reference concentration (RfC) and reference dose (RfD) for benzene are undergoing review by an EPA Workgroup (IRIS 1996).

The International Agency for Cancer Research (IARC) classifies benzene as a Group 1 carcinogen (carcinogenic to humans) (IARC 1987). The U.S. EPA assigns Class A (human carcinogen) (IRIS 1996), and the National Toxicology Programs lists benzene as a "substance known to be carcinogenic," that is, a substance for which the evidence from human studies indicates that there is a causal relationship between exposure to the substance and human cancer (NTP 1994).

Benzene is on the list of chemicals in "The Emergency Planning and Community Right-to-Know Act of 1986" (EPA 19888). Section 313 of Title III of the Superfund Amendments and Reauthorization Act (SARA) requires owners and operators of certain facilities that manufacture, import, process, or otherwise use the chemicals on this list to report annually any release of those chemicals to any environmental media over a specified threshold level (U.S. Congress 1986).

OSHA requires employers of workers who are occupationally exposed to benzene to institute engineering controls and work practices to reduce and maintain employee exposure at or below permissible exposure limits (PEL). If the employer can document that benzene is used in the workplace less than 30 days per year, the employer can use any combination of engineering controls, work practice controls, or respirators to reduce employee exposure to or below the (PEL) of 1 ppm.

However, the employer must use engineering and work practice controls, if feasible, to reduce exposure to or below an 8-hour TWA of 10 ppm. Respirators must be provided and used during the time period necessary to install or implement feasible engineering and work practice controls, or where controls are not yet sufficient. Respirators are also required when the employer determines that compliance with the TWA or PEL is not feasible with engineering or work practice controls, such as maintenance and repair activities, vessel cleaning, or other operations where exposures are intermittent and limited in duration, and in emergencies (OSHA 1987b).

Benzene is regulated by the Clean Water Effluent Guidelines in 40 CFR Part 401. For each point source category, benzene may be regulated as part of a group of chemicals controlled as total toxic organics, may have a specific regulatory limitation, or may have a zero discharge limitation. The point source categories for which benzene is controlled as a total toxic organic include electroplating (EPA 1981a), metal finishing (EPA 1983b), metal molding and casting (1985c), and copper forming (EPA 1983c). The point source categories for which benzene has a specific regulatory limitation include organic chemicals (EPA 1993a), and iron and steel manufacturing (EPA 1982a).

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), food tolerance restrictions are not required for benzene when it is used in accordance with good agricultural practices as an inert (or occasionally active) ingredient i formulations applied to growing crops only (EPA 1985h).
FDA has restricted benzene in food packaging for use only as a component of adhesives in packaging, transporting, or holding food. Adhesives are separated from the food by a functional barrier. The quantity of adhesive that contacts packaged dry food shall not exceed the limits of good manufacturing practices. The quantity of adhesive that contacts packaged fatty and aqueous foods and is found at seams and at the exposed edge between packaging laminates shall not exceed the limits of good manufacturing practices. Under normal conditions of use, the packaging seams or laminates will remain firmly bonded without visible separation. The label of the finished adhesive container must bear the statement "food-packaging adhesive" (FDA 1977a).

Table 7-1. Regulations and Guidelines Applicable to Benzene

Agency	Description	Information	References
INTERNATIONAL			
IARC	Carcinogenic Classification	Group 1[a]	IARC 1987
WHO	Drinking Water Guidelines	10 μg/L	WHO 1984
NATIONAL			
Regulations:			
a. Air:			
OSHA	PEL TWA	1 ppm	29 CFR 1910.1028 OSHA 1987a
	STEL (15-minute average)	5 ppm	
	Respirators and/or Engineering Controls Required if Compliance with TWA is Not Feasible	Yes	
	Limits for Industry Segments Excluded in 29 CFR 1910.1028		29 CFR 1910.1000 OSHA 1974
	PEL TWA (8 hr)	10 ppm[b]	
	Ceiling Limit	25 ppm	
	Maximum Peak Above the Acceptable Ceiling Concentration for an 8 hr Shift	50 ppm (10 min)[b]	
	Coke Oven Emissions[b]		
	8 hr PEL	<150 μg/m^3	29 CFR 1910.1029(c) OSHA 1976
	Types of Respiratory Protection (NPRM)	<150 to <7500 μg/m^3	59 CFR 58884 OSHA1994
	NRC Recommendations Concerning Chemical Hygiene in Labs (non-mandatory)	Yes	29 CFR 1910.1450, Appendix A OSHA 1990
EPA OAR	Listed as a Hazardous Air Pollutant Under Section 112 of the Clean Air Act	Yes	40 CFR 61.01 EPA 1977a
	Approval and Promulgation of Implementation Plans	No	40 CFR 52 EPA 1972
	New Source Performance Standards -		
	Subpart VV: SOCMI VOC Equipment Leaks - chemicals produced by affected facilities	Yes	40 CFR 60.489 EPA 1983a
	Subpart NNN: SOCMI Distillation Operation Volatile Organic Compound (VOC) Emissions - chemicals produced by affected facilities	Yes	40 CFR 60.667 EPA 1990a
	Subpart RRR: SOCMI Reactor Processes VOC Emissions - affected chemicals	Yes	40 CFR 60.707 EPA 1993a
	SOCMI Wastewater (proposed)	Yes	59 FR 46780 EPA 1994x

7. REGULATIONS AND ADVISORIES

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
	National Emission Standards for Hazardous Air Pollutants (NESHAPs)		
	List of Pollutants and Applicability of Part 61	Yes	40 CFR 61.01 EPA 1985a
	Subpart J - Equipment Leaks (Fugitive Emission Sources) of Benzene	Yes	40 CFR 61.110-61.112 EPA 1984a
	Subpart L - Benzene Emissions From Coke By-product Recovery Plants	Yes	40 CFR 61.130-61.137 EPA 1989a
	- Process vessels, storage tanks and tar-intercepting sumps	>500 ppm benzene above background concentration	40 CFR 61.132 EPA 1989a
	- Light-oil sumps	>500 ppm above background concentration	40 CFR 61.133 EPA 1989a
	- Naphthalene processing, final coolers, and final cooler cooling towers	No ("zero") emission	40 CFR 61.134 EPA 1989a
	- Standards for equipment leaks	Yes	40 CFR 61.135 EPA 1989a
	- Compliance provisions and alternative means of emissions limitation	Yes	40 CFR 61.136 EPA 1989a
	- Test methods and procedures	Yes	40 CFR 61.137 EPA 1989a
	- Provisions for alternative means for process vessels, storage tanks, and tar-intercepting sumps	Yes	40 CFR 61.139 EPA 1991k
	Subpart V - Equipment Leaks (Fugitive Emission Sources)		40 CFR 61.240-61.242 EPA 1984b
	Pressure relief valve Closed-vent systems and control devices	Leak = ≥10,000 ppm Leak = ≥ 500 ppm	
	Subpart Y - Benezene Emissions From Benzene Storage Vessels	Yes	40 CFR 61.270-61.273 EPA 1989b
	Subpart BB - Benzene Emissions From Benzene Transfer Operations	Yes	40 CFR 61.300-61.304 EPA 1990b

7. REGULATIONS AND ADVISORIES

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
	Subpart FF - Benzene Waste Operations		40 CFR 61.340-.353 EPA 1990c
	- Equipment standard	No detectable emissions <500 ppmv	40 CFR 61.340 EPA 1990c
	- Standards for tanks	<500 ppm above background	40 CFR 61.343 EPA 1990c
	- Surface impoundments	<500 ppm above background	40 CFR 61.344 EPA 1990c
	- Containers	<500 ppm above background	40 CFR 61.345 EPA 1990c
	- Individual drain systems	<500 ppm above background	40 CFR 61.346 EPA 1990c
	- Oil-water separator	<500 ppm above background	40 CFR 61.347 EPA 1990c
	- Closed-vent systems and control devices	Reduce organic emissions vented to it by ≥95 weight percent; achieve total organic compound concentration of 20 ppm (enclosed combustion devices) Recover or control organic emissions vented to it with an efficiency of ≥95 weight percent or the benzene emissions with an efficienty of ≥98 weight percent (vapor recovery system and other control devices except flares)	40 CFR 61.349 EPA 1990c
	- Performance standard	≥ 99% removal or destruction or removal to < 10 ppmw in waste	40 CFR 61.348 EPA 1990c
	- Alternative standards and emissions limitations	Yes	40 CFR 61.351-353 EPA 1990c
	NESHAPs for Source Categories		
	Subpart A - General Provisions	Yes	40 CFR 63.1 EPA 1994a
	Subpart B - Requirements for Control Technology Determinations for Major Sources in Accordance with Clean Air Act Sections 112(g) and 112(j)	Yes	40 CFR 63.51 EPA 1994b

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
	Subpart D - Regulations Governing Compliance Extensions for Early Reductions of Hazardous Air Pollutants — Demonstration of Early Reduction	Yes	40 CFR 63.74 EPA 1992a
	Subpart F - Synthetic Organic Chemical Manufacturing Industry (SOCMI)		
	Table 1 — SOCMI Chemicals	Yes	40 CFR 63.100 EPA 1994c
	Table 2 — Organic HAPs	Yes	40 CFR 63.100 EPA 1994d
	Subpart G - Synthetic Organic Chemical Manufacturing Industry for Process Vents, Storage Vessels, Transfer Operations, and Wastewater - Applicability		40 CFR 63.110 EPA 1994e
	Table 8 — Organic HAPs Subject to the Wastewater Provisions for Process Units at New Sources	Yes	40 CFR 63, Appendix EPA 1994f
	Table 9 — Organic HAPs Subject to Wastewater Provisions for Process Units at New and Existing Sources and Corresponding Fraction Removed (FR) Values	$F_R = 0.99$	
	Subpart H - Equipment Leaks - Applicability	Yes	40 CFR 63.160 EPA 1994g
	Subpart L - Coke Oven Batteries		
	Alternative Standards for Coke Oven Doors Equipped with Sheds	Yes	40 CFR 63.305 EPA 1993b
	Subpart R - Gasoline Distribution	Yes	59 FR 64303 EPA 1994h
	Subpart CC — Petroleum Refineries	Yes	60 FR 43244 EPA 1995f
	NESHAPs		
	Constructed, Reconstructed, or Modified Major Sources (proposed)	No	59 FR 15504 EPA 1994k
	Off-Site Waste Operations (proposed)	Yes	59 FR 51913 EPA 1994m
	Subpart Y — Marine Tank Vessel Loading and Unloading Operations (proposed)	Yes	60 FR 48388 EPA 1995g
	Subpart X — Secondary Lead Smelters	Yes	40 CRF 63 60 FR 32587 EPA 1995d

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
	Regulation of Fuels and Fuel Additives		
	Subpart D - Reformulated Gasoline	Yes	40 CFR 80 EPA 1994i
	Standards for Reformulated and Conventional Gasoline - Revisions	Yes	40 CFR 80 59 FR 36944 EPA 1994j
	NPRM	Yes	60 FR 40009 EPA 1995e
	Standards for Reformulated Gasoline (Proposed)	Yes	58 FR 11722 EPA 1993k
	Renewable Oxygenate Requirement for Reformulated Gasoline (proposed)	Yes	58 FR 68343 EPA 1993c
	New and In-Use Motor Vehicles (proposed)	Yes	60 FR 52769 EPA 1995h
	New Gasoline Spark-Ignition and Diesel Compression-ignition Marine Engines (NPRM)	Yes	59 FR 55930 EPA 1994n
	Emission Standards for New Non-Road-Spark-Ignition Engines at or Below 19 KW (proposed)	Yes	59 FR 25399 EPA 1994y
b. Water			
EPA ODW	National Primary Drinking Water Standards		
	MCLs	0.005 mg/L	40 CFR 141.61 EPA 1991a
	BAT	Yes	40 CFR 141.61 EPA 1991b
	Guidelines Establishing Test Procedures for the Analysis of Pollutants: New Test Methods — Required Container, Preservation Technique and Holding (Proposed)	Yes	60 FR 53988 EPA 1995i
	National Primary Drinking Water Regulations Implementation - Variances and Exemptions from MCLs	Yes	40 CFR 142.62 EPA 1991c
	Hazardous Waste Injection Restrictions	Yes	40 CFR 148.10 EPA 1988a
EPA OW	Designation of Hazardous Substances	Yes	40 CFR 116.4 EPA 1978a
	Determination of Reportable Quantities for Hazardous Substances	Yes	40 CFR 117.3 EPA 1985b
	Form 2C - Application for Permit to Discharge Wastewater	Yes	40 CFR 125 EPA 1984c
	Guidelines Establishing Test Procedures		

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
	Table 1C-List of Approved Test Procedures for Non-Pesticide Organic Compounds	Yes	40 CFR 136.3 EPA 1984f
	Method 601 - Purgeable Hydrocarbons	Yes	
	Method 602 - Purgeable Aromatics	Yes	
	Method 624 - Purgeables	Yes	
	Method 1624 Revision B - VOCs by Isotope Dilution GC/MS	Yes	
	Method 1625 Revision B - Semivolatile Organic Compounds by Isotope Dilution GC/MS	Yes	40 CFR 136, App. A EPA 1984d
	Effluent Guidelines and Standards Toxic Pollutants	Yes	40 CFR 401.15 EPA 1979b
	General Pretreatment Regulations for Existing and New Sources of Pollution		
	65 Toxic Pollutants	Yes	40 CFR 403, App. B EPA 1986a
	Standards for Use or Disposal of Sewage Sludge, Appendix G — Pollutants Eligible for a Removal Credit: Table II — Additional Pollutants Eligible (Proposed)	Land Application = 16 kg/g Surface Disposal Unlined = 140 mg/kg Unlined = 3,400 mg/kg	60 FR 54771 EPA 1995j
	Electroplating Point Source Category - General Definitions	Yes	40 CFR 413.02 EPA 1981a
	Organic Chemicals, Plastics and Synthetic Fibers		
	Subpart C - Other Fibers		40 CFR 414.35 EPA 1993d
	Maximum 1-day	136 µg/L	
	Maximum monthly average	37 µg/L	
	Subpart D - Thermoplastic Resins		40 CFR 414.45 EPA 1993e
	Maximum 1-day	136 µg/L	
	Maximum monthly average	37 µg/L	
	Subpart F - Commodity Organic Chemicals		40 CFR 414.65 EPA 1993f
	Maximum 1-day	134 µg/L	
	Maximum monthly average	37 µg/L	
	Subpart I - Direct Discharge Point Sources That Use End-0f-Pipe Biological Treatment		40 CFR 414.91 EPA 1987a
	Maximum (1 day)	136 µg/L	
	Maximum (monthly average)	37 µg/L	
	Subpart J - Direct Discharge Point Sources That Do Not Use End-Of-Pipe Biological Treatment		40 CFR 414.101 EPA 1993g
	Maximum (1 day)	134 µg/L	
	Maximum (monthly average)	57 µg/L	

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
	Subpart K - Indirect Discharge Point Sources		40 CFR 414.111 EPA 1993h
	Maximum (1 day)	134 µg/L	
	Maximum (monthly average)	57 µg/L	
	Non-complexed Metal-Bearing Waste Streams and Cyanide-Bearing Waste Streams (Zinc)	Yes	40 CFR 414, App. A EPA 1987b
	Iron and Steel Manufacturing Point Source Category		
	General Definitions	Yes	40 CFR 420.02 EPA 1982a
	BAT Effluent Limits for By-Product Cokemaking		40 CFR 420.13 (b) EPA 1982b
	iron and steel	3.19×10^{-5} Kg/kkg of product	
	maximum (1 day) with physical chemical treatment system	2.15×10^{-5} Kg/kkg of product	
	merchant	3.55×10^{-5} Kg/kkg of product	
	maximum (1 day) with physical chemical	2.5×10^{-5} Kg/kkg of product	
	NSPS for By-product Cokemaking		40 CFR 420.14(a) EPA 1982c
	iron and steel		
	maximum (1 day)	3.19×10^{-5} Kg/kkg of product	
	merchant	3.55×10^{-5} kg/kkg of product	
	maximum (1 day)		
	Steam Electric Power Generating Point Source Category		
	126 Priority Pollutants	Yes	40 CFR 423, App. A EPA 1982d
	Centralized Waste Treatment Industry (Proposed)		
	Monthly Average	0.011-6.8 mg/L	60 FR 5464
	Maximum for One Day	0.014-9.0 mg/L	EPA 1995a
	Pharmaceutical Manufacturing (Proposed)		60 FR 21592 EPA 1995c
	Definition of Minimal Level	10 µg/L	
	Subpart A — Fermentation		
	Monthly Average	212-268 µg/L	
	Maximum for One Day	573-796 µg/L	
	Subpart B — Extraction		
	Monthly Average	17-268 µg/L	
	Maximum for One Day	40-796 µg/L	
	Subpart C — Chemical Synthesis		
	Monthly Average	212-268 µg/L	
	Maximum for One Day	573-796 µg/L	

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			

	Subpart D — Mixing, Compounding, and Formulation
	    Monthly Average
	    Maximum for One Day
		17-268 μg/L
		40-796 μg/L	

	Metal Finishing Point Source Category Definition of Total Toxic Organics (TTO)	>0.01 mg/L	40 CFR 433.11 EPA 1983b
	Metal Molding and Casting Point Source Category TTO Definition	>0.01 mg/L	40 CFR 464.11 EPA 1985c
	Copper Forming Point Source Category TTO Definition	>0.01 mg/L	40 CFR 468.02 EPA 1983c
c. Food: FDA	Indirect Food Additive For Use Only as Component of Adhesives	Yes	21 CFR 175.105 FDA 1977a
d. Other: DOT	Hazardous Materials Table	Yes	49 CFR 172.101 DOT 1990
EPA OW	Ocean Dumping - Limits Established	Waste concentration < solubility Limit in sea water	40 CFR 227.7 EPA 1977b
EPA OSW	Solid Waste Disposal: Appendix I - MCLs	0.005 mg/L	40 CFR 257, App. I EPA 1991d
	Municipal Solid Waste Landfills		
	Design Criteria	0.005 mg/L	40 CFR 258.40 EPA 1991e
	App. I - Constituents for detection monitoring		40 CFR 258 EPA 1991f
	App. II - List of hazardous inorganic and organic constituents		40 CFR 258, App. II EPA 1991g
	Identification and Listing of Hazardous Waste		
	Toxicity Characteristic	0.5 mg/L	40 CFR 261.24 EPA 1990d
	Exemption for Petroleum — Contaminated Media and Debris	Yes	58 FR 8504 EPA 1993l
	Hazardous Wastes from Non-specific Sources	Yes	40 CFR 261.31 EPA 1981b
	Hazardous Wastes From Specific Sources	Yes	40 CFR 261.32 EPA 1981c
	Additional Petroleum Refining Wastes (Proposed)	Yes	60 FR 57747 EPA 1995k

7. REGULATIONS AND ADVISORIES

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
	Discarded Commercial Chemical Products, Off-specification Species, Container Residues, and Spill Residues Thereof	Yes	40 CFR 261.33(f) EPA 1980c
	Basis for Listing Hazardous Wastes (F024, F025, K085, K104, K105, K141, K142, K143, K144, K145, K147, K151 (Final); K170, K171, K172 [Proposed])	Yes	40 CFR 261, Appendix VII EPA 1981d
	Hazardous Constituents	Yes	40 CFR 261, Appendix VIII EPA 1988b
	Wastes Excluded Under 260.20 and 260.22	Yes	40 CFR 261, Appendix IX EPA 1984e
	Risk-Based Exit Levels (Proposed)		60 FR 66344, Appendix X EPA 1995l
	MCL Benchmark Option		
	Wastewater — Totals	0.021 mg/L	
	Nonwastewater		
	Totals	110 mg/kg	
	Leach	0.009 mg/L	
	Toxicity Benchmark Option		
	Wastewater — Totals	0.018 mg/L	
	Nonwastewater		
	Totals	110 mg/kg	
	Leach	0.0054.mg/L	
	Risk-Based Conditional Exit Levels (Proposed)		60 FR 66344, Appendix XI EPA 1995l
	MCL Benchmark Option		
	Wastewater — Totals		
	Nonwastewater	0.02 mg/L	
	Totals		
	Leach	250 mg/kg 0.039 mg/L	
	Toxicity Benchmark Option		
	Wastewater — Totals	0.018 mg/L	
	Nonwastewater		
	Totals	250 mg/kg	
	Leach	0.023 mg/L	
	Standards for Owners and Operators of Hazardous Wate TSDF		–
	Subpart AA - Air Emission Standards for Process Vents	Yes	40 CFR 264.1030 EPA 1990e
	Subpart BB - Air Emission Standards for Equipment Leaks	Yes	40 CFR 264.1050 EPA 1990f
	Subpart CC - Organic Air Emission Standards for Tanks, Surface Impoundments, and Containers	Yes	40 CFR 264 59 FR 62896 EPA 1994p

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information		References
NATIONAL (cont.)				
	Groundwater Monitoring List	PQL = 2µg/L		40 CFR 264, Appendix IX EPA 1987c
	Interim Status Standards for Owners and Operators of Hazardous Waste TSDF	Yes		
	Subpart AA - Air Emission Standards for Process Vents	Yes		40 CFR 265.1030 EPA 1990e
	Subpart BB - Air Emission Standards for Equipment Leaks	Yes		40 CFR 265.1050 EPA 1990f
	Subpart CC - Organic Air Emission Standards for Tanks, Surface Impoundments, and Containers	Yes		40 CFR 265 59 FR 62896 EPA 1994o
	Health-Based Limits for Exclusion of Waste - Derived Residues	Yes		40 CFR 266, Appendix VII EPA 1991h
	Appendix VIII - Potential PICs for Determination of Exclusion of Waste - Derived Residues	Yes		40 CFR 266, Appendix VIII EPA 1991i
	Land Disposal Restrictions			
	Identification of Wastes to be Evaluated by August 8, 1988	Yes		40 CFR 268.10 EPA 1986b
	Waste Specific Prohibitions - Third Wastes	Yes		40 CFR 268.35 EPA 1990g
	Treatment Standards for Hazardous Wastes			40 CFR 268.40 59 FR 47982 EPA 1994q
		ww	non ww	
	F001 - F005 (spent solvents)	0.14 mg/L	10 mg/kg	
	F037, F038, K048, K049, K051, K052	0.14 mg/L	10 mg/kg	
	F039	0.14 mg/L	10 mg/kg	
	K011, K013	0.14 mg/L	10 mg/kg	
	K060	0.14 mg/L	10 mg/kg	
	K083	0.14 mg/L	10 mg/kg	
	K085, K105	0.14 mg/L	10 mg/kg	
	K087	0.14 mg/L	10 mg/kg	
	K104	0.14 mg/L	10 mg/kg	
	K141, K142, K143, K144, K145, K147, K151	0.14 mg/L	10 mg/kg	
	K171, K172 (Proposed)	0.14 mg/L	10 mg/kg	40 CFR 261 60 FR 57747 EPA 1995k

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Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information		References
NATIONAL (cont.)				
	D018, K156, K158, K159 (Proposed)	0.14 mg/L	10 mg/kg	60 FR 11702 EPA 1995b
	Treatment Standards Expressed as Specified Technologies	Yes		40 CFR 268.42 59 FR 47982 EPA 1994r
	Treatment Standards Expressed as Waste Concentrations	Yes		40 CFR 268.43 59 FR 47982 EPA 1994s
		ww	non ww	
	F001 - F005 (spent solvents)	0.070 mg/L	3.7 mg/kg	
	F037, F038, K048, K049, K051	0.14 mg/L	14 mg/kg	
	F039, U019	0.14 mg/L	36 mg/kg	
	K011, K014	0.02 mg/L	0.03 mg/kg	
	K060	0.17 mg/L	0.071 mg/kg 6.6 - mg/kg	
	K083	0.14 mg/L	4.4 mg/kg	
	K085, K105	0.14 mg/L	6.0 mg/kg	
	K103, K104	0.15 mg/L		
	Universal Treatment Standards	0.14 mg/L	10 mg/kg	40 CFR 268.48 59 FR 47982 EPA 1994t
	Minimize Threat Levels Without Management Requirements (Proposed) — Non Wastewater Standard	110 mg/kg 0.0054 mg/L		60 FR 66344 EPA 1995l
	Effective Dates of Surface Disposed Wastes (Non-soil and Debris) Regulated in the LDRs - Comprehensive List	Yes		40 CFR 268, Appendix VII EPA 1991j
	Basis for Listing Hazardous Waste (proposed)	Yes		59 FR 9808 EPA 1994u
EPA OERR	Toxic Chemical Release Reporting			
	List of Hazardous Substances and Reportable Quantities			
	Benzene and Benzene-containing Wastes (K085, K104, K105, K151)	10 lb		40 CFR 302.4 EPA 1989c
	Benzene-containing Wastes (F024, F025, K141, K142, K143, K144, K145, K147, K170, K171, K172) (Proposed)	1 lb		60 FR 57747 EPA 1995k
	TRI Reporting Thresholds	10,000 lb		40 CFR 372.25 EPA 1988g
	Specific Toxic Chemical Listings	Yes		40 CFR 372.65 EPA 1988c
EPA OPTS	Chemical Fate Testing Guidelines			
	Boiling point/boiling range	80.1°C		40 CFR 796.1220 EPA 1985d
	Partition Coefficient (n-Octanol-water) (log k_{ow})	Yes		40 CFR 796.1570 EPA 1985e

7. REGULATIONS AND ADVISORIES

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
DOT Coast Guard	Facilities Transferring Oil or Hazardous Materials in Bulk	Yes	33 CFR 154 CG 1990
	Natural Resource Damage Assessments (proposed)	Yes	59 FR 1062 NOAA 1994
Guidelines:			
a. Air:			
EPA	RfC (8-hr inhalation)	Pending[e]	IRIS 1996
ACGIH	TLV TWA	10 ppm, 32 mg/m^{3c}	ACGIH 1996
	Carcinogen classification	A2 = Suspected human carcinogen	
	Biological Exposure Indices Total phenol in urine at end of shift In exhaled air prior to next shift Mixed-exhaled End-exhaled	50 mg/g creatinine[d] 0.8 ppm 0.12 ppm	
NIOSH	REL (8-hr TWA)	0.1 ppm	NIOSH 1992
	Ceiling (15 minutes)	1 ppm	
b. Water:			
EPA OWRS	Health Advisories 1-day (recommended) (child) 10-day (child) Long-term (child & adult) Lifetime (child & adult) DWEL	0.2 mg/L 0.2 mg/L None[f] None[f] None[f]	EPA 1995m
	Ambient Water Quality Criteria For Protection of Human Health[g] Ingesting water and organisms 10^{-5} 10^{-6} 10^{-7} Ingesting organisms only 10^{-5} 10^{-6} 10^{-7} Drinking water 10^{-4} 10^{-5} 10^{-6}	6.6 µg/L 0.66 µg/L 0.066 µg/L 400 µg/L 40.0 µg/L 4.0 µg/L 70 µg/L 7.0 µg/L 0.7 µg/L	45 FR 79318 EPA 1980b
	Public Notification	Yes	40 CFR 141.32(e)(5) EPA 1987d
	MCLGs	0 mg/L	40 CFR 141.50 EPA 1985f
	Water Quality Guidance for Great Lakes System (proposed)	Yes	59 FR 44678 EPA 1994z
c. Other			
EPA	EEGL (1-hr) (preliminary) EEGL (24-hr) (preliminary)	50 ppm 2 ppm	NAS/NRC 1993
	Cancer Classification	A	IRIS 1996

7. REGULATIONS AND ADVISORIES

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
NTP	Weight of Evidence	Clear evidence	NTP 1995
EPA	Unit Risk (air)	8.3×10^{-6} per $\mu g/m^3$	IRIS 1996
	Unit Risk (water)	8.3×10^{-7} per $\mu g/L$	IRIS 1996
	q_1^* cancer slope factor	2.9×10^{-2} per mg/kg-day	IRIS 1996
NIOSH	Carcinogen Classification	Ca[h]	NIOSH 1992
STATE			
Regulations and Guidelines:			
a. Air:	Acceptable ambient air concentrations (average time)		NATICH 1990
AZ	1 hour	$6.30 \times 10^{+2}$ $\mu g/m^3$	
	24 hour	$5.10 \times 10^{+1}$ $\mu g/m^3$	
	Annual	1.40×10^{-1} $\mu g/m^3$	
CT	8 hour	$1.50 \times 10^{+2}$ $\mu g/m^3$	
FL-FTLDLF	8 hour	3.00×10^{-1} mg/m^3	
FL-Pinella	8 hour	$3.00 \times 10^{+1}$ $\mu g/m^3$	
	24 hour	7.20 $\mu g/m^3$	
	Annual	1.20×10^{-1} $\mu g/m^3$	
IN	Annual	1.20×10^{-1} $\mu g/m^3$	
	8 hour	$1.60 \times 10^{+2}$ $\mu g/m^3$	
IN-Innap.	Annual	1.45×10^{-1} $\mu g/m^3$	
KS	Annual	1.20×10^{-1} $\mu g/m^3$	
KS-KC	Annual	1.20×10^{-1} $\mu g/m^3$	
LA	Annual	$1.20 \times 10^{+1}$ $\mu g/m^3$	
MA	24 hour	1.74 $\mu g/m^3$	
	Annual	1.20×10^{-1} $\mu g/m^3$	
ME	Year	1.20×10^{-1} $\mu g/m^3$	
MI	Annual	1.40×10^{-1} $\mu g/m^3$	
ND	NA	0.00 LAER	
NV	8 hour	7.14×10^{-1} mg/m^3	
NY	1 year	$1.00 \times 10^{+2}$ $\mu g/m^3$	
OK	24 hour	3.20 $\mu g/m^3$	
PA - Phil.	1 year	$7.20 \times 10^{+1}$ $\mu g/m^3$	
	Annual	$2.40 \times 10^{+1}$ ppb	
RI	Annual	1×10^{-1} $\mu g/m^3$	
SC	24 hour	$1.50 \times 10^{+2}$ $\mu g/m^3$	
TX	30 minutes	$3.00 \times 10^{+1}$ $\mu g/m^3$	
	Annual	3.00 $\mu g/m^3$	
VA	24 hour	$3.20 \times 10^{+2}$ $\mu g/m^3$	
VT	Annual	1.20×10^{-1} $\mu g/m^3$	
WA-SWEST	Annual	1.20×10^{-1} $\mu g/m^3$	
KY	Significant Emission Levels of Toxic Air Pollutants	7.654×10^{-3} lb/hour	401 KAR 63:021 KAR 1991a

7. REGULATIONS AND ADVISORIES

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
STATE (cont.)			
WI	Hazardous Air Contaminants Without Acceptable Ambient Concentrations Requiring Application of Lowest Achievable Emission Rates	300 lb/year	DNR 445.04 WAC 1988
b. Water:			
	State Water Quality Standards Summaries		EPA 1988d
IA	Class B - Upper value	0.01 mg/L	
	- 2° upper limit	0.0012 mg/L	
	Class C - Upper Value	0.01 mg/L	
ID	Domestic Upper Value	0.01 mg/L	
IL	General Use Water - Upper value		
	Public Food - *Upper value	0.15 mg/L	
	2° Upper Value		
IN	Yes-. site-specific		
KS	Agri. Upper Value	0.1 mg/L	
	Agri. Upper Value	0.2 mg/L	
KY	Warm Water Aquatic - upper value	4.0 µg/L	
	- 2° upper limit	12 µg/L	
MN	Domestic Consumption - Upper value	0.01 mg/L	
MO	Irrigation - upper value	10 µg/L	
	Protxn of w. - upper value	12µg/L	
	Coldwater Sport - upper value	1.2 µg/L	
	Drinking - upper value	10 µg/L	
MS	Public Water Supply - upper value	0.01 mg/L	
MT	Yes-.-site specific		
NC	Fresh Sfc. w. (WS-I) - upper value	0.4 µg/L	
	- 2° upper limit	2.0 µg/L	
	Fresh Sfc. Water (WS-II) - upper value	0.4 µg/L	
	- 2° upper limit	0.2 µg/L	
	Fresh Sfc. Water (WS-III) - upper value	0.4 µg/L	
	- 2° upper limit	2.0 µg/L	
	Fresh Sfc. Water (Class B) - upper value	0.4 µg/L	
	- 2° upper limit	0.2 µg/L	
	Fresh Sfc. Water (Class C) - upper value	0.4 µg/L	
	- 2° upper limit	0.2 µg/L	
	Tidal Salt Water (Class SA) - upper value	5 µg/L	
	Tital Salt Water (Class SB) - upper value	5 µg/L	
	Tidal Salt Water (Class SC) - upper value)	5 µg/L	
ND	Class I Streams - upper value	0.01 mg/L	
	Class IA Streams - upper value	0.01 mg/L	
	Class II Streams - upper value	0.01 mg/L	
	Class III Streams - upper value	0.01 mg/L	
NE	Water Supply - upper value	0.01 mg/L	
NJ	Fresh water FW2 - upper value	10 µg/L	

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Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
STATE (cont.)			
NV	All Classes - upper value	0.0004 mg/L	
NY	Class A - upper value	10 µg/L	
	2° upper limit	Yes	
	Class B-D funct.	NA	
	Class AA Special Class I - upper value	10 µg/L	
	- 2° upper limit	Yes	
	Class A Special Class II - upper value	10 µg/L	
	- 2° upper limit	Yes	
	Class A Special - upper value	10 µg/L	
	Class A Special - upper limit	Yes	
OH	Warm water Habit - upper value	Yes- site specific	
	Limited Warm Water - upper value	Yes-site specific	
	Exceptional Warm Water - upper value	Yes- site specific	
	Seasonal Salmon - upper value	Yes -site specific	
	Cold water - upper value	Yes- site specific	
	Public Water Supply - upper value	10 µg/L max.	
	Agricultural - upper value	50 µg/L	
OK	Public and Private - upper value	0.020 mg/L	
	Fish and Wildlife - upper value	Yes- site specific	
OR	All Classes - upper value	Yes- site specific	
PR	Class SA - upper value	Yes- site specific	
	Class SB - upper value	5.0 µg/L	
	Class SC - upper value	5.0 µg/L	
	Class SD - upper value	5.0 µg/L	
	Class SE - upper value	5.0 µg/L	
RI	Fresh water Class SB 2° upper limit	Yes	
	Fresh water Class C	Yes	
	Fresh water Class D	Yes	
	Sea Water Class SA upper value	59 µg/L	
	Sea Water Class SA 2° upper limit	4.5 µg/L	
	Sea Water Class SB upper value	59 µg/L	
	Sea Water Class SB 2° upper limit	4.5 µg/L	
	Sea Water Class SC upper value	59 µg/L	
	Sea Water Class SC 2° upper limit	4.5 µg/L	
SD	Domestic water - upper value	0.01 mg/L	
TN	Domestic water - upper value	10 µg/L	
UT	Domestic Purpose - Class IA - upper value	0.01 mg/L	
	Domestic Purpose - Class IB	0.01 mg/L	
	Domestic Purpose - Class IC	0.01 mg/L	
	Recreation and Class 2A - upper value	Yes	
	Recreation and Class 2B - upper value	Yes	
	Aquatic Class 3A - upper value	0.004 mg/L	
	Aquatic Class 3B - upper value	0.04 mg/L	
	Aquatic Class 3C - upper value	Yes	
	Aquatic Class 3D - upper value	Yes	
	Agricultural Use Class 4 - upper value	0.01 mg/L	
	Agricultural Use Class 5 - upper value	Yes	
VA	Surface Public - upper value	0.01 mg/L	
	Protection of Surface Public	9.3 µg/L	

7. REGULATIONS AND ADVISORIES

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
STATE (cont.)			
WV	Public Water Su. Category A - upper value	Yes (10 mg/L)	
AZ	Drinking Water	1.3 µg/L	FSTRAC 1990
CT	Drinking Water	1 µg/L	
ME	Drinking Water	5 µg/L	
MN	Drinking Water	7 µg/L	
RI	Drinking Water	0.7 µg/L	
	Water Quality Criteria: Human Health		
AZ	Concentration Domestic/Drinking	0.005 mg/L 1.2 µg/L	CELDs 1994 Sittig 1994b
AL	Concentration	0.005 mg/L	CELDs 1994
AZ	Domestic Water Source	5 µg/L	
AZ	Fish Consumption	120 µg/L	
AZ	Partial Body Contact	470 µg/L	
CO	Maximum Contaminant Level	0.005 mg/L	
CT	Quantification Limit (Organic Chemical Parameters) Domestic/Drinking	1 mg/L 1 µg/L	 Sittig 1994b
CA	Maximum Contaminant Level	0.005 mg/L	CELDS 1994
DE	Organic Chemical Conc. Level	0.005 mg/L	
DC	Numeric Stds for Water Quality	0.8 µg/L	
FL	Maximum Contaminant Level Detection Limit	0.001 mg/L 0.5 µg/L	 Sittig 1994b
GA	Maximum Contaminant Level	0.005 mg/L	CELDs 1994
IA	Maximum Contaminant Level	0.005 mg/L	
ID	Maximum Contaminant Level Goal	zero	
ID	Maximum Contaminant Level	0.005 mg/L	
IN	Continuous Criterion Conc. (Avg. 4 days) Outside Mixing Zone	400 µg/L	
IN	Continuous Criterion Conc. (Avg. 4 days) Point of Water Intake	6.6 µg/L	
IL	Maximum Contaminant Level	0.005 mg/L	
KY	Water Quality Conc. from Consumption of Fish Tissue	.40 µg/L	
KY	Domestic Water Supply (MCL)	.66 µg/L	
LA	Drinking Water Supply	1.10 µg/L	
LA	Drinking Water Supply	12.50 µg/L	

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
STATE (cont.)			
MA	Maximum Contaminant Levels	0.005 mg/L	
ME	Domestic/Drinking	5 µg/L	Sittig 1994b
MI	Domestic/Drinking Detection Limit	1.2 µg/L 1 µg/L	
MD	Toxic Sub. Criteria-Drinking Water	5 µg/L	CELDs 1994
MD	Toxic Sub. Criteria-Fish Consumption	400 µg/L	
MT	Maximum Volatile Organic Chemical Contaminant Level	0.005 mg/L	
MO	Maximum Contaminant Level for VOs	0.005 mg/L	
MN	Maximum Contaminant Level for VOs	0.005 mg/L	
MO	Toxic Sub.Criteria - Human Health Fish Consumption	71 µg/L	
MO	Toxic Sub. Criteria - Drinking Water	5 µg/L	
NE	Maximum Contaminant Levels	0.005 mg/L	
MN	Maximum Contaminant Levels	0.002 mg/L	
ND	Maximum Contaminant Levels	0.005 mg/L	
NJ	Domestic/Drinking	1 µg/L	Sittig 1994b
NY	Water Quality Stds. Health	.7 µg/L	CELDS 1994
NC	Maximum Contaminant Levels	0.005 mg/L	
NC	Water Quality Stds. for Class GS Waters	0.001 mg/L	
OH	Maximum Contaminant Levels	0.005 mg/L	
OK	Maximum Allowable Levels	0.005 mg/L	
OR	Domestic/Drinking	3 µg/L	Sittig 1994b
PR	Maximum Allowable Conc. (Class SB & SC)	400 µg/L	CELDs 1994
PR	Maximum Allowable Conc. (Class SD)	5 µg/L	
PR	Maximum Allowable Conc. (Class SBI)	5 µg/L	
PR	MCL Goals	0.005 mg/L	
RI	Maximum Contaminant Levels	0.005 mg/L	
SC	Maximum Contaminant Levels	0.005 mg/L	
SD	Volatile Organic Chemical Level	0.005 mg/L	

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
STATE (cont.)			
TN	Maximum Contaminant Levels	0.005 mg/L	
TX	Maximum Contaminant Levels	0.005 mg/L	
VA	Maximum Contaminant Levels	0.005 mg/L	
VT	Class A or B Criteria	0.66 µg/L	
VT	Class C Waters Criteria	40 mg/L	
UT	Volatile Organic Contaminants	0.005 mg/L	
WA	Domestic/Drinking	5 µg/L	Sittig 1994b
WV	Maximum Synthetic Organic Contaminant	0.005 mg/L	CELDS 1994
WI	Maximum Contaminant Levels for VOs	0.005 mg/L	
WI	Human Cancer Criteria in Warm Water Sport Fish Commun.	5 mg/L	
WI	Human Cancer Criteria in Coldwater Commun.	5 mg/L	
WI	Human Cancer Criteria in Great Lakes Commun.	5 mg/L	
WI	Human Cancer Criteria in Warm Water Sport Fish Commun. (non-public WS)ublic WS)	140 mg/L	
WI	Human Cancer Criteria in Cold Water Commun. (non-public WS)	45 mg/L	
WI	Human Cancer Criteria in Warm Water Forage & Limited Forage (non-public WS)	1,300 mg/L	
WI	Interim Std. for those landfill and impoundments that accepted waste between 11-19-80 -- 7-26-82	0.005 mg/L	
WV	All water stds. criteria (B1, B2, B3)	40 mg/L	
WV	All water stds. criteria (A1)	0.66 mg/L	
WI	Maximum Contaminant Levels Goals Less than Maximum Contaminant Levels	0.001 mg/L	
WI	Human Cancer Criteria Public water supply Warm water sport fish communities Cold water communities	 5 µg/L 5 µg/L 5 µg/L	DNR 105.09 WAC1989a
	Water Quality Criteria: Aquatic Life		CELDS 1994
AZ	(Acute) Cold Water Fishery	2,700 µg/L	
AZ	(Acute) Warm Water Fishery	2,700 µg/L	

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
STATE (cont.)			
AZ	(Acute) Effluent Dominated Water	11,000 µg/L	
AZ	(Acute) Ephemeral	NS	
AZ	(Chronic) Cold Water Fishery	180 µg/L	
AZ	(Chronic) Warm Water Fishery	180 µg/L	
AZ	(Chronic) Effluent Dominated Water	700 µg/L	
AZ	(Chronic) Ephemeral	NS	
CO	(Acute) Organic Compounds to Second Power Std.	5,300 µg/L	
DC	Numeric Standards for Water Quality	1,000 µg/L	
FL	For Mixing Zone Pollutants (Maximum Conc. Levels)	4 mg/L	
HI	(Acute) Freshwater	1,800 µg/L	
HI	(Chronic) Freshwater	NS	
HI	(Acute) Saltwater	1,700 µg/L	
HI	(Chronic) Saltwater	NS	
HI	Fish Consumption	13 µg/L	
LA	Acute Freshwater	2,249 µg/L	
LA	Acute Marinewater	2700	
LA	Chronic Freshwater	1,125 µg/L	
LA	Chronic Marinewater	1,350 µg/L	
NC	Chronic - Stds. for Carcinogens in Fish and Shellfish	71.4 µg/L	
NY	Domestic/Drinking	6 µg/L	Sittig 1994b
OH	(Outside Mixing Zone) Maximum Concentration	1,100 µg/L	CELDS 1994
OH	30 Day Average - Coldwater	560 µg/L	
OH	(Inside Mixing Zone) Maximum Concentration Aquatic Habitat	2,100 µg/L	
OH	(Outside Mixing Zone) Aquatic Habitat Avg. 30 days Human Health	710 µg/L	
WI	Nonpublic water supply		DNR 105.09
	Warm water sport fish communities	140 µg/L	WAC 1989b
	Cold water forage and limited forage fish	45 µg/L	
	communities and limited aquatic life	1,300 µg/L	
	Groundwater Quality Standards		CELDS 1994
CO	Carcinogenic Chemical Standard	5 µg/L	

7. REGULATIONS AND ADVISORIES

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
STATE (cont.)			
KY	Maximum Contaminant Levels for Underground Drinking Water	0.005 mg/L	
MO	Toxic Criteria-Groundwater Quality	5 µg/L	
MN	Groundwater Standards	0.01 mg/L	
NY	Groundwater Effluent Standards	0.7 µg/L	
OK	Numerical Water Quality Criteria for Groundwater	0.2 µg/L	
OK	Numerical Groundwater Quality Ref.	0.005 mg/L	
UT	Groundwater Quality Stds.	0.005 mg/L	
WI	Groundwater Quality Enforced Std.	5 mg/L	
WI	Groundwater Quality Preventative Action Limit	0.5 mg/L	
WI	Maximum Conc. of Constituents for Groundwater Prot.	0.005 mg/L	
WI	Public Health Groundwater Quality Standards		
	Enforcement standard	5 µg/L	DNR 140.10 WAC 1994a
	Preventive action limit	0.05 µg/L	DNR 140.10 WAC 1994b
	Groundwater Quality Monitoring Parameters		CELDS 1994
AL	Suggested Methods	8020, 8240	
CO	Suggested Methods	8020, 8240	
CA	Suggested Methods	8020, 8240	
IL	Suggested Methods	8020, 8240	
LA	Suggested Methods	8020, 8240	
KY	Suggested Methods	8020, 8240	
MT	Suggested Methods	8020, 8240	
NY	Suggested Methods	8020, 8240	
OH	Suggested Methods	8020, 8240	
SC	Suggested Methods	8020, 8240	
VA	Suggested Methods	8020, 8240	
WI	Suggested Methods	8020, 8240	
	Hazardous Waste Constituents		CELDS 1994
AL	Maximum Concentration	0.5 mg/L	
CA	Hazardous Waste Max. Conc. of Tox. Characteristics	0.5 mg/L	
CA	Recyclable Hazardous Waste Type	NA	

7. REGULATIONS AND ADVISORIES

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
STATE (cont.)			
CO	Maximum Concentration	0.5 mg/L	
IL	Maximum Concentration of Tox. Characteristics	0.5 mg/L	
KY	Maximum Concentration of Cont. for Tox. Characteristics	0.5 mg/L	
LA	Reportable Quantities for Selected Air Cont.	100 lbs.	
LA	Effluent Limitations for Produced Water Discharge	0.0125 mg/L	
LA	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
ME	Minimum Detection Unit	≤5 mg/kg	
MA	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
MD	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
MN	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
NJ	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
NE	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
ND	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
PA	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
OH	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
SC	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
VA	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
VT	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
WI	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
WY	Maximum Conc. of Cont. for Tox. Characteristics	0.5 mg/L	
	Maximum Leachable Concentrations		CELDS 1994
TX	Maximum Leachable Conc.	0.50 mg/L	
TX	MCL	0.005 mg/L	

Table 7-1. Regulations and Guidelines Applicable to Benzene (continued)

Agency	Description	Information	References
STATE (cont.)			
c. Other			
CA	Hazardous Substance Subject to Minimum Standard of Management of Hazardous and Extremely Hazardous Wastes	Yes	66260.24 CA 1993a
CA	Regulatory Level	0.50 µg/day	66260.24 CA 1993b
KY	Defined as Hazardous Waste	Yes	401 KAR 31:170 KAR 1991b

[a] Group 1=Carcinogenic to humans.
[b] Coke oven emissions have been demonstrated to contain benzene.
[c] Notice of intended change for benzene would lower the TWA to 0.l ppm and STEL to 0.3 mg/m^3; the carcinogen classification would be changed to Category A1 (confirmed Human Carcinogen).
[d] The Biological Exposure Indices for benzene are under study for possible change.
[e] Under review by an EPA work group.
[f] "None" indicates that these values have not been calculated for benzene because of its potent carcinogenicity.
[g] Because of its carcinogenic potential, the EPA-recommended concentration for benzene in ambient water is zero. However, because attainment of this level may not be possible, levels that correspond to upper-bound incremental lifetime cancer risks of 10^{-5}, 10^{-6}, and 10^{-7} are estimates.
[h] Ca = Carcinogenic

ACGIH = American Conference of Governmental Industrial Hygienists; BAT = Best Available Technology; CELDS = Computer-assisted Environmental Legislative Database; CFR = Code of Federal Regulations; DWEL = Drinking Water Equivalent Level; EEGL = Emergency Exposure Guidance Level; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; FSTRAC = Federal State Toxicology and Regulatory Alliance Committee; GA = ground water best used as sources of drinking water; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; KAR = Kentucky Administrative Regulations; LAER = Lowest Achievable Emission Rate; LDR = Land Disposal Restrictions; MCL = Maximum Contaminant Level; MCLG = Maximum Contaminant Level Goal; NA = Not applicable; NAS = National Academy of Sciences; NATICH = National Air Toxics Information Clearinghouse; NESHAP = National Emission Standard for Hazardous Air Pollutants; NIOSH = National Institute for Occupational Safety and Health; NPRM = Notice of Proposed Rulemaking; NR = Natural Resources; NRC = National Research Council; NS = not specified; NSPS = New Source Performance Standards; NTP = National Toxicology Program; OAR = Office of Air and Radiation; ODW = Office of Drinking Water; OERR = Office of Emergency and Remedial Response; OPTS = Office of Pesticides and Toxic Substances; OSHA = Occupational Safety and Health Administration; OSW = Office of Solid Waste; OWRS = Office of Water Regulations and Standards; PEL = Permissible Exposure Limit; PICs = Products of Incomplete Combustion; PQL = Principal Quantitation Limit; PSES = Pretreatment Standards for Existing Sources; PSNS - Pretreatment Standards for New Sources; REL = Recommended Exposure Limit; RfC = Reference Concentrations; RfD = Reference Dose; SDWA = Safe Drinking Water Act; SNARL = Suggested-No-Adverse-Response Level; STEL = Short Term Exposure Limit; TLV = Threshold Limit Value; TRI = Toxics Release Inventory; TTO = Total Toxic Organic; TWA = Time-Weighted Average; VHAP = Volatile Hazardous Air Pollutant; WAC = Wisconsin Administrative Code; WHO = World Health Organization; ww = wastewater

8. REFERENCES

*Abraham N. 1996. Hematopoietic effects of benzene inhalation assessed by long term bone marrow culture. Environ Health Perspect 104 (Suppl 6): 1277-1282.

*ACGIH. 1996. Threshold limit values for chemical substances and physical agents and biological exposure indices. American Conference of Governmental Industrial Hygienists. Cincinnati, OH.

*Adlkofer F, Scherer G, Conze C, et al. 1990. Significance of exposure to benzene and other toxic compounds through environmental tobacco smoke. J Cancer Res Clin Oncol 116(6):591-598.

*Ahmad S, Singh V, Rao GS. 1994. Antioxidant potential in serum and liver of albino rats exposed to benzene. Indian J Exp Biol 32(3):203-206.

*Ahmad S, Singh V, Rao GS. 1995. Release of iron from ferritin by 1,2,4-benzenetriol. Chemico-Biological Interactions 96:103-l11.

*Aksoy M. 1980. Different types of malignancies due to occupational exposure to benzene: A review of recent observations in Turkey. Environ Res 23: 181-190.

*Aksoy M. 1987. Chronic lymphoid leukaemia and hairy cell leukaemia due to chronic exposure to benzene: report of three cases. Br J Haematol 66:209-211.

*Aksoy M. 1989. Hematotoxicity and carcinogenicity of benzene. Environ Health Perspect 82:193-197.

*Aksoy M. 1991. Hematotoxicity, leukemogenicity, and carcinogenicity of chronic exposure to benzene. In: E. Arinc, J. B. Schenkman and E. Hodgson, eds. Molecular aspects of monooxygenases and bioactivation of toxic compounds. NATO advanced science institutes series a: life sciences, vol. 202. New York, NY: Plenum Press. 415-434.

*Aksoy M, Dincol K, Akgun T, et al. 1971. Haematological effects of chronic benzene poisoning in 217 workers. Br J Ind Med 28:296-302.

*Aksoy M, Dincol K, Erdem S, et al. 1972. Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Br J Ind Med 29:56-64.

*Aksoy M, Erdem S. 1978. Followup study on the mortality and the development of leukemia in 44 pancytopenic patients with chronic benzene exposure. Blood 52:285-292.

*Aksoy M, Erdem S, Dincol G, et al. 1984. Anemia due to chemicals and drugs: A study of 108 patients. Sex Trans Disp Suppl 11, 347.

*Aksoy M, Erdem S, Dincol G. 1974. Leukemia in shoe-workers exposed chronically to benzene. Blood 44:837-841.

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*Cited in text

*Aksoy M, Erdem S, Erdogan G, et al. 1976. Combination of genetic factors and chronic exposure to benzene in the aetiology of leukaemia. Hum Hered 26:149-153.

*Aksoy M, Ozeris S, Sabuncu H, et al. 1987. Exposure to benzene in Turkey between 1983 and 1985: A haematological study on 231 workers. Br J Ind Med 44:785-787.

*Allen PD. 1987. Residential population exposure to ambient benzene in California. California Air Resources Board. ARB/TS-87-001.

*Anbar M, Neta P. 1967. A compilation of specific bimolecular rate constants for the reaction of hydrated electrons, hydrogen atoms and hydroxyl radicals with inorganic and organic compounds in aqueous solutions. International Journal of Applied Radiation and Isotopes 18:493-523.

*Andersen ME, Krishnan K. 1994. Relating *in vitro* to *in vivo* exposures with physiologically-based tissue dosimetry and tissue response models. In: Current concepts and approaches on animal test alternatives. H. Salem, ed. U.S. Army Chemical Research Development and Engineering Center, Aberdeen Proving Ground, Maryland.

*Andersen ME, MacNaughton MG, Clewell HJ, et al. 1987. Adjusting exposure limits for long and short exposure periods using a physiological pharmacokinetic model. Am Ind Hyg Assoc J 48(4):335-343.

*Anderson D, Richardson CR. 1981. Issues relevant to the 'assessment of chemically induced chromosome damage *in vivo* and their relationship to chemical mutagenesis. Mutat Res 90:261-272.

*Anderson D, Yu TW, Phillips BJ, et al. 1995. An investigation of the DNA damaging ability of benzene and its metabolites in human lymphocytes, using the comet assay. BIBRA Toxicology International, Woodmansteme Rd, German Cancer Research Center, Im Neuenheimer Feld 280, Heidelberg, Germany. (abstract CREMS Abstract)

*Andrews LS, Lee EW, Witmer CM, et al. 1977. Effects of toluene on metabolism, disposition, and hematopoietic toxicity of [^{3}H] benzene. Biochem Pharmacol 26:293-300.

*Andrews LS, Snyder R. 1986. Toxic effects of solvents and vapors. In: Casarett LJ, Doull J, eds. Toxicology, the Basic Science of Poisons. 3rd ed. New York, NY: MacMillan Publishing Co., 641.

*Angelosanto FA, Blackbum GR, Schreiner CA, et al. 1996. Benzene induces a dose-dependent increase in micronuclei in Zymbal gland cells of rats. Environ Health Perspect 104 (Supp 6): 113l-1136.

*Angerer J, Scherer G, Schaller KH, et al. 1991. The determination of benzene in human blood as an indicator of environmental exposure to volatile aromatic compounds. Fresenius J Anal Chem 339(10):740-742.

*Angley JT, Brusseau ML, Miller WL, et al. 1992. Nonequilibrium sorption and aerobic biodegradation of dissolved alkylbenzenes during transport in aquifer material: Column experiments and evaluation of a coupled-process model. Environ Sci Technol 26(7):1404-1410.

*Anonymous. 1994. Facts and figures for the chemical industry at a glance. Chemical and Engineering News 72(27): 28-36.

*Antoine SR, DeLeon IR, O'Dell-Smith RM. 1986. Environmentally significant volatile organic pollutants in human blood. Bull Environ Contam Toxicol 36:364-371.

*Aoyama K. 1986. Effects of benzene inhalation on lymphocyte subpopulations and immune response in mice. Toxicol Appl Pharmacol 85:92-101.

*Appel BR, Guirguis G, Kim IS, et al. 1990. Benzene, benzo(a)pyrene, and lead in smoke from tobacco products other than cigarettes. Am J Public Health 80(5):560-564.

*Arinc E, Adali 0, Iscan M, et al. 1991. Stimulatory effects of benzene on rabbit liver and kidney microsomal cytochrome P-450 dependent drug metabolizing enzymes. Arch Toxicol 65(3): 186-190.

*Armstrong MJ, Galloway SM. 1993. Micronuclei induced in peripheral blood of E u-PIM-1 transgenic mice by chronic oral treatment with 2-acetylaminofluorene or benzene but not with diethyl-nitrosamine or 1,2-dichloroethane. Mutat Res 302(1):61-70.

*Arvin E, Jensen BK, Gundersen AT. 1989. Substrate interactions during aerobic biodegradation of benzene. Appl Environ Microbial 55:3221-3225.

*Ashley DL, Bonin MA, Cardinali FL, et al. 1994. Blood concentrations of volatile organic compounds in a nonoccupationally exposed US population and in groups with suspected exposed. Clin Chem 40(7):1401-1404.

*Ashley DL, Bonin MA, Cardinali FL. 1992. Determining volatile organic compounds in the human blood from a large sample population by using purge and trap gas chromatography/mass spectrometry. Anal Chem 64:1021-1029.

*Astier A. 1992. Simultaneous high-performance liquid chromatographic determination of urinary metabolites of benzene, nitrobenzene, toluene, xylene and styrene. J Chromatogr 573(2): 318-322.

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

*ATSDR. 1991. Benzene groundwater exposure study Nesmith, South Carolina. Final report Technical assistance to South Carolina Dept. of Health and Environmental Control. Agency for Toxic Substance and Disease Registry. Division of Health Assessment and Consultation. Atlanta, GA.

*ATSDR. 1992. Case studies in environmental medicine. Benzene toxicity. US Dept. of Health & Human Services. Agency for Toxic Substance and Disease Registry. Division of Health Education. Atlanta, GA.

*ATSDR. 1995. National Exposure Registry Benzene Subregistry baseline technical report. US Dept. of Health and Human Services l-l14. Agency for Toxic Substance and Disease Registry Division of Health Studies. Atlanta. GA.

Au WW, Anwar W, Paolini M, et al. 1991b. Mechanism of clastogenic and co-clastogenic activity of chromophor with benzene in mice. Carcinogenesis (Eynsham) 12(1):53-58.

*Au WW, Anwar WA, Hanania E, et al. 1990. Antimutagenic effects of dimethyl sulfoxide on metabolism and genotoxicity of benzene *in vivo*. Basic Life Sci 52:389-393.

*Au WW, Ramanujam VM, Ward JB Jr, et al. 1991a. Chromosome aberrations in lymphocytes of mice after sub-acute low-level inhalation exposure to benzene. Mutat Res 260(2):219-224.

*Aubrecht J, Rugo R, Schiestl RH. 1995. Carcinogens induce intrachromosomal recombination in human cells. Carcinogenesis 16(11): 2841-2846.

*Avis SP, Hutton CJ. 1993. Acute benzene poisoning: a report of three fatalities. J Forensic Sci 38(3):599-602.

*Baarson K, Snyder CA, Green J, et al. 1982. The hematotoxic effects of inhaled benzene on peripheral blood, bone marrow, and spleen cells are increased by ingested ethanol. Toxicol Appl Pharmacol 64:393-404.

*Baarson KA, Snyder CA. 1991. Evidence for the disruption of the bone marrow microenvironment by combined exposures to inhaled benzene and ingested ethanol. Arch Toxicol 65(5):414-420.

*Baarson KA, Snyder CA, Albert RE. 1984. Repeated exposures of C57BL mice to inhaled benzene at 10 ppm markedly depressed erythropoietic colony formation. Toxicol Lett 20:337-342.

*Bandow H, Washida N, Akimoto H. 1985. Ring-cleavage reactions of aromatic hydrocarbons studied by FT-IR spectroscopy: I. Photooxidation of toluene and benzene in the NO_x-Air System. Bull Chem Sot Jpn 58:2531-2540.

*Barale R, Giorgelli F, Migliore L, et al. 1985. Benzene induces micronuclei in circulating erythrocytes of chronically treated mice. Mutat Res 144: 193-196.

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

Barrefors G, Petersson G. 1995. Assessment by gas chromatography and gas chromatography-mass spectrometry of volatile hydrocarbons from biomass burning. J Chromatogr 710:71-77.

*Barrett RH. 1985. Assays for unscheduled DNA synthesis in HeLa S3 cells. In: Ashby J, de Serres FJ, Draper M, et al., eds. Progress in mutation research 5. Evaluation of short-term tests for carcinogens: Report of the International Program on Chemical Safety's collaborative study on *in vitro* assays. Amsterdam, Netherlands: Elsevier Science Publisher, 347-352.

*Bartczak A, Kline SA, Yu R, et al. 1994. Evaluation of assays for the identification and quantitation of muconic acid, a benzene metabolite in human urine. J Toxicol Environ Health 42: 245-258.

*Baslo A, Aksoy M. 1982. Neurological abnormalities in chronic benzene poisoning: A study of six patients with aplastic anemia and two with preleukemia. Environ Res 27:457-465.

*Bayer CW, Black MS, Galloway LM. 1988. Sampling and analysis techniques for trace volatile organic emissions from consumer products. J Chromatogr Sci 26:168-173.

*BCCR. 1990. Barclays California Code of Regulations. Article 5.5. Primary Standards-Organic Chemicals; Article 7. No Significant Risk Levels. Title 22. Register 90, No.26-31. San Francisco, California.

*Bechtold WE, Henderson RF. 1993. Biomarkers of human exposure to benzene. J Toxicol Environ Health 40:377-386.

*Bechtold WE, Lucier G, Bimbaum LS, et al. 1991. Muconic acid determinations in urine as a biological exposure index for workers occupationally exposed to benzene. Am Ind Hyg Assoc J 52:473-478.

*Bechtold WE, Sabourin PJ, Henderson RF. 1988. A reverse isotope dilution method for determining benzene and metabolites in tissues. J Anal Toxicol 12:176-179.

*Bechtold WE, Strunk MR. 1996. S-Phenylcysteine in albumin as benzene biomarker. Environ Health Perspect 104 (Suppl 6): 1147-l149.

*Bechtold WE, Sun JD, Bimbaum LS, et al. 1992a. S-phenylcysteine formation in hemoglobin as a biological exposure index to benzene. Arch Toxicol 66(5):303-309.

*Bechtold WE, Willis JK, Sun JD, et al. 1992b. Biological markers of exposure to benzene: S-phenylcysteine in albumin. Carcinogenesis 13(7):1217-1220.

*Bennett GF. 1987. Air quality aspects of hazardous waste landfills. Haz Waste Haz Mat 4:119-138.

*Berlin M, Gage JC, Gullberg B, et al. 1980. Breath concentration as an index to the health risk from benzene. Stand J Work Environ Health 6: 104-111.

*Black FM, High LE, Lang JM. 1980. Composition of automobile evaporative and tailpipe hydrocarbon emissions. J Air Pollut Control Assoc 30:1216-1221.

*Black H. 1995. Like sugar for poison: glucose as a substitute for benzene [news]. Environ Health Perspect 103(6):564-566.

*Blanchard RD, Hardy JK. 1986. Continuous monitoring device for the collection of 23 volatile organic priority pollutants. Anal Chem 58:1529-1532.

*Blank IH, McAuliffe DJ. 1985. Penetration of benzene through human skin. J Invest Dermatol 85:522-526.

*Bleasdale C, Kennedy G, MacGregor JO, et al. 1996. Chemistry of muconaldehydes of possible relevance to the toxicology of benzene. Environ Health Perspect 104 (Suppl 6): 1201-1209.

*Bloch P, Kulig A, Paradowski M, et al. 1990. The toxicodynamics of benzene, ethanol, and benzene plus ethanol based on the histopathological examination of selected organs in the rat. Pol J Occup Med 3(1):69-82.

*BNA. 1990. California hazardous waste management regulations (California Administrative Code, Title 22, Division 4, Chapter 30). Washington, DC: The Bureau of National Affairs, Inc.

Bock CW, George P, Greenberg A, et al. 1994. An *ab initio* computational molecular orbital study of the conformers of muconaldehyde, and the possible role of 2-formyl-2h-pyran in bringing about the conversion of a (z,z)-muconaldehyde structure into an (e,z)-muconaldehyde structure. Chemical Research In Toxicology 7 (4):534-543.

*Bock FG, King DW. 1959. A study of the sensitivity of the mouse forestomach toward certain polycyclic hydrocarbons. J Nat1 Cancer Inst 23:833-839.

*Bodell WJ, Levay G, Pongracz K. 1993. Investigation of benzene-DNA adducts and their detection in human bone marrow. Environ Health Perspect 99:241-244.

*Bodell WY, Pathak DN, Levay G, et al. 1996. Investigation of the DNA adducts formed in B6C3F$_1$ mice treated with benzene: Implications for molecular dosimetry. Environ Health Perspect 104 (Suppl 6):1189-1193.

*Bois FY, Jackson E, Pekari K. 1996. Population benzene toxicokinetics of benzene in humans. Environ Health Perspect 104 (Suppl 6): 1405-1411.

*Bois FY, Paxman DG. 1992. An analysis of exposure rate effects for benzene using a physiologically based pharmacokinetic model. Regul Toxicol Pharmacol 15(2):122-136.

*Bois FY, Smith MT, Spear RC. 1991a. Mechanisms of benzene carcinogenesis: application of a physiological model of benzene pharmacokinetics and metabolism. Toxicol Lett 56(3):283-298.

*Bois FY, Woodruff TJ, Spear RC. 1991b. Comparison of three physiologically based pharmacokinetic models of benzene disposition. Toxicol Appl Pharmacol 110(1):79-88.

*Bond AE, Thompson VL, Ortman GC, et al. 1986a. Self service station vehicle refueling exposure study. In: Proceedings of the 1986 EPA/APCA symposium on measurements of toxic air pollutants. Pittsburgh, PA: Air Pollution Control Association, 458-466.

*Bond GG, McLaren EA, Baldwin CL, et al. 1986b. An update of mortality among chemical workers exposed to benzene. Br J Ind Med 43:685-691.

*Boogaard PJ, van Sittert NJ. 1995. Biological monitoring of exposure to benzene: a comparison between S-phenylmercapturic acid, truns,trans-muconic acid, and phenol. Occup Environ Med 52:61l-620.

*Boogaard PJ, van Sittert NJ. 1996. Suitability of S-phenyl mercapturic acid and *trans,trans*-muconic acid as biomarkers for exposure to low concentrations of benzene. Environ Health Perspect 104 (Suppl 6):1151-1157.

*Bordarier C, Robain 0, Ponsot G. 1991. Bilateral porencephalic defect in a newborn after injection of benzol during pregnancy. Brain Dev 13(2):126-129.

*Bradley EB, Frenzel CA. 1970. On the exploitation of laser Raman spectroscopy for the detection and identification of molecular water pollutants. Water Res 4:124-128.

*Bradley MO. 1985. Measurement of DNA single-strand breaks by alkaline elution in rat hepatocytes. Progress in Mutation Research 5:353-357.

*Brass HJ, Feige MA, Halloran T, et al. 1977. The national organic monitoring survey: Samplings and analyses for purgeable organic compounds. In: Pojasek RB, ed. Drinking water quality enhancement through source protection. Ann Arbor, MI: Ann Arbor Science Publishers, 393-416.

*Brett SM, Rodricks JV, Chinchilli VM. 1989. Review and update of leukemia risk potentially associated with occupational exposure to benzene. Environ Health Perspect 82:267-281.

*Brief RS, Lynch J, Bernath T, et al. 1980. Benzene in the workplace. Am Ind Hyg Assoc J 41:616-623.

*Brondeau MT, Ducos P, Gaudin R, et al. 1992. Evaluation of the interaction of benzene and toluene on the urinary excretion of t,t-muconic acid in rats. Toxicol Lett 61(2-3):311-316.

*Bronstein AC, Currance PL. 1988. Emergency care for hazardous materials exposure. Guideline 69. The C.V. Mosby Company. Washington, DC. 221-222.

*Brown RH. 1988a. Determination of benzene, toluene and xylene in industrial air by charcoal tube, solvent desorption and gas chromatography. In: Fishbein L, O'Neill JK, eds. Environmental carcinogens method of analysis and exposure measurement: Vol. 10--benzene and alkylated benzene. New York, NY: Oxford University Press, 225-233.

*Brown RH. 1988b. Determination of benzene, toluene, and xylene in industrial air by porous polymer adsorption tube, thermal desorption and gas chromatography. In: Fishbein L, O'Neill JK, eds. Environmental carcinogens method of analysis and exposure measurement: Vol. 10--benzene and alkylated benzene. New York, NY: Oxford University Press, 235-242.

*Brown-Woodman PD, Webster WS, Huq F, et al. 1992. Assessment of interactive effects of the aromatic hydrocarbons toluene, xylene, and benzene on rat embryonic development *in vitro*. Teratology 45(3):326.

*Brown-Woodman PD, Webster WS, Picker K, et al. 1994. *In vitro* assessment of individual and interactive effects of aromatic hydrocarbons on embryonic development of the rat. Reprod Toxicol 8(2):121-135.

*Brown-Woodman PD, Webster WS, Ritchie H, et al. 1991. Effect of the aromatic hydrocarbons toluene, xylene and benzene on rat embryonic development *in vitro*. Teratology 44(4):479.

*Brugnone F, Perbellini L, Faccini GB, et al. 1989. Benzene in the blood and breath of normal people and occupationally exposed workers. Am J Ind Med 16:385-399.

*Brugnone F, Perbellini L, Maranelli G, et al. 1992. Reference values for blood benzene in the occupationally unexposed general population. Int Arch Occup Environ Health 64(3):179-184.

*Brunnemann KD, Kagan MR, Cox JE, et al. 1989. Determination of benzene, toluene and 1,3-butadiene in cigarette smoke by GC-MSD. Exp Pathol 37:108-l 13.

*Brunnemann KD, Kagan MR, Cox JE, et al. 1990. Analysis of 1,3-butadiene and other selected gas-phase components in cigarette mainstream and sidestream smoke by gas chromatography-mass selective detection. Carcinogenesis 11: 1863-1868.

*Bryce-Smith D, Gilbert A. 1976. The organic photochemistry of benzene. I. Tetrahedron 32:1309-1326.

*Buchet JP. 1988. Determination of phenol and its glucurono- and sulfoconjugates in urine by gas chromatography. In: Fishbein L, O'Neill JK, eds. Environmental carcinogens method of analysis and exposure measurement: Vol. 10--benzene and alkylated benzene. IARC Scientific Publications No. 85. Lyon, France: World Health Organization, International Agency for Research on Cancer, 281-286.

*Buckley TJ, Lindstrom AB, Highsmith VR, et al. 1992. The time-course and sensitivity of muconic acid as a biomarker for human environmental exposure to benzene. In: Proceedings of the 1992 US Environmental Protection Agency / Air and Waste Management Association symposium on measurement of toxic and related air pollutants. 981-986.

*Budnick LD, Sokal DC, Falk H, et al. 1984. Cancer and birth defects near the Drake superfund site, Pennsylvania. Arch Environ Health 39:409-413.

*Bull RJ, Robinson M, Laurie RD. 1986. Association of carcinoma yield with early papilloma development in SENCAR mice. Environ Health Perspect 68:11-17.

*Burback BL, Perry JJ. 1993. Biodegradation and biotransformation of groundwater pollutant mixtures by mycobacterium vaccae. Appl Environ Microbial 59(4):1025-1029.

Burford MD, Hawthorne SB, Miller DJ. 1994. Analysis of volatile organics by supercritical fluid extraction coupled to gas chromatography II. Quantitation of petroleum hydrocarbons from environmental sample. J Chromatogr A 685:95-l11.

Burg JA, Gist GL. 1995. The National Exposure Registry: Procedures for establishing a registry of persons environmentally exposed to hazardous substances. Toxicol Ind Health 11(2):231-247.

*Burgess JL, Crutchfield CD. 1995. Tucson fire fighter exposure to products of combustion: a risk assessment. Applied Occupational And Environmental Hygiene, Vol. 10, No. 1, January 1995.

*Burmaster DE. 1982. The new pollution-groundwater contamination. Environ 24:6-13, 33-36.

*Byrd GD, Fowler KW, Hicks RD, et al. 1990. Isotope dilution gas chromatography-m&s spectrometry in the determination of benzene, toluene, styrene and acrylonitrile in mainstream cigarette smoke. J Chromatogr 503:359-368.

*C&EN. 1994. Top 50 chemicals production rose modestly last year. Chemical and Engineering News. 72(15):12-13.

*C&EN. 1995. Production soared in most chemical sectors. Chemical and Engineering News. 73(26):38-40.

*C&EN. 1987. Key chemicals: Benzene. Chemical and Engineering News. February 9, 1987, 65:ll.

*CA. 1993a. California hazardous waste management regulations. 66260, Appendix VIII. Hazardous Constituents.

*CA. 1993b. California hazardous waste management regulations. 66260.24. Characteristics of Toxicity. Table I. Maximum concentration of contaminants for the toxicity characteristic. Regulatory Level.

*Cao XL, Hewitt CN. 1991. Evaluation of Tenax-GR adsorbent for the passive sampling of volatile organic compounds at low concentrations. Atmos Environ Part A 27A 1865-1872.

*Carbonnelle P, Lison D, Leroy JY, et al. 1995. Effect of the benzene metabolite, hydroquinone, on interleukin-1 secretion by human monocytes *in vitro*. Toxicol Appl Pharmacol Jun 1995 132(2):220-226.

*Carpenter CP, Shaffer CB, Weil CS, et al. 1944. Studies on the inhalation of 1:3-butadiene; with a comparison of its narcotic effect with benzol, toluol, and styrene, and a note on the elimination of styrene by the human. J Ind Hyg Toxicol 26:69-78.

*Cassidy MK, Houston JB. 1984. *In vivo* capacity of hepatic and extra hepatic enzymes to conjugate phenol. Drug Metab Dispos 12: 619-624.

*CDC. 1994. Continued use of drinking water wells contaminated with hazardous chemical substances Virgin Islands and Minnesota 1981-1993. Morbid Mortal Weekly Rep 43(5):89-92.

*CEH. 1983. Centers for Environmental Health. Benzene contamination in well water, Perdido, Alabama. Public Health Service-CDC-Atlanta EPI-82-90-2.

*CELDS. 1994. Computer-aided environmental legislative data systems. University of Illinois, Urbana, IL. June 28, 1994.

*CG. 1990. Facilities transferring oil or hazardous material in bulk. U.S. Coast Guard. Code of Federal Regulations. 33 CFR 154.

*Ghan C-C, Lin S-H, Her G-R. 1993. Student's exposure to volatile organic compounds while commuting by motorcycle and bus in Taipei City. J Air Waste Manage Assoc 43(9):1231-1238.

*Ghan C-C, Lin S-H, Her G-R. 1994. Office worker's exposure to volatile organic compounds while commuting and working in Taipei city. Atmospheric Environment 28(14):2351-2359.

*Chancy AM, Carlson GP. 1995. Comparison of pulmonary and hepatic microsomal metabolism of benzene in rats. In: Abstracts of the 34th annual meeting. The Toxicologist 15(1):263-264.

*Chang M-K, Voice TC, Criddle CS. 1993. Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene, and p-xylene by two Pseudomonas isolates. Biotechnol Bioeng 41(11):1057-1065.

*Chang RL, Wong CQ, Kline SA, et al. 1994. Mutagenicity of trans, trans-muconaldehyde and its metabolites in V79 cells. Environmental and Molecular Mutagenesis 24: 112-115.

*Chao TC, Lo DST, Koh J, et al. 1993. Glue sniffing deaths in Singapore-volatile aromatic hydrocarbons in post-mortem blood by headspace gas chromatography. Med Sci Law 33(3):253-260.

*Chapman DE, Namkung MJ, Juchau MR. 1994. Benzene and benzene metabolites as embryotoxic agents: effects on cultured rat embryos. Toxicol Appl Pharmacol 128 (1):129-137.

*Chen H, Rupa DS, Tomar RR, et al. 1994. Chromosomal loss and breakage in mouse bone marrow and spleen cells exposed to benzene *in vivo*. Cancer Research 54:3533-3539.

*Chen H, Eastmond DA. 1995. Topoisomerase inhibition by phenolic metabolites: A potential mechanism for benzene's clastogenic effects. Carcinogenesis 16(10):2301-2307.

*Chenna A, Hang B, Rydberg B, et al. 1995. The benzene metabolite p-benzoquinone forms adducts with dna bases that are excised by a repair activity from human cells that differs from an ethenoadenine glycosylase. Proc Nat1 Acad Sci U S A 92(13):5890-5894.

*Chepiga TA, Yang CS, Snyder R. 1990. Benzene metabolism by two purified, reconstituted rat hepatic mixed function oxidase systems. Adv Exp Med Biol 283:261-265.

*Chertkov JL, Lutton JD, Jiang S, et al. 1992. Hematopoietic effects of benzene inhalation assessed by murine long-term bone marrow culture. J Lab Clin Med 119:412-419.

*Chiang CY, Salanitro JP, Chai EY, et al. 1989. Aerobic biodegradation of benzene, toluene, and xylene in a sandy aquifer-data analysis and computer modeling. Ground Water 27:823-834.

*Choy WN, MacGregor JT, Shelby MD, et al. 1985. Induction of micronuclei by benzene in $B6C3F_1$ mice: Retrospective analysis of peripheral blood smears from the NTP carcinogenesis bioassay. Mutat Res 143:55-59.

*Ciccone G, Mirabelli D, Levis A, et al. 1993. Myeloid leukemias and myelodysplastic syndromes: chemical exposure, histologic subtype and cytogenetics in a case-control study. Cancer Genet Cytogenet 68(2):135-139.

*Ciranni R, Barale R, Adler ID. 1991. Dose-related clastogenic effects induced by benzene in bone marrow cells and in differentiating spermatogonia of Swiss CD1 mice. Mutagenesis 6(5):417-421.

*Ciranni R, Barale R, Marrazzini A, et al. 1988. Benzene and the genotoxicity of its metabolites I. Transplacental activity in mouse fetuses and in their dams. Mutat Res 208:61-67.

*Clark AI, McIntyre AE, Lester JN, et al. 1984. A comparison of photoionisation detection gas chromatography with a Tenax GC sampling tube procedure for the measurement of aromatic hydrocarbons in ambient air. J Environ Anal Chem 17:315-326.

*Clark SC, Kamen R. 1987. The human hematopoietic colony-stimulating factors. Science 236:1229-1237.

*Clewell HJ III, Andersen ME. 1985. Risk assessment extrapolations and physiological modeling. Toxicol Ind Health l (4):lll-131.

*Cline PV, Viste DR. 1985. Migration and degradation patterns of volatile organic compounds. Waste Management and Research 3:35l-360.

*Coate WB, Hoberman AM, Durloo RS. 1984. Inhalation teratology study of benzene in rats. Adv Mod Environ Toxicol 6:187-198.

*Cody RP, Strawderman WW, Kipen HM. 1993. Hematologic effects of benzene. Job-specific trends during the first year of employment among a cohort of benzene-exposed rubber workers. J Occup Med 35(8):776-782.

*Cohen MA, Ryan PB, Yanagisawa Y, et al. 1989. Indoor/outdoor measurements of volatile organic compounds in Kanawha Valley of West Virginia. J Air Pollut Control Assoc 39:1086-1093.

*Colenutt BA, Davies DN. 1980. The sampling and gas chromatographic analysis of organic vapours in landfill sites. Int J Environ Anal Chem 7:223-229.

*Colenutt BA, Thorbum S. 1980. Gas chromatographic analysis of trace hydrocarbon pollutants in water samples. Int J Environ Stud 15:25-32.

*Colinas R J, Burkart P T, Lawrence D A. 1995. The effects of interleukin-l-beta and tumor necrosis factor-alpha on *in vitro* colony formation by human hematopoietic progenitor cells exposed to doxorubicin or hydroquinone. Experimental Hematology (Charlottesville) 23(12): 1247-1255.

*Collins JJ, Conner P, Friedlander BR, et al. 1991. A study of the hematologic effects of chronic low-level exposure to benzene. J Occup Med 33(5):619-626.

*Cornish HH, Ryan RC. 1965. Metabolism of benzene in nonfasted, fasted, and aryl-hydroxylase inhibited rats. Toxicol Appl Pharmacol 7:767-771.

*Cox LA Jr. 1991. Biological basis of chemical carcinogenesis: insights from benzene. Risk Anal 11(3):453-464.

*Cox LA Jr. 1996. Reassessing benzene risks using internal doses and Monte-Carlo uncertainty analysis. Environ Health Perspect 104 (Suppl 6): 1413-1429.

*Cox LA Jr., Ricci PF. 1992. Reassessing benzene cancer risks using internal doses. Risk Anal 12(3):401-410.

*Crawford DW, Bonnevie NL, Wenning RJ. 1995. Sources of pollution and sediment contamination in Newark Bay, New Jersey. Ecotoxicol Environ Safety 30:85-100.

*Crebelli R, Bellincampi D, Conti G, et al. 1986. A comparative study on selected chemical carcinogens for chromosome malsegregation, mitotic crossing-over and forward mutation induction in *Aspergillus nidulans.* Mutat Res 172:139-149.

*CRISP. 1996. Computer Retrieval of Information on Scientific Projects. National Library of Medicine, Toxicology Information Program, Bethesda, MD. March 1996.

*Cronin HJ. 1924. Benz01 poisoning in the rubber industry. Boston Medical and Science Journal 191:1164-1166.

*Cronkite EP. 1986. Benzene hematotoxicity and leukemogenesis. Blood Cells 12:129-137.

*Cronkite EP, Bullis JE, Inoue T, et al. 1984. Benzene inhalation produces leukemia in mice. Toxicol Appl Pharmacol 75:358-361.

*Cronkite EP, Drew RT, Inoue T, et al. 1985. Benzene hematotoxicity and leukemogenesis. Am J Ind Med 7:447-456.

*Cronkite EP, Drew RT, Inoue T, et al. 1989. Hematotoxicity and carcinogenicity of inhaled benzene. Environ Health Perspect 82:97-108.

*Cronkite EP, Inoue T, Carsten AL, et al. 1982. Effects of benzene inhalation on murine pluripotent stem cells. J Toxicol Environ Health 9:411-421.

*Crump KS. 1994. Risk of benzene-induced leukemia: a sensitivity analysis of the pliofilm cohort with additional follow-up and new exposure estimates. J Toxicol Environ Health 42(2):219-242.

*Crump KS. 1996. Risk of benzene-induced leukemia predicted from the pliofilm cohort. Environmental Health Perspect 104 (Suppl 6):1437-1441.

*Crump KS, Allen BC, Clewell H. 1993. Limitations to benzene cancer risk assessment by Cox and Ricci [letter]. Risk Anal 13(2):145-146.

*Daiker DH, Moslen MT, Ward JB. 1995. Effects of chronic, low dose benzene on liver enzymes involved in benzene metabolism. Fund Appl Toxicol Suppl. The Toxicologist 15(1):261.

Daisey JM, Hodgson AT, Fisk WJ, et al. 1994. Volatile organic compounds in twelve California office buildings: classes, concentrations and sources. Atmospheric Environ 28(22):3557-3562.

Davidson KA, Faust RA. 1996. Oral carcinogen potency factors for benzene and some of its metabolites. Fund Appl Toxicol Suppl. The Toxicologist 30(1):114.

*Davies EM, Murray HE, Liehr JG, et al. 1981. Technical note: Basic microbial degradation rates and chemical byproducts of selected organic compounds. Water Res 15: 1125-l 127.

Davis JW, Klier NK, Carpenter CL. 1994. Natural biological attenuation of benzene in ground water beneath a manufacturing facility. Ground Water 32(2):215-226.

*Dayal H, Gupta S, Trieff N, et al. 1995. Symptom clusters in a community with chronic exposure to chemicals in two superfund sites. Archives Of Environmental Health 50 (2):108-l 11.

*Dees C, Travis C. 1994. Hyperphosphorylation of ~53 induced by benzene, toluene, and chloroform. Cancer Lett 84(2): 117- 123.

*De Flora S, Zanacchi P, Camoirano A, et al. 1984. Genotoxic activity and potency for 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat Res 133:161-198.

de Gandarias JM, Casis 0, Irazusta J, et al. 1992. Effect of subacute benzene exposure on the activity of two neuropeptide-degrading enzymes in the rat brain. Arch Environ Contam Toxicol 22(3):345-348.

*DeLeon IR, Antoine SR. 1985. Clinical screening test for toxic volatile organic chemicals in blood. Clinical Ecology 3:108-109.

*Delfino JJ, Miles CJ. 1985. Aerobic and anaerobic degradation of organic contaminants in Florida groundwater. Proceedings Soil and Crop Science Society of Florida 44:9-14.

*Demirgian J. 1992. Continuous emission monitor for incinerators. Govt Reports Announcements & Index (GRA&I), Issue 23, 1992.

*Dempster AM, Evans HL, Snyder CA. 1984. The temporal relationship between behavioral and hematological effects of inhaled benzene. Toxicol Appl Pharmacol 76: 195-203.

*Dempster AM, Snyder CA. 1990. Short term benzene exposure provides a growth advantage for granulopoietic progenitor cells over erythroid progenitor cells. Arch Toxicol 64(7):539-544.

*Dempster AM, Snyder CA. 1991. Kinetics of granulocytic and erythroid progenitor cells are affected differently by short-term, low-level benzene exposure. Arch Toxicol 65(7):556-561.

*Diaz M, Reiser A, Braier L, et al. 1980. Studies on benzene mutagenesis: I. The micronucleus test. Experientia 36:297-299.

*Dibben MJ, Thomas TC, Anderson MP. 1989. Is benzene a problem in fuel operations? A new analytical method to overcome potential interferences in the present method. LC-GC International 2:54-58.

*Ding X-J, Li Y, Ding Y, et al. 1983. Chromosome changes in patients with chronic benzene poisoning. Chinese Med J (Peking Engl Ed) 96:681-685.

*Dar F, Le Moullec Y, Festy B. 1995. Exposure of city residents to carbon monoxide and monocyclic aromatic hydrocarbons during commuting trips in the paris metropolitan area. Journal Of The Air And Waste Management Association 45(2):103-l10.

*Dosemeci M, Li GL, Hayes RB, et al. 1994. Cohort study among workers exposed to benzene in china: ii. exposure assessment. Am J Ind Med 26(3):401-411.

*Doskin VA. 1971. [Effect of age on the reaction to a combination of hydrocarbons.] Hyg Sanit 36:379-384. (Russian)

*DOT. 1990. Hazardous materials table. U. S. Department of Transportation. Code of Federal Regulations. 49 CFR 172.101.

*Douglas GR, Blakey DH, Liu-Lee VW, et al. 1985. Alkaline sucrose sedimentation, sister-chromatid exchange and micronucleus assays in CHO cells. Progress in Mutation Research 5:359-366.

*Dow. 1980. Initial submission: Risks of long-term health effects of benzene absorbed via skin in laboratory animals with cover letter dated 01/20/92.EPA/OTS Dot #88-920000700.

*Dow. 1992. Initial submission: Effects of benzene vapor in the pig and rat pertaining to hematology and immunology with cover letter dated 05/14/92. EPA/OTS Dot #88-920003196.

*Dowty BJ, Carlisle DR, Laseter JL. 1975. New Orleans drinking water sources tested by gas chromatography-mass spectrometry. Environ Sci Technol 9:762-765.

*Dowty BJ, Laseter JL, Storer J. 1976. The transplacental migration and accumulation in blood of volatile organic constituents. Pediatr Res 10:696-701.

*Dragan YP, Sargent L, Xu YD, et al. 1993. The initiation-promotion-progression model of rat hepatocarcinogenesis. Proc Sot Exp Biol Med 202(1):16-24.

*Drew RT, Fouts JR. 1974. The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. Toxicol Appl Pharmacol 27: 183- 193

*Drummond L, Luck R, Afacan AS, et al. 1988. Biological monitoring of workers exposed to benzene in the coke oven industry. Br J Ind Med 45:256-261.

*Ducos P, Gaudin R, Be1 J, et al. 1992. *trans,trans*-Muconic acid, a reliable biological indicator for the detection of individual benzene exposure down to the ppm level. Int Arch Occup Environ Health 64(5):309-313.

*Ducos P, Gaudin R, Robert A, et al. 1990. Improvement in HPLC analysis of urinary trans, truns-muconic acid, a promising substitute for phenol in the assessment of benzene exposure. Int Arch Occup Environ Health 62:(7):529-534.

*Eastmond DA. 1993. Induction of micronuclei and aneuploidy by the quinone-forming agents benzene and *o*-phenylphenol. Toxicol Lett 67(1-3):105-l18.

*Eastmond DA, Rupa DS, Hasegawa LS. 1994. Detection of hyperdiploidy and chromosome breakage in interphase human lymphocytes following exposure to the benzene metabolite hydroquinone using multicolor fluorescence *in situ* hybridization with dna probes. Mutat Res 322(1):9-20.

*Eastmond DA, Smith MT, Irons RD. 1987. An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene exposure. Toxicol Appl Pharmacol 91:85-95.

*Eastmond DA, Smith MT, Ruzo LO, et al. 1986. Metabolic activation of phenol by human myeloperoxidase and horseradish peroxidase. Molecular Pharmacol 30(6):674-679.

*Edgerton SA, Shah JJ. 1992. Assessing total exposures to gasoline vapor using the source exposure model. J Exposure Anal Environ Epidemiol 2(1):109-115.

*Edwards EA, GrbiC-GaliE D. 1992. Complete mineralization of benzene by aquifer microorganisms under strictly anaerobic conditions. Appl Environ Microbial 58(8):2663-2666.

*Eisenreich SJ, Looney BB, Thornton JD. 1981. Airborne organic contaminants in the Great Lakes ecosystem. Environ Sci Technol 15:30-38.

*Ellenhorn MJ, Barceloux DG. 1988. Medical toxicology: diagnosis and treatment of human poisoning. New York, NY: Elsevier. 947-950.

EPA. 1972. Approval and promulgation of implementation plans. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 FR Part 52.

*EPA. 1977a. List of pollutants and applicability of part 61. National emission standards for hazardous air pollutants. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 61.01.

*EPA. 1977b. Limits established for specific wastes or waste constituents. Criteria for the evaluation of permit applications for ocean dumping of materials. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 227.7.

*EPA. 1978a. Designation of hazardous substances. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 116.4.

*EPA. 1978b. Assessment of human exposure to atmospheric benzene. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Air and Waste Management, Office of Air Quality Planning and Standards. EPA 450/378-031.

*EPA. 1979a. Environmental monitoring benzene. Washington, DC: U.S. Environmental Protection Agency, Office of Toxic Substances. EPA 560/679/006.

*EPA. 1979b. Toxic pollutants. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 401.15.

*EPA. 1980a. Ambient air monitoring for benzene: 24-hr integrated sampling in six cities. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory. EPA 600/4-80-027.

EPA. 1980b. Ambient water quality criteria. U. S. Environmental Protection Agency. Federal Register. 45 FR 79318.

*EPA. 1980c. Discarded commercial chemical products, off-specification species, container residues, and spill residues thereof. Identification and listing of hazardous waste. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 261.33(f).

*EPA. 1981a. General definitions. Electroplating point source category. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 413.02.

*EPA. 1981b. Hazardous wastes from non-specific sources. Identification and listing of hazardous waste. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 261.31.

*EPA. 1981c. Hazardous wastes from specific sources. Identification and listing of hazardous waste. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 261.32.

*EPA. 1981d. Basis for listing hazardous waste. Identification and listing of hazardous waste. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 261, Appendix VII.

*EPA. 1982a. General definitions. Iron and steel manufacturing point source category. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 420.02.

*EPA. 1982b. Effluent limitations representing the degree of effluent reduction attainable by the application of the best available technology economically available. By-product cokemaking - iron and steel. Iron and steel manufacturing point source category. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 420.13(b).

EPA. 1982c. New source performance standards. By-product cokemaking - iron and steel. Iron and steel manufacturing point source category. U. S. Environmental Protection Agency. Code of Federal Regulations 40 CFR 420.14(a).

*EPA. 1982d. 126 Priority pollutants. Steam electric power generating point source category. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 423, Appendix A.

*EPA. 1982e. An exposure and risk assessment for benzene. Washington, DC: U.S. Environmental Protection Agency. EPA-440/4-85006, PB 85-212017, 3-l - 3-32.

*EPA. 1982f. U.S. EPA management of hazardous waste leachate EPA contract No. 68-03-2766. E-42, E-96, E-l11, E-142, E-143. (as cited in HSDB 1996).

*EPA. 1983a. List of chemicals produced by affected facilities. Standards of performance for new stationary sources. U.S. Environmental Protection Agency. Subpart VV - Standards of Performance for Equipment Leaks of VOC in the Synthetic Organic Chemical Manufacturing Industry (SOCMI) Industry. Code of Federal Regulations. 40 CFR 60.489.

*EPA. 1983b. Specialized definitions. Metal finishing point source category. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 433.11.

*EPA. 1983c. Specialized definitions. Copper forming point source category. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 468.02.

*EPA. 1984a. Applicability and designation of sources. National emission standards for hazardous air pollutants. U.S. Environmental Protection Agency. Subpart J - National Emission Standard for Equipment Leaks (Fugitive Emission Sources) of Benzene. Code of Federal Regulations. 40 CFR 61.110-112.

*EPA. 1984b. Definitions. National emission standards for hazardous air pollutants. U.S. Environmental Protection Agency. Subpart V - National Emission Standard for Equipment Leaks (Fugitive Emission Sources). Code of Federal Regulations. 40 CFR 61.240-61.242.

*EPA. 1984c. Forrn 2C - Application for Permit to Discharge Wastewater. Criteria and Standards for the National Pollutant Discharge Elimination System. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 125.

*EPA. 1984d. Method 1625 Revision B - Semivolatile organic compounds by isotope dilution GC/M5. Methods for organic chemical analysis of municipal and industrial wastewater. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 136, Appendix A.

*EPA. 1984e. Wastes Excluded Under 260.20 and 260.22. Identification and Listing of Hazardous Waste. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 261, Appendix IX.

*EPA. 1984f. Guidelines for establishing test procedures for the analysis of pollutants under the Clean Water Act. U.S. Environmental Protection Agency. Federal Register 49:43234, 43255-43256, 432272-43280, 43373-43384, 43407-43415.

*EPA. 1985a. Lists of pollutants and applicability of part 61. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 61.01.

*EPA. 1985b. Determination of reportable quantities. Determination of reportable quantities for hazardous substances. U.S. Environmental Protection Agency. Subpart A - General Provisions. Code of Federal Regulations. 40 CFR 117.3.

*EPA. 1985c. Specialized definitions. Metal molding and casting point source category. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 464.11.

*EPA. 1985d. Boiling point/boiling range. Chemical fate testing guidelines. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 796.1220.

*EPA. 1985e. Partition coefficient (n-Octano/water) - Estimation by Liquid Chromatography. Chemical Fate Testing Guidelines. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 796.1570.

*EPA. 1985f. Maximum contaminant level goals for organic contaminants. National primary drinking water regulations. U.S. Environmental Protection Agency. Subpart F - Maximum Contaminant Level Goals. Code of Federal Regulations. 40 CFR 141.50.

*EPA. 1985g. Drinking water criteria document on benzene. Final draft. Washington;DC: U.S. Environmental Protection Agency, Office of Drinking Water. PB86-118122.

*EPA. 1985h. Exemptions from tolerances. U.S. Environmental Protection Agency Code of Federal Regulations. 40 CFR 180.1001.

*EPA. 1986a. 65 Toxic Pollutants. General Pretreatment Regulations for Existing and New Sources of Pollution. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 403, Appendix B.

*EPA. 1986b. Identification of Wastes to be Evaluated by August 8, 1988. Land Disposal Restrictions. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 268.10.

*EPA. 1987a. Toxic Pollutant Effluent Limitations and Standards for Direct Discharge Point Sources That Use End-Of-Pipe Biological Treatment. Organic Chemicals, Plastics, and Synthetic Fibers. Subpart I - Direct Discharge Point Sources That Use End-of-Pipe Biological Treatment. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 414.91.

*EPA. 1987b. Non-Complexed Metal-Bearing Waste Streams and Cyanide-Bearing Waste Streams (Zinc). Organic Chemicals, Plastics, and Synthetic Fibers. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 414, Appendix A.

*EPA. 1987c. Ground-Water Monitoring List. Standards for Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 264, Appendix IX.

*EPA. 1987d. Public Notification. National Primary Drinking Water Regulations. U.S. Environmental Protection Agency. Subpart D - Reporting, Public Notification, and Recordkeeping. Code of Federal Regulations. 40 CFR 141.32.

*EPA. 1987e. June-September, 6-9 AM, ambient air benzene concentrations in 39 U.S. cities, 1984-l 986. Research Triangle Park, NC: U.S. Environmental Protection Agency, Atmospheric Sciences Research Lab. EPA/600/D-87/160.

*EPA. 1988a. Waste Specific Prohibitions - Solvent Wastes. Hazardous Waste Injection Restrictions. U.S. Environmental Protection Agency. Subpart B - Prohibitions on Injection. Code of Federal Regulations. 40 CFR 148.10.

*EPA. 1988b. Hazardous Constituents. Identification and Listing of Hazardous Waste. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 261, Appendix VIII.

*EPA. 1988c. Chemicals and Chemical Categories To Which This Part Applies. Toxic Chemical Release Reporting: Community Right-To-Know. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 372.65.

*EPA. 1988d. State Water Quality Standards Summaries. U.S. Environmental Protection Agency. Office of Water Regulations and Standards.

*EPA. 1988e. Recommendations for and documentation of biological values for use in risk assessment. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Environmental Protection Agency, Cincinnati, OH. NTIS No. PB88-179874.

EPA. 1988f. Compendium of methods for the determination of toxic organic compounds in ambient air. EPA 600/4-89/017.

*EPA. 19888. Thresholds for Reporting. Toxic Chemical Release Reporting: Community Right-To-Know. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 372.25.

*EPA. 1989a. Definitions. National Emission Standards for Hazardous Air Pollutants. U.S. Environmental Protection Agency. Subpart L - National Emission Standard for Benzene Emissions from Coke By-Product Recovery Plants. Code of Federal Regulations. 40 CFR 61.131-40 CFR 61.137.

*EPA. 1989b. Applicability and Designation of Sources. National Emission Standards for Hazardous Air Pollutants. U.S. Environmental Protection Agency. Subpart Y - National Emission Standard for Benzene Emissions from Benzene Storage Vessels. Code of Federal Regulations. 40 CFR 61.270-61.273.

*EPA. 1989c. Designation of Hazardous Substances. Designation, Reportable Quantities, and Notification. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 302.4.

EPA 1989d. National Emission Standards for Hazardous Air Pollutants; Benzene Emissions from Maleic Anhydride Plants, Ethylbenzene/Styrene Plants, Benzene Storage Vessels, Benzene Equipment Leaks, and Coke By-Products Recovery Plants. U.S. Environmental Protection Agency. 54 FR 38044.

*EPA. 1990a. Chemicals Affected by Subpart NNN. Standards of Performance for New Stationary Sources. U.S. Environmental Protection Agency. Subpart NNN - Standards of Performance for Volatile Organic Compound Emissions from Synthetic Organic Chemical Manufacturing Industry (SOCMI) Distillation Operations. Code of Federal Regulations. 40 CFR 60.667.

*EPA. 1990b. Applicability. National Emission Standards for Hazardous Air Pollutants. U.S. Environmental Protection Agency. Subpart BB - National Emission Standard for Benzene Emissions from Benzene Transfer Operations. Code of Federal Regulations. 40 CFR 61.300-61.304.

*EPA. 1990c. Applicability. National emission standards for hazardous air pollutants. U.S. Environmental Protection Agency. Subpart FF - National Emission Standard for Benzene Waste Operations. Code of Federal Regulations. 40 CFR 61.340-353

*EPA. 1990d. Toxicity Characteristic. Identification and Listing of Hazardous Waste. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 261.24.

*EPA. 1990e. Applicability. Standards for Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities. U.S. Environmental Protection Agency. Subpart AA - Air Emission Standards for Process Vents. Code of Federal Regulations. 40 CFR 264.1030.

*EPA. 1990f. Applicability. Standards for Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities. U.S. Environmental Protection Agency. Subpart BB - Air Emission Standards for Equipment Leaks. Code of Federal Regulations. 40 CFR 264.1050.

*EPA. 1990g. Waste Specific Prohibitions - Third Wastes. Land Disposal Restrictions. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 268.35.

*EPA. 1990h. Interim methods for development of inhalation reference concentrations. Office of Research and Development. U. S. Environmental Protection Agency. EPA 600 8-90/066A.

*EPA. 1991a. Maximum Contaminant Levels for Organic Contaminants. National Primary Drinking Water Regulations. U.S. Environmental Protection Agency. Subpart G - National Revised Primary Drinking Water Regulations: Maximum Contaminant Levels. Code of Federal Regulations. 40 CFR 141.61.

*EPA. 1991b. BAT for Organic Contaminants Listed in 141.61. National Primary Drinking Water Regulations. U.S. Environmental Protection Agency. Subpart G - National Revised Primary Drinking Water Regulations: Maximum Contaminant Levels. Code of Federal Regulations. 40 CFR 141.61.

*EPA. 1991c. Variances and Exemptions from the Maximum Contaminant Levels for Synthetic Organic Chemicals and Exemptions from the Treatment Technique for Lead and Copper. National Primary Drinking Water Regulations Implementation. U.S. Environmental Protection Agency. Subpart G - Identification of Best Technology, Treatment Techniques or Other Means Generally Available. Code of Federal Regulations. 40 CFR 142.62.

*EPA. 1991d. Maximum Contaminant Levels. Criteria for Classification of Solid Waste Disposal Facilities and Practices. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 257, Appendix I.

*EPA. 1991e. Design Criteria. Criteria for Municipal Solid Waste Landfills. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 258.40.

*EPA. 1991f. Constituents for Detection Monitoring. Criteria for Municipal Solid Waste Landfills. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 258, Appendix I.

*EPA. 1991g. List of Hazardous Inorganic and Organic Constituents. Criteria for Municipal Solid Waste Landfills. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 258, Appendix II.

*EPA. 1991h. Health-Based Limits for Exclusion of Waste-Derived Residues. Standards for the Management of Specific Hazardous Wastes and Specific Types of Hazardous Waste Management Facilities. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 266, Appendix VII.

*EPA. 1991i. Potential PICs for Determination of Exclusion of Waste-Derived Residues. Standards for the Management of Specific Hazardous Wastes and Specific Types of Hazardous Waste Management Facilities. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 266, Appendix VIII.

*EPA. 1991j. Effective Dates of Surface Disposed Wastes (Non-Soil and Debris) Regulated in the LDRs - Comprehensive List. Land Disposal Restrictions. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 268, Appendix VII.

EPA. 1991k. National emission standard for benzene emissions from coke by products recovery plants. Provisions for alternative means for process vessels, storage tanks, and tar-intercepting sumps. U. S. Environmental Protection Agency. CFR 61.139.

*EPA. 1992a. Demonstration of Early Reduction, Table 1. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart D - Regulations Governing Compliance Extensions for Early Reductions of Hazardous Air Pollutants. Code of Federal Regulations. 40 CFR 63.74.

EPA. 1992b. Methods for the determination of organic compounds in drinking water, Supplement II. Environmental Monitoring Systems Laboratory. 600/R-92/129.

*EPA. 1993a. Chemicals affected by Subpart RRR. Standards of Performance for New Stationary Sources. U.S. Environmental Protection Agency. Subpart RRR - Standards of Performance for Volatile Organic Compound (VOC) Emissions from the Synthetic Organic Chemical Manufacturing Industry Reactor Processes. Code of Federal Regulations. 40 CFR 60.707.

*EPA. 1993b. Alternative standards for Coke Oven doors equipped with sheds. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart L - National Emission Standards for Coke Oven Batteries. Code of Federal Regulations. 40 CFR 63.305.

*EPA. 1993c. Regulation of fuel and fuel additives. U.S. Environmental Protection Agency. Renewable Oxygenate Requirements for Reformulated Gasoline: Proposed Rule. 58 FR 68343.

*EPA. 1993d. Pretreatment standards for existing sources. Organic chemicals, plastics, and synthetic fibers. Subpart C - Other Fibers. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 414.35

*EPA. 1993e. Pretreatment standards for existing sources. Organic Chemicals, Plastics, and Synthetic Fibers. Subpart D - Thermoplastic Resins. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 414.45.

*EPA. 1993f. Pretreatment standards for existing sources. Organic chemicals, plastics, and synthetic fibers. Subpart F - Commodity Organic Chemicals. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 414.65.

*EPA. 1993g. Toxic pollutant effluent limitations and standards for direct discharge point sources that do not use end-of-pipe treatment. Organic chemicals, plastics, and synthetic fibers. Subpart J - direct discharge point sources that do not use end-of-pipe biological treatment. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 414.101.

*EPA. 1993h. Toxic Pollutant Standards for Indirect Discharge Point Sources. Organic Chemicals, Plastics, and Synthetic Fibers. Subpart K - Indirect Discharge Point Sources. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 414.111.

*EPA. 1993i. Water Quality Guidance for the Great Lakes System: Proposed Rule. U.S. Environmental Protection Agency. 58 FR 20802.

*EPA. 1993j. Superfund record of decision: Rhinehart Tire fire dump. NTIS PB 93-9639211GAR

*EPA. 1993k. Standards for Reformulated Gasoline: U.S. Environmental Protection Agency. 58 FR 11722.

*EPA. 1993l. Identification and Listing of Hazardous Waste, Exemption of Petroleum Contaminated Media and Debris. U.S. Environmental Protection Agency. 58 FR 8504.

*EPA. 1994a. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart A - General Provisions. Code of Federal Regulations. 40 CFR 63.1. Applicability.

*EPA. 1994b. Applicability. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart B - Requirements for Control Technology Determinations for Major Sources in Accordance with Clean Air Act Sections 112(g) and 112(i). Code of Federal Regulations. 40 CFR 63.51.

*EPA. 1994c. Applicability and Designation of Source, Table 1. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart F - National Emission Standards for Organic Hazardous Air Pollutants from the Synthetic Organic Chemical Manufacturing Industry. Code of Federal Regulations. 40 CFR 63.100

*EPA. 1994d. Applicability and Designation of Source, Table 2. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart F - National Emission Standards for Organic Hazardous Air Pollutants from the Synthetic Organic Chemical Manufacturing Industry. Code of Federal Regulations. 40 CFR 63.100

*EPA. 1994e. Applicability. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart G - National Emission Standards for Organic Hazardous Air Pollutants from the Synthetic Organic Chemical Manufacturing Industry for Process Vents, Storage Vessels, Transfer Operations, and Wastewater. Code of Federal Regulations. 40 CFR 63.110

*EPA. 1994f. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart G - National Emission Standards for Organic Hazardous Air Pollutants from the Synthetic Organic Chemical Manufacturing Industry for Process Vents, Storage Vessels, Transfer Operations, and Wastewater, Tables 8 and 9. 40 CFR 63, Appendix to Subpart G.

*EPA. 1994g. Applicability and Designation of Source. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart H - National Emission Standards for Organic Hazardous Pollutants for Equipment Leaks. Code of Federal Regulations. 40 CFR 63.160.

*EPA. 1994h. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart R - National Emission Standards for Stage I Gasoline Distribution Facilities. 59 FR 64303.

*EPA. 1994i. Standards for Reformulated Gasoline. Regulation of Fuels and Fuel Additives. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR Part 80, Subpart D.

*EPA. 1994j. Regulation of Fuels and Fuel Additives. U.S. Environmental Protection Agency. 40 CFR Part 80, Subpart D. Standards for Reformulated and Conventional Gasoline. Direct Final Rule. Federal Regulations. 59 FR 36944.

*EPA. 1994k. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. Subpart D - Regulations Governing Constructed, Reconstructed or Modified Major Sources: Proposed Rule. 59 FR 51913.

*EPA. 1994l. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. National Emission Standards for Petroleum Refineries: Proposed Rule. 59 FR 36130.

*EPA. 1994m. National Emission Standards for Hazardous Air Pollutants for Source Categories. U.S. Environmental Protection Agency. National Emission Standards for Off-Site Waste and Recovery Operations: Proposed Rule. 59 FR 66336.

*EPA. 1994n. Control of Air Pollution. U.S. Environmental Protection Agency. Emission Standards for New Nonroad Spark-ignition Engines at or Below 19 Kilowatts: Proposed Rule. 59 FR 55930.

*EPA. 1994o. Standards for Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities. U.S. Environmental Protection Agency. Subpart CC - Organic Air Emission Standards for Tanks, Surface Impoundments, and Containers. 59 FR 62896.

*EPA. 1994p. Interim Status Standards for Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities. U.S. Environmental Protection Agency. Subpart CC - Organic Air Emission Standards for Tanks, Surface Impoundments, and Containers. 59 FR 62896.

*EPA. 1994q. Applicability of Treatment Standards. Land Disposal Restrictions Phase II - Universal Treatment Standards and Treatment Standards for Organic Toxicity Characteristic Wastes and Newly Listed Wastes. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 268.40.

*EPA. 1994r. Treatment Standards Expressed as Specified Technologies. Land Disposal Restrictions Phase II - Universal Treatment Standards and Treatment Standards for Organic Toxicity Characteristic Wastes and Newly Listed Wastes. U.S. Environmental Protection Agency. 59 FR 47982. Code of Federal Regulations. 40 CFR 268.42.

*EPA. 1994s. Land Disposal Restrictions Phase II - Universal Treatment Standards and Treatment Standards for Organic Toxicity Characteristic Wastes and Newly Listed Wastes. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 268.43. Treatment Standards Expressed as Waste Concentrations. Federal Regulations. 59 FR 47982.

*EPA. 1994t. Universal Treatment Standards. Land Disposal Restrictions Phase II - Universal Treatment Standards and Treatment Standards for Organic Toxicity Characteristic Wastes and Newly Listed Wastes. U.S. Environmental Protection Agency. 59 FR 47982. Code of Federal Regulations. 40 CFR 268.48.

*EPA. 1994u. Identification and Listing of Hazardous Waste: Proposed Rule. U.S. Environmental Protection Agency. Code of Federal Regulations. 40 CFR 261, Appendix VII. Basis for Listing Hazardous Waste. Federal Regulations. 59 FR 9808.

*EPA. 1994v. Drinking Water Regulations and Health Advisories. U.S. Environmental Protection Agency. Office of Water.

*EPA. 1994w. Test Methods for Evaluating Solid Waste Method 8021a. Halogenated volatiles by gas chromatography using photoionization and electrolytic conductivity detectors in series: Capillary column technique.

*EPA. 1994x. Standards of performance for new stationary sources: volatile organic compounds (VOC) emissions for SOC in wastewater. 59 FR 46780.

EPA 1994y. Emission Standards for New-Road-Spark-Ignition Engines At or Below 19kw (Proposed) 59 FR 25399.

EPA 1994z. Water Quality Guidance for Great Lakes Systems (Proposed) 59 FR 44678

*EPA. 1995a. Effluent limitations guidelines pretreatment standards, and new source performance standards: Source performance standards centralized waste treatment category. 60 FR 5464.

*EPA. 1995b. Land disposal restrictions-phase III. Decharacterized wastewaters caramante and organobromine wastes, and spent potliners. Environmental Protection Agency. 60 FR 11702.

*EPA. 1995c. Effluent limitations guidelines pretreatment standards, and new sources performance standards pharmaceutical manufacturing category. Environmental Protection Agency. 60 FR 21592.

*EPA. 1995d. National emission standards for hazardous air pollutants from secondary lead smelting. Environmental Protection Agency. 60 FR 32587.

*EPA. 1995e. Regulation of fuels and fuel additives standards for reformulated and conventional gasoline. Environmental Protection Agency. 60 FR 40009.

*EPA. 1995f. National emission standards for hazardous air pollutants petroleum refineries. Environmental Protection Agency. 60 FR 43244.

*EPA. 1995g. Federal standards for marine tank vessel loading operations and national emission standards for hazardous air pollutants for marine tank vessel loading operations. Environmental Protection Agency. 60 FR 48388.

*EPA. 1995h. Control of air pollution from new motor vehicles and new motor vehicle engines: Voluntary standards for light density vehicles. Environmental Protection Agency. 60 FR 52769.

*EPA. 1995i. Guidelines establishing test procedures for the analysis of pollutants: New methods. Environmental Protection Agency. 60 FR 53988.

*EPA. 1995j. Standard for the use or disposal of sewage sludge. Environmental Protection Agency. 60 FR 54771.

*EPA. 1995k. Hazardous waste management system; identification and listing of hazardous waste petroleum refining process wastes; land disposal restrictions for newly identified wastes ad CERCLA hazardous substances designation and reportable quantities. Environmental Protection Agency. 60 FR 57747.

*EPA. 1995l Hazardous waste management system; identification and listing of hazardous waste, hazardous waste identification rule (HWIR). Appendix X-XI, Environmental Protection Agency. 60 FR 66344.

EPA 1995m. Drinking Water Regulations and Health Advisories. U.S. Environmental Protection Agency. Office of Water. May 1995.

*Erdogan G, Aksoy M. 1973. Cytogenetic studies in thirteen patients with pancytopenia and leukaemia associated with long-term exposure to benzene. New Istanbul Contrib Clin Sci 10:230-247.

*Erexson GL, Wilmer JL, Steinhagen WH, et al. 1986. Induction of cytogenetic damage in rodents after short-term inhalation of benzene. Environ Mutagen 8:29-40.

*Erf LA, Rhoads CP. 1939. The hematological effects of benzene (benzol) poisoning. J Ind Hyg Toxicol 20:421-435.

*Etzel RA, Ashley DL. 1994. Volatile organic compounds in the blood of persons in Kuwait during the oil fires. Int Arch Occup Environ Health 66: 125-129.

*Eutermoser M, Rusch GM, Kuna RA, et al. 1986. A method for repeated evaluation of benzene uptake in rats and mice during a six hour inhalation period. Am Ind Hyg Assoc J 47:37-40.

*Evans HL, Dempster AM, Snyder CA. 1981. Behavioral changes in mice following benzene inhalation. Neurobehav Toxicol Teratol 3:481-485.

*Eveleth WT, ed. 1990. Kline guide to the U.S. Chemical Industry. 5th ed. Fairfield, NJ: Kline & Company, Inc. 2-2-2-34.

*Exxon. 1986. Initial submission: Determination of material toxicity & fetal toxicity of benzene in rats following oral exposure (final report) with cover letter (sanitized). EPA/OTS; Dot #88-9200010293.

*Fan XH. 1992. Effect of exposure to benzene on natural killer (NK) cell activity and interleukin-2 (IL-2) production of C57BL/6 mice. J Nippon Med Sch 59(5):393-399.

*Farm Chemicals Handbook. 1993. Pesticides Dictionary, United States of America. Meister Publishing Co. Willoughby, OH C 46.

*Farris GM, Everitt JI, Irons RD, et al. 1993. Carcinogenicity of inhaled benzene in CBA mice. Fundam Appl Toxicol 20(4):503-507.

*Fan-is GM, Robinson SN, Gaido KW, et al. 1996. Effects of low concentrations of benzene on mouse hematopoietic cells *in vivo*. A preliminary report. Environ Health Perspect 104 (Suppl 6):1275-1276.

*Farris GM, Shah RS, Wong VA, et al. 1995. Benzene-induced hematotoxicity in mice evaluated by flow cytometry. Fund App. Toxicol Suppl. The Toxicologist 15(1):222-223.

*FDA. 1977a. Indirect food additives: Adhesives and components of coatings. Food and Drug Administration. Code of Federal Regulations. 21 CFR 175.105.

*FDA. 1977b. Indirect food additives: Adhesives and components of coatings. Food and Drug Administration. Federal Register 42: 14534-14554.

*FEDRIP. 1996. Federal Research in Progress. On-line retrieval of federal research in progress database. March 1996.

*Ferrario JB, Lawler GC, DeLeon IR, et al. 1985. Volatile organic pollutants in biota and sediments of Lake Pontchartrain. Bull Environ Contam Toxicol 34:246-255.

Fiserova-Bergerova 1955. Modeling of Inhalation Exposure to Vapors: Uptake, Distribution, and Elimination Volume 1 and II. CRC Press, Inc. Boca Raton, Florida.

*Fishbeck WA, Townsend JC, Swank MG. 1978. Effect of chronic occupational exposure to measured concentrations of benzene. J Occup Med 20:539-542.

*Fishbein L. 1988a. Benzene: Uses, occurrence, and exposure. In: Fishbein L, O'Neill IK, eds. Environmental carcinogens methods of analysis and exposure measurement: Vol. lO--benzene and alkylated benzene. IARC Scientific Publications No. 85. Lyon, France: World Health Organization, International Agency of Research on Cancer. 67-96.

*Fishbein L. 1992. Exposure from occupational versus other sources. Stand J Work Environ Health 18(Suppl 1):5-16.

*Fleming AF. 1990. Benzene in petrol: a continuing hazard [letter; comment]. Lancet 336(8722):1076-1077.

*Flury F. 1928. [II. Toxicities in modem industry. IIa. Pharmacological-toxicological aspects of intoxicants in modern industry.] Archives fuer Experimentelle Pathologie und Pharmakologie 138:65-82. (German)

*Forni A. 1996. Benzene-induced chromosome aberrations: a follow-up study. Environ Health Perspect 104 (Suppl 6):1309-1312.

*Forni A, Cappellini A, Pacific0 E, et al. 1971a. Chromosome changes and their evolution in subjects with past exposure to benzene. Arch Environ Health 23:385-391.

*Forni A, Moreo L. 1967. Cytogenetic studies in a case of benzene leukaemia. Eur J Cancer 3:251-255.

*Forni A, Moreo L. 1969. Chromosome studies in a case of benzene-induced erythroleukaemia. Eur J Cancer 5:459-463.

*Forni AM, Pacifico E, Limonta A. 1971b. Chromosome studies in workers exposed to benzene or toluene or both. Arch Environ Health 22:373-378.

*Frantik E, Homychova M, Horvath M. 1994. Relative acute neurotoxicity of solvents: Isoeffective air concentrations of 48 compounds evaluated in rats and mice. Environ Res 66: 173-185.

*Frantz CE, Eastmond DA. 1996. Inhibition of human topoisomerase II *in vitro* by diphenoquinone, a peroxidase-activated metabolite of benzene. The Toxicologist 30(1):230.

*Frantz CE, Chen H, Eastmond DA. 1996. Inhibition of human topoisomerase II *in vitro* by bioactive benzene metabolites. 104:(Suppl 6):1319-1323.

*Franz TJ. 1984. Percutaneous absorption of benzene. In: MacFarland HN, Holdsworth CE, MacGregor JA, et al., eds. Advances in modem environmental toxicology. Vol. VI. Applied toxicology of petroleum hydrocarbons. Princeton, NJ: Princeton Scientific Publishers, Inc., 61-70.

*Frash VN, Yushkov BG, Karaulov AV, et al. 1976. Mechanism of action of benzene on hematopoiesis (investigation of hematopoietic stem cells). Bull Exptl Biol Med 82:985-987.

*Froom P, Dyerassi L, Cassel A, et al. 1994. Erythropoietin-independent colonies of red blood cells and leukocyotisis in a worker exposed to low levels of benzene. Stand J Work Environ Health 20:306-308.

*FSTRAC. 1990. Summary of State and Federal Drinking Water Standards and Guidelines. U.S. Environmental Protection Agency. Federal-State Toxicology and Regulatory Alliance Committee. Report 7. Detailed Standards and Guidelines, by Chemical.

*Fujie K, Ito Y, Maeda S. 1992. Acute cytogenetic effect of benzene on rat bone marrow cells in vivo and the effect of inducers or inhibitors of drug-metabolizing enzymes. Mutat Res 298(2):81-90.

*Furies-Cravioto F, Zapata-Gayon C, Kolmodin-Hedman B, et al. 1977. Chromosome aberrations and sister chromatid exchange in workers in chemical laboratories and a rotoprinting factory and in children of women laboratory workers. Lancet 2:322-325.

*Fung KK, Wright BJ. 1986. Monitoring of benzene in ambient air with organic vapor badges. J Air Pollut Control Assoc 36:819-821.

*Fuselli S, Benigni R, Conti L. 1995. Volatile organic compounds (vocs) and air mutagenicity: results of one year monitoring at an urban site. International Journal Of Environmental Health Research 5(2):123-132.

*Fustinoni S, Buratti M, Giampiccolo R, et al. 1995. Biological and environmental monitoring of exposure to airborne benzene and other aromatic hydrocarbons. in Milan traffic wardens. Toxicol Letters 77:387-392.

*Gad-El Karim MM, Ramanujam VMS, Legator MS. 1985. *trans,* trans-Muconic acid, an open chain urinary metabolite of benzene in mice: Quantification by high-pressure liquid chromatography. Xenobiotica 15:21l-220.

*Gaffney JS, Levin SZ. 1979. Predicting gas phase organic molecule reaction rates using linear free-energy correlations: I. O(^{3}P) and QH addition and abstraction reactions. Int J Chem Kinet 11:1197-1209.

Gaido KW, Wierda D. 1987. Suppression of bone marrow stromal cell function by benzene and hydroquinone is ameliorated by indomethacin. Toxicol Appl Pharmacol 89:378-390.

*Ganousis LG, Goon D, Zyglewska T, et al. 1992. Cell-specific metabolism in mouse bone marrow stroma: studies of activation and detoxification of benzene metabolites. Mol Pharmacol 42(6):1118-l125.

*Geiselhart LA, Freed BM. 1996. Inhibition of human T cell activation by p-benzoquinone. The Toxicologist. 30(1):60.

*Geist CR, Drew KL, Schoenheit CM, et al. 1983. Learning impairments following postnatal exposures to benzene. Percept Mot Skills 57:1083-1086.

*Gemer-Smidt P, Friedrich U. 1978. The mutagenic effect of benzene, toluene and xylene studied by the SCE technique. Mutat Res 58:313-316.

*Geyer H, Politzki G, Freitag D. 1984. Prediction of ecotoxicological behavior of chemicals: relationship between n-octanol/water coefficient and bioaccumulation of organic chemicals by alga Chlorella. Chemosphere 13(2):269-284.

*Ghantous H, Danielsson BRG. 1986. Placental transfer and distribution of toluene, xylene and benzene, and their metabolites during gestation in mice. Biol Res Pregnancy 7:98-105.

*Ghittori S, Fiorentino ML, Maestri L, et al. 1993. Urinary excretion of unmetabolized benzene as an indicator of benzene exposure. J Toxicol Environ Health 38(3):233-243.

*Ghittori S, Maestri L, Fiorentino ML, et al. 1995. Evaluation of occupational exposure to benzene by urinalysis. Int Arch Occup Environ Health 67:195-200.

*Ghoos Y, Claus D, Geypens B, et al. 1994. Screening method for the determination of volatiles in biomedical samples by means of an off-line closed-loop trapping system and high-resolution gas chromatography-ion trap detection. J Chromatogr 665:333-345.

*Gibson DT. 1977. Biodegradation of aromatic petroleum hydrocarbons. In: Wolfe DA, ed. Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. New York, NY: Pergamon, 36-46.

*Gibson DT. 1980. Microbial metabolism. In: Hutzinger 0, ed. The handbook of environmental chemistry. New York, NY: Springer-Verlag, 161-192.

*Gill DP, Ahmed AE. 1981. Covalent binding of [14C]benzene to cellular organelles and bone marrow nucleic acids. Biochem Pharmacol 30: 1127-l 131.

*Gill DP, Jenkins VK, Kempen RR, et al. 1980. The importance of pluripotential stem cells in benzene toxicity. Toxicology 16:163-171.

*Gill DP, Kempen RR, Nash JB, et al. 1979. Modifications of benzene myelotoxicity and metabolism by phenobarbital, SKF-525A and 3-methylcholanthrene. Life Sciences 25:1633-1640.

*Gilli G, Scursatone E, Bono R. 1994. Benzene, toluene and xylenes in air, geographical distribution in the Piedmont region (Italy) and personal exposure. Sci Total Environ 148(1):49-56.

*Glass LR, Connor TH, Thiess JC, et al. 1986. Genotoxic evaluation of the offgassing products of particle boards. Toxicol Lett 31:75-83.

*Glatt H, Padykula R, Berchtold GA, et al. 1989. Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. Environ Health Perspect 82:81-89.

*Glatt H, Witz G. 1990. Studies on the induction of gene mutations in bacterial and mammalian cells by the ring-opened benzene metabolites *trans,trans*-muconaldehyde and *trans,trans*-muconic acid. Mutagenesis 5:263-266.

*Glauert HP, Kennan WS, Sattler GL, et al. 1985. Assays to measure the induction of unscheduled DNA synthesis in cultured hepatocytes. Progress in Mutation Research 5:371-373.

*Gofmekler VA. 1968. [Effect on embryonic development of benzene and formaldehyde in inhalation experiments.] Hygiene Sanitation 33:327-332. (Russian)

*Gold LS, Slone TH, Stern BR, et al. 1993. Comparison of target organs of carcinogenicity for mutagenic and non-mutagenic chemicals. Mutat Res 286(1):75-100.

*Goldman LR, Paigen B, Magnant MM, et al. 1985. Low birth weight, prematurity and birth defects in children living near the hazardous waste site, Love Canal. Hazardous Waste and Hazardous Materials 2:209-223.

*Goldstein BD. 1985. Risk assessment and risk management of benzene by the Environmental Protection Agency. In: Risk quantitation and regulatory policy, Banbury Report 19:293-304.

*Goldstein BD. 1988. Benzene toxicity: Occupational medicine. State of the Art Reviews 3:541-554.

*Goldstein BD, Warren J. 1993. HEI's mobile air toxics project. Environ Health Perspect 101(3):256-258.

*Goldwater LJ. 1941. Disturbances in the blood following exposure to benzol. J Lab Clin Med 26:957-973.

*Gonasun LM, Witmer C, Kocsis J, et al. 1973. Benzene metabolism in mouse liver microsomes. Toxicol Appl Pharmacol 26:398-406.

*Gonzalez J, Levine SP. 1986. The development and evaluation of a thermally-desorbable miniature passive dosimeter for the monitoring of organic vapors. Am Ind Hyg Assoc J 47:339-346.

*Goon D, Matsuura J, Ross D. 1993. Metabolism and cytotoxicity of *trans,trans-* muconaldehyde and its derivatives: potential markers of benzene ring cleavage reactions. Chem-Biol Interact 88(1):37-53.

*Gordian ME, Guay G. 1995. Benzene in Alaska. J Alaska Medicine 37(1):25-36

*Gossett RW, Brown DA, Young DR. 1983. Predicting the bioaccumulation of organic compounds in marine organisms using octanoVwater partition coefficients. Marine Poll Bull 14 (10):387-392.

*Graedel TE. 1978. Chemical compounds in the atmosphere. New York, NY: Academic Press, 105-107.

*Grassman J, Haas R. 1993. Development of an immunoassay to detect hemoglobin adducts formed by benzene exposure. Int Arch Occup Environ Health 65(1 Suppl):147-150.

*Grbic-Galic D, Vogel TM. 1987. Transformation of toluene and benzene by mixed methanogenic cultures. Appl Environ Microbial 53(2):254-260.

*Greek BF. 1990. Prices and demand for aromatics reverse earlier gains. Chemical and Engineering News 68:11-12.

*Green JD, Leong BKJ, Laskin S. 1978. Inhaled benzene fetotoxicity in rats. Toxicol Appl Pharmacol 46:9-18.

*Green JD, Snyder CA, LoBue J, et al. 1981a. Acute and chronic dose/response effects of inhaled benzene on multipotential hematopoietic stem (CFU-S) and granulocyte/macrophage progenitor (GM-CFU-C) cells in CD-l mice. Toxicol Appl Pharmacol 58:492-503.

*Green JD, Snyder CA, LoBue J, et al. 1981b. Acute and chronic dose/response effect of benzene inhalation on the peripheral blood, bone marrow, and spleen cell of CD-l male mice. Toxicol Appl Pharmacol 59:204-214.

*Greenburg L. 1926. Benz01 poisoning as an industrial hazard. Public Health Reports 41: 1357-1375.

*Greenburg L, Mayers MR, Goldwater L, et al. 1939. Benzene (benzol) poisoning in the rotogravure printing industry in New York City. J Ind Hyg Toxicol 21:395-420.

*Greenland S, Salvan A, Wegman DH, et al. 1994. A case-control study of cancer mortality at a transformer-assembly facility. Int Arch Occup Environ Health 66:49-54.

*Grab K, Frauenfelder C, Artho A. 1990. Uptake by foods of tetrachloroethylene, trichlorethylene, toluene, and benzene from air. Z. Lebensm Unters Forsch 191:435-441.

*Grotz VL, Ji S, Kline SA, et al. 1994. Metabolism of benzene and trans,trans-muconaldehyde in the isolated perfused rat liver. Toxicol Lett 70 (3):281-290.

*Grotz VL, Witz G. 1995. Strain comparison studies on the metabolism of benzene and trans, trans-muconaldehyde by liver cell fractions from DBA/2N and C57BL/6 mice. Toxicologist 15(1):154.

*Gruenke LD, Craig JC, Wester RC, et al. 1986. Quantitative analysis of benzene by selected ion monitoring/gas chromatography/mass spectrometry. J Anal Toxicol 10:225-232.

*Guerra G, Lemma A, Lerda D, et al. 1995. Benzene emissions from motor vehicle traffic in the urban area of Milan: hypothesis of health impact assessment. Atmospheric Environment 29(23):3559-3569.

*Gulati DK, Witt K, Anderson B, et al. 1989. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*: III: Results with 27 chemicals. Environ Mol Mutagen 13:133-193.

*Gut I, Nedelcheva V, Soucek P, et al. 1996. Cytochromes P450 in benzene metabolism and involvement of their metabolites and reactive oxygen species in toxicity. Environ Health Perspect 104 (Suppl 6):1211-1218.

*Gut I, Terelius Y, Frantik E, et al. 1993. Exposure to various benzene derivatives differently induces cytochromes P450 2Bl and P450 2El in rat liver. Arch Toxicol 67(4):237-243.

*Guy RL, Dimitriadis EA, Hu PD, et al. 1990. Interactive inhibition of erythroid ^{59}Fe utilization by benzene metabolites in female mice. Chem-Biol Interact 74(l-2):55-62.

*Haddad LM, Winchester JF. 1990. Clinical management of poisoning and drug overdose. W.B Saunders Company. Philadelphia. 1222-1232.

*Hadeishi T, Pollard M, McLaughlin R, et al. 1985. Development of an optical monitor for toxic organic compounds in air. Research Triangle Park, NC: U.S. Environmental Protection Agency, ORD, Environmental Monitoring Systems Laboratory EPA/600/4-85-043. NTISPB85-225027

*Haider K, Jagnow G, Kohnen R, et al. 1981. Degradation of chlorinated benzene, phenols and cyclohexane derivatives by benzene- and phenol-utilizing soil bacteria under aerobic conditions. In: Overcash MR, ed. Decomposition of toxic and nontoxic organic compounds in soils. Ann Arbor, MI: Ann Arbor Science, 207-223.

*Hajimiragha H, Ewers U, Brockhaus A, et al. 1989. Levels of benzene and other volatile aromatic compounds in the blood of non-smokers and smokers. Int Arch Occup Environ Health 61:513-518.

*Hallberg LM, Grossman L, Hedayati MA, et al. 1995. DNA repair activities in lymphocytes of benzene exposed workers. 26th Annual Meeting Of The Environmental Mutagen Society, St. Louis, Missouri, USA, March 12-16, 1995. Environmental And Molecular Mutagenesis; 25 (Suppl. 25) 22.

*Hallenbeck WH, Flowers RE. 1992. Risk analysis for worker exposure to benzene. Environ Manage 16(3):415-420.

*Hamilton A. 1922. The growing menace of benzene (benzol) poisoning in American industry. J Am Med Assoc 78:627-630.

*Hammers WE, Bosman HFPM. 1986. Quantitative evaluation of a simple dynamic headspace analysis technique for non-polar pollutants in aqueous samples at the ng/kg level. J Chromatogr 360:425-432.

*Hammond JW, Hermann ER. 1960. Industrial hygiene features of a petro-chemical benzene plant design and operation. Am Ind Hyg Assoc J 21:173-177.

*Hanke J, Dutkiewicz T, Piotrowski J. 1961. [The absorption of benzene through the skin in men]. Medycyna Pracy 12:413-426. (Polish)

*Hanzlick R. 1995. National association of medical examiners pediatric toxicology (pedtox) registry report 3: case submission summary and data for acetaminophen, benzene, carboxyhemoglobin, dextromethorphan, ethanol, phenobarbital, and pseudoephedrine. American Journal of Forensic Medicine and Pathology 16 (4):270-277.

*Harayama S, Timmis KN. 1992. Aerobic biodegradation of aromatic hydrocarbons by bacteria. In: Sigel, H. and A. Sigel (ed.). Metal ions in biological systems, vol. 28: Degradation of environmental pollutants by microorganisms and their metalloenzymes. Marcel Dekker, Inc.: New York, New York, USA.

*Harkov R, Gianti SJ Jr, Bozzelli JW, et al. 1985. Monitoring volatile organic compounds at hazardous and sanitary landfills in New Jersey. J Environ Sci Health A20:491-501.

*Harland BJ, Whitby FJ, Comber MHI. 1985. Measurement of volatile aromatic hydrocarbons in an industrial estuary. Int J Environ Anal Chem 20:295-311.

*Harper BL, Sadagopa Ramanujam VM, Gad-El-Karim MM, et al. 1984. The influence of simple aromatics on benzene clastogenicity. Mutat Res 128: 105-114.

Harrison I, Leader RU, Higgo JJW, et al. 1994. Determination of organic pollutants in small samples of groundwaters by liquid-liquid extraction and capillary gas chromatography. J Chromatogr A688:181-188.

*Hartwich G, Schwanitz G, Becker J. 1969. Chromosome anomalies in a case of benzene leukaemia. German Medical Monthly 14:449-450.

*Hatakeyama Y, Nakajima E, Atai H, et al. 1992. Effects of benzene in a micronucleus test on peripheral blood utilizing acridine orange-coated slides. Mutat Res 278(2-3):193-195.

*Hattemer-Frey HA, Travis CC, Land ML. 1990. Benzene: Environmental partitioning and human exposure. Environ. Res. 53:221-232.

*Hawkins NC. 1991. Conservatism in maximally exposed individual (MEI) predictive exposure assessments: a first-cut analysis. Regul Toxicol Pharmacol 14(2):107-l17.

*Hayashi M, Norppa H, Sofuni T, et al. 1992. Flow cytometric micronucleus test with mouse peripheral erythrocytes. Mutagenesis 7(4):257-264.

*Hayes RB. 1992. Biomarkers in occupational cancer epidemiology: considerations in study design. Environ Health Perspect 98:149-154.

*Hayes RB, Yin SN, Dosemeci M, et al. 1996. Mortality among benzene-exposed workers in China. Environ Health Perspect 104 (Suppl 6):1349-1352.

*HazDat. 1996. Database. Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA.

*Hazel B, O'Connor A, Niculescu R, Kalf G. et al. 1996. Induction of granlocytic differentiation in a mouse model by benzene and hydroquinone. Environ Health Perspect 104 (Suppl 6): 1257-1264.

*Hazel BA, O'Connor A, Niculescu R, et al. 1995. Benzene and its metabolite, hydroquinone, induce granulocytic differentiation in myeloblasts by interacting with cellular signaling pathways activated by granulocyte colony-stimulating factor. Stem Cells 13(3): 295-310.

*Heath CW. 1983. Field epidemiologic studies of populations exposed to waste dumps. Environ Health Perspect 48:3-7.

*Heavner DL, Morgan WT, Ogden MW. 1995. Determination of volatile organic compounds and ETS apportionment in 49 homes. Environ Int 21(1):3-21.

*Hedli CC, Nimmagudda R, Reuhl KR, et al. 1995. DNA adducts and granulocytic differentiation in HL-60 cells following treatment with benzene metabolites. In: Abstracts of the 34th annual meeting. Toxicologist 15(1):153.

*Hedli CC, Snyder R, Witmer CM. 1990. Bone marrow DNA adducts and bone marrow cellularity following treatment with benzene metabolites *in vivo.* Adv Exp Med Biol 283:745-748.

*Hedli CC, Yurkow E, Snyder R. 1996. The in vitro hematotoxicity of 1,2,4-benzenetriol (BT) to female BALB/C mice. The Toxicologist 30(1):339.

*Hellmer L, Bolcsfoldi G. 1992a. An evaluation of the *E. coli* K-12 *uvrB/recA* DNA repair host-mediated assay: II. *in vivo* results for 36 compounds tested in the mouse. Mutat Res 272(2):161-173.

*Hellmer L, Bolcsfoldi G. 1992b. An evaluation on the *E. coli* k-12 *uvrB/recA* DNA repair host-mediated assay: I. *in vitro* sensitivity of the bacteria to 61 compounds. Mutat Res 272(2):145-160.

*Henderson RF. 1996. Species differences in the metabolism of benzene. Environ Health Perspect 104 (Suppl 6):1173-l175.

*Henderson RF, Bechtold WE, Strunk MR, et al. 1995. Analysis of benzene metabolites and glutathione in the bone marrow of B6C3F$_1$ mice. Toxicologist 15(1):222.

*Henderson RF, Sabourin PJ, Bechtold WE, et al. 1989. The effect of dose, dose rate, route of administration, and species on tissue and blood levels of benzene metabolites. Environ Health Perspect 82:9-17.

*Henderson RF, Sabourin PJ, Medinsky MA, et al. 1992. Benzene dosimetry in experimental animals: Relevance for risk assessment. In: D'Amato R, Slaga TJ, Farland WH, et al. Relevance of animal studies to the evaluation of human cancer risk. New York, NY: Wiley-Liss Inc. 93-105.

*Hendrickson HP, Sahafayen M, Bell MA, et al. 1994. Relationship of flavonoid oxidation potential and effect on rat hepatic microsomal metabolism of benzene and phenol. J Pharm Biomed Anal 12(3):335-341.

*Henschler R, Glatt HR. 1995. Induction of cytochrome p4501al in haemopoietic stem cells by hydroxylated metabolites of benzene. Toxicology *in vitro* 9(4):453-457.

*Hite M, Pecharo M, Smith I, et al. 1980. The effect of benzene in the micronucleus test. Mutat Res 77:149-155.

*Hoechst. 1977. Initial submission: mutagenicity evaluation of benzene in Salmonella typhimurium, Saccharomyces cerevisiae, and mice (final report) with attachment EPA/OTS; Dot #88-920002878.

*Holdren MW, Smith DL, Smith RN. 1985. Comparison of ambient air sampling techniques for volatile organic compounds. U.S. Environmental Protection Agency. EPA 6OO/S4-85-067. PB86-120953/GAR.

*Holmberg B, Lundberg P. 1985. Benzene: Standards, occurrence, and exposure. Am J Ind Med 7:375-383.

*Hopper DJ. 1978. Microbial degradation of aromatic hydrocarbons. In: Watkinson RJ, ed. Developments in biodegradation of hydrocarbons. London, England: Applied Science, 85-l12.

HSDB. 1996. Hazardous Substances Data Bank. National Library of Medicine, National Toxicology Program (via TOXNET), Bethesda, MD. January 1996.

*Hsieh GC, Parker RDR, Sharma RP, et al. 1990. Subclinical effects of groundwater contaminants. III. Effects of repeated oral exposure to combinations of benzene and toluene on immunologic responses in mice. Arch Toxicol 64(4):320-328.

*Hsieh GC, Parker RDR, Sharma RP. 1988a. Subclinical effects of groundwater contaminants: II. Alteration of regional brain monoamine neurotransmitters by benzene in CD-1 mice. Arch Environ Contam Toxicol 17:799-805.

*Hsieh GC, Sharma RP, Parker RDR. 1988b. Subclinical effects of groundwater contaminants: I. Alteration of humoral and cellular immunity by benzene in CD-l mice. Arch Environ Contam Toxicol 17:151-158.

*Hsieh GC, Sharma RP, Parker RD. 1991. Hypothalamic-pituitary-adrenocortical axis activity and immune function after oral exposure to benzene and toluene. Immunopharmacology 21(1):23-31.

*Huff JE, Haseman JK, DeMarini DM, et al. 1989. Multiple-site carcinogenicity of benzene in Fischer 344 rats and $B6C3F_1$ mice. Environ Health Perspect 82:125-163.

*Hunter CG. 1968. Solvents with reference to studies on the pharmacodynamics of benzene. Proc R Sot Med 61:913-915.

*Hunting KL, Longbottom H, Kalavar SS, et al. 1995. Haematopoietic cancer mortality among vehicle mechanics. Occupational Environmental Medicine 52:673-678.

*Hurley JF, Cherrie JW, Maclaren W. 1991. Exposure to benzene and mortality from leukaemia: results from coke oven and other coal product workers [letter]. Br J Ind Med 48(7):502-503.

*Hustert K, Mansour H, Korte F. 1981. A method to determine the photochemical decomposition of organic compounds in aquatic systems.

*Hutt AM, Kalf GF. 1996. Inhibition of human DNA topoisomerase II by hydroquinone and p-benzoquinone, reactive metabolites of benzene. Environ Health Perspect 104 (Suppl 6): 126l-1269.

*IARC. 1982. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 29: Some industrial chemical and dyestuffs. Benzene. Lyon, France: World Health Organization, International Agency for Research on Cancer 93-148.

*IARC. 1987. IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes 1 to 42. Suppl 7. Lyons, France: World Health Organization, International Agency for Research on Cancer, 38-74.

*Ikeda M, Ohtsuji H. 1971. Phenobarbital-induced protection against toxicity of toluene and benzene in the rat. Toxicol Appl Pharmacol 20:30-43.

*Ikeda M, Ohtsuji H, Imamura T. 1972. *In vivo* suppression of benzene and styrene oxidation by co-administered toluene in rats and effects of phenobarbital. Xenobiotica 2: 101-106.

*Infante PF. 1978. Leukemia among workers exposed to benzene. Tex Rep Biol Med 37:153-161.

*Infante PF, Rinsky RA, Wagoner JK, et al. 1977. Leukemia in benzene workers. Lancet 2:76-78.

*Inoue O, Seiji K, Kasahara M, et al. 1986. Quantitative relation of urinary phenol levels to breathzone benzene concentrations: a factory survey. Br J Ind Med 43:692-697.

*Inoue O, Seiji K, Kasahara M, et al. 1988a. Determination of catechol and quinol in the urine of workers exposed to benzene. Br J Ind Med 45:487-492.

*Inoue O, Seiji K, Nakatsuka H, et al. 1989. Urinary t,t-muconic acid as an indicator of exposure to benzene. Br J Ind Med 46: 122-127.

*Inoue O, Seiji K, Watanabe T, et al. 1988b. Mutual metabolic suppression between benzene and toluene in man. Int Arch Occup Environ Health 60: 15-20.

*IRIS. 1996. Integrated Risk Information System. National Institute for Occupational Safety and Health.

*Irons RD. 1985. Quinones as toxic metabolites of benzene. J Toxicol Environ Health 16:673-678

*Irons RD, Dent JG, Baker TS, et al. 1980. Benzene is metabolized and covalently bound in bone marrow *in situ*. Chem-Biol Interact 30:241-245.

*Irons RD, Heck Hd'A, Moore BJ, et al. 1979. Effects of short-term benzene administration on bone marrow cell cycle kinetics in the rat. Toxicol Appl Pharmacol 51:399-409.

*Irons RD, Stillman WS. 1993. Cell proliferation and differentiation in chemical leukemogenesis. Stem Cells 11:235-242.

*Irons RD, Stillman WS. 1996. Impact of benzene metabolites on differentiation of bone marrow progenitor cells. Environ Health Perspect 104 (Suppl 6): 1247-1250.

*Irons RD, Stillman WS, Colagiovanni DB, et al. 1992. Synergistic action of the benzene metabolite hydroquinone on myelopoietic stimulating activity of granulocyte/macrophage colony-stimulating factor *in vitro.* Proc Nat1 Acad Sci 89(9):3691-3695.

IRPTC. 1985. Treatment and disposal methods for waste chemicals. Data Profile Series No. 5. International Register of Potentially Toxic Chemicals. Geneva, Switzerland. United Nations Environment Programme.

*ITII. 1975. Toxic and hazardous industrial chemicals safety manual for handling and disposal, with toxicity and hazard data. Tokyo, Japan: International Technical Information Institute, 62-64.

*Jablonicka A, Vargova M, Karelova J. 1987. Cytogenetic analysis of peripheral blood lymphocytes in workers exposed to benzene. J Hyg Epidemiol Microbial Immunol 31:127-l 33.

*Jakobsson R, Ahlbom A, Bellander T, et al. 1993. Acute myeloid leukemia among petrol station attendants. Arch Environ Health 48(4):255-259.

*Jen J-F, Tsai M-Y. 1994. Determination of phenol in urine by high-performance liquid chromatography with on-line precolumn enzymatic hydrolysis of the conjugates. J Chromatogr 658:87-92.

*Jerina D, Daly J, Witkop B, et al. 1968. Role of arene oxide-oxepin system in the metabolism of aromatic substances. I. *In vitro* conversion of benzene oxide to a premercapturic acid and a dihydrodioi. Arch Biochem Biophys 128:176-183.

*Jirka AM, Bourne S. 1982. Gas-chromatographic analysis for benzene in blood. Clin Chem 28:1492-1494.

*Johansson I, Ingelman-Sundberg M. 1988. Benzene metabolism by ethanol-, acetone-, and benzene-inducible cytochrome P-450(IIEl) in rat and rabbit liver microsomes. Cancer Res 48:5387-5390.

*Johnson ES, Lucier G. 1992. Perspectives on risk assessment impact of recent reports on benzene. Am J Ind Med 21(5):749-757.

*Jongeneelen FJ, Dirven HAAM, Leijdekkers C-M, et al. 1987. S-phenyl-n-acetylcysteine in urine of rats and workers after exposure to benzene. J Anal Toxicol 11:100-104.

*Kaden DA, Hites RA, Thilly WG. 1979. Mutagenicity of soot and associated polycyciic aromatic hydrocarbons to *Salmonella typhimurium.* Cancer Res 39:4152-4159.

*Kahn H, Muzyka V. 1973. The chronic effect of benzene on porphyrin metabolism. Work Environ Health 10:140-143.

*Kale PG, Baum JW. 1983. Genetic effects of benzene in Drosophila melanogaster males. Environ Mutagen 5:223-226.

*Kalf GF, Post GB, Snyder R. 1987. Solvent toxicology: Recent advances in the toxicology of benzene, the glycol ethers, and carbon tetrachloride. Ann Rev Pharmacol Toxicol 27:399-427.

*Kalf GF, Rushmore T, Snyder R. 1982. Benzene inhibits RNA synthesis in mitochondria from liver and bone marrow. Chem-Biol Interact 42:353-370.

*Kalf GF, Schlosser MJ, Renz JF, et al. 1989. Prevention of benzene-induced myelotoxicity by nonsteroidal anti-inflammatory drugs. Environ Health Perspect 8257-64.

*Kalf G, Shurina R, Rem J, et al. 1990. The role of hepatic metabolites of benzene in bone marrow peroxidase-mediated myelo- and genotoxicity. Adv Exp Med Biol 283:443-455.

*Kanada M, Miyagawa M, Sato M, et al. 1994. Neurochemical profile of effects of 28 neurotoxic chemicals on the central nervous system in rats (1) effects of oral administration on brain contents of biogenic amines and metabolites. Ind Health 32:145-164.

*KAR. 1991a. Threshold Ambient Limits and Significant Emission Levels of Toxic Air Pollutants. Kentucky Administrative Regulations (KAR). 401 KAR 63:021. Existing Sources Emitting Toxic Air Pollutants. Appendix B.

*KAR. 1991b. Appendix on Hazardous Waste Constituents. Kentucky Hazardous Waste Management Regulations. 401 KAR 31: 170.

*Karacic V, Skender L, Prpic -Majic D. 1987. Occupational exposure to benzene in the shoe industry. Am J Ind Med 12:531-536.

*Karacic V, Skender L, Bosner-Cucancic B, et al. 1995. Possible genotoxicity in low level benzene exposure. Am J Ind Med 27:379-388.

*Karickhoff SW. 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. Chemosphere 10:833-846.

*Karlson U, Frankenberger WT Jr. 1989. Microbial degradation of benzene and toluene in groundwater. Bull Environ Contam Toxicol 43:505-5 10.

*Kate S, Onda M, Matsukura N. 1995. Cytochrome p4502El (CYP2El) genetic polymorphism in a case-control study of gastric cancer and liver disease. Pharmacogenetics 5:141-144.

*Kawai T, Yamaoka K, Uchida Y, et al. 1990. Benzene exposure in a Japanese petroleum refinery. Toxicol Lett 52(2):135-139.

*Kawai T, Yamaoka K, Uchida Y, et al. 1991. Exposure to vapors of benzene and other aromatic solvents in tank truck loading and delivery. Bull Environ Contam Toxicol 46(1): l-8.

*Keener WK, Arp DJ. 1994a. Transformations of aromatic compounds by Nitrosomonas europaea. Appl Environ Microbial 60(6): 1914-1920.

*Keener WK, Arp DJ. 1994b. Biotransformations of aromatic compounds by Nitrosomonas europaea. Abstracts of the general meeting of the American Society for Microbiology 94:446.

*Keller KA, Snyder CA. 1986. Mice exposed *in utero* to low concentrations of benzene exhibit enduring changes in their colony forming hematopoietic cells. Toxicology 42: 17l-l81.

*Keller KA, Snyder CA. 1988. Mice exposed *in utero* to 20 ppm benzene exhibit altered numbers of recognizable hematopoietic cells up to seven weeks after exposure. Fundam Appl Toxicol 10:224-232.

*Kelly TJ, Callahan PJ, Pleil J, Evans GF. 1993. Method development and field measurements for polar volatile organic Compounds in Ambient Air. Environ Sci Technol 27(6):1146-l152.

*Kenaga EE. 1980. Predicted bioconcentration factors and soil sorption coefficients of pesticides and other chemicals. Ecotoxicol Environ Safety 4:26-38.

*Kennedy LG, Hutchins SR. 1992. Applied Geologic, Microbiological, and Engineering Constraints of In-situ BTEX Bioremediation. Remediation 3:83-l10.

*Kenyon EM, Medinsky MA. 1995. Incorporation of heterogeneous enzyme distribution into a physiological model for benzene, phenol and hydroquinone. In: Abstracts of the 34th annual meeting. Toxicologist 15(1):49.

*Kiang PH, Grob RL. 1986. A headspace technique for the determination of volatile compounds in soil. J Environ Sci Health, Part A 21:71-100.

*Kimmel CA, Wilson JG. 1973. Skeletal deviations in rats: Malformations or variations? Teratology 8:309-3 16.

*Kipen HM, Cody RP, Goldstein BD. 1989. Use of longitudinal analysis of peripheral blood counts to validate historical reconstructions of benzene exposure. Environ Health Perspect 82:199-206.

*Kirschner EM. 1995. Production of top 50 chemicals increased substantially in 1994. Chemical and Engineering News April 10, 1995. 16-20.

*Kissling M, Speck B. 1972. Further studies on experimental benzene induced aplastic anemia. Blut 25:97-103.

*Kissling M, Speck B. 1973. Chromosome aberrations in experimental benzene intoxication. Helv. med. Acta 36:59-66.

*Klan MJ, Adams DO, Lewis JG. 1990. Effects of exposure to benzene *in vivo* on the murine mononuclear phagocyte system. Toxicol Appl Pharmacol 103(2):198-205.

*Kok PW, Ong CN. 1994. Blood and urinary benzene determined by headspace gas chromatography with photoionization detection: application in biological monitoring of low-level nonoccupational exposure. Int Arch Occup Environ Health 66(3):195-201.

*Kolachana P, Subrahmanyam VV, Meyer KB, et al. 1993. Benzene and its phenolic metabolites produce oxidative DNA damage in HL60 cells *in vitro* and in the bone marrow *in vivo*. Cancer Res 53(5):1023-1026.

*Koop DR, Laethem CL. 1992. Inhibition of rabbit microsomal cytochrome P-450 2El-dependent p-nitrophenol hydroxylation by substituted benzene derivatives. Drug Metab Dispos 20(5):775-777.

*Korde VM, Phelps TJ, Bienkowski PR, et al. 1993. Biodegradation of chlorinated aliphatics and aromatic compounds in total-recycle expanded-bed biofilm reactors. Appl Biochem Biotechnol 39-40:631-641.

*Korte F, Klein W. 1982. Degradation of benzene in the environment. Ecotoxicol Environ Safety 6:31l-327.

*Kowalski JD, Shkiya JM, Tsou G. 1985. Ambient sampling and analysis of halogenated hydrocarbons and benzene. Air Pollution Control Association, Detroit, MI. APCA Annual Meeting: June 16-21, 2-13.

*Kozioski RP. 1985. Determination of benzene and toluene in soils and plant material by azeotropic distillation. Bull Environ Contam Toxicol 34:10-16.

*Kraut A, Lilis R, Marcus M, et al. 1988. Neurotoxic effects of solvent exposure on sewage treatment workers. Arch Environ Health 43:263-268.

*Krishnan K, Andersen ME. 1994. Physiologically-based pharmacokinetic modeling in toxicology. In: Principles and methods of toxicology. 3rd edition, Wallace Hayes, ed. Raven Press, Ltd. New York, NY.

*Krishnan K, Andersen ME, Clewell HJ III, et al. 1994. Physiologically-based pharmacokinetic modeling of chemical mixtures. In: Toxicology of chemical mixtures, R.S.A. Yang, ed. Academic Press, New York, NY.

*Kuna RA, Kapp RW. 1981. Embryotoxic/teratogenic potential of benzene vapor in rats. Toxicol Appl Pharmacol 57: l-7.

*Kuna RA, Nicolich MJ, Schroeder RE, et al. 1992. A female rat fertility study with inhaled benzene. J Am Co11 Toxicol 11(3):275-282.

*Kunisaki TA, Augenstein WL. 1994. Drug-and toxin-induced seizures. Emergency Medicine Clinics Of North America; 12(4):1027-10.56.

*Lagorio S, Forastiere F, Iavarone I, et al. 1994b. Mortality of filling station attendants. Scand J Work Environ Health 20:331-338.

*Lagorio S, Tagesson C, Forastiere F, et al. 1994a. Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. Occupational and Environmental Medicine 5 1:739-743.

*Laitinen J, Kangas J, Pekari K, et al. 1994. Short time exposure to benzene and gasoline at garages. Chemosphere 28: 197-205.

*Lakhanisky TH, Hendrickx B. 1985. Induction of DNA single-strand breaks in CHO cells in culture. In: Ashby J, de Serres FJ, Draper M, et al., eds. Progress in Mutation Research 5:367-370.

*Lange A, Smolik R, Zatonski W, et al. 1973a. Leukocyte agglutinins in workers exposed to benzene, toluene and xylene. Int Arch Arbeitsmed 31:45-40.

*Lange A, Smolik R, Zatonski W, et al. 1973b. Serum immunoglobulin levels in workers exposed to benzene, toluene and xylene. Int Arch Arbeitsmed 31:37-44.

*Lansari A, Lindstrom AB, Templeman BD, et al. 1992. Multizonal mass balance modeling of benzene dispersion in a private residence. Proceedings of the 1992 US Environmental Protection Agency/Air and Waste Management Association Symposium on Measurement of Toxic and Related Air Pollutants, May 1992.

*Larson TV, Koenig JQ. 1994. Wood smoke: Emissions and noncancer respiratory effects. Annu Rev Public Health 15:133-156.

*Laskin DL, Heck DE, Punhabi CJ, et al. 1996. Role of nitric oxide in benzene-induced bone marrow toxicity. Environ Health Perspect 104 (Suppl 6):1283-1287.

*Laskin JD, Rao NR, Punjabi CJ, et al. 1995. Distinct actions of benzene and its metabolites on nitric oxide production by bone marrow leukocytes. J Leukoc Biol 57:422-426.

*Latriano L, Goldstein BD, Witz G. 1986. Formation of muconaldehyde, an open-ring metabolite of benzene, in mouse liver microsomes: An additional pathway for toxic metabolites. Proc Nat1 Acad Sci USA 83:8356-8360.

*Lauwerys RR, Buchet JP, Adrien F. 1994. Muconic acid in urine: A reliable indicator of occupational exposure to benzene. Am J Ind Med 25:297-300.

*Lee B-L, New A-L, Kok P-W, et al. 1993. Urinary *trans,trans*-muconic acid determined by liquid chromatography: application in biological monitoring of benzene exposure. Clin Chem 39: 1788-1792.

*Lee EW, Gamer CD. 1991. Effects of benzene on DNA strand breaks *in vivo* versus benzene metabolite-induced DNA strand breaks *in vitro* in mouse bone marrow cells. Toxicol Appl Pharmacol 108(3):497-508.

*Lee EW, Gamer CD, Johnson JT. 1988. A proposed role played by benzene itself in the induction of acute cytopenia: Inhibition of DNA synthesis. Res Commun Chem Pathol Pharmacol 60:27-46.

*Lee EW, Johnson JT, Gamer CD. 1989. Inhibitory effect of benzene metabolites on nuclear DNA synthesis in bone marrow cells. J Toxicol Environ Health 26:277-291.

*Lee J-Y, Roh J-R, Kim H-S. 1994. Metabolic engineering of *Pseudomonas putida* fo; the simultaneous biodegradation of benzene, toluene, and p-xylene mixture. Biotechnol Bioengin 43(1I):1146-l 152.

*Legathe A, Hoener BA, Tozer TN. 1994. Pharmacokinetic interaction between benzene metabolites, phenol and hydroquinone, in B6C3F$_1$ mice. Toxicol Appl Pharmacol 124:131-138.

Leo A, Hansch C, Elkins D. 1971. Partition coefficients and their uses. Chemical Reviews 71(6):525, 569.

*Leung H. 1993. Physiologically-based pharmacokinetic modeling. In: General and applied toxicology. Vol. I. Ballantine B, Marro T, Turner T, eds. Stockton Press, New York, NY. 153-164.

*Levay G, Bodell WJ. 1992. Potentiation of DNA adduct formation in HL-60 cells by combinations of benzene metabolites. Proc Nat1 Acad Sci USA 89(15):7105-7109.

*Levy A. 1973. The photochemical smog reactivity of organic solvents. In: Solvent theory and practices. Adv Chem Ser 124:70-94.

*Lewis JG, Odom B, Adams DO. 1988b. Toxic effects of benzene metabolites on mononuclear phagocytes. Toxicol Appl Pharmacol 92:246-254.

*Lewis JG, Stewart W, Adams DO. 1988a. Role of oxygen radicals in induction of DNA damage by metabolites of benzene. Cancer Res 48:4762-4765.

*Lewis RJ. 1993. Benzene. Hawley's condensed chemical dictionary. Twelfth Edition. 128-129.

*Li G-L, Linet MS, Hayes RB, et al. 1994. Gender differences in hematopoietic and lymphoproliferative disorders and other cancer risks by major occupational group among workers exposed to benzene in China. J Occup Med 36(8):875-881.

*Li G-L, Yin S-N, Watanabe T, et al. 1986. Benzene-specific increase in leukocyte alkaline phosphatase activity in rats exposed to vapors of various organic solvents. J Toxicol Environ Health 19:581-589.

*Li G, Wang C, Xin W, et al. 1996. Tissue distribution of DNA adducts and their persistence in the blood of mice exposed to benzene. Environ Health Perspect 104(Suppl 6):1337-1338.

*Li L, Sun W, Gong Z, et al. 1992. Effect of low benzene exposure on neurobehavioral function, AChE in blood and brain and bone marrow picture in mice. Biomed Environ Sci 5(4):349-354.

*Li Q, Freed BM. 1996. Role of iron in hydroquinone- and catechol-induced inhibition of IL-2 dependent lymphoblast proliferation. The Toxicologist 30(1):4.

*Li Q, Geiselhart L, Freed BM. 1995. Inhibitory effect of hydroquinone on IL-2 dependent cell proliferation. In: Abstracts of the 34th annual meeting. The Toxicologist 15(1):104-105.

*Li Y, Kuppusamy P, Zweier JL, et al. 1995. ESR evidence for the generation of reactive oxygen species from the copper-mediated oxidation of the benzene metabolite, hydroquinone: roie in DNA damage. Chem Biol Interact 94(2):101-120.

*Lide DR, ed. 1990. CRC Handbook of Chemistry and Physics. 71 st ed. Ann Arbor, MI: CRC Press, Inc., 3-75.

*Lin T-F, Little JC, Nazaroff WW. 1994. Transport and sorption of volatile organic compounds and water vapor within dry soil grains. Environ Sci Technol 28:322-330.

*Lindstrom AB, Highsmith VR, Buckley TJ, et al. 1994. Gasoline-contaminated ground water as a source of residential benzene exposure: A case study. Journal of Exposure Analysis and Environmental Epidemiology 4(2): 183-l95.

*Linet MS, Yin S-N, Travis LB, et al. 1996. Clinical features of hematopoietic malignancies and related disorders among benzene-exposed workers in China. Environ Health Perspect 104(Suppl 6): 1353-1364.

*Lioy PJ, Pellizzari E. 1995. Conceptual framework for designing a national survey of human exposure. Journal Of Exposure Analysis And Environmental Epidemiology 1995 5(3):425-444.

*Loden M. 1986. The *in vitro* permeability of human skin to benzene, ethylene glycol, formaldehyde, and n-hexane. Acta Pharmacol Toxicol 58:382-389.

*Longacre SL, Kocsis JJ, Snyder R. 1981a. Influence of strain differences in mice on the metabolism and toxicity of benzene. Toxicol Appl Pharmacol 60:398-409.

*Longacre SL, Kocsis JJ, Witmer CM, et al. 1981b. Toxicological and biochemical effects of repeated administration of benzene in mice. J Toxicol Environ Health 7:223-237.

*Low LK, Lambert CE, Meeks JR. 1995. Tissue-specific metabolism of benzene in zymbal gland and other solid tumor target tissues in rats. Journal Of The American College Of Toxicology 14(1):40-60.

*Low LK, Meeks JR, Norris KJ, et al. 1989. Pharmacokinetics and metabolism of benzene in Zymbal gland and other key target tissues after oral administration in rats. Environ Health Perspect 821215-222.

*Lowry WT, Juarez L, Petty CS, et al. 1985. Studies of toxic gas production during actual structural fires in the Dallas area. J Forensic Sci 30:59-72.

*Lox C. 1995. Alterations in coagulant activity following exposure to industrial solvents a possible marker of toxic chemical induced pathogenesis. Keystone Symposium On Molecular Toxicology, Copper Mountain, Colorado, USA, January 9-15, 1995. Journal Of Cellular Biochemistry Supplement; 0 (19a) 197.

*Ludwig RC, Eksteen R. 1988. LC column switching for the rapid analysis of benzene in unleaded gasoline. LC-GC 6:250-252.

*Luke CA, Tice RR, Drew RT. 1988a. The effect of exposure regimen and duration on benzene-induced bone-marrow damage in mice: I. Sex comparison in DBA/2 mice. Mutat Res 203:251-271.

*Luke CA, Tice RR, Drew RT. 1988b. The effect of exposure regimen and duration on benzene-induced bone marrow damage in mice: II. Strain comparisons involving B6C3F$_1$, C57BL/6 and DBA/2 male mice. Mutat Res 203:273-295.

*Lumley M, Barker H, Murray JA. 1990. Benzene in petrol [letter; comment]. Lancet 336(8726):1318-1319.

*Lunte SM, Lunte CE. 1990. Electroanalytical approaches to understanding benzene metabolism. J Pharm Biomed Anal 8(2):131-136.

*Lutz WK, Schlatter CH. 1977. Mechanism of the carcinogenic action of benzene: Irreversible binding to rat liver DNA. Chem-Biol Interact 18:241-245.

*Lyman WJ. 1982. Atmospheric residence time. In: Lyman WJ, Reehl WF, Rosenblatt DH, eds. Handbook of chemical property estimation methods: Environmental behavior of organic compounds. New York. NY: McGraw-Hill.

*Lysyj I, Perkins G, Farlow JS. 1980. Trace analysis for aromatic hydrocarbons in natural waters. Environ Int 4:407-416.

*MacEachem L, Laskin DL. 1992. Increased production of tumor necrosis factor-α by bone marrow leukocytes following benzene treatment of mice. Toxicol Appl Pharmacol 113(2):260-266.

*MacEachem L, Snyder R, Laskin DL. 1992. Alterations in the morphology and functional activity of bone marrow phagocytes following benzene treatment of mice. Toxicol Appl Pharmacol 117(2):147-154.

*Macintosh DL, Xue J, Ozkaynak H, et al. 1995. Population-based exposure model for benzene. Journal Of Exposure Analysis And Environmental Epidemiology 5(3):375-403.

*Mackay D, Leinonen PJ. 1975. Rate of evaporation of low-solubility contaminants from water bodies to atmosphere. Environ Sci Technol 9:1178-l180.

*Magos GA, Lorenzana-Jimenez M, Vidrio H. 1990. Toluene and benzene inhalation influences on ventricular arrhythmias in the rat. Neurotoxicol Teratol 12(2): 119-124.

*Maibach HI, Anjo DM. 1981. Percutaneous penetration of benzene and benzene contained in solvents in the rubber industry. Arch Environ Health 36:256-260.

*Major DW, Mayfield CI, Barker JF. 1988. Biotransformation of benzene by denitrification in aquifer sand. Ground Water 26:8-14.

*Major J, Jakab M, Kiss G, et al. 1994. Chromosome aberration, sister-chromatid exchange, proliferative rate index, and serum thiocyanate concentration in smokers exposed to low-dose benzene. Environ Mol Mutagen 23: 137-142.

*Major J, Kemeny G, Tompa A. 1992. Genotoxic effects of occupational exposure in the peripheral blood lymphocytes of pesticide preparing workers in Hungary. Acta Med Hung 49(1-2):79-90.

*Malachowsky KJ, Phelps TJ, Teboli AB, et al. 1994. Aerobic mineralization of trichloroethylene, vinyl chloride, and aromatic compounds by Rhodococcus species. Appl Environ Microbial 60(2):542-548.

*Maltoni C, Ciliberti A, Cotti G, et al. 1989. Benzene, an experimental multipotential carcinogen: Results of the long-term bioassays performed at the Bologna Institute of Oncology. Environ Health Perspect 82: 109- 124.

*Maltoni C, Conti B, Cotti G. 1983. Benzene: A multipotential carcinogen: Results of long-term bioassays performed at the Bologna Institute of Oncology. Am J Ind Med 4589-630.

*Maltoni C, Conti B, Cotti G, et al. 1985. Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology: Current results and ongoing research. Am J Ind Med 7:415-446.

*Maltoni C, Cotti G, Valgimigli L, et al. 1982a. Hepatocarcinomas in Sprague-Dawley rats following exposure to benzene by inhalation: First experimental demonstration. Med Lav 4:446-450.

*Maltoni C, Cotti G, Valgimigli L, et al. 1982b. Zymbal gland carcinomas in rats following exposure to benzene by inhalation. Am J Ind Med 3: 1 l-1 6.

*Mangino J, Jones JW. 1994. Mobile source hazardous air pollutant emissions in the Seattle-Tacoma Urban area. U. S. Department of Commerce. NTIS PB95-174660.

*Manning BW, Adams DO, Lewis JG. 1994. Effects of benzene metabolites on receptor-mediated phagocytosis and cytoskeletal integrity in mouse peritoneal macrophages. Toxicol Appl Pharmacol 126(2):214-223.

*Mannino DM, Schreiber J, Aldous K, et al. 1995. Human exposure to volatile organic compounds: a comparison of organic vapor monitoring badge levels with blood levels. Int Arch Occup Environ Health 67:59-64.

*Marina ML, Garica MA, Pastor M, et al. 1995. A statistical study of the correlation between k' or log k' and log P,, for a group of benzene and naphthalene derivatives in micellar liquid chromatography using a C- 18 column. Chromatographia 40(3/4): 185- 191.

*Mattie DR, Bates G D JR, Jepson GW, et al. 1994. Determination of skin:air partition coefficients for volatile chemicals: experimental method and applications. Fundamental Applied Toxicology 22(1):51-57.

*Mayer GJ, Cheng IS, Pau P, et al. 1994. Emissions of air toxics from wastewater treatment plants. Water Environ Res 66:140-144.

*McAllister PM, Chiang CY. 1994. A practical approach to evaluating natural attenuation of contaminants in ground water. Ground Water Monitoring and Remediation 14:161-173.

*McDonald TA, Yeowell-O'Connell K, Rappaport SM. 1994. Comparison of protein adducts of benzene oxide and benzoquinone in the blood and bone marrow of rats and mice exposed to [^{14}C/^{13}C$_6$] benzene. Cancer Res 54:4907-4914.

*McDougal JN, Jepson GW, Clewell HJ III, et al. 1990. Dermal absorption of organic-chemical vapors in rats and humans. Fund Appl Toxicol 14:299-308.

*McMahon TF, Birnbaum LS. 1991. Age-related changes in disposition and metabolism of benzene in male C57BL/6N mice. Drug Metab Dispos 19(6):1052-1057.

*McMahon TF, Medinsky MA, Bimbaum LS. 1994. Age-related changes in benzene disposition in male C57BL/6N mice described by a physiologically based pharmacokinetic model. Toxicol Lett 74(3):241-253.

*McMurry ST, Lochmiller RL, Vestey MR, et al. 1991. Acute effects of benzene and cyclophosphamide exposure on cellular and humoral immunity of cotton rats, Sigmodon hispidus. Bull Environ Contam Toxicol 46(6):937-945.

*McMurry ST, Lochmiller RL, Vestey MR, et al. 1994a. Cellular immune responses of nutritionally stressed juvenile cotton rats (Sigmodon hispidus) during acute benzene exposure. Arch Environ Contam Toxicol 27(1):14-19.

*McMurry ST, Lochmiller RL, Vestey MR, et al. 1994b. Immunological responses of weanling cotton rats (Sigmodon hispidus) to acute benzene and cyclophosphamide exposure. Bull Environ Contam Toxicol 52(1):155-162.

*McNeal TP, Nyman PJ, Diachenko GW, et al. 1993. Survey of benzene in foods by using headspace concentration techniques and capillary gas chromatography. Journal of AOAC International 76:1213-1219.

*Medinsky M A. 1995. The application of physiologically based pharmacokinetic-phaimacodynamic (PBPK-PD) modeling to understanding the mechanism of action of hazardous substances. Toxicology Letters 79:185-191.

*Medinsky MA, Kenyon E, Seaton MJ, et al. 1996. Mechanistic considerations in benzene physiological model development. Environ Health Perspect 104 (Suppl 6):1399-1404.

*Medinsky MA, Sabourin PJ, Henderson RF, et al. 1989b. Differences in the pathways for metabolism of benzene in rats and mice simulated by a physiological model. Environ Health Persp 82:43-49.

*Medinsky MA, Sabourin PJ, Lucier G, et al. 1989a. A physiological model for simulation of benzene metabolism by rats and mice. Toxicol Appl Pharmacol 99:193-206.

*Medinsky MA, Sabourin PJ, Lucier G, et al. 1989c. A toxicokinetic model for simulation of benzene metabolism. Exp. Pathol. 37:150-154.

*Melikian AA, Prahalad AK, Hoffmann D. 1993. Urinary *trans,trans*-muconic acid as an indicator of exposure to benzene in cigarette smokers. Cancer Epidemiol Biomarkers Prev 2(1):47-51.

*Melikian AA, Prahalad AK, Seeker-Waker RH. 1994. Comparison of the levels of the urinary benzene metabolite *trans*,trans,-muconic acid in smokers and nonsmokers, and the effects of pregnancy. Cancer Epidemiology 3:239-244.

*Merck Index. 1989. Merck index: an encyclopedia of chemicals, drugs, and biologicals. 1lth ed. Budavari S, ed. Rahway NJ: Merck & Co., Inc.

*Meyne J, Legator MS. 1980. Sex-related differences in cytogenetic effects of benzene in the bone marrow of Swiss mice. Environ Mutagen 2:43-50.

*Michael LC, Erickson MD, Parks SP, et al. 1980. Volatile environmental pollutants in biological matrices with a headspace purge technique. Anal Chem 52: 1836-l 841.

*Michael LC, Pellizzari ED, Wiseman RW. 1988. Development and evaluation of a procedure for determining volatile organics in water. Environ Sci and Technol 22:565-570.

*Michon S. 1965. [Disturbances of menstruation in women working in an atmosphere polluted with aromatic hydrocarbons.] Pol Tyg Lek 20:1648-1649. (Polish)

*Midzenski MA, McDiarmid MA, Rothman N, et al. 1992. Acute high dose exposure to benzene in shipyard workers. Am J Ind Med 22:553-565.

*Mikesell L MD, Kukor JJ, Olsen RH. 1993. Metabolic diversity of aromatic hydrocarbon-degrading bacteria from a petroleum-contaminated aquifer. Biodegradation 4(4):249-259.

*Miller AC, Schattenberg DG, Malkinson AM, et al. 1994. Decreased content of the ill alpha processing enzyme calpain in murine bone marrow-derived macrophages after treatment with the benzene metabolite hydroquinone. Toxicol Lett 74 (2): 177-184.

*Miller MM, Wasik SP, Huang G-L, et al. 1985. Relationships between octanol-water partition coefficients and aqueous solubility. Environ Sci Technol 19:522-529.

*Mills NE, Hayes RB, Fishman CL, et al. 1994. Ras mutations in Chinese patients with benzene-associated and de novo acute leukemia. Abstracts Submitted To The 36th Annual Meeting Of The American Society Of Hematology, Nashville, Tennessee, USA, December 2-6, 1994. Blood 84(10 Suppl. 1) 47a.

*Moen BE, Hollund BE, Berntsen M, et al. 1995a. Occupational exposure of deck crews to carcinogenic agents on crude oil tankers. American Journal Of Industrial Medicine 27(4): 555-564.

*Moen BE, Hollund BE, Berntsen M, et al. 1995b. Exposure of the deck crew to carcinogenic agents on oil product tankers. Ann Occup Hyg 39 (3):347-361.

*Moeschlin S, Speck B. 1967. Experimental studies on the mechanism of action of benzene on the bone marrow (radioautographic studies using ^{3}H-thymidine). Acta Haematol 38: 104-l 11.

*Money CD, Gray CN. 1989. Exhaled breath analysis as a measure of workplace exposure to benzene ppm. Ann Occup Hyg 33:257-262.

*Morikawa M, Imanaka T. 1993. Isolation of a new mixotrophic bacterium which can fix CO_2 and assimilate aliphatic and aromatic hydrocarbons anaerobically. J Ferment Bioengin 76(4):280-283.

*Morimoto K. 1976. Analysis of combined effects of benzene with radiation on chromosomes in cultured human leukocytes. Jpn J Ind Health 18:23-34.

*Morimoto K. 1983. Induction of sister chromatid exchanges and cell division delays in human lymphocytes by microsomal activation of benzene. Cancer Res 43:1330-1334.

*Morimoto K, Wolff S, Koizumi A. 1983. Induction of sister-chromatid exchanges in human lymphocytes by microsomal activation of benzene metabolites. Mutat Res 119:355-360.

*Mukhametova IM, Vozovaya MA. 1972. [Reproductive power and the incidence of gynecological affections in female workers exposed to the combined effect of benzene and chlorinated hydrocarbons.] Gig Tr Prof Zabol 16:6-9. (Russian)

*Mullin AH, Rando R, Esmundo F, et al. 1995. Inhalation of benzene leads to an increase in the mutant frequencies of a 1acZ transgene in lung and spleen tissues of mice. Mutat Res 327:121-129.

*Murray FJ, John JA, Rampy LW, et al. 1979. Embryotoxicity of inhaled benzene in mice and rabbits. Am Ind Hyg Ass J 40:993-998.

*Mushrush GW, Mose DG, Sullivan KT. 1994. Soil vapor and groundwater analysis from a recent oil spill. Bull Environ Contam Toxicol 52:31-38.

*Naft BN. 1992. Removing Benzene from Wastewater. Environ Protect 3(2):47-54.

*Nahum LH, Hoff HE. 1934. The mechanism of sudden death in experimental acute benzol poisoning. J Pharmacol Exp Ther 50:336-345.

*Nakajima T, Okino T, Sato A. 1987. Kinetic studies on benzene metabolism in rat liver-possible presence of three forms of benzene metabolizing enzymes in the liver. Biochem Pharmacol 36:2799-2804.

*Nakajima T, Okuyama S, Yonekura I, et al. 1985. Effects of ethanol and phenobarbital administration on the metabolism and toxicity of benzene. Chem-Biol Interact 55:23-38.

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

*NAS/NRC. 1993. Compilation of Current EEGLs and CEGLs. Nuclear Regulatory Commission and National Academy of Science.

*NATICH 1990. NATICH Data Base Report of Federal, State, and Local Air Toxics Activities. Table 4-1. Acceptable Ambient Concentration Guidelines or Standards. U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards. Washington, DC. September 17, 1990.

*Nerland DE, Pierce WM Jr. 1990. Identification of N-acetyl-S-(2,5-dihydroxyphenyl)-L-cysteine as a urinary metabolite of benzene, phenol, and hydroquinone. Drug Metab Dispos 18(6):958-961.

*NESCAUM. 1989. Evaluation of the health effects from exposure to gasoline and gasoline vapors. Final Report. Northeast States for Coordinated Air Use Management, Air Toxics Committee. 3-l - 3-16, 5-l - 5-33.

*Nesnow S, Argus M, Bergman H, et al. 1986. Chemical carcinogens: A review and analysis of the literature of selected chemicals and the establishment of the gene-tox carcinogen data base. Mutat Res 185:1-195.

*Neun DJ, Penn A, Snyder CA. 1992. Evidence for strain-specific differences in benzene toxicity as a function of host target cell susceptibility. Arch Toxicol 66(l):ll-17.

*Neun DJ, Penn A, Snyder CA. 1994. Erythroid progenitor cells that survive benzene exposure exhibit greater resistance to the toxic benzene metabolites benzoquinone and hydroquinone. Arch Toxicol 68535-540.

NFPA. 1994. Benzene. Fire Protection guide to hazardous materials. 11th edition. National Fire Protection Association. One Batterymarch Park, Quincy, MA.

*Niculescu R, Kalf GF. 1995. A morphological analysis of the short-term effects of benzene on the development of the hematological cells in the bone marrow of mice and the effects of interleukin-1 alpha on the process. Arch Toxicol 69(3):141-148.

*Nielsen PH, Bjerg PL, Nielsen P, et al. 1996. *In situ* and laboratory determined first-order degradation rate constants of specific organic compounds in an aerobic aquifer. Environ Sci Technol 30:31-37.

*Ning H, Kado NY, Kuzmicky PA, et al. 1991. Benzene-induced micronuclei formation in mouse fetal liver blood, peripheral blood, and maternal bone marrow cells. Environ Mol Mutagen 18(1):1-5.

*NIOSH. 1974. Criteria for a recommended standard. Occupational exposure to benzene. Washington, DC: U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health. NIOSH 74-137.

*NIOSH. 1984. NIOSH manual of analytical methods, Vol. 2, 3rd ed. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, 1500-l - 1501-7.

*NIOSH. 1987. NIOSH manual of analytical methods, Vol. 1, 3rd ed. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, 3700-l - 3700-3.

*NIOSH. 1988. Occupational safety and health guideline for benzene: Potential human carcinogen Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Division of Standards Development and Technology Transfer. NIOSH publication no. 89-104, Supp. II.

*NIOSH 1989. National Occupational Exposure survey 1981-83. U.S. Department of Health and Human Services. National Institute for Occupational Safety and Health, Cincinnati, OH.

*NIOSH. 1990. NIOSH pocket guide to chemical hazards. Washington, DC: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health. DHHS (NIOSH) publication no. 90-117.

*NIOSH. 1992. NIOSH Recommendations for Occupational Safety and Health: Compendium of Policy Documents and Statements. Table 1. NIOSH Recommended Safety and Health Standards for Hazardous Agents in the Workplace.

*NOAA. 1994. Natural Resource Damage Assessments: Proposed Rule. National Oceanic and Atmospheric Administration. 59 FR 1062.

*NOES. 1990. National Occupational Exposure Survey 1981-83. U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, Cincinnati, OH. July 1, 1990.

*Nojima K, Fukaya K, Fukui S, et al. 1975. Studies on photochemistry of aromatic hydrocarbons: II. The formation of nitrophenols and nitrobenzene by the photochemical reaction of benzene in the presence of nitrogen monoxide. Chemosphere 2:77-82.

*Nomiyama K, Nomiyama H. 1974a. Respiratory retention, uptake and excretion of organic solvents in man: Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int Arch Arbeitsmed 32:75-83.

*Nomiyama K, Nomiyama H. 1974b. Respiratory elimination of organic solvents in man: Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int Arch Arbeitsmed 32:85-91.

*Norpoth KH, Mtiller G, Schell CH et al. 1996. Phenylguanine found in urine after benzene exposure. Environ Health Perspect 104 (Suppl 6): 1159-l163.

*Norpoth K, Stuker W, Krewet E, et al. 1988. Biomonitoring of benzene exposure by trace analyses of phenylguanine. Int Arch Occup Environ Health 60:163-168.

*Norwalk CK, Howland MA. 1994. Hydrocarbons. In: Goldfrank's Toxicologic Emergencies, 5th ed. 1231-1244.

*NRC. 1980a. Drinking water and health. Vol. 3. Washington, DC: National Academy of Sciences, National Research Council, National Academy Press, 70, 80-86.

*NRC. 1986. Emergency and continuous exposure guidance levels for selected airborne contaminants. Vol 6. Washington, DC: National Research Council, National Academy Press, 7-33

*NREPC. 1986. Proposed Regulation. Frankfort, KY: Department for Environmental Protection, Natural Resources and Environment Protection Cabinet. 401 KAR 63:021.

*NREPC. 1988. Kentucky Waste Management Regulations. Frankfort, KY: Department for Environmental Protection, Natural Resources and Environment Protection Cabinet. 401 KAR 31:040.

*NTP. 1986. National Toxicology Program--technical report series no. 289. Toxicology and carcinogenesis studies of benzene (CAS No. 71-43-2) in F344/N rats and B6C3F$_1$ mice (gavage studies). Research Triangle Park, NC: U.S. Department of Health and Human Services;Public Health Service, National Institute of Health. NIH publication no. 86-2545.

*NTP. 1994. Seventh Annual Report on Carcinogens: 1994 Summary. National Toxicology Program. Benzene. USDHHS PHS.

NTP 1995. Management Status Report. Printed long-term technical reports and short-term toxicity study reports. NTP Division of Toxicological Research and Testing

*Nutmagul W, Cronn DR. 1985. Determination of selected atmospheric aromatic hydrocarbons at remote continental and oceanic locations using photoionization/flame-ionization detection. J Atmos Chem 2:415-433.

*O'Bryan TR, Ross RH. 1988. Chemical scoring system for hazard and exposure identification. J Toxicol Environ Health 1: 119- 134.

*Oberly TJ, Bewsey BJ, Probst GS. 1984. An evaluation of the L5178Y TK+P mouse lymphoma forward mutation assay using 42 chemicals. Mutat Res 125:291-306.

*Ogata M, Fujisawa K, Ogino Y, et al. 1984. Partition coefficients as a measure of bioconcentration potential of crude oil compounds in fish and shellfish. Bull Environ Contam Toxicol 33:561-567.

*Ogata M, Taguchi T. 1987. Quantitation of urinary metabolites of toluene, xylene, styrene, ethylbenzene, benzene and phenol by automated high performance liquid chromatography. Int Arch Occup Environ Health 59:263-272.

*OHM/TADS 1990. Oil and Hazardous Materials/Technical Assistance Data System. Chemical Information Systems, Inc. Baltimore, MD.

*Olsen J. 1983. Organic solvents as possible risk factors of low birthweight. J Occup Med 25:854-855.

*Olsen RH, Mikesell MD, Kukor JJ. 1994. Enumeration and characterization of btex-degrading bacteria from hypoxic environments functional with mixed electron acceptors. Res microbial 145(1):47-49.

*Ong CN, Kok PW, Lee BL, et al. 1995. Evaluation of biomarkers for occupational exposure to benzene. Occup Environ Med 52 (8):528-533.

*Ong CN, Lee BL. 1994. Determination of benzene and its metabolites: application in biological monitoring of environmental and occupational exposure to benzene. J Chromatogr B Biomed Appl 660 (l):l-22.

*Orzechowski A, Schwarz L R, Schwegler U et al. 1995. Benzene metabolism in rodent hepatocytes: role of sulphate conjugation. Xenobiotica 25(10):1093-1102.

*OSHA. 1974. Table 2-2. Occupational Safety and Health Standards. Air Contaminants. U.S. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1000.

*OSHA. 1976. Permissible Exposure Limit. Occupational Safety and Health Standards. Coke Oven Emissions. U.S. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1029(c).

*OSHA. 1977. Final environmental impact statement: Benzene. Washington, DC: U.S. Department of Labor, Occupational Safety and Health Administration. Federal Register 42:22516-22529, 27460-27464.

*OSHA. 1985. Occupational exposure to benzene. Proposed rule and notice of hearing. U.S. Department of Labor, Occupational Safety and Health Administration. Federal Register 50505 12-505 17, 50540, 50528-50529, 50577-50582.

*OSHA. 1987a. Benzene. U.S. Department of Labor. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1028.

*OSHA. 1987b. Occupational exposure to benzene. Final Rule. Federal Register 52:34460-34578.

*OSHA. 1989. Air contaminants. Final rule. Occupational Safety and Health Administration. Federal Register 54:2332-2960.

*OSHA. 1990. National Research Council Recommendations Concerning Chemical Hygiene in Laboratories (Non-Mandatory). Occupational Safety and Health Standards. U.S. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1450, Appendix A.

OSHA. 1994. Respiratory Protection. Occupational Safety and Health Administration. 59 FR 58884.

*Ott MG, Townsend JC, Fishbeck WA, et al. 1978. Mortality among workers occupationally exposed to benzene. Arch Environ Health 33:3-10.

*Page BD, Conacher HBS, Salminen J, et al. 1993. Survey of bottled drinking water sold in Canada. II. Selected volatile organic compounds. J Assoc Off Anal Chem Int 76:26-31.

*Pagnotto LD, Elkins HB, Brugsch HG, et al. 1961. Industrial benzene exposure from petroleum naphtha: I. Rubber coating industry. Am Ind Hyg Assoc J 22:417-421.

*Painter RB, Howard R. 1982. The HeLa DNA-synthesis inhibition test as a rapid screen for mutagenic carcinogens. Mutat Res 92:427-437.

*Pandya KP, Rao GS, Khan S, et al. 1990. Accumulation of low molecular weight (bleomycin detectable) iron in bone marrow cells of rats after benzene exposure. Arch Toxicol 64(4):339-342.

*Parke DV. 1989. Introduction: Session on metabolism. Environ Health Perspect 82:7-8.

*Parke DV, Williams RT. 1953b. Studies in Detoxification 54. The metabolism of benzene. (a) The formation of phenylglucuronide & phenylsulphuric acid from [^{14}C] benzene (b) the metabolism of [^{14}C$_1$]phenol. Biochem J 54:337-340.

*Parke DV, Williams RT. 1953a. Studies in detoxication. 49. The metabolism of benzene containing [^{14}C$_1$] benzene. Biochem J 54:231-238.

*Pate CT, Atkinson R, Pitts JN Jr. 1976. The gas phase reaction of O_3 with a series of aromatic hydrocarbons. J Environ Sci Health AII: l-10.

*Pathak DN, Levay G, Bode11 WJ. 1995. DNA adduct formation in the bone marrow of B6C3F$_1$ mice treated with benzene. Carcinogenesis 16(8): 1803- 1808.

*Patil SF, Lonkar ST. 1992. Thermal desorption-gas chromatography for the determination of benzene, aniline, nitrobenzene and chlorobenzene in workplace air [published erratum appears in J Chromatogr 1992 Nov 20;625(2):402]. J Chromatogr 600(2):344-3.5 1.

*Paustenbach DJ, Price PS, Ollison W, et al. 1992. Reevaluation of benzene exposure for the Pliofilm (rubberworker) cohort (1936-1976). J Toxicol Environ Health 36(3):177-231.

*Pawar SS, Mungikar AM. 1975. Changes in the activities of hepatic drug metabolizing enzymes and lipid peroxidation caused by benzene and toluene. Indian J Biochem Biophys 12:133-134.

*Paxman D, Rappaport SM. 1990. Analysis of OSHA's short-term-exposure limit for benzene. Regul Toxicol Pharmacol 11(3):275-287.

*Paxton MB. 1996. Leukemia risk associated with benzene exposure in the pliofilm cohort. Environ Health Perspect 104 (Suppl 6):1431-1436.

*Paxton MB, Chinchilli VM, Breet SM, et al. 1994a. Leukemia risk associated with benzene exposure in the pliofilm cohort: I. mortality update and exposure distribution. Risk Analysis 14(2):147-154.

*Paxton MB, Chinchilli VM, Brett SM, et al. 1994b. Leukemia risk associated with benzene exposure in the pliofilm cohort. II. risk estimates. Risk Anal 14(2):155-161.

*Pekari K, Riekkola M-L, Aitio A. 1989. Simultaneous determination of benzene and toluene in blood using head-space gas chromatography. J Chromatogr 491:309-320.

*Pekari K, Vainiotalo S, Heikkila P, et al. 1992. Biological monitoring of occupational exposure to low levels of benzene. Stand J Work Environ Health 18(5):317-322.

*Pellack-Walker P, Blumer JL. 1986. DNA damage in L5178YS cells following exposure to benzene metabolites. Mol Pharmacol 30:42-47.

*Pellizzari ED, Lioy P, Quackenboss J, et al. 1995. Population-based exposure measurements in EPA region 5: a phase i field study in support of the national human exposure assessment survey. Journal Of Exposure Analysis and Environmental Epidemiology 15(3):327-358.

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

*Pellizzari ED, Zweidinger RA, Sheldon LS. 1988. Determination of benzene, toluene and xylene in breath samples by gas chromatography/mass spectrometry. In: Fishbein L, O'Neill JK, eds. Environmental carcinogens method of analysis and exposure measurement: Vol. lo-benzene and alkylated benzene. IARC Scientific Publication No. 85. Lyons, France: World Health Organization, International Agency for Research on Cancer, 267-279.

*Perbellini L, Faccini GB, Pasini F, et al. 1988. Environmental and occupational exposure to benzene by analysis of breath and blood. Br J Ind Med 45:345-352.

*Pereira WE, Hughes BA. 1980. Determination of selected volatile organic priority pollutants in water by computerized gas chromatography-quadrupole mass spectrometry. J Am Water Works Assoc 72:220-230.

*Philip P, Jensen MK. 1970. Benzene induced chromosome abnormalities in rat bone marrow cells. Acta Pathol Microbial Stand Sect A 78:489-490.

*Picciano D. 1979. Cytogenetic study of workers exposed to benzene. Environ Res 19:33-38.

*Plappert U, Barthel E, Raddatz K, et al. 1994a. Early effects of benzene exposure in mice. haematological versus genotoxic effects. Arch Toxicol 68:284-290.

*Plappert U, Barthel E, Seidel HJ, et al. 1994b. Reduction of benzene toxicity by toluene. Environ Mol Mutagen 24:283-292.

*Plumb RH Jr. 1987. A comparison of groundwater monitoring data from CERCLA and RCRA sites. Ground Water Monit Rev 7:94-100.

*Poole SK, Furton KG, Poole CF. 1988. Determination of benzene and toluene in gasoline by gas chromatography using a liquid organic salt column. J Chromatogr Sci 26:67-73.

*Popp W, Rauscher D, Muller G, et al. 1994. Concentrations of benzene in blood and s-phenylmercapturic and t,t-muconic acid in urine in car mechanics. Int Arch Occup Environ Health 1994 66(1):1-6.

*Popp W, Vahrenholz C, Yaman S, et al. 1992. Investigations of the frequency of DNA strand breakage and cross-linking and of sister chromatid exchange frequency in the lymphocytes of female workers exposed to benzene and toluene. Carcinogenesis 13(1):57-61.

*Post GB, Snyder R, Kalf GF. 1985. Inhibition of RNA synthesis and interleukin-2 production in lymphocytes *in vitro* by benzene and its metabolites, hydroquinone and p-benzoquinone. Toxicol Lett 29:161-167.

*Preston WD. 1986. Executive Summary. In: Benzene in Florida groundwater: An assessment of the significance to human health. Tallahassee, FL: Florida Petroleum Council, American Petroleum Institute.

*Probst GS, Hill LE. 1985. Tests for the induction of DNA-repair synthesis in primary cultures of adult rat hepatocytes. Progress in Mutation Research 5:381-386.

*Punjabi CJ, Laskin JD, Hwang SM, et al. 1994. Enhanced production of nitric oxide by bone marrow cells and increased sensitivity to macrophage colony-stimulating factor (csf) and granulocyte-macrophage csf after benzene treatment of mice. Blood Jun 1 1994 83(11):3255-3263.

*Purcell KJ, Cason GH, Gargas ML, et al. 1990. *In vivo* metabolic interactions of benzene and toluene. Toxicol Lett 52(2):141-152.

*Purcell WP. 1978. Benzene. In: Grayson M, Eckroth D, eds. Kirk-Othmer Encyclopedia of Chemical Technology. 3rd ed. Vol. 3. New York, NY: John Wiley and Sons, 744-771.

*Raabe GK, Wong O. 1996. Leukemia mortality by cell type in petroleum workers with potential exposure to benzene. Environ Health Perspect 104 (Suppl 6): 1381-1392.

*Rajganesh B, Selvaraj PT, Manning FS, et al. 1995. Biotreatment of produced water for removal of sulfides, organics, and toxicity. Appl Biochem Biotech 51/52:735-746.

*Ramsey JC, Andersen ME. 1984. A physiologically based description the inhalation pharmacokinetics of styrene in rats and humans. Toxicol Appl Pharmacol 73: 159-175.

*Rae GS. 1991. Hematin catalyzed autoxidation of hydroquinone or 1,2,4-benzenetriol. Chem Biol Interactions 80:339-347.

*Rao GS. 1996. Glutathionyl hydroquinone: a potent pro-oxidant and a possible toxic metabolite of benzene. Toxicology 106:49-54.

*Rae GS, Pandya KP. 1989. Release of 2-thiobarbituric acid reactive products from glutamate or deoxyribonucleic acid by 1,2,4-benzenetriol or hydroquinone in the presence of copper ions. Toxicology 59: 59-65.

*Rae RR, Snyder R. 1995. Oxidative modifications produced in HL-60 cells on exposure to benzene metabolites. J Appl Toxicol 15(5):403-409.

*Rauscher D, Lehnert G, Angerer J. 1994. Biomonitoring of occupational and environmental exposures to benzene by measuring truns,trans-muconic acid in urine. Clin Chem 40:1468-1470.

*Reddy MV, Blackbum GR, Irwin SE, et al. 1989a. A method for *in vitro* culture of rat Zymbal gland: Use in mechanistic studies of benzene carcinogenesis in combination with ^{32}P-postlabeling. Environ Health Perspect 82:239-247.

*Reddy MV, Blackbum GR, Schreiner CA, et al. 1989b. 32P Analysis of DNA adducts in tissues of benzene-treated rats. Environ Health Perspect 82:253-257.

*Reddy, MR, Schultz SC, Blackbum GR, et al. 1994. Lack of DNA adduct formation in mice treated with benzene. Mutat Res 325(4): 149-155.

*Reineke FJ, Bachmann K. 1985. Gas chromatographic determination of C_2-C_8 hydrocarbons and halocarbons in ambient air by simultaneous use of three detectors. J Chromatogr 323:323-329.

*Reisch MS. 1992. Top 50 chemicals production stagnated last year. Chemical and Engineering News April 13: 16-20.

*Reisch MS. 1993. Top 50 chemicals production recovered last year. Chemical and Engineering News April 12: 10-13.

*Reisch MS. 1994. Top 50 chemicals production rose modestly last year. Chemical and Engineering News April 11:12-l 5.

*Renz JF, Kalf GF. 1991. Role for interleukin-1 (IL-l) in benzene-induced hematotoxicity: inhibition of conversion of pre-IL-l alpha to mature cytokine in murine macrophages by hydroquinone and prevention of benzene-induced hematotoxicity in mice by IL-l alpha. Blood 78(4):938-944.

*Rickert DE, Baker TS, Bus JS, et al. 1979. Benzene disposition in the rat after exposure by inhalation. Toxicol Appl Pharmacol 49:417-423.

*Rinsky RA, Homung RW, Landrigan PJ, et al. 1989. Re: "Benzene and leukemia: A review of the literature and a risk assessment." Am J Epidemiol 129:1084-1086.

*Rinsky RA, Smith AB, Homung R, et al. 1987. Benzene and leukemia: An epidemiological risk assessment. N Eng J Med 316:1044-1050.

*Rinsky RA, Young RJ, Smith AB. 1981. Leukemia in benzene workers. Am J Ind Med 2:217-245.

*Rithidech K, Au WW, Sadagopa Ramanujam VM, et al. 1987. Induction of chromosome aberrations in lymphocytes of mice after subchronic exposure to benzene. Mutat Res 188:135-140.

*Rithidech K, Au WW, Ramanujam VMS, et al. 1988. Persistence of micronuclei in peripheral blood normochromatic erythrocytes of subchronically benzene-treated male mice. Environ Mol Mutagen 12:319-329.

*Roberts JM, Fehsenfeld FC, Liu SC, et al. 1984. Measurements of aromatic hydrocarbon ratios and NO_x concentrations in the rural troposphere: Observation of air mass photochemical aging and NO_x removal. Atmos Environ 18:2421-2432.

*Roberts JM, Hutte RS, Fehsenfeld FC, et al. 1985. Measurements of anthropogenic hydrocarbon concentration ratios in the rural troposphere: Discrimination between background and urban sources. Atmos Environ 19: 1945-l 950.

*Robertson ML, Eastmond DA, Smith MT. 1991a. Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes. Mutat Res 249(1):201-210.

*Robertson ML, Zhang L, Smith MT. 1991b. Induction of micronuclei by the benzene metabolite 1,2,4- benzenetriol in human lymphocytes and HL-60 cells. Environ Mol Mutagen 17 (Suppl 19):61-62. (Abstract)

*Rosenstock L, Cullen M. 1994. Textbook of clinical occupational and environmental medicine. WB Saunders Co. Philadelphia, PA. 562-564.

*Rosenthal GJ, Snyder CA. 1985. Modulation of the immune response to *Listeria rnonocytogenes* by benzene inhalation. Toxicol Appl Pharmacol 80:502-510.

*Rosenthal GJ, Snyder CA. 1987. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. Toxicol Appl Pharmacol 88:35-43.

*Ross D, Siegel D, Schattenberg D. 1996a. Cell-specific activation and detoxification of benzene metabolites in mouse and human bone marrow: Identification of target cells and a potential role for modulation of apoptosis in benzene toxicity. Environ Health Perspect 104 (Suppl 6): 1177-l 182.

*Ross D, Siegel D, Schattenberg DG et al. 1996b. Cell-specific metabolism in benzene toxicity. A metabolic basis for benzene-induced toxicity at the level of the progenitor cell in human bone marrow. The Toxicologist 30(1):339.

*Rothman N, Smith MT, Hayes RB, et al. 1996. An epidemiological study of early biologic effects in Chinese workers. Environ Health Perspect 104 (Suppl 6):1365-1370.

*Rothman N, Haas R, Hayes RB, et al. 1995. Benzene induces gene-duplicating but not geneinactivating mutations at the glycophorin A locus in exposed humans. Proc Nat1 Acad Sci 92:4069-4073.

*Roush GJ, Ott MG. 1977. A study of benzene exposure versus urinary phenol levels. Am In Hyg Assoc J 38:67-75.

*Rozen MG, Snyder CA. 1985. Protracted exposure of C57BL/6 mice to 300 ppm benzene depresses B- and T-lymphocyte numbers and mitogen responses. Evidence for thymic and bone marrow proliferation in response to the exposures. Toxicology 37:13-26.

*Rozen MG, Snyder CA, Albert RE. 1984. Depression in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol Lett 20:343-349.

*Ruiz MA, August0 LGS, Vassallo J, et al. 1994. Bone marrow morphology in patients with neutropenia due to chronic exposure to organic solvents (benzene): early lesions. Path Res Pratt 190:151-154.

*Ruiz MA, Vassallo J, De Souza CA. 1991. Morphologic study of the bone marrow of neutropenic patients exposed to benzene of the metallurgical industry of Cubatao, Sao Paulo, Brazil [letter]. J Occup Med 33(1):83.

*Ruppert T, Scherer G, Tricker AR, et al. 1995. Determination of urinary *trans*, trans-muconic acid by gas chromatography-mass spectrometry. J Chromatogr B 666:71-76.

*Rushmore T, Snyder R, Kalf G. 1984. Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria *in vitro*. Chem-Biol Interact 49:133-154.

*Rushton L. 1996. Benzene exposure in the petroleum distribution industry associated with leukemia in the United Kingdom: Overview of the methodology of a case-control study. Environ Health Perspect 104 (Suppl 6):1371-1374.

*Sabourin PJ, Bechtold WE, Birnbaum LS, et al. 1988. Differences in the metabolism and disposition of inhaled [^{3}H]benzene by F344/N rats and B6C3F$_1$ mice. Toxicol Appl Pharmacol 94:128-140.

*Sabourin PJ, Bechtold WE, Griffith WC, et al. 1989a. Effect of exposure concentration, exposure rate, and route of administration on metabolism of benzene by F344 rats and B6C3F$_1$ mice. Toxicol Appl Pharmacol 99:421-444.

*Sabourin PJ, Chen BT, Lucier G, et al. 1987. Effect of dose on the absorption and excretion of [^{14}C]benzene administered orally or by inhalation in rats and mice. Toxicol Appl Pharmacol 87:325-336.

*Sabourin PJ, Muggenburg BA, Couch RC, et al. 1992. Metabolism of [^{14}C]benzene by cynomolgus monkeys and chimpanzees. Toxicol Appl Pharmacol 114(2):277-284.

*Sabourin PJ, Sun JD, Bimbaum LS, et al. 1989b. Effect of repeated benzene inhalation exposures on subsequent metabolism of benzene. Exp Pathol 37: 155-l57.

*Sabourin PJ, Sun JD, MacGregor JT, et al. 1990. Effect of repeated benzene inhalation exposures on benzene metabolism, binding to hemoglobin, and induction of micronuclei. Toxicol Appl Pharmacol 103:452-462.

*Saito FU, Kocsis JJ, Snyder R. 1973. Effect of benzene on hepatic drug metabolism and ultrastructure. Toxicol Appl Pharmacol 26:209-217.

*Salanitro JP. 1993. The role of bioattenuation in the management of aromatic hydrocarbon plumes in aquifers. Ground Water Monitoring and Remediation 13: 150-161.

*Sammett D, Lee EW, Kocsis JJ, et al. 1979. Partial hepatectomy reduces both the metabolism and toxicity of benzene. J Toxicol Environ Health 5:785-792.

*Sandmeyer EE. 1981. Aromatic hydrocarbons. In: Clayton GD, Clayton FE, eds. Patty's industrial hygiene and toxicology. Vol. 2B. 3rd rev. ed. New York, NY: John Wiley & Sons, 3253-3283.

*Sardas S, Karakaya AE, Furtun Y. 1994. Sister chromatid exchanges in workers employed in car painting workshops. Int Arch Occup Environ Health 66:33-35.

*Sasiadek M. 1992. Nonrandom distribution of breakpoints in the karyotypes of workers occupationally exposed to benzene. Environ Health Perspect 97:255-257.

*Sasiadek M, Jagielski J. 1990. Genotoxic effects observed in workers occupationally exposed to organic solvents. Pol J Occup Med 3(1):103-108.

*Sasiadek M, Jagielski J, Smolik R. 1989. Localization of breakpoints in the karyotype of workers professionally exposed to benzene. Mutat Res 224:235-240.

*Sato A, Nakajima T. 1979a. Dose-dependent metabolic integration between benzene and toluene in viva and *in vitro*. Toxicol Appl Pharmacol 48:249-256.

*Sato A, Nakajima T. 1979b. Partition coefficients of some aromatic hydrocarbons and ketones in water, blood and oil. Br J Ind Med 36:231-234.

*Sato A, Nakajima T. 1985. Enhanced metabolism of volatile hydrocarbons in rat liver following food deprivation, restricted carbohydrate intake, and administration of ethanol, phenobarbital, polychlorinated biphenyl and 3-methylcholanthrene: A comparative study. Xenobiotica 15:67-75.

*Sato A, Nakajima T, Fujiwara Y, et al. 1975. Kinetic studies on sex differences in susceptibility to chronic benzene intoxication - with special reference to body fat content. Br J Ind Med 32:321-328.

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

*Sawahata T, Neal RA. 1983. Biotransformation of phenol to hydroquinone and catechol by rat liver microsomes. Mol Pharmacol 23:453-460.

*Sax NI, Lewis RJ. 1987. Hawley's Condensed Chemical Dictionary. 11th ed. New York, NY: Van Nostrand Reinhold Co., 129.

*Saxton J, Narkus-Kramer M. 1975. EPA findings of solid waste from industrial chemicals. Chemical Engineering 82:107-112.

*Schad H, Schafer F, Weber L, et al. 1992. Determination of benzene metabolites in urine of mice by solid-phase extraction and high-performance liquid chromatography. J Chromatog 593:147-151.

*Schafer F, Schad H, Weber L. 1993. Determination of phenylmercapturic acid in urine of benzene-exposed BDF-1 mice. J Chromatog 620:239-242.

*Schattenberg DG, Stillman WS, Gruntmeir JJ, et al. 1994. Peroxidase activity in murine and human hematopoietic progenitor cells: potential relevance to benzene-induced toxicity. Mol Pharmacol 46(2):346-351.

*Scheding S, Loeffler M, Schmitz S, et al. 1992. Hematotoxic effects of benzene analyzed by mathematical modeling. Toxicology 72:265-279.

*Scherer G, Ruppert T, Daube H, et al. 1995. Contribution of tobacco smoke to environmental benzene exposure in Germany. Environ Int 21(6):779-789.

*Scheunert I, Topp E, Schmitzer J, et al. 1985. Formation and fate of bound residues on [^{14}C]benzene and [^{14}C]chlorobenzenes in soil and plants. Ecotox Environ Safety 9: 159-170.

*Schlosser PM, Bond JA, Medinsky MA. 1993. Benzene and phenol metabolism by mouse and rat liver microsomes. Carcinogenesis 14:2477-2486.

*Schoenfeld HA, Radbourne R, Goldstein BD et al. 1996. Mechanistic studies on benzene toxicity: formation of DNA-protein crosslinks induced by benzene in mice and muconaldehyde in HL60 cells. Fundamental and Applied Toxicology. The Toxicologist 30(1):232.

*Schoeters G E R, Vander Plaetse F, Leppens H, et al. 1995. Haemopoietic and osteogenic toxicity testing *in vitro* using murine bone marrow cultures. Toxicology *in vitro* 9 (4): 421-428.

*Schrenk D, Bock KW. 1990. Metabolism of benzene in rat hepatocytes. Influence of inducers on phenol glucuronidation. Drug Metab Dispos 18(5):720-725.

*Schrenk D, Ingelman-Sundberg M, Bock KW. 1992. Influence of P-4502El induction on benzene metabolism in rat hepatocytes and on biliary metabolite excretion. Drug Metab Dispos 20(2): 137-141.

*Schrenk D, Orzechowski A, Schwarz LR. 1996. Phase II metabolism of benzene. Environ Health Perspect 104 (Suppl 6):1183-1188.

*Schrenk HH, Yant WP, Pearce SJ, et al. 1941. Absorption, distribution and elimination of benzene by body tissues and fluids of dogs exposed to benzene vapor. J Ind Hyg Toxicol 23:20-34.

*Schuberth J. 1994. Joint use of retention index and mass spectrum in post-mortem test for volatile organics by headspace capillary gas chromatography with ion-trap detection. J Chromatogr A 674:63-71.

*Schultz B, Kjeldsen P. 1986. Screening of organic matter in leachates from sanitary landfills using gas chromatography combined with mass spectrometry. Water Res 20:965-970.

*Seaton MJ, Asgharian B, Farris G. 1996. Effects of benzene and unleaded gasoline coexposures on benzene metabolism and toxicity. Fundamental and applied toxicology. The Toxicologist. 30(1):219.

*Seaton MJ, Schlosser PM, Bond JA, et al. 1995. The potential influence of interindividual variation in phase I and phase II metabolism of benzene on benzene toxicity. In: Abstracts of the 34th annual meeting. Toxicologist 15(1):58-59.

*Seidel HJ, Bader R, Weber L, et al. 1990. The influence of ethanol on the stem cell toxicity of benzene in mice. Toxicol Appl Pharmacol 105(1):13-18.

*Seidel HJ, Barthel E, Schafer F, et al. 1991. Action of benzene metabolites on murine hematopoietic colony-forming cells *in vitro.* Toxicol Appl Pharmacol 111(l): 128- 13 1.

*Seidel HJ, Barthel E, Zinser D. 1989a. The hematopoietic stem cell compartments in mice during and after long-term inhalation of three doses of benzene. Exp Hematol 17:300-303.

*Seidel HJ, Beyvers G, Pape M, et al. 1989b. The influence of benzene on the erythroid cell system in mice. Exp Hematol 17:760-764.

*Seidenberg JM, Anderson DG, Becker RA. 1986. Validation of an *in vivo* developmental toxicity screen in the mouse. Teratog Carcinog Mutagen 6:361-374.

*Seiji K, Jin C, Watanabe T, et al. 1990. Sister chromatid exchanges in peripheral lymphocytes of workers exposed to benzene, trichloroethylene, or tetrachloroethylene, with reference to smoking habits. Int Arch Occup Environ Health 62:171-176.

*Seixas GM, Andon BM, Hollingshead PG, et al. 1982. The aza-arenes as mutagens for *Salmonella typhimurium.* Mutat Res 102:201-212.

*Selling L. 1916. Benz01 as leucotoxin--Studies on the degeneration and regeneration of the blood hematopoietic organs. Johns Hopkins Hosp Rep 17:83-142.

*Sellyei M, Kelemen E. 1971. Chromosome study in a case of granulocytic leukemia with "pelgerisation" 7 years after benzene pancytopenia. Eur J Cancer 7:83-85.

*Serraino D, Franceschi S, LeVecchio C, et al. 1992. Occupation and soft-tissue sarcoma in northeastern Italy. Cancer Causes and Controls 3:25-30.

*Shah JJ, Singh HB. 1988. Distribution of volatile organic chemicals in outdoor and indoor air. Environmental Science and Technology 22: 1381-1388.

*Shamy MY, El Gazzar RME, El Sayed MA, et al. 1994. Study of some biochemical changes among workers occupationally exposed to phenol, alone or in combination with other organic solvents. Ind Health 32:207-214.

*Shankar U, Kumar A, Rao GS, et al. 1993. Modulation of benzene induced toxicity by protein A. Biochem Pharmacol 46(3):517-524.

*Sharma RK, Jacobsen-Kram D, Lemmon M, et al. 1985. Sister-chromatid exchange and cell replication kinetics in fetal and maternal cells after treatment with chemical teratogens. Mutat Res 158:217-231.

*Shelby MD, Erexson GL, Hook GJ, et al. 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. Environ Mol Mutagen 21(2):160-179.

*Shelby MD, Witt KL. 1995. Comparison of results from mouse bone marrow chromosome aberration and micronucleus test. Environ Mol Mutagen 25:302-313.

*Shell. 1980. Initial Submission: A dominant-lethal inhalation study with benzene in rats (final report) with attachments and cover letter dated 041492 EPA/OTS; Dot 88-920002041.

*Shell. 1992. Initial Submission: Draft Report on immunosuppression of B6C3F$_1$ female mice following subchronic Exposure to benzene from drinking water EPA/OTS; Dot #88-920002020.

*Sherwood RJ. 1972. Benzene: The interpretation of monitoring results. Ann Occup Hyg 15:409-421.

*Sherwood RJ. 1988. Pharmacokinetics of benzene in a human after exposure at about the permissible limit. Ann NY Acad Sci 534:635-647.

*Sherwood RJ, Carter FWG. 1970. The measurement of occupational exposure to benzene vapour. Ann Occup Hyg 13:125-146.

Shuckrow AJ, Pajak AP, Touhill CJ. 1982. Management of hazardous waste leachate. Contract No. 68-03-2766. Municipal Environmental Research Laboratory, Office of Research and Development, EPA, Cincinnati. OH

*Sidhu KS, Chadzynski L. 1994. Risk communication: Health risks associated with environmentally contaminated private wells versus chloroform in a public water supply. J Environ Health 56(10):13-16.

*Singh GB, Salas LJ, Cantrell BK, et al. 1985. Distribution of aromatic hydrocarbons in ambient air. Atmos Environ 19:1911-1919.

*Singh V, Ahmad S, Rao GS. 1994. Prooxidant and antioxidant properties of iron-hydroquinone and iron-1,2,4-benzenetriol complex. implications for benzene toxicity. Toxicology 89(1):25-33.

*Siou G, Conan L, el Haitem M. 1981. Evaluation of the clastogenic action of benzene by oral administration with 2 cytogenetic techniques in mouse and Chinese hamster. Mutat Res 90:273-278. Sittig M, ed. 1994a. Pesticide manufacturing and toxic materials control encyclopedia. Park Ridge, NJ: Noyes Data Corporation, 366-373.

Sittig M. 1994b. Worldwide limits for toxic and hazardous changes in air, water, and soil. Park Ridge, NJ: Noyes Publications, page 78.

*Skowronski GA, Turkall RM, Abdel-Rahman MS. 1988. Soil adsorption alters bioavailability of benzene in dermally exposed male rats. Am Ind Hyg Assoc J 49:506-511.

*Smith MT, Rothman N, Zhang L. et al. 1996. Molecular cytogenetics of humans exposed to benzene. Fund Applied Toxicology. The Toxicologist. 30(1):178.

*Smith MT, Yager JW, Steinmetz KL, et al. 1989. Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. Environ Health Perspect 82:23-29.

*Smith MT, Zhang L, Rothman N, et al. 1995. Interphase cytogenetics of workers exposed to benzene. In: Abstracts of the 34th annual meeting. Toxicologist 15(1):87.

*Smyth HF, Carpenter CP, Weil CS, et al. 1962. Range-finding toxicity data: List VI. J Ind Hyg Assoc 23:95-107.

*Snyder CA. 1987. Benzene. In: Snyder R, ed. Ethyl Browning's toxicity and metabolism of industrial solvents, 2nd ed., Vol. 1: Hydrocarbons.

*Snyder CA, Erlichman MN, Laskin S, et al. 1981b. The pharmacokinetics of repetitive benzene exposures at 300 and 100 ppm in AKR mice and Sprague-Dawley rats. Toxicol Appl Pharmacol 57:164-171.

*Snyder CA, Goldstein BD, Sellakumar A. 1978a. Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. J Toxicol Environ Health 4:605-618.

*Snyder CA, Goldstein BD, Sellakumar AR. 1984. Evidence for hematotoxicity and tumorigenesis in rats exposed to 100 ppm benzene. Am J Ind Med 5:429-434.

*Snyder CA, Goldstein BD, Sellakumar AR, et al. 1980. The inhalation toxicology of benzene: Incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. Toxicol Appl Pharmacol 54:323-33 1.

*Snyder CA, Green JD, LoBue J, et al. 1981~. Protracted benzene exposure causes a proliferation of myeloblasts and/or promyelocytes in CD-l mice. Bull Environ Contam Toxicol 27:17-22.

*Snyder CA, Sellakumar AR, James DJ, et al. 1988. The carcinogenicity of discontinuous inhaled benzene exposures in CD-l and C57BL/6 mice. Arch Toxicol 62:331-335.

*Snyder R, Hedli CC. 1996. An overview of benzene metabolism. Environ Health Perspect 104 (Suppl 6):1165-1171.

*Snyder R, Chepiga T, Yang CS, et al. 1993a. Benzene metabolism by reconstituted cytochromes P450, 2B1, and 2El and its modulation by cytochrome b5, microsomal epoxide hydrolase, and glutathione transferases: evidence for an important role of microsomal epoxide hydrolase in the formation of hydroquinone. Toxicol Appl Pharmacol 122(2): 172-181.

*Snyder R, Dimitriadis E, Guy R, et al. 1989. Studies on the mechanism of benzene toxicity. Environ Health Perspect 82:3 l-35.

*Snyder R, Jowa L, Witz G, et al. 1987. Formation of reactive metabolites from benzene. Arch Toxicol 60:61-64.

*Snyder R, Kalf GF. 1994. A perspective on benzene leukemogenesis. Crit Rev Toxicol 1994 24(3):177-209.

*Snyder R, Kocsis JJ. 1975. Current concepts of chronic benzene toxicity. CRC Crit Rev Toxicol 3:265-288.

*Snyder R, Lee EW, Kocsis JJ. 1978b. Binding of labeled metabolites to mouse liver and bone marrow. Res Commun Chem Pathol Pharmacol 20: 19 1- 194.

*Snyder R, Witz G, Goldstein BD. 1993b. The toxicology of benzene. Environ Health Perspect 100:293-306.

*Sole F, Caballin MR, Co11 MD, et al. 1990. Acute lymphoblastic leukemia with t(4;ll) in a patient previously exposed to a carcinogen. Cancer Genet Cytogenet 49(1):133-136.

*Spear RC, Bois FY, Woodruff T, et al. 1991. Modeling benzene pharmacokinetics across three sets of animal data: parametric sensitivity and risk implications. Risk Anal 11(4):641-654.

*Sporstol S, Urdal K, Drangsholt H, et al. 1985. Description of a method for automated determination of organic pollutants in water. Int J Environ Anal Chem 21:129-138.

*Srbova J, Teisinger J, Skramovsky S. 1950. Absorption and elimination of inhaled benzene in man. Arch Ind Hyg Occup Med 2:1-8.

*SRI 1993. 1993 Directory of Chemical Producers. United States of America. Stanford Research Institute International. Menlo Park, CA. p 471-472.

*SRI 1994. 1994 Directory of Chemical Producers. United States of America. Stanford Research Institute International. Menlo Park, CA. 472.

*SRI 1995. 1995 Directory of Chemical Producers. United States of America. Stanford Research Institute International. Menlo Park. CA. 466.

*St-Germain F, Mamer 0, Brunet J, et al. 1995. Volatile organic compound analysis by an inertial spray extraction interface coupled to an ion trap mass spectrometer. Anal Chem 67:4536-4541.

*Staples CA, Werner AF, Hoogheem TJ. 1985. Assessment of priority pollutant concentrations in the United States using STORET database. Environmental Toxicology and Chemistry 4: 13l-142.

*Steineck G, Plato N, Gerhardsson M, et al. 1990. Increased risk of urothelial cancer in Stockholm during 1985-87 after exposure to benzene and exhausts. Int J Cancer 45:1012-1017.

*Stetter JR, Jurs PC, Rose SL. 1986. Detection of hazardous gases and vapors: Pattern recognition analysis of data from an electrochemical sensor array. Anal Chem 58:860-866.

*Stolze K, Nohl H. 1994. Effect of xenobiotics on the respiratory activity of rat heart mitochondria and the concomitant formation of superoxide radicals. Environmental Toxicology And Chemistry; 13 (3):499-502.

*Stommel P, Muller G, Stucker W. 1989. Determination of S-phenylmercapturic acid in the urine an improvement in the biological monitoring of benzene exposure. Carcinogenesis 10(2):279-282

*Stoner RD, Drew RT, Bernstein DM. 1981. Benzene-inhalation effects upon tetanus antitoxin responses and leukemogenesis in mice. In: Mahlum DD, Gray RH, Felix WD, eds. Coal conversion and the environment. Oak Ridge, TN: U.S. Department of Energy, Technical Information Center, 445-461.

*Storck WJ. 1991. Better times ahead for chemicals industry. Chemical and Engineering News, December 9:37-39.

*Stuart JD, Lute HD, Smith SR, et al. 1984. Detection and effects of aperiodic leachate discharges from landfills. Technical report. Storrs, CT: Institute of Water Resources, University of Connecticut. NTIS PB 85-242949.

*Stucker I, Mandereau L, Aubert-Berleur MP, et al. 1994. Occupational paternal exposure to benzene and risk of spontaneous abortion. Occupational Environ Med 5 1:475-478.

*Stutz DR, Janusz SJ. 1988. Hazardous Materials Injuries: A Handbook for Pre-Hospital Care. Bradford Communications Corporation, Beltsville, MD. 298-299.

*Styles JA, Richardson CR. 1984. Cytogenetic effects of benzene: Dosimetric studies on rats exposed to benzene vapour. Mutat Res 135:203-209.

*Subrahmanyam VV, Doane-Setzer P, Steinmetz KL, et al. 1990. Phenol-induced stimulation of hydroquinone bioactivation in mouse bone marrow *in vivo*: possible implications in benzene myelotoxicity. Toxicology 62(1): 107-l 16.

*Subrahmanyam VV, Kolachana P, Smith MT. 1991. Hydroxylation of phenol to hydroquinone catalyzed by a human myeloperoxidase-superoxide complex: possible implications in benzene-induced myelotoxicity. Free Rad Res Commun 15(5):285-296.

*Sullivan J, Krieger G., eds. 1972. Hazardous material toxicology. Clinical Principles of environmental health. Williams and Wilkins. Baltimore, Md. 725-731

*Sun. 1971. Initial Submission: Subacute inhalation study of benzene on monkeys (final report) with attachment and cover letter dated 022892 EPA/OTS; doc #:88-920001855.

*Sun JD, Medinsky MA, Birnbaum LS, et al. 1990. Benzene hemoglobin adducts in mice and rats: Characterization of formation and physiological modeling. Fundam Appl Toxicol 15:468-475.

*Susten A, Dames B, Burg J, et al. 1985. Percutaneous penetration of benzene in hairless mice: An estimate of dermal absorption during tire-building operations. Am J Ind Med 7:323-335.

*Suzuki S, Atai H, Hatakeyama Y, et al. 1989. Administration-route-related differences in the micronucleus test with benzene. Mutat Res 223:407-410.

*Swenberg JA, Petzold GL, Harbach PR. 1976. *In vitro* DNA damage/alkaline elution assay for predicting carcinogenic potential. Biochem Biophys Res Commun 72:732-738.

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

Tabershaw IR, Lamm SH, Infante PF, et al. 1977. Benzene and leukemia. Lancet (2): 868-869.

*Tarringher M, Perrotta A, Malacarne D, et al. 1995. Lack of significant promoting activity by benzene in the rat liver model of carcinogenesis. J Toxicol Environ Health 45:481-488.

*Tannoka H. 1977. Development and application of *Bacillus substilis* test systems for mutagens, involving DNA-repair deficiency and suppressible auxotrophic mutations. Mutat Res 42: 19-32.

*Tatrai E, Rodics K, Ungvary G. 1980a. Embryotoxic effects of simultaneously applied exposure of benzene and toluene. Folia Morphol (Praha) 28:286-289.

*Tatrai E, Ungvary GY, Hudak A, et al. 1980b. Concentration dependence of the embryotoxic effects of benzene inhalation in CFY rats. J Hyg Epidemiol Microbial hnmunol 24:363-371.

*Tauber J. 1970. Instant benzol death. J Occup Med 12:91-92.

*Taylor L, Morris SS. 1995. Final report on the operations and findings of the Anchorage volatile organic compounds (VOV) monitoring project. Department of Health and Human Services, Environmental Services Division, Municipality of Anchoriage, Anchorage Air Pollution Control Agency, Anchorage, Alaska.

*Teisinger J, Fiserova-Bergerova B. 1955. Valeus Camparee de la determination des sulfates et du phenol contenus dans l'urine pour l'evaluation de la concentration du benzene dans l'air. Archives des Maladies Professionnelles de Medecine du Travail et de Securite Sociale 16:221-232. (French)

*Thienes H, Haley TJ. 1972. Clinical toxicology. 5th ed. Philadelphia, PA: Lea & Febiger, 124-127.

*Thomas KW, Pellizzari ED, Clayton CA, et al. 1993. Temporal variability of benzene exposures for residents in several New Jersey homes with attached garages or tobacco smoke. J Expo Anal Environ Epidemiol 3(1):49-73.

*Thomas KW, Pellizzari ED, Cooper SD. 1991. A canister-based method for collection and GC/M5 analysis of volatile organic compounds in human breath. J Anal Toxicol 15:54-59.

*Thrall, K. 1997. Personal communication with Robert Chessin, RTI. Richland, Washington.
*Tice RR, Costa DL, Drew RT. 1980. Cytogenetic effects of inhaled benzene in murine bone marrow: Induction of sister chromatid exchanges, chromosomal aberrations, and cellular proliferation inhibition in DBA/2 mice. Proc Nat1 Acad Sci USA 77:2148-2152.

*Tice RR, Vogt TF, Costa DL. 1982. Cytogenetic effects of inhaled benzene in murine bone marrow. In: Genotoxic effects of airborne agents. Environ Sci Res 25:257-275.

*Taft K, Olofsson T, Tunek A, et al. 1982. Toxic effects on mouse bone marrow caused by inhalation of benzene. Arch Toxicol 51:295-302.

*Tompa A, Major J, Jakab MG. 1994. Monitoring of benzene-exposed workers for genotoxic effects of benzene: improved-working-condition-related decrease in the frequencies of chromosomal aberrations in peripheral blood lymphocytes. Mutat Res 304 (2):159-165.

*Tondel M, Persson B, Carstensen J. 1995. Myelofibrosis and benzene exposure. Occup Med 45(1):51-52.

*Topham JC. 1980. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutat Res 74:379-387.

*Topp E, Scheunert I, Korte F. 1989. Kinetics of the uptake of ^{14}C-labeled chlorinated benzene from soil by plants. Ecotox Environ Safety 17:157-166.

*Tough IM, Court Brown WM. 1965. Chromosome aberrations and exposure to ambient benzene. Lancet 1:684.

*Tough IM, Smith PG, Brown WMC. 1970. Chromosome studies on workers exposed to atmospheric benzene. Europ J Cancer 6:49-55.

*Townsend JC, Ott MG, Fishbeck WA. 1978. Health exam findings among individuals occupationally exposed to benzene. J Occup Med 20:543-548.

*Travis C, Bowers J. 1990. Altered pharmacokinetics of soil-adsorbed benzene administered orally in the rat. Bull Environ Contam Toxicol 45(2):197-207.

*Travis CC, Fox MT, Simmons WM, et al. 1992. Co-exposure to gasoline vapor decreases benzene metabolism in Fischer-344 rats. Toxicol Lett 62(2-3):231-240.

*Travis CC, Quillen JL, Arms AD. 1990. Pharmacokinetics of benzene. Toxicol Appl Pharmacol 102:400-420.

Travis LB, Li CY, Zhang ZN, et al. 1994. Hematopoeitic malignancies and related disorders among benzene-exposed workers in China. Leukemia and Lymphoma 14:91-102.

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

*TRI92. 1994. Toxic Chemical Release Inventory. National Library of Medicine, National Toxicology Information Program, Bethesda, MD.

*TRI93. 1995. Toxic Chemical Release Inventory. National Library of Medicine, National Toxicology Information Program, Bethesda, MD.

*Tsai SP, Wen CP, Weiss NS, et al. 1983. Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries. J Occup Med 25:685-692.

*Tsuruta H. 1989. Skin absorption of organic solvent vapors in nude mice *in vivo*. Ind Health 27:37-47.

*Tucker WA, Huang C, Bra1 JM. 1986. Validation of transport model. In: Benzene in Florida groundwater: An assessment of the significance to human health. Tallahassee, FL: Florida Petroleum Council, American Petroleum Institute, 93-108.

*Tunca BT, Egeli U. 1996. Cytogenetic findings of shoe workers exposed long-term to benzene. Environ Health Perspect 104 (Suppl 6):1313-1317.

*Tunek A, Olofsson T, Berlin M. 1981. Toxic effects of benzene and benzene metabolites on granulopoietic stem cells and bone marrow cellularity in mice. Toxicol Appl Pharmacol 59: 149-156.

*Turk R, Fuchs R, Momcilovic B. 1991. Granulocyte reserve in chronic experimental benzene poisoning in rats. Arh Hig Rada Toksikol 42(1):37-42.

*Turkall RM, Skowronski G, Gerges S, et al. 1988. Soil absorption alters kinetics and bioavailability of benzene in orally exposed male rats. Arch Environ Contam Toxicol 17: 159-164.

*Turkel B, Egeli U. 1994. Analysis of chromosomal aberrations in shoe workers exposed long term to benzene. Occupational and Environmental Medicine 51:50-53.

U.S. Congress. 1986. Superfund Amendments and Reauthorization Act of 1986. 99th Congress, 2nd Session, October, 17, 1986.

*U.S. Congress. 1990. Clean Air Act Amendments of 1990. Conference Report to Accompany S. 1630. U.S. House of Representatives. 10lst Congress, 2d Session. Report No. 101-952.

*Uchrin CG, Mangels G. 1987. Sorption equilibria of benzene and toluene on two New Jersey coastal plain ground water aquifer solids. J Environ Sci Health [A] 22:743-758.

*Ungvary G, Tatrai E. 1985. On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats and rabbits. Arch Toxicol Suppl 8:425-430.

*USDOC. 1994. U.S. Merchandise Import and Export Trade. Retrieved from National Trade Data bank. United States Department of Commerce, Bureau of Census, June 29, 1994.

*USDOC. 1995. U.S. Merchandise Import and Export Trade. Retrieved from National Trade Data Bank. United States Department of Commerce, Bureau of Census.

*USITC. 1995. Preliminary report on U.S. production of selected synthetic organic chemicals (including synthetic plastics and resin materials) first quarter, second quarter and cumulative totals, 1994.

*Utterback DF, Rinsky RA. 1995. Benzene exposure assessment in rubber hydrochloride workers: a critical evaluation of previous estimates. Am J Ind Med May 1995 27(5):661-676.

*Vaalavirta L, Tahti H. 1995. Effects of selected organic solvents on the astrocyte membrane atpase *in vitro*. Clin Exp Pharmacol Physiol 22(4):293-294.

*Vacha J, Znojil V, Seidel HJ, et al. 1990. Ferrokinetics and erythropoiesis in mice after long-term inhalation of benzene. Blut 60(1):41-47.

*Vaishnav DD, Babeu L. 1987. Comparison of occurrence and rates of chemical biodegradation in natural waters. Bull Environ Contam Toxicol 39:237-244.

*Valentine JL, Seaton MJ, Asgharian B. 1996. Benzene metabolism and toxicity in transgenic CYP2El knockout mice. Fundamental and applied toxicology. The Toxicologist. 30(1):73.

*Van den Berghe H, Louwagie A, Broeckaert-Van Orshoven A, et al. 1979. Chromosome analysis in two unusual malignant blood disorders presumably induced by benzene. Blood 53:558-566.

*Van Emon JM, Gerlach CL. 1995. A status report on field-portable immunoassay. Environ Sci Tech 29(7):312-317.

*van Sittert NJ, Boogaard PJ, Beulink GD. 1993. Application of the urinary S-phenylmercapturic acid test as a biomarker for low levels of exposure to benzene in industry. Br J Ind Med 50(5):460-469,

*van Sittert NJ, de Jong G. 1985. Biomonitoring of exposure to potential mutagens and carcinogens in industrial populations. Fd Chem Toxic 23:23-31.

*van Wijnen JH, Verhoeff AP, Jans H WA, et al. 1995. The exposure of cyclists, car drivers and pedestrians to traffic-related air pollutants. International Archives of Occupational and Environmental Health 67(3):187-193.

*Vara P, Kinnunen O. 1946. Benzene toxicity as a gynecologic problem. Acta Obstet Gynecol Stand 26:433-452.

*Verschueren K. 1983. Handbook of environmental data on organic chemicals. 2nd ed. New York, NY: Van Nostrand Reinhold Co., 236-242.

*Vigliani EC, Forni A. 1976. Benzene and leukemia. Environ Res 11:122-127.

*Vogel TM, Grbic-Galic D. 1986. Incorporation of oxygen from water into toluene and benzene during anaerobic fermentative transformation. Appl Environ Microbial 52:200-202.

*Van Oettingen WF. 1940. Toxicity and potential dangers of aliphatic and aromatic hydrocarbons: A critical review of the literature. Washington, DC: U.S. Public Health Service, Division of Industrial Hygiene, National Institute of Health, Public Health Bulletin no. 255, 66-97, 125-135.

*Voytek PE, Thorslund TW. 1991. Benzene risk assessment: status of quantifying the leukemogenic risk associated with the low-dose inhalation of benzene. Risk Anal 11(3):355-357.

*WAC. 1985. Groundwater quality. Wisconsin Administrative Code, Chapter NR 140. Department of Natural Resources.

*WAC. 1988. Table 3. Hazardous Air Contaminants without Acceptable Ambient Concentrations Requiring Application of Lowest Achievable Emission Rate for Sources of Group A Hazardous Air Contaminants. Wisconsin Administrative Code. Hazardous Air Pollutants Emissions Standards. DNR 445.04.

*WAC. 1989a. Table 9. Human Cancer Criteria for Public Water Supply. Wisconsin Administrative Code. Water Quality Standards. DNR 105.09.

*WAC. 1989b. Table 9. Human Cancer Criteria for Nonpublic Water Supply. Wisconsin Administrative Code. Water Quality Standards. DNR 105.09.

*WAC. 1994a. Enforcement Standard. Wisconsin Administrative Code. Groundwater Quality Standards. DNR 140.10.

*WAC. 1994b. Preventative Action Limit. Wisconsin Administrative Code. Groundwater Quality Standards. DNR 140.10.

*Wallace LA. 1986. The total exposure assessment methodology (TEAM) study: Part I: Summary and analysis: Part II: Protocols for environmental and human sampling and analysis. Final report to the Environmental Protection Agency.

*Wallace LA, Pellizzari ED, Hartwell TD, et. al. 1986. Total exposure assessment methodology (TEAM) study: Personal exposures, indoor-outdoor relationships, and breath levels of volatile organic compounds in New Jersey. Environment International 12:369-387.

*Wallace LA. 1989a. Major sources of benzene exposure. Environ Health Perspect 82: 165-169.
*Wallace LA. 1989b. The exposure of the general population to benzene. Cell Biol Toxicol 5:297-314.

*Wallace LA. 1995. Human exposure to environmental pollutants: a decade of experience. Clinical and Experimental Allergy 25:4-9.

*Wallace LA, Nelson WC, Ziegenfus R, et al. 1991. The Los Angeles Team Study: Personal Exposures, Indoor-Outdoor air Concentrations, and Breath Concentrations of 25 Volatile Organic Compounds. J Exp Anal Environ Epidemiol 1(2):157-192.

*Wallace LA, Pellizzari ED. 1986. Personal air exposure and breath concentrations of benzene and other volatile hydrocarbons for smokers and nonsmokers. Toxicol Lett 35:113-l 16.

*Wallace LA, Pellizzari ED, Hartwell TD, et al. 1985. Personal exposures, indoor-outdoor relationships, and breath levels of toxic air pollutants measured for 355 person in New Jersey. Atmos Environ 19:1651-1661.

*Wallace LA, Pellizzari ED, Hartwell TD, et al. 1987. Exposures to benzene and other volatile compounds from active and passive smoking. Arch Environ Health 42:272-279.

*Ward CO, Kuna RA, Snyder NK, et al. 1985. Subchronic inhalation toxicity of benzene in rats and mice. Am J Ind Med 7:457-473.

*Ward JB Jr, Ammenheuser MM, Ramanujam VMS, et al. 1992. The mutagenic effects of low level sub-acute inhalation exposure to benzene in CD-1 mice. Mutat Res 268(1):49-57.

*Watanabe KH, Bois FY, Daisey JM, et al. 1994. Benzene toxicokinetics in humans: exposure of bone marrow to metabolites. Occup Environ Med 51(6):414-420.

*WDNR. 1987. Surface water quality criteria for toxic substances. Order of the State of Wisconsin Natural Resources Board Repealing, Renumbering, Renumbering and Amending, Amending, Repealing and Recreating, and Creating Rules. Section 27, Chapter NR 105. Department of Natural Resources, Wisconsin.

*Weisel C, Yu R, Roy A, et al. 1996. Biomarkers of environmental benzene exposure. Environ Health Perspect 104 (Suppl 6):1141-l146.

*Weisel CP, Yu R. 1995. Exhaled breath and urinary muconic acid as biomarkers of environmental benzene exposures. 209th American Chemical Society National Meeting, Anaheim, California, USA, April 2-6, 1995. Abstracts Of Papers American Chemical Society; 209:(1-2) Agro 91.

*Wells MS, Nerland DE. 1991. Hematotoxicity and concentration-dependent conjugation of phenol in mice following inhalation exposure to benzene. Toxicol Lett 56(l-2):159-l 66.

*Wester RC, Maibach HI, Gruenke LD, et al. 1986. Benzene levels in ambient air and breath of smokers and nonsmokers in urban and pristine environments. J Toxicol Environ Health 18:567-573.

*White MC, Johnson CA, Ashley, et al. 1993. Exposure to methyl tertiary-butyl ether from oxygenated gasoline in Stamford, Connecticut. Archives of Environmental Health 50(3):183-l 89.

*WHO. 1984. Guidelines for drinking water quality. Vol. 2: Health criteria and other supporting information. Geneva, Switzerland: World Health Organization.

*WHO. 1991. Guidelines for Drinking Water Quality. Vol. I. Recommendations. World Health Organization.

*Williams GM, Tong C, Ved Brat S. 1985. Tests with the rat hepatocyte primary culture/DNA-repair test. Progress in Mutation Research 5:341-345.

*Wilson BH, Smith GB, Rees JF. 1986. Biotransformation of selected alkylbenzenes and halogenated hydrocarbons in methanogenic aquifer material: A microcosm study. Environ Sci Technol 20:997- 1002.

*Winek CL, Collom. WD. 1971. Benzene and toluene fatalities. J Occup Med 13:259-261.

*Winek CL, Collom WD, Wecht CH. 1967. Fatal benzene exposure by glue sniffing. Lancet (March 25):683.

*Witz G, Zhang Z, Goldstein BD. 1996. Reactive ring-opened aldehyde metabolites in benzene toxicity. Environ Health Perspect 104 (Suppl 6): 1195-l 199.

*Witz G, Kirley TA, Maniara WM, et al. 1990. The metabolism of benzene to muconic acid, a potential biological marker of benzene exposure. Biological Reactive Intermediates IV 283:613-618.

*Witz G, Latriano L, Goldstein BD. 1989. Metabolism and toxicity of truns,trans-muconaldehyde, and open-ring microsomal metabolite of benzene. Environ Health Perspect 82:19-22.

*Witz G, Maniara W, Mylavarapu V, et al. 1990. Comparative metabolism of benzene and truns, trans-muconaldehyde to *trans,* trans-muconic acid in DBA/2N and C57BL/6 mice. Biochem Pharmacol 40:1275-1280.

*Witz G, Rao GS, Goldstein BD. 1985. Short-term toxicity of *trans,trans*-mucondialdehyde. Toxicol Appl Pharmacol 80:511-516.

*Wolf MA, Rowe VK, McCollister DD, et al. 1956. Toxicological studies of certain alkylated benzene and benzene. AMA Arch Ind Health 14:387-398.

*Wolff SP. 1992. Correlation between car ownership and leukaemia: is non-occupational exposure to benzene from petrol and motor vehicle exhaust a causative factor in leukaemia and lymphoma? Experientia 48(3):301-304.

*Wong O. 1987a. An industry wide mortality study of chemical workers occupationally exposed to benzene: I. General results. Br J Ind Med 44:365-381.

*Wong O. 1987b. An industry wide mortality study of chemical workers occupationally exposed to benzene: II. Dose response analyses. Br J Ind Med 44:382-395.

*Wong O. 1995. Risk of acute myeloid leukaemia and multiple myeloma in workers exposed to benzene. Occupational and Environmental Medicine 52:380-384.

*Wong O, Morgan RW, Whorton MD. 1983. Comments on the NIOSH study of leukemia in benzene workers. Technical report submitted to Gulf Canada Ltd., by Environmental Health Associates, August 3 1.

*Wong O, Raabe GK. 1995. Cell-Type-Specific leukemia analysis in a combined cohort of more than 208,000 petroleum workers in the United States and the United Kingdom, 1937-1989. Regulatory Toxicology and Pharmacology 21:307-321.

*Wood JA, Porter ML. 1987. Hazardous pollutants in Class II landfills. J Air Pollut Control Assoc 37:609-615.

*Woodruff TJ, Bois FY. 1993. Optimization issues in physiological toxicokinetic modeling: A case study with benzene. Toxicol Lett 69(2):181-196.

*Woodruff TJ, Bois FY, Auslander D, et al. 1992. Structure and parameterization of pharmacokinetic models: their impact on model predictions. Risk Anal 12(2):189-201.

*Xia Z-L, Xi-Peng J, Pei-Lian L, et al. 1995. Ascertainment corrected prevalence rate (ACPR) of leukopenia in workers exposed to benzene in small-scale industries calculated wit capture-recapture methods. Biomed Environ Sci 8:30-34.

*Xing SG, Shi X, Wu ZL, et al. 1992. Transplacental genotoxicity of triethylenemelamine, benzene, and vinblastine in mice. Teratogenesis Carcinog Mutagen 12(5):223-230.

*Yager JW, Eastmond DA, Robertson ML, et al. 1990. Characterization of micronuclei induced in human lymphocytes by benzene metabolites. Cancer Res 50(2):393-399.

*Yardley-Jones A, Anderson D, Jenkinson PC, et al. 1988. Genotoxic effects in peripheral blood and urine of workers exposed to low level benzene. Br J Ind Med 45:694-700.

*Yardley-Jones A, Anderson D, Love11 DP, et al. 1990. Analysis of chromosomal aberrations in workers exposed to low level benzene. Br J Ind Med 47(1):48-51.

*Yeowell-O'Connell K, McDonald TA, Rappaport SM. 1996. Use of protein adducts to investigate the reactive metabolites of benzene in rats. Fundamental and applied toxicology. The toxicologist 30(1): 279-280

*Yin S, Li G, Hu Y, et al. 1987b. Symptoms and signs of workers exposed to benzene, toluene or the combination. Ind Health 25:113-130.

*Yin S, Li Q, Liang Y. 1982. Significance of leukocyte alkaline phosphatase in the diagnosis of chronic benzene poisoning. Reg Toxicol Pharmacol 2:209-212.

*Yin SN, Linet MS, Hayes RB, et al. 1994. Cohort study among workers exposed to benzene in china: i. general methods and resources. American Journal Of Industrial Medicine 26 (3): 383-400.

*Yin SN, Hayes RB, Linet MS, et al. 1996. An expanded cohort study of cancer among benzene-exposed workers in China. Environ Health Perspect 104 (Suppl 6): 1339-1341.

*Yin SN, Li GL, Tain FD, et al. 1987a. Leukemia in benzene workers: A retrospective cohort study. Br J Ind Med 44:124-128.

*Yin S-N, Li G-L, Tain F-D, et al. 1989. A retrospective cohort study of leukemia and other cancers in benzene workers. Environ Health Perspect 82:207-213.

*Yin SN, Li Q, Liu Y, et al. 1987c. Occupational exposure to benzene in China. Br J Ind Med 44:192-195.

*Young RJ, Rinsky RA, Infante PF, et al. 1978. Benzene in consumer products. Science 199:248.

*Zenz C. 1978. Benzene--attempts to establish a lower exposure standard in the United States: A review. Stand J Work Environ Health 4:103-l13.

*Zenz C, Dickerson OB, Horvath EP, eds. 1994. Benzene. In: Occupational medicine, third edition. St. Louis, MO: Mosby, 721-722, 747-748.

Zhang L, Robertson ML, Kolachana P, et al. 1993. Benzene metabolite, 1,2,4-Benzenetriol, induces micronuclei and oxidative DNA damage in human lymphocytes and HL60 cells. Environ Mol Mutagen 21:339-348.

*Zhang LP, Rothman N, Wang Y, et al. 1996. Interphase cytogenetics of workers exposed to benzene. Environ Health Perspect 104 (Suppl 6):1325-1329.

*Zhang Z, Goldstein BD, Witz G. 1995a. Iron-stimulated ring-opening of benzene in a mouse liver microsomal system. Mechanistic studies and formation of a new metabolite. Biochem Pharmacol 50(10):1607-1617.

*Zhang Z, Schafer F, Schoenfeld H, et al. 199%. The hematotoxic effects of 6-hydroxy-trans, trans-2,4-hexadienal, a reactive metabolite of trans, trans-muconaldehyde, in cd-l mice. Toxicol Appl Pharmacol 132(2):213-219.

*Zhang Z, Xiang Q, Glatt H, et al. 1995b. Studies of pathways of ring opening of benzene in a fenton system. Free Radical Biology & Medicine; 18 (3):411-419.

*Zhu H, Li Y, Trush MA. 1995. Differences in xenobiotic detoxifying activities between bone marrow stromal cells from mice and rats: implications for benzene-induced hematotoxicity. J Toxicol Environ Health 46 (2):183-201.The reference list is being compiled and printed from a database. It will be converted to WordPerfect in a later draft. A reference list is attached.

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_{ow}) -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.

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.

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 g-hour shift.

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

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 (I) 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.

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

ATSDR MINIMAL RISK LEVEL

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99-4991, requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1-14 days), intermediate (15-364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for

establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as a hundredfold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agencywide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road, Mailstop E-29, Atlanta, Georgia 30333.

MINIMAL RISK LEVEL WORKSHEET

Chemical name(s):   Benzene
CAS number(s):   7l-43-2
Date: September 12, 1997
Profile status: Final
Route: [X] Inhalation [ ] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to figure: 46
Species: mouse

MRL: <u>0.05</u>   [ ] mgkg/day [Xl ppm [ ] mg/m^3

<u>Reference</u>: Rozen et al. 1984

<u>Experimental design</u>:

Male C57BL/6J mice (7-Wgroup) were exposed to benzene (0, 10.2, 31, 100 or 301 ppm) in wholebody
dynamic inhalation chambers for 6 hours/day for 6 consecutive days. Control mice were
exposed to filtered, conditioned air only. Erythrocyte counts were depressed in C57BL/6 mice only at
100 and 301 ppm. At exposures of 10.2 and 31 ppm, respectively, depressions of the proliferative
responses of bone-marrow-derived B-cells and splenic T-cells occurred in C57BL/6J mice without
causing a concomitant depression in number of splenic T- or B- cells. Peripheral lymphocyte counts
were depressed at all levels. No correlation was made of reduced lymphocytes with general effects.
These results demonstrates that short-term inhaled benzene even at low exposure concentrations can
cause reduction in certain immune associated processes.

<u>Effects noted in study and corresponding doses</u>:

10.2 ppm =   No adverse effect on erythrocytes, depressed peripheral lymphocytes and
                           mitogen-induced blastogenesis of femoral B-lymphocytes (Less Serious
                           LOAEL) .

31 ppm =   No adverse effect on erythrocytes, depression of mitogen-induced blastogenesis
                           of splenic T-cells.

100 ppm =   Depressed erythrocyte counts.

<u>Dose end point used for MRL derivation:</u> 10.2 ppm
[ ] NOAEL [X]LOAEL

<u>Uncertainty factors used in MRL derivation: 300</u>

[ ] 1 [ ] 3 [X] 10 (for use of a LOAEL)
[ ] 1 [X] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [ ] 3 [X] 10 (for human variability)

<u>Was a conversion factor used from ppm in food or water to a mg/body weight dose?</u>
If so, explain: None

<u>Was a conversion used from intermittent to continuous exposure? If so, explain:</u>

The ratio of the blood/gas partition coefficients was assumed to be 1. The concentration was adjusted for intermittent exposure by multiplying the LOAEL (10.2 ppm) by 6/24 to correct for less than a full day of exposure. The resulting number (adjusted LOAEL) is 2.50 ppm.

<u>If an inhalation study in animals, list conversion factors used in determining human equivalent dose:</u>

The human equivalent concentration (HEC) was calculated using Formula 4-l1, from Interim Methods for Development of Inhalation Reference Concentrations, EPA 1990h:

$$\text{LOAEL}_{[HEC]} \ (mg/m^3 \text{ or ppm}) = \text{LOAEL}_{[ADJ]} \ (mg/m^3 \text{ or ppm}) \ x \ \frac{(V_A/BW)_A}{(V_A/BW)_H}$$

where:

$\text{LOAEL}_{[HEC]}$ =   the LOAEL human equivalent concentration

$\text{LOAEL}_{[ADJ]}$ =   the LOAEL adjusted for duration (see above)

$\dfrac{(V_A/BW)_A}{(V_A/BW)_H}$ =   the ratio of the alveolar ventilation rate (mL/min or L/hr) divided by Body weight (kg) of the animal species to the same parameters for humans.

Values for this ratio were taken from EPA 1988e, as follows:

$(V_A)_A$ = ventilation rate for male B6C3F$_1$ mice, subchronic,
                           0.053 m^3/day x 1,000 L/m^3 x 1 day/24 hr= 2.208 L/hr = 2.21 L/hr.

$(BW)_A$ = body weight for male B6C3F$_1$ mice, subchronic,
                           0.0316 kg

$(V_A)_H$ = ventilation rate for human adult male,
                           20 m^3/day x 1,000 L/m^3 x 1 day/24 hr= 833.33 L/hr = 833 L/hr.

$(BW)_H$ = body weight for human adult male, 70 kg

2.50 ppm x $\dfrac{2.21 \text{ L/hr}}{0.0316 \text{ kg}}$ / $\dfrac{833 \text{ L/hr}}{70 \text{ kg}}$ =   14.7 ppm

<u>Other additional studies or pertinent information that lend support to this MRL</u>: Increased number of MN-PCEs, decreased numbers of granulopoietic stem cells (Toft et al. 1982) lymphopenia (Cronkite et al. 1985), lymphocyte depression, and increased susceptibility to bacterial infection (Rosenthal and Snyder 198.5) are among the adverse hematological and immunological effects observed in several other acute-duration inhalation studies. The study by Rozen et al. (1984) shows benzene immunotoxicity (reduced mitogen-induced lymphocyte proliferation) at a slightly lower exposure level than these other studies. C57BI/6J mice were exposed to 0, 10.2, 31, 100, and 301 ppm benzene for 6 days at 6 hrs/day. Control mice were exposed to filtered, conditioned air only. Lymphocyte counts were depressed at all exposure levels while erythrocyte counts were elevated at 10.2 ppm, equal to controls at 31 ppm and depressed at 100 and 301 ppm. Femoral B-lymphocyte and splenic B-lymphocyte numbers were reduced at 100 ppm. Levels of circulating lymphocytes and mitogeninduced blastogenesis of femoral B-lymphocytes were depressed after exposure to 10.2 ppm benzene for 6 days. Mitogen-induced blastogeneses of splenic T-lymphocytes were depressed after exposure to 31 ppm of benzene for 6 days. In another study, mice exhibited a 50% decrease in the population of erythroid progenitor cells (CFU-E) after exposure to 10 ppm benzene for 5 days, 6 hours per day (Dempster and Snyder 1991). In a study by Wells and Nerland (1991), groups of 4-5 male Swiss-Webster mice were exposed to 0, 3, 25, 55, 105, 199, 303, 527, 1,150, or 2,290 ppm benzene for 6 hours/day for 5 days. The number of leukocytes in peripheral blood and spleen weights were significantly decreased compared with untreated controls at all concentrations ≥25 ppm. Therefore, 3 ppm was the NOAEL and 25 ppm was the LOAEL for these effects. Other endpoints were not monitored in this study. These data support the choice of Rozen et al. (1984) as a critical study.

<u>Agency Contact (Chemical Manager): Sharon Wilbur</u>

APPENDIX A

MINIMAL RISK LEVEL WORKSHEET

Chemical name(s):   Benzene
CAS number(s):   71-43-2
Date: June 30, 1997
Profile status: Final
Route: [X] Inhalation [ ] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Key to figure: 133
Species: mouse

MRL: 0.004   [ ] mg/kg/day [X] ppm [ ] mg/m3

Reference: Li et al. 1992

Experimental design:

Forty adult Kunming male mice were divided into 4 groups (5/group) and exposed to 0, 0.78, 3.13 and 12.52 ppm benzene for 2 hours/day, 6 days/week for 30 days. Control mice were exposed to air. Benzene level was monitored by gas chromatography every 30 minutes for 3 days at the beginning of the experiment. Locomotor activity was measured in 15-minute sessions. A Y-maze, designed with a starting point and safety area, was used to measure rapid response. Motivation of the mice was accomplished by electrical shock. Fore limb grip strength of mice was determined by persistent time (in seconds) while grasping a vertical iron stick. Body weight, food consumption, relative organ weight, blood and brain acetylcholinesterase activity, and bone marrow panel were determined. Body weight and food consumption were not affected. Significant differences in organ to body weight ratios occurred only with the liver and spleen, but not for the kidney. A 12% increase in liver weight and a 26% decrease in spleen weight were observed at 12.52 ppm. The increase in liver weight was not considered to be adverse. There was decreased grip strength at 12.52 ppm, but increased grip strength occurred at the lower doses. Low level exposure also affected frequency of rapid response; at 0.78 ppm, the response was increased, whereas at the higher doses, the response was depressed. All of the changes were significantly different from the control values. Locomotor activity increased at the low dose, was similar to control values at the mid dose, and decreased at the high dose. However, these changes were not significantly different from the control value. A significant decrease of AChE activity in the brain was observed at 12.52 ppm, but the decrease was not large enough to be considered an adverse effect. Bone marrow picture showed decreased percentages of precursors of leukocytes and erythrocytes, with increased concentrations of inhaled benzene; this effect was most consistent at the high dose. Myelocytes, premyelocytes, myeloblasts, and metamyelocytes were among the cell types most affected.

Effects noted in study and corresponding doses:

0.78 ppm =   Increased rapid response time (Less Serious minimal LOAEL). No adverse
                 effect on immunological/lymphoreticular indices, including spleen weight and
                 bone marrow panel, or body, liver, or kidney weight. No effect on blood or
                 brain acetylcholinesterase.

3.13 ppm =   Increased forelimb grip strength, decreased rapid response time. No adverse
                  effect on immunological/lymphoreticular indices, including spleen weight and
                  bone marrow panel, or body, liver, or kidney weight. No effect on blood or
                  brain acetylcholinesterase.

12.52 ppm =   Decreased forelimb grip strength, decreased rapid response time, decreased brain
                  acetylcholinesterase activity, 26% decrease in relative spleen weight, decrease in
                  myelocytes, premyelocytes, myeloblasts, and metamyeloblasts in the bone
                  marrow.

Dose end point used for MRL derivation: 0.78 ppm

[ ] NOAEL [X]LOAEL

Uncertainty factors used in MRL derivation: 90

[ ] 1 [X] 3 [ ] 10 (for use of a minimal LOAEL)
[ ] 1 [X] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [ ] 3 [X] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose?
If so, explain: None

Was a conversion used from intermittent to continuous exposure? If so, explain:

The ratio of the blood/gas partition coefficients was assumed to be 1. The concentration was adjusted
for intermittent exposure by multiplying the LOAEL (0.78 ppm) by 2/24 and by 6/7 days to adjust to
continuous exposure. The resulting number (adjusted LOAEL) is 0.056 ppm.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose:

The human equivalent concentration (HEC) was calculated using Formula 4-l1, from Interim Methods
for Development of Inhalation Reference Concentrations, EPA 1990h:

$$\text{LOAEL}_{[HEC]} \ (\text{mg/m}^3 \text{ or ppm}) = \text{LOAEL}_{[ADJ]} \ (\text{mg/m}^3 \text{ or ppm}) \times \frac{(V_A/BW)_A}{(V_A/BW)_H}$$

where:

$\text{LOAEL}_{[HEC]}$ = the LOAEL human equivalent concentration

$\text{LOAEL}_{[ADJ]}$ = the LOAEL adjusted for duration (see above)

$\frac{(V_A/BW)_A}{(V_A/BW)_H}$ = the ratio of the alveolar ventilation rate (mL/min or L/hr) divided by
                body weight (kg) of the animal species to the same parameters for
                humans.

Values for this ratio were taken from EPA 1988e, as follows:

$(V_A)_A$ = ventilation rate for male $B6C3F_1$ mice (default strain), subchronic,
$\quad\quad\quad$ 0.053 m^3/day x 1,000 L/m^3 x 1 day/24 hr= 2.208 L/hr = 2.21 L/hr.

$(BW)_A$ = body weight for male $B6C3F_1$ mice (default strain), subchronic,
$\quad\quad\quad$ 0.0316 kg

$(V_A)_H$ = ventilation rate for human adult male,
$\quad\quad$ 20 m3/day x 1,000 L/m^3 x 1 day/24 hr= 833.33 L/hr = 833 L/hr.

$(BW)_H$ = body weight for human adult male, 70 kg

0.056 ppm $\quad\quad$ x $\quad\quad$ $\dfrac{2.21\ \text{L/hr}}{0.0316\ \text{kg}}$ $\quad\quad$ / $\quad\quad$ $\dfrac{833\ \text{L/hr}}{70\ \text{kg}}$ $\quad\quad$ = $\quad\quad$ 0.33 ppm

Other additional studies or pertinent information that lend support to this MRL: Li et al. (1992) observed neurological changes at doses that were lower than those that caused the expected changes in bone marrow cells. Based on the observation of neurological changes in a study that also identified classic indicators of benzene toxicity, it seems reasonable to use the neurological effects observed by Li et al. (1992) as a basis for an intermediate-duration MRL. Benzene is known to cause adverse neurological effects in both humans and animals. Following acute inhalation of benzene, humans exhibited symptoms indicative of central nervous system effects (Cronin 1924; Flury 1928; Greenburg 1926; Midzenski et al. 1992). These symptoms, reported to occur at levels ranging from 300 to 3,000 ppm, included drowsiness, dizziness, headache, vertigo, tremor, delirium, and loss of consciousness. Acute exposure (5-10 minutes) to higher concentrations of benzene (approximately 20,000 ppm) can result in death, which has been associated with vascular congestion in the brain (Avis and Hutton 1993; Flury et al. 1928). Lethal exposures are also associated with neurological symptoms similar to those reported for nonlethal exposures. These symptoms are similar to the consequences of exposure to multiple organic solvents and are reversible when symptomatic workers are transferred from the problem area (Kraut et al. 1988; Yin et al. 1987b). In reports of cases of benzene poisoning, subjects exhibited headaches, nausea, tremor, convulsions, and unconsciousness, among other neurological effects (Cronin 1924; Davies 1929; Greenburg 1926; Midzenski et al. 1992; Tauber 1970).

Chronic exposure to benzene has been reported to produce neurological abnormalities in humans. Of eight patients (6 with aplastic anemia and 2 with preleukemia) with previous occupational exposure to adhesives and solutions containing 9-88% benzene, 4 of the 6 patients with aplastic anemia showed neurological abnormalities (global atrophy of lower extremities and distal neuropathy of -upper extremities) (Baslo and Aksoy 1982). Air concentrations in the workplace were reported to have reached levels of 210 ppm or higher. These findings suggest that benzene may induce toxic effects on the nervous system involving peripheral nerves and/or spinal cord. However, the limitations of this study are that benzene exposure levels were not monitored and that there was a possibility of an additional exposure to toluene (6.37-9.25%).

Chronic exposure to benzene and toluene was studied in 121 workers exposed to benzene for 2-9 years (Kahn and Muzyka 1973). The air concentration of benzene between 1962 and 1965 was

6-15.6 ppm (20-50 mg/m^3), while the toluene vapors did not exceed the 5 mg/m^3 level. Subsequently (the authors do not specify when), the air levels of both benzene and toluene have not exceeded the 5 mg/m^3 level. Seventy-four of the examined workers complained of frequent headaches (usually at the end of the work day), became tired easily, had difficulties sleeping, and complained of memory loss. The limitations of this study are that workers were exposed to both benzene and toluene and that the precise dose and duration of exposure are not known.

The neurotoxicity of benzene has not been studied extensively in animals, Male albino SPF rats from a Wistar-derived strain exposed to benzene for 4 hours in glass chambers (dose not specified) exhibited depression of evoked electrical activity in the brain; the authors calculated the 30% effect level (depressed activity) as 929 ppm (Frantik et al. 1994). When female H strain mice were exposed to benzene for 2 hours, the 30% effect level for depression of evoked electrical activity in the brain was 856 ppm (Frantik et al. 1994). In rabbits, symptoms that occurred 3.7 minutes following acute exposure to benzene (45,000 ppm) were relaxation and light narcosis (Carpenter et al. 1944). As the time after exposure progressed so did the symptoms to include excitation and tremors (after 5 minutes), loss of pupillary reflex to strong light (after 6.5 minutes), pupillary contraction (after 12 minutes), and involuntary blinking (after 15.6 minutes). Insufficient information was provided in the same study on narcosis and death that occurred in rabbits after exposure to 45,000 ppm for 30 minutes. Behavioral tests of C57BL/6 mice showed significant increase in licking of sweetened milk after 1 week of exposure to 300 ppm; a 90% decrease in hind limb grip strength after one exposure to 1,000 or 3,000 ppm (data for 100 ppm were not reported); and tremors after one exposure to 3,000 ppm that subsided 30 minutes after the exposure (Dempster et al. 1984). In another study, designed to reflect occupational exposure, male CD-l and C57BL/6 mice were exposed to 300 or 900 ppm of benzene 6 hours per day for 5 days followed by 2 weeks of no exposure after which the exposure regimen was repeated for an unspecified amount of time (Evans et al. 1981). The following seven categories of behavioral activities were monitored in exposed and control animals: stereotypic behavior, sleeping, resting, grooming, eating, locomotion, and fighting. Only minimal and insignificant differences were observed between the two strains of mice. Increased behavioral activity was observed after exposure to benzene in both strains of mice. Mice exposed to 300 ppm of benzene had a greater increase than those exposed to 900 ppm, probably because of narcosis-like effects induced at the higher exposure level (Evans et al. 1981). It is not known if benzene induces behavioral changes by directly acting upon the central nervous system. It is also not known whether these changes occur before or after hematological changes.

Agency Contact (Chemical Manager): Sharon Wilbur

APPENDIX B

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

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 and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upperbound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 2-1 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 exists, 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-l) and oral (LSE Figure 2-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.

(2)   Exposure Period Three exposure periods - acute (less than 15 days), intermediate (15-364 days), and chronic (365 days or more) are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3)   Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4)   Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the 2 "18r" data points in Figure 2-l).

(5)   Species The test species, whether animal or human, are identified in this column. Section 2.5, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 2.3, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6)   Exposure Frequency/Duration The duration of the study and the weekly and daily exposure 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 1,1,2,2-tetrachloroethane via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 1981.

(7)   System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

(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 dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

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

(11) <u>CEL</u> A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) <u>Footnotes</u> 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 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 concentrations for particular exposure periods.

(13) <u>Exposure Period</u> 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) <u>Health Effect</u> These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

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

(16) <u>NOAEL</u> In this example, 1% NOAEL is the critical endpoint for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates to 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) <u>CEJ</u> Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) <u>Estimated Upper-Bound Human Cancer Risk Levels</u> This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates-of the slope of the cancer dose response curve at low dose levels (ql*).

(19) <u>Key to LSE Figure</u> The Key explains the abbreviations and symbols used in the figure.

SAMPLE

[1] →

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

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	

[2] → INTERMEDIATE EXPOSURE

[3] → Systemic   [5]↓   [6]↓   [7]↓   [8]↓   [9]↓   [10]↓

[4] →

| 18 | Rat | 13 wk 5d/wk 6hr/d | Resp | 3[b] | 10 (hyperplasia) | | Nitschke et al. 1981 |

CHRONIC EXPOSURE

                                                        [11]

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.

[12] →

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

SAMPLE

13 ⟶ **Figure 2-1. Levels of Significant Exposure to [Chemical X] – Inhalation**

Acute
(≤14 days)

Intermediate
(15-364 days)

Systemic

Systemic

14 ⟶

15 ⟶ (ppm)

Death Respiratory Hematological Death Respiratory Hematological Hepatic Reproductive Cancer*

10000

1000

100 ● ● 16r 17r ◐ 24g ◐ 18r ◐ 18r ○ 20m ◐ ◐ 20m 22g 21r ● 30r 31r ◐ 28m ◐ 22m ◐ 29r ○ 37m ○ 35m ◐ 34r ◐ 27r ◆ 38r ◆ 40m ◆ 39r

○ 33r ○ 34r

16 ⟶ 10 ○ 18r

17 ⟵

1

0.1

0.01

0.001

0.0001

0.00001

0.000001

0.0000001

10⁻⁴

10⁻⁵ } Estimated Upper Bound Human Cancer Risk Levels ⟵ 18

10⁻⁶

10⁻⁷

19 ⟶

Key

r   Rat
m   Mouse
h   Rabbit
g   Guinea Pig
k   Monkey

● LOAEL for serious effects (animals)
◐ LOAEL for less serious effects (animals)
○ NOAEL (animals)
◆ CEL - Cancer Effect Level

Ɩ Minimal risk level for effects
other than cancer

The number next to each point
corresponds to entries in the
accompanying table.

* Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply
the existence of a threshold for the cancer end point.

Chapter 2 (Section 2.5)

Relevance to Public Health

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

      1.   What effects are known to occur in humans?

      2.   What effects observed in animals are likely to be of concern to humans?

      3 .   What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section covers end points in the same order they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotdxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

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

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MRLs) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2.5, "Relevance to Public Health," contains basic information known about the substance. Other sections such as 2.7, "Interactions with Other Substances," and 2.8, "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 the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint 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. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

APPENDIX C

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, Distribution, Metabolism, and Excretion
AML	acute myeloid leukemia
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BEI	Biological Exposure Index
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
Ci	curie
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
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

APPENDIX C

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
MA	trans,trans-muconic acid
mCi	millicurie
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mm Hg	millimeters of mercury
mmol	millimole
mo	month
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NCE	normochromatic erythrocytes
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
PCE	polychromatic erythrocytes
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
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
UMDNJ	University of Medicine and Dentistry New Jersey
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
α	alpha
β	beta
δ	delta
γ	gamma
μm	micrometer
μg	microgram